LIFESTYLE INTERVENTION STRATEGIES FOR DIABETES MANAGEMENT

A thesis submitted for the Degree of

Doctor of Philosophy

by

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This thesis is dedicated to my grandparents,

Mr Tay Kheng Yong & Mdm Lee Guat Eng,

You instilled in me a love for reading, writing and research,

Mr Tan Tiong Tai & Mdm Pan Ah Yoke,

Diligence, tenacity and resolve are traits that I have learnt from you.

And my parents,

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You are my pillars of strength. Thank you for your agápē love.

For the glory of God

Thank you Jesus, the author and perfecter of our faith (Hebrews 12:2)

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ABSTRACT

The inexorable rise of type 2 diabetes (T2D) worldwide is a serious public health problem with significant health and socioeconomic costs. Diabetes- related complications are underpinned by poor glycaemic control that is greatly influenced by diet composition. Sustainable lifestyle modifications in diet and physical activity form the cornerstone of T2D prevention and management. Energy- restricted, high unrefined carbohydrate, low fat (HC) diets have traditionally been recommended for the dietary management of T2D. However, accumulating nutrition research indicate that carbohydrate restriction and higher intakes of protein and unsaturated fats, improve glycaemic control and reduce cardiovascular disease (CVD) risk markers. Based on this evidence, a novel dietary approach incorporating a very low carbohydrate, high unsaturated fat/ low saturated fat (LC) diet was designed. This LC diet was nutritionally adequate, with the potential to improve glycaemic control and mitigate CVD risk to a greater extent than the traditional HC diet. This thesis discusses the findings of a large, well- controlled, randomised, clinical trial that compared the long- term effects of consuming a traditional HC diet with an energy- matched LC diet, on a range of health outcomes including glycaemic control and CVD risk markers. Both diets were delivered as part of a holistic lifestyle intervention that included a structured exercise program. After one year, both diets achieved substantial weight loss, and reduced blood pressure, HbA1c, fasting glucose and LDL-C. However, the LC diet sustained greater reductions in diabetes medication and glycaemic variability, as well as triglycerides (TAG), and greater increases in HDL-C. Both diets had similar changes in renal and cognitive outcomes, suggesting that the LC diet did not adversely affect renal or cognitive function. These results have important implications for the lifestyle management of T2D with direct relevance to achieving better health outcomes and reducing healthcare costs.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Tay Jiahui (Jeannie Tay)

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GLOSSARY OF ABBREVIATIONS

ACCORD	Action to Control Cardiovascular Risk in Diabetes trial
ACR	Albumin creatinine ratio
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation trial
CBT	Cognitive behavioural therapy
CGMS	Continuous glucose monitoring systems
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration equation
CV	Cardiovascular
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DKD	Diabetic kidney disease
DSST	Digit Symbol Substitution Test
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
FBG	Fasting blood glucose
FMD	Flow-mediated dilatation
GL	Glycaemic load
GV	Glycaemic variability

HbA1c	Glycated haemoglobin A1c
HC diet	High carbohydrate, low fat diet
HDL-C	High density lipoprotein cholesterol
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LC diet	Very low carbohydrate diet
LDL-C	Low density lipoprotein cholesterol
MES	Medication effect score
MIND- ACCORD	Memory in Diabetes study of the ACCORD trial
MUFA	Monounsaturated fats
PPG	Postprandial glucose
RDA	Recommended dietary allowance
RCT	Randomised controlled trial
ROS	Reactive oxygen species
SCr	Serum creatinine
SMBG	Self- monitoring of blood glucose
TAG	Triglycerides
T1D	Type 1 diabetes
T2D	Type 2 diabetes

UKPDS	UK Prospective Diabetes Study
VADT	Veterans Affairs Diabetes Trial
VLDL-C	Very low density lipoprotein cholesterol

LIST OF PUBLICATIONS ARISING FROM THESIS

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Tay J, Thompson CH and Brinkworth GD. **Glycemic Variability: Assessing Glycemia Differently and the Implications for Dietary Management of Diabetes.** Annu Rev Nutr 2015;35:389-424

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CHAPTER 1. LITERATURE REVIEW

1.1 Background and introduction: obesity and type 2 diabetes

Obesity has reached epidemic proportions worldwide and represents one of the biggest public health challenges of the 21st century. Overweight is defined as a body mass index (BMI) of 25 kg/m^2 to 29.9 kg/m^2 and obesity as a BMI $\geq$ 30 kg/m^2 (1). Notwithstanding the limitations of these BMI categories (such as the inability to reflect visceral adiposity and the limited generalisability to diverse populations), these cut-offs broadly reflect morbidity and mortality risk in overweight and obese adults. In the past three decades, obesity rates have more than doubled (2). In 2014, nearly 2 billion (39%) adults (≥18 years of age) were overweight and 600 million (13%) were obese (2).

Obesity is pervasive, affecting all socioeconomic groups in both industrialised and developing nations. Seismic changes in lifestyles associated with rapid urbanisation, industrialisation, economic development and socioeconomic transition have led to 30% greater increases in childhood obesity rates in developing compared to developed countries (2). The effect of these changes on obesity rates reflect the nature of obesity being a multifaceted health problem with biological, behavioural and environmental causes and underscores the importance of lifestyle and diet interventions for obesity prevention.

Obesity induces multiple metabolic abnormalities leading to diabetes, hypertension, dyslipidaemia, cardiovascular disease (CVD), stroke and some cancers (1). As a major risk factor, obesity is blamed for the rising incidence of these non-communicable diseases (1). Obesity also increases the risk of musculoskeletal disorders and chronic conditions such as osteoarthritis, sleep apnoea, gallbladder disease and respiratory problems (1). These downstream health consequences are associated with increased morbidity and mortality, reduced quality of life, and impose a significant burden on both the individual and society.

As the risk of these chronic diseases increase in parallel with weight gain, there is much similarity in the strategies for obesity prevention and those for the prevention of chronic diseases, particularly type 2 diabetes (T2D) (3).

At the nucleus of the obesity problem is energy imbalance with decreasing energy expenditure from physical inactivity and excessive energy intake from the consumption of high calorie diets. Obesity complicates the management of T2D by augmenting insulin resistance and blood glucose concentrations (4). Consequently, lifestyle modification forms the cornerstone for obesity and T2D prevention and management, involving increased physical activity, and reduction in portion sizes and consumption of energy dense foods to achieve energy balance and weight control (5).

1.2 Diabetes mellitus and its clinical classifications

Diabetes Mellitus is a chronic, metabolic disease that is fundamentally characterised by deficient insulin production. Insulin is a hormone produced by the pancreas that regulates blood glucose levels by transporting glucose from the bloodstream into the body's cells for use as energy. The insulin deficiency that occurs in Diabetes may be caused by pancreatic β-cell failure and/or ineffective insulin action due to insulin resistance (6). The resulting elevated blood glucose concentration is a key metabolic abnormality that traditionally characterises diabetes and serves as a diagnostic marker of the disease. Uncontrolled diabetes results in persistent hyperglycaemia over time which damages nerves and blood vessels in the body. The duration of glycaemic burden is a strong predictor of the development of macrovascular and microvascular complications. These complications include diabetic retinopathy an important cause of blindness; nephropathy leading to renal failure, peripheral neuropathy which produces dysesthesias and loss of protective sensation thus increasing the risk of foot ulcers, infection, amputations and Charcot arthropathy; autonomic neuropathy manifested as orthostasis without an anticipated increase in heart rate, resting tachycardia, gastrointestinal and genitourinary disturbances; as well as cerebrovascular and CVD (5). These diabetes complications and associated ramifications are discussed in greater detail in Section 1.2.3.

Diabetes is one of the fastest growing non- communicable diseases globally, driven in part by population growth and aging. Based on international survey and epidemiological data (7), the number of people with diabetes has increased from 153 million in 1980, to 347 million in 2008. The global prevalence of diabetes in adults has increased in men (8.3% to 9.8%) and women (7.5% to 9.2%) during the same period. According to the International Diabetes Federation (8), diabetes now affects 415 million adults worldwide, including 193 million who are undiagnosed. A further 318 million or 1 in 15 adults have impaired

glucose tolerance, a form of prediabetes which increases their risk of developing diabetes. By 2040, an estimated 642 million people or 1 in 10 adults are expected to have diabetes. These steadily escalating prevalence rates highlight the severity of the problem.

There are two major forms of diabetes. In Type 1 diabetes (T1D) which accounts for 5-10% of diabetes, autoimmune destruction of pancreatic β- cells leads to absolute insulin deficiency and dependence on exogenous insulin for survival (5). While some forms of T1D are idiopathic, it has several genetic predispositions and has been associated with some environmental factors. In contrast, Type 2 diabetes (T2D) accounts for 90-95% of all diabetes and is often referred to as a "lifestyle disease" (8). In T2D, the problem is compounded by the concurrent presentation of defective insulin secretion due to progressive loss of β-cell function leading to insulin deficiency, on a background of insulin resistance rendering the insulin produced ineffective (5). While T2D has a strong genetic predisposition, its exact aetiology is not known. Excessive dietary intakes combined with physical inactivity, and excess weight gain leading to overweight and obesity are stoking a surge in T2D rates with 44% of diabetes worldwide being attributed to overweight and obesity (2). The paradigm for T2D has been evolving rapidly in recent decades. T2D was formerly considered an "adult- onset diabetes", associated with the socioeconomic elite, and occurred predominantly in western countries (9, 10). However, T2D is now increasingly diagnosed in children and adolescents (11). Asia now accounts for 60% of the world's diabetes population and the prevalence of T2D has increased in both developing and developed countries (8, 12).These changes have implications on the treatment approach for T2D, which will be discussed in Section 1.2.5.

Gestational diabetes (GDM) is a form of glucose intolerance diagnosed after the first trimester of pregnancy which has similar risk factors to T2D (5). Women with GDM have a 35-60% increased risk of developing T2D, and the hyperglycaemia in GDM complicates 2-10% of pregnancies (13, 14). Other rarer forms of diabetes (<5% cases) such as maturity-

onset diabetes of youth (i.e. monogenic diabetes), or latent autoimmune diabetes in adults, among others, may be caused by genetic defects in β-cell function or insulin action, endocrinopathies, diseases of the exocrine pancreas (e.g. cystic fibrosis) or medications (e.g. from glucocorticoid use) (5).

1.2.1 Diagnostic criteria for type 2 diabetes

Diabetes is clinically diagnosed based on glycated haemoglobin A1c (HbA1c, ≥6.5%) or plasma glucose criteria which include fasting plasma glucose (FPG, ≥7.0mmol/L) or 2-h plasma glucose after a 75g oral glucose tolerance test (2h- OGTT, ≥11.1mmol/L) (5). The HbA1c test should be performed using a standardised method traceable to the Diabetes Control and Complications Trial (DCCT) reference assay (15). In the absence of a measureable biological marker that differentiates between individuals with and without diabetes, the diagnostic thresholds for these tests are based on their association with an increased risk of developing diabetic retinopathy in epidemiologic studies and reflect the curvilinear continuum of risk (16).

There are pros and cons associated with each test and imperfect agreement between these criteria. The FPG test is less costly and more convenient and the 2h-OGTT test is a more sensitive assay compared to the HbA1c criterion which has been shown to detect lower diabetes prevalence compared to the glucose criteria (17). However, fasting is not essential for the HbA1c assay which is also less affected by interday fluctuations secondary to stress or illness. Although the HbA1c test is relatively costlier and has a limited availability in some developing countries (5). Moreover, HbA1c may inaccurately reflect glycaemia in conditions that affect erythrocyte lifespan such as iron deficiency, haemolytic anaemia and haemoglobianopathies.

These tests are also used to identify individuals with prediabetes, an intermediate state of impaired glucose homeostasis where plasma glucose is elevated above the normal range but below that of clinical diabetes and represents an increased risk of developing diabetes and CVD (5, 18). According to cut-offs defined by the American Diabetes Association (5), these individuals are deemed to have impaired fasting glucose (IFG; FPG 5.6-6.9mmol/L) or impaired glucose tolerance (IGT; 2h-OGTT 7.8-11.0mmol/L). IFG and IGT are commonly associated with the constellation of cardiometabolic aberrations constituting the metabolic syndrome that predispose to T2D and CVD- hyperglycaemia and insulin resistance, abdominal and visceral obesity, hypertension, raised triglycerides (TAG) and low high density lipoprotein cholesterol (HDL-C) (18).

1.2.2 Pathogenesis of type 2 diabetes

Progressive β-cell failure and insulin resistance primarily in the muscle and liver represent the core pathophysiologic defects and underlying cause of T2D (19-21). These abnormalities occur early in the pathogenesis of T2D with data suggesting that individuals in the upper tertile of IGT have lost over 80% of their β-cell function and are almost maximally insulin resistant (21). The pathophysiology of T2D is complex and multiple metabolic aberrations such as incretin deficiency and resistance in the gastrointestinal tract, hyperglucagonemia from α-cells, increased renal glucose reabsorption and deranged adipocyte metabolism are associated with the development of glucose intolerance in T2D (21).

A variety of environmental, genetic and lifestyle factors such as early life events including low birth- weight, intrauterine programming, nutrition and the expression of epigenetic

changes in adult life, physical inactivity, certain dietary factors, overweight and obesity have also been implicated in the genesis of diabetes and disease progression (10, 22-25).

While individuals may be genetically predisposed to insulin resistance, obesity and physical inactivity are insulin resistant states that add further strain to pancreatic β-cells. Both states cause the β-cells to increase insulin secretion to compensate for the further decrease in insulin sensitivity (26-28). These factors are associated with the initial development of insulin resistance, the transition from insulin resistance to impaired glucose metabolism (prediabetes) and eventually to overt T2D (19).

In the initial stage, insulin resistance stimulates the β-cells to increase insulin production to maintain normoglycaemia and the resultant hyperinsulinemia is a recognised indicator of insulin resistance (10). Insulin resistance is manifested by the impaired suppression of gluconeogenesis in the liver and by impaired glucose uptake by the muscles following ingestion of dietary carbohydrates. This insulin resistance occurs despite hyperinsulinemia and leads to hyperglycaemia (29, 30). As the disease progresses through IGT to T2D, insulin production and secretion fall following an inverted-U-shaped relationship. Consequently, glucose tolerance deteriorates due to progressive β-cell dysfunction and decreased insulin gene transcription (19, 31-33). The resultant chronic hyperglycaemia or glycotoxicity further induces β-cell apoptosis. This reduction in β-cells is not compensated by an increase in β-cell proliferation and neogenesis, thus potentiating the problem (31). Therefore this progressive loss in β-cell function determines the rate of T2D disease progression (21).

1.2.3 Diabetes complications and mortality rates

Diabetes is an independent and major risk factor for CVD such as heart failure, atrial fibrillation, coronary heart disease, myocardial infarction (MI), angina, coronary or other arterial revascularization, stroke, transient ischaemic attack and peripheral arterial disease of atherosclerotic origin (34). CVD is recognised as the main cause of morbidity and mortality in people with diabetes. CVD and cerebrovascular disease account for 50-60% of all mortality in people with diabetes (35) and half of all people with T2D are expected to develop heart failure, a heterogeneous syndrome related to multiple causes including atherosclerosis, hypertension and renal disease (36). In fact, diabetes is considered a "CV risk equivalent" because people with diabetes and without prior MI have as high a risk of an MI as individuals without diabetes but with a previous MI, after adjustment for age and gender (37).

Diabetic kidney disease (DKD) is the leading cause of end- stage renal disease (ESRD) and affects 20-40% of people with diabetes (5). Factors that increase the likelihood of a progression in DKD include a family history of kidney disease, raised lipids and/ or uric acid concentrations, increasing albuminuria or blood pressure, declining estimated glomerular filtration rate (eGFR) and the concurrent presence of macrovascular disease or retinopathy(38).

Diabetic retinopathy is another microvascular complication of diabetes that is the most common cause of new incidence of blindness in adults (5). Clinically significant macular oedema and proliferative diabetic retinopathy are complications that can lead to severe vision loss. Factors that increase the risk of diabetic retinopathy include the duration of diabetes, prolonged hyperglycaemia, nephropathy, hypertension and dyslipidaemia (39-42).

Diabetic neuropathies affect various aspects of the nervous system and have clinically diverse manifestations (43). Sensorimotor distal polyneuropathy and autonomic neuropathy are two of the most common neuropathies (43). Foot ulcers and amputations are late sequelae of diabetic peripheral neuropathy. Neuropathy and peripheral arterial disease are involved in the etiopathogenesis of foot ulceration that develop from a combination of internal (e.g. high foot pressures) and external factors (e.g. ill-fitting shoe) (43).

People with diabetes have about double the risk of mortality compared to the general population (44, 45). In 2015, diabetes was the direct cause of 5 million deaths worldwide (8). Total deaths from diabetes are expected to increase by >50% in the next decade (46) and diabetes is predicted to become the 7th leading cause of mortality worldwide by 2030 (47). Diabetes increases the risk of CVD and stroke, which is a major cause (50-80%) of death in people with diabetes (48). Moreover, people with diabetes often have comorbidities such as obesity, hypertension and dyslipidaemia which increase CVD risk and contribute disproportionately to mortality rates in people with T2D (49). The increased mortality observed in individuals with T2D is also attributed to other diabetes- related complications such as renal disease (48).

1.2.4 Economic impact and healthcare costs of diabetes

Diabetes imposes a hefty financial burden on individuals and their families and also has a significant economic impact on national health budgets and healthcare systems. The health expenditure for diabetes consists of direct costs incurred from the increased use of health services and medical care for T2D management. For most countries, the treatment of long term- diabetes complications such as CVD, stroke, kidney failure and blindness are the

largest contributors to the economic burden of diabetes (50). High incidences of complications, disability and premature mortality also cause indirect costs to society in the form of loss of productivity if affected individuals were actively contributing to the economy before diabetes onset (6, 8).

Compared with people without diabetes, the healthcare expenditures for people with diabetes are about two to three fold higher (51-54). Globally, healthcare expenditure for diabetes totalled US$673- 1,197 billion in 2015, equivalent to 12% of total health spending, which translates to an average of US$ 1,622- 2,886 per person spent on diabetes (8). The bulk of this expenditure on diabetes (75%) was for people in the age range from 50-79 years (8). This trend highlights the greater prevalence of diabetes and related complications in this age group, and the importance for treatment strategies targeting it. Most countries spend between 5-20% of their national health expenditure on diabetes and global spending on diabetes is predicted to increase 19% by 2040 to US$802- 1,452 billion. The increase in spending is fuelled by rising diabetes prevalence driven in turn by aging populations, population growth particularly in low- and middle- income countries, as well as increasing urbanisation and lifestyle changes (8).

The annual estimated cost of managing diabetes in the US has increased 41% from US$174 billion in 2007 to US$245 billion in 2012, comprising US$176 billion from direct medical costs and US$69 billion from lost productivity resulting from complications, and accounting for more than 1 in 5 healthcare dollars (45, 55). The main components of medical costs were inpatient care (43%), medications for treatment of diabetes complications (18%), anti- diabetes agents and supplies (12%) (55). Thus the increasing costs may be partially attributed to the advent of novel therapies and management tools for diabetes, including newer and costlier forms of insulin, insulin pump therapy, new classes of oral anti-hyperglycaemic medications, and intensive blood glucose monitoring devices (56).

China has the highest number of people living with diabetes in the word (114 million) (57), where the prevalence of T2D has more than tripled in the last decade (58). This exponential increase in diabetes rates has fuelled a 20% annual growth in drug sales and placed a strain on health services to provide basic care (58). In 2010, China had an annual diabetes- related medical expenditure of about US$26 million, which accounts for 13% of total healthcare spending (59). Latest estimates from the International Diabetes Federation suggest this figure has almost doubled to US$ 51 billion (8). The losses in national income from largely preventable deaths from diabetes and CVD between 2005- 2015 are estimated to reach US$ 558 billion in China (60).Given that only about 40% of people diagnosed with diabetes in China achieve adequate glycaemic control and the prevalence of prediabetes is 50% (57), medical costs are expected to increase rapidly if preventative measures are not applied as a growing number of Chinese with undiagnosed diabetes seek medical care and those who have been diagnosed with diabetes start developing preventable complications such as stroke, blindness and kidney disease (8, 59). Moreover, it may take years before more effective pharmacological treatments are added to healthcare reimbursement lists making these medications affordable and available to the masses. Potential barriers include governments being hesitant of accepting high costs reimbursement structures that overstretch national healthcare budgets (58).

Besides the rising costs, the healthcare expenditures of the various countries discussed above highlight another problem- the economic burden of diabetes affects less economically developed countries disproportionately. The high costs of treating and managing diabetes represent a considerable proportion of the national healthcare budgets of developing countries. Diabetes is thus a significant challenge to sustainable economic development in these emerging economies. Disparities in healthcare spending also mean that only 19% of the global health expenditure on diabetes was spent in low- to middle income- countries where some 75% of people with diabetes live (8).China has almost four

times as many people with diabetes than the US, yet a key difference is that an average of US$194 a year is spent treating each diabetes patient in China, compared to more than $7000 in developed countries such as the US (8, 55, 59). As much as a quarter of a family's income may be spent on diabetes care in a low-income family with one diabetic adult in India (50). People from low- and middle income countries may bear a larger proportion of medical costs because of the lack of access to health insurance and high quality care due to inadequate public health infrastructures (8, 61). Countries with high population growth are also often the ones with the lowest per capita spending for diabetes (8). The combined spending of US, China and Germany, three countries with the highest diabetes- related spending, made up 60% of the total global health expenditure on diabetes. Whereas India spent less than 3% of the global total expenditure on diabetes despite having the second highest number of people with diabetes in the world (69 million) (8).

In addition, emotional well- being is a key determinant of diabetes care and self-management (5). Beyond financial costs, psychosocial factors such as depression, pain, inconvenience, discrimination, diabetes- related distress and anxiety also exert intangible costs and lower the quality of life of people with diabetes (50).

In summary, diabetes incurs high healthcare costs, labour productivity losses and slower rates of economic growth. The rising costs of diabetes are unsustainable and underscore the importance of investing in cost- effective lifestyle interventions for preventing and managing T2D to stem the tide of the diabetes epidemic. These therapeutic options are discussed in greater detail in the next 2 sections (Sections 1.2.5 and 1.2.6).

1.2.5 Therapeutic options and treatment strategies for type 2 diabetes

Diabetes can be treated and managed by lifestyle strategies that include healthful eating and regular physical activity to achieve a healthy body weight. Treatment for diabetes may also include pharmacological approaches with anti- hyperglycaemic medications and insulin to control blood glucose levels.

The optimisation of glucose control is a key and consistent treatment goal in T2D to reduce the risk or slow the progression of diabetes- related complications, especially microvascular diseases such as diabetic nephropathy, neuropathy and retinopathy (5). Intensive diabetes management to achieve near- normoglycaemia in large, prospective randomised controlled trials (RCTs) in T2D such as the UK Prospective Diabetes Study (UKPDS), Action to Control Cardiovascular Risk in Diabetes (ACCORD) and Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trials have been shown to delay the onset or progression of albuminuria, a surrogate marker for DKD, and reduce the risk of microvascular complications including diabetic retinopathy (62-66). Optimal glycaemic control has also been shown to slow the progression of neuropathy in T2D (64, 67). Observational data suggest that neuropathic symptoms may improve with circumventing extreme blood glucose fluctuations (68).

1.2.5.1 Lifestyle management

An energy- controlled diet, regular physical activity and maintenance of a healthy weight play important roles in the prevention and treatment of T2D. The pathophysiology of T2D mandates that treatment should be based upon the reversal of known pathogenic abnormalities (e.g. insulin resistance), besides reducing HbA1c, and therapy should begin early to prevent/ slow the progressive β-cell failure that is already well established in

prediabetes individuals (21). To this effect, there is strong and consistent epidemiologic evidence that T2D can be delayed or prevented by a healthier lifestyle. Weight loss through caloric restriction alleviates and even reverses insulin resistance (69), and the addition of an exercise program further improves insulin sensitivity (70-72). Several clinical RCTs conducted in diverse ethnic and racial groups have demonstrated that diet and lifestyle modification are highly effective in preventing T2D (73-79) with 28-58% reduction in diabetes risk reported and effects sustained up to 20 years after the active intervention period.

Lifestyle modification is recognised as the cornerstone of diabetes care and should serve as the foundation for other diabetes treatment strategies such as pharmacology or bariatric surgery. Weight loss through diet, physical activity and behavioural therapy is recommended in overweight and obese individuals with T2D (5, 80). This recommendation, which has been the mainstay of diabetes management since time immemorial is based primarily on short- term studies that demonstrate the benefits of modest and sustained weight loss on improving glycaemic control and quality of life, as well as reducing CVD risk factors, obesity- related comorbidities and medications to control glucose, blood pressure and lipids (1, 81). Intentional weight loss by overweight/ obese adults with T2D has also demonstrated a significant benefit on mortality in a meta-analysis of cohort studies (82).

The long- term effects of intensive lifestyle intervention focusing on weight loss achieved through caloric restriction and increased physical activity in overweight/ obese adults with T2D, such as in the Action for Health in Diabetes (Look AHEAD) trial include greater improvements in glycaemic control (HbA1c) and CVD risk factors (e.g. systolic blood pressure, HDL-C) compared to the control program of diabetes support and education (83-86). Although the trial did not show that CVD morbidity and mortality were reduced with intensive lifestyle intervention and the differences in CVD risk factors between groups

attenuated over time (87), it demonstrated the feasibility of achieving safe, clinically meaningful (≥5%) and sustainable long term weight loss maintenance in 50% of patients after at least 8 years of intervention (88). At the end of the trial which was stopped after a median follow-up of 9.6 years, the average weight loss was 6% in the intervention group vs. 3.5% in the control group (87). Participants randomised to the intensive lifestyle group also achieved comparable CVD risk factor reductions with lower use of antihypertensive medications, statins and insulin than those in the standard care group (87). These reductions in medications have implications on healthcare costs.

The assiduous attention to CVD risk factor management that is part of standard care in both groups may have made the relative advantage of the intensive lifestyle intervention more difficult to establish (86, 89). This is demonstrated in a comparison of Look AHEAD with the Steno-2 trial, a smaller study of adults with T2D with moderately increased microalbuminuria conducted in the early 1990s that compared conventional therapy at that time with an intensified multifactorial intervention involving stepwise implementation of lifestyle modification, pharmacological therapy targeting hyperglycaemia, hypertension, dyslipidaemia, and microalbuminuria, before such an approach targeting concomitant risk factors became part of standard clinical practice (90). In contrast with Look AHEAD, Steno- 2 reported a long- term clinical benefit of lifestyle interventions combined with pharmacotherapy on the reduction in the development of nephropathy, progression of retinopathy and autonomic neuropathy, as well as macrovascular complications and mortality after ~13 years of follow-up (90, 91). The resulting difference in event rates in the 2 studies (the all cause- mortality rate in the control group for Look AHEAD was 8% compared to 50% in Steno-2) further illustrate the effect of improvements in standard care in lowering overall risk (87, 91). The disparity in event rates between the 2 studies also underscore the significance of the lower use of medications such statins, metformin and angiotensin- converting- enzyme inhibitors in Look AHEAD. The reduction in these

medications was an outcome that occurred in the intervention group in Look AHEAD because CVD risk factors improved with weight loss. Furthermore, based on evidence from the UKPDS, risk reductions for MI (-15-33%) and all-cause mortality (-13-27%) favouring intensive glucose therapy in people with T2D only emerged during the 10 years post- trial monitoring. These reductions in risk occurred despite the early loss in glycemic differences between groups after the first year (92). These results suggest that lifestyle interventions may only have a modest effect on macrovascular outcomes that require more than 10 years to become apparent as more events occur (92, 93). In addition, as the Look AHEAD trial tested a specific lifestyle intervention that focused on weight loss, it is unclear whether differences in dietary composition, such as with a very low carbohydrate (LC) diet, could affect CVD endpoints differently.

Other benefits observed in the intervention group included a greater likelihood of partial diabetes remission (94), reductions in depression (95), obstructive sleep apnoea (96), urinary incontinence (97), as well as improvements in physical functioning (98), mobility (99), health- related quality of life and physical fitness (100). It is important to note that these health outcomes were achieved in a study where the level of intervention was more intensive compared to other lifestyle intervention trials in diabetes such as the Diabetes Prevention Program (DPP) (101) which has implications for clinical practice. Look AHEAD had higher individual weight-loss goals (10%), lower calorie and dietary fat targets based on initial body weight, higher physical activity goals (≥175 minutes moderate intensity activities / week) including strength training, the option of using a weight loss medication orlistat for a short period, more frequent, ongoing contact comprising group and individual sessions, stepped- care protocols and use of more structured dietary approaches from inception including meal replacements to improve dietary adherence and combined fat and calorie counting (102, 103).

Large- scale lifestyle intervention trials such as the Look AHEAD and DPP incorporate several behavioural interventions that target multiple health behaviours including physical activity and dietary modifications with a focus on weight loss or management (104). These trials provide some insight on important features required for the successful implementation of lifestyle interventions, various aspects of which are reflected in the most recent American College of Cardiology/ American Heart Association/ The Obesity Society guidelines for the management of overweight and obesity in adults (1). For achieving weight loss, the guidelines (1) recommend comprehensive lifestyle interventions that comprise a moderately reduced- calorie diet, a program of increased physical activity and the use of behavioural strategies to enhance adherence. These interventions should be provided in- person by trained interventionists from multidisciplinary teams of medical, nutrition and behaviour health professionals, with regular contact (≥14 sessions in 6 months) in individual or group settings. In contrast, the strategies for long term weight loss maintenance after successful weight loss differ from the strategies for achieving weight loss (1). For individuals who have lost weight successfully, participation in comprehensive long- term weight maintenance programs and the readiness to try different approaches have been shown to produce better long- term results (1). As continued contact is associated with better weight loss maintenance, these programs should provide long- term support with at least monthly in- person or telephone contact after the initial weight loss treatment. Other strategies associated with the better long- term results include ongoing self-monitoring of body weight (at least weekly), consumption of a hypocaloric diet (portion control), participation in high levels of exercise (200—300 min/ week) as well as weight maintenance counselling (1, 80).

1.2.5.2 Pharmacological therapy

Lifestyle modification to lose weight and improve glycaemic control is usually the first step for T2D management. Pharmacological therapy is generally initiated as an adjunct to lifestyle modification to augment lifestyle improvements when the latter alone is insufficient to achieve and maintain treatment goals. A meta-analysis found that HbA1c would be lowered by 0.9-1.1% for every new class of non- insulin agent added to initial therapy (105). However, cost effectiveness models have suggested that some newer anti-hyperglycaemic agents may offer comparatively lower value due to higher cost and modest glycaemic efficacy (56).

This is particularly important because multiple- drug therapy may be required to achieve treatment goals and cost considerations, formulary restrictions and potential side effects serve as barriers to medication adherence (5). Consequently, there are benefits if blood pressure, lipid and glycaemic goals can be achieved with fewer drugs and minimal side effects. With lipid lowering drugs, statin/ fibrate combination therapy is associated with an increased risk of abnormal transaminase levels, myositis and rhabdomyolysis (106). Statin use has also been shown to lead to a small increase in risk of incident diabetes although this is offset by a reduction in CVD event rate (107). Polypharmacy of diabetes medications as well as insulin and insulin secretagogues such as sulphonylureas are associated with potential adverse effects such as hypoglycaemia and weight gain (108). Phase 4 clinical trials evaluating the effect of oral anti-hyperglycaemic agents on heart failure and mortality outcomes have showed varied results. Long- term use of thiazolidinediones has been found to be consistently associated with a significantly increased risk of heart failure (109, 110). However, a recently approved diabetes treatment, empagliflozin, an inhibitor of sodium-glucose cotransporter 2, reduced the composite cardiovascular (CV) outcome of MI, stroke and CV death by 14%. This overall

reduction in CV outcome was ascribed to a 38% relative risk reduction in CV death, in patients with T2D at high risk for CVD (57% had diabetes for >10years and 70% had a previous history of a stroke or MI) (111).The empagliflozin group also had a 32% relative risk reduction in all-cause mortality compared to the placebo group (111).

1.2.5.3 Bariatric surgery

Bariatric surgery is now acknowledged as a treatment option for obese adults (BMI>35kg/m^2) with T2D, and particularly for individuals with diabetes or comorbidities refractory to lifestyle or pharmacological therapy (5). In the Swedish Obesity study, a non-randomised cohort study comprising patients with T2D who had under gone bariatric surgery, diabetes remission was achieved in 72% of patients 2 years following surgery compared with 16% in a matched control group treated with lifestyle and pharmacological agents (112). After 15 years of follow- up, diabetes remission rates decreased to 7% in the control group and 30% in the surgery group (112). Micro- and macrovascular complications including MI rates were also lower in the surgery group (112, 113). Some studies have also showed that bariatric surgery may be cost- effective for people with T2D (114, 115). However, these conclusions are based on assumptions about the effectiveness and safety of the surgical procedures and there are concerns about the potential long- term adverse effects of bariatric surgery such as nutrient deficiencies, dumping syndrome, osteoporosis, severe hypoglycaemia from insulin hypersecretion and an increased risk of substance abuse (5, 116). Therefore, lifestyle modification remains the primary therapeutic approach for T2D management.

1.2.6 Treatment and management of co-existing cardiovascular risk factors: hypertension and dyslipidaemia

CVD risk is increased in diabetes and prediabetes, and the presentation of T2D is often clustered with other CVD risk factors. Individuals with T2D have a prevalence of 71-85% for hypertension, 65-80% for elevated LDL-C, and 60-70% for obesity (45, 117, 118). Another critical component of diabetes management therefore involves multifactorial CVD risk reduction through blood pressure and lipid management.

As discussed in section 1.2.5.1, lifestyle strategies have been shown to improve glycaemia, blood pressure and lipid control. The management of hypertension consists of weight loss if overweight or obese, and involves a combination of lifestyle and pharmacological therapy. Hypertensive individuals with T2D have a 66-100% higher risk of vascular complications than with either condition in isolation (119). A systematic review and meta-analysis showed that every 10 mmHg reduction in systolic blood pressure (SBP) in adults with T2D was associated with significantly lower risk of mortality, CVD events, coronary heart disease, albuminuria and retinopathy (120).

With regards to lipid management, CVD mortality risk rises exponentially as a function of serum cholesterol levels (121). Statins, which are regarded as the first- line pharmacological agents for lowering low density lipoprotein cholesterol (LDL-C), have been shown to reduce the CVD risk of people with diabetes (5). Although the LDL-C lowering response to statins can be highly variable (122), clinical trials in people with diabetes have demonstrated significant primary and secondary prevention of CVD events and coronary heart disease mortality with statin therapy (123, 124). The reduction of CVD events with statins has been shown to be strongly correlated with the absolute reduction of LDL-C achieved (125). A meta-analysis of 18 686 individuals with diabetes from 14 randomised trials of statin therapy (mean follow-up 4.3 years) showed a 9% proportional

reduction in all- cause mortality and 13% reduction in vascular mortality for each mmol/L reduction in LDL-C (126). A Cochrane review found reductions in all-cause mortality, major vascular events and revascularisations with no excess of adverse events in trials that included individuals with T2D without CVD, treated with statins (127).

The most prevalent pattern of dyslipidaemia in T2D is a combination of high TAG, low HDL-C and increased concentration of small dense LDL-C particles which is ascribed to increased free fatty acid flux due to insulin resistance (121). However, the evidence for using pharmacological therapy to target these lipid fractions in mixed dyslipidaemia is considerably weaker than for statin therapy (5, 128). There is also limited evidence to support aggressively increasing HDL-C levels beyond what can be achieved by lifestyle modification alone which has been shown to increase HDL-C by 20-30% (128). Furthermore, diet and lifestyle modifications including alcohol abstinence, which lead to improvements in glycaemic control have been found to benefit patients with elevated TAG (5, 129).

With regards to assessing residual atherogenicity in statin- treated individuals with T2D and greater propensity to present with small LDL-C particles by measuring non-HDL-C, lipoprotein particles or apolipoprotein B, However, there are currently no clear treatment guidelines for these alternative lipoprotein goals which limits their clinical applicability (130)

Furthermore, lipoprotein abnormalities associated with insulin resistance and T2D include a predominance of small, dense LDL-C particles which are an independent predictor of CVD (131-133). These small dense LDL-C are not detected by standard lipid testing and may be present in patients despite normal LDL-C (130, 132). This residual atherogenicity may be assessed by measuring non-HDL-C, apolipoprotein B, lipoprotein particle size and

density. However, there are currently no clear treatment guidelines for these alternative lipoprotein goals which limits their clinical applicability (130)

Taken together, lifestyle modifications encompassing an energy restricted diet and increased physical activity with a consequent reduction in weight, are effective primary and secondary prevention strategies for T2D. These lifestyle strategies also confer additional health benefits beyond lessening the impact of diabetes and its complications by reducing obesity, CVD and some cancers (50).

1.3 Assessment of glycaemic control in type 2 diabetes

Glycated haemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) are typically used to assess glycaemic control in current clinical practice.

1.3.1 Conventional markers of glycaemic control

FPG is a measure of plasma glucose levels after an 8-12 hour fast, reflecting blood glucose regulation in the absence of dietary glucose input (134). Advantages of FBG include its relatively lower cost and ease of measurement to provide immediate information. However, single, isolated FBG measurements provide inadequate characterisations of diurnal blood glucose trajectories and do not accurately reflect long-term glucose concentrations (135-137). FPG correlates modestly with indices of hyperglycaemia and poorly predicts HbA1c and postprandial glucose (PPG) (137, 138). The exclusive use of FBG has also failed to identify individuals at increased risk of postprandial hyperglycaemia- associated mortality (139, 140).

The HbA1c assay is a well- established predictor of diabetes complications risk (141, 142). It is often considered the "gold standard" for assessing whether glycaemic targets have been met and maintained and for determining the effectiveness of therapy (108, 143). HbA1c reflects long- term glucose control and provides an indication of the mean level of glycaemia in the preceding 2-3 months, corresponding to the half-life of erythrocytes (144). However, as previously described in Section 1.2.1, the HbA1c test is subject to several limitations including inaccuracy of the test in haemoglobinopathic conditions and its relatively higher cost.

In addition, growing evidence suggests that the onset and progression of diabetes complications may be influenced by other glycaemic markers beyond HbA1c and FPG. Glycaemic variability (GV), a measure of the amplitude, frequency and duration of

glycaemic fluctuations around mean blood glucose is poorly characterised by HbA1c (145-147) but is now recognised to contribute to diabetes-related complications (148). This has raised interest for the use and assessment of GV in clinical diabetes management and in therapeutic approaches that target and modify GV. Section 1.3.3 and Chapter 2 of this thesis provide further discourse on the concept and evidence for GV.

1.3.2 Glycaemic targets and their relationship to clinical outcomes

The role of hyperglycaemia, a salient feature of diabetes in the pathogenesis of micro- and macro- vascular complications, is well- established and the goal of nutrition and medical therapy in diabetes is to prevent these by maintaining good glycaemic control (5). Importantly, there exists a continuum of risk for diabetes complications with all glycaemic measures which increases with increasing hyperglycaemia (141). The following section provides a summary of the evidence in this area and Chapter 2 of this thesis provides a more comprehensive discussion of the topic.

The increased use of HbA1c to monitor long- term glycaemic control in diabetes is largely derived from data originating from landmark diabetes trials such as the Diabetes Control and Complications Trial (DCCT) and UKPDS that showed HbA1c is strongly correlated with diabetic complication risks in people with T1D and T2D, respectively (141, 142). Data from the UKPDS demonstrated a risk reduction of 21% for diabetes- related deaths, 14% for myocardial infarction, and 37% for microvascular complications, for every 1% reduction in HbA1c in newly diagnosed individuals with T2D (141). These reductions in microvascular complications achieved with intensive glycaemic control early in the UKPDS persisted during the 10 years of post-trial follow- up where significant reductions in all- cause mortality and MI were also observed (92).

The HbA1c targets currently used in clinical practice are based on decreased rates of onset and progression of microvascular complications observed in several large scale randomised controlled trials such as UKPDS, ACCORD, ADVANCE and the Veterans Affairs Diabetes Trial (VADT) (64, 65, 92, 149). Although there are no specific glycaemic thresholds for particular diabetes complications, the attainment of near normal HbA1c levels in people with T2D is recommended. However, this should be balanced with consideration of the risk of hypoglycaemia (141). In general, the HbA1c goal for adults with diabetes is <7% which may be individualised depending on the patient's health status (e.g. comorbidities, life expectancy, history of severe hypoglycaemia). In epidemiological associations, further lowering of HbA1c to 6% was still associated with additional reductions in microvascular complication risk but also with a significantly increased risk of hypoglycaemia (141).

In contrast, intensive glycaemic control that achieved mean HbA1c levels between 6.4-6.9% in ACCORD, ADVANCE and VADT did not produce significant reductions in CVD events (65, 149, 150). 6- year follow- up in ADVANCE also did not show long- term benefits with regards to macrovascular events (151), although 10- year follow- up in the VADT did show a reduction in the risk of CV events, but without an improvement in overall survival (152). Differences in the glycaemic targets achieved, treatment approaches and study population characteristics in these trials may explain the differences observed (153). Unlike in the UKPDS, the participants in ACCORD, ADVANCE and VADT had more advanced T2D and either known CVD or multiple CVD risk factors. Collectively, these results suggest that the rate of increase in risk for microvascular disease with hyperglycaemia may be greater than that for macrovascular disease and intensive glycaemic treatment to lower CVD risk may be more effective early in disease development, such as in individuals newly diagnosed with T2D and similar to the UKPDS (153).

PPG is another glycaemic target that contributes to GV and is usually measured 1-2 hours after meals, corresponding to the time to peak glucose in individuals with diabetes (154). Elevated PPG characterised by postprandial hyperglycaemia and acute glucose excursions may occur even in individuals with apparently satisfactory glycaemic control (HbA1c<7%) (136, 155, 156), and is involved in the pathogenesis of diabetes complications (157-159) including CVD (160). This is supported by evidence from epidemiological (139, 161-171) and interventional studies (172-175) that show elevated PPG is an independent predictor and risk factor for CVD and all-cause mortality. The target for post- meal glucose is 9.0 mmol/l, provided hypoglycaemia is avoided (176).

1.3.3 Glycaemic variability

GV is an emerging target for diabetes management with a growing body of evidence showing a clinically significant relationship between GV and the development of diabetes complications (177-182). Evidence for the contribution of GV towards diabetes- related complication risk beyond PPG is provided by studies that show greater hyperglycaemia-induced endothelial dysfunction when initial basal blood glucose levels are lower and the resultant oscillation responses are larger (183), and declines in oxidative stress markers following reductions in hyperglycaemic excursions that lower GV (184). These results suggest that the deleterious effects on endothelial function and oxidative stress caused by oscillating glucose levels may be more harmful for the CV system than chronic sustained hyperglycaemia (179, 182).

The prognostic significance of GV would be further clarified with more data regarding the role of GV on the incidence or progression of clinical end points (such as myocardial infarction, stroke, retinopathy, etc.). Most studies at present have investigated the effect of GV on surrogate markers of CVD and diabetes complications (such as flow- mediated

dilatation, urinary excretion of 8-iso-PGF2α- a recognised marker of oxidative stress, left ventricular mass, etc.). The absence of a uniformly accepted standard of measuring GV further confounds interpretation of the literature. However, technological advancements in continuous glucose monitoring systems (CGMS) will permit more precise assessment of the relationship between GV and vascular damage, clinical end-points or diabetes complications. By providing continuous information on blood glucose dynamics in real life, CGMS addresses some of the limitations of single capillary blood glucose measurements such as with self- monitoring of blood glucose (SMBG).
Section 1.3.4 discusses SMBG and CGMS in greater detail and Chapter 2 further expounds on the pathophysiological mechanisms linking GV with the development of diabetes complications.

1.3.3.1 Nutritional management of glycaemic variability

Insulin deficiency may increase GV (185), which suggests that improvements in insulin sensitivity from diet- induced weight loss may stimulate corresponding improvements in GV (186). A variety of factors including poor matching of carbohydrate intake with insulin availability, irregularity in meals and snacking, gastroparesis, delayed or missed administration of prandial insulin, inadequate matching of basal insulin, or need for insulin pump therapy have also been implicated in increasing GV (185). Chapter 2 describes the effect of several nutrition factors on GV. These factors include carbohydrate quality, quantity and distribution, protein and fibre which can serve as targets in nutrition therapy to reduce GV and optimise glycaemic control. These findings accentuate the importance of considering dietary factors beyond energy restriction in diabetes and GV management.

1.3.4 Glucose monitoring in diabetes care

The day- to –day effectiveness of a diabetes management plan on glycaemic control can be assessed by SMBG which enables an individual to evaluate if glycaemic targets are being met. SMBG is thus a useful tool for facilitating timely intervention to achieve and maintain glycaemic control through adjustments in medication regimens, nutrition therapy and physical activity when the blood glucose information obtained is integrated into self-management and clinical plans (5). More frequent and structured SMBG has been associated with better glycaemic control in people with T2D (187, 188).

However, the computation of GV from SMBG readings requires the patient to collect sufficient capillary blood glucose samples to obtain an adequate representation of a typical diurnal pattern. The invasiveness and inconvenience posed to patients from frequent blood glucose measurements may pose a constraint to getting sufficient blood samples . Therefore, SMBG may only provide limited information based on irregular blood glucose measurements, potentially missing hyperglycaemic and hypoglycaemic fluxes in actual glucose trends (148).

1.3.4.1 Continuous glucose monitoring

Recent technological advancements have led to the development and increased use of CGMS which are holter-like sensor systems that continuously measure subcutaneous interstitial glucose levels at 5 minute intervals, 24 hours a day, 7-days a week (189). CGMS provides ongoing feedback on an individual's response to therapy including the effects of medication, meals, stress, exercise and other factors that affect glucose levels, in a continuous manner. Comprehensive diurnal blood glucose profiles can be generated under free-living conditions using CGMS and this data can be used for the computation of

GV parameters. Compared to SMBG, CGMS confers greater precision and enables differentiation of the effects of a reduction in sustained chronic hyperglycaemia from those of acute glucose fluctuations. Results from a meta- analysis have also showed that, compared with SMBG, real- time CGM achieved a greater reduction in HbA1c of 0.26%, without any difference in severe hypoglycaemia (190). Although most studies were small, of short duration and conducted in people with T1D.

However, as CGMS measures interstitial glucose which may lag behind actual blood glucose measurements by several minutes due to physiological factors, calibration with SMBG is still required particularly for making acute treatment decisions (5). Nonetheless, CGMS is recognized as a useful adjunct to SMBG particularly in those who experience frequent hypoglycaemia or hypoglycaemia unawareness (191).

Both SMBG and CGMS are important components of diabetes management although the utility of both methods is dependent on patients receiving training for the correct use of both devices, interpreting test results and adjusting their treatments accordingly to achieve good glycaemic control (5). Information on the different GV parameters derived from CGMS data, including their characteristics, advantages and disadvantages are discussed in Chapter 2.

1.4 Lifestyle management of type 2 diabetes

The lifestyle management of T2D centres on the need to promote lifelong healthy dietary patterns with regular exercise to maintain a healthy weight throughout life. The current literature supports the effectiveness of nutrition therapy and physical activity in preventing and managing T2D (192-195). Weight loss is recommended as a strategy for managing glucose control for people with T2D who are overweight or obese, especially in those early in the disease process (94, 196).

1.4.1 Physical activity guidelines for type 2 diabetes

Successful approaches in the lifestyle management of T2D have included regular physical activity and behavioural interventions to help sustain improved lifestyles (197). Physical activity has an important role in diabetes management and exercise, as an adjunct to dietary advice, has been shown to improve HbA1c in people with T2D at 6 and 12 months (198). Being a key determinant of daily energy expenditure, physical activity is also fundamental to energy balance and weight control. A recent large meta- analysis of RCTs showed that exercise significantly improved cardiorespiratory fitness and cardiometabolic biomarkers such as lipids, HbA1c and insulin sensitivity, with greater benefits in people with T2D (199). Increased fitness is also associated with a lower risk of developing T2D independent of demographic characteristics and baseline risk factors (200).

Adults with T2D are recommended to undertake ≥150 min/ week of moderate- intensity aerobic physical activity (50-70% of maximum heart rate), spread over at least 3 days/ week with no more than 2 consecutive days without exercise (5, 201). Resistance training involving all major muscle groups is recommended twice/ week (5, 201). Combined aerobic and resistance training has been shown to confer additive benefits on HbA1c (202,

203). A meta- analysis reported that structured exercise interventions lasting at least 8 weeks reduced HbA1c by 0.66%, even in the absence of significant weight reductions (204). Prolonged exercise durations and higher exercise intensities have been associated with further improvements in insulin action, HbA1c and cardiorespiratory fitness (205-208).

The beneficial effects of regular physical training on insulin action and overall glucose tolerance are mediated by several factors, including increased muscle mass, augmented blood perfusion to the muscle and capillary area, improved mitochondrial oxidative enzyme capacity, and activation of the glucose transport system leading to augmented glycogen synthesis to supplement intramuscular glycogenolysis, enhanced glucose transport and accelerated muscular glucose disposal (28, 204, 209, 210). Both aerobic and resistance training mediate their effect by different synergistic mechanisms to improve glycaemic control therefore providing support for the benefits of combined training for achieving more effective glycaemic management (206). Aerobic exercises stimulate glucose uptake via enhanced whole- body insulin action, independent from changes in muscle mass or aerobic capacity as well as by muscle contraction- mediated insulin-independent pathways not impaired by T2D or insulin resistance (211, 212). Whereas resistance training builds muscle mass which increases muscular blood glucose uptake separate from the muscles' inherent insulin sensitivity (211, 213). The resulting additive increase in glucose tolerance from combined training may persist for up to 72 hours after exercise (212, 214, 215).

Furthermore, physical training also stimulates fat oxidation and reduces hepatic and visceral lipids, even without weight loss (72, 216, 217). A meta- analysis showed that physical training in adults with T2D reduces LDL-C by ~5% and greater benefits on lipid profiles are observed when exercise is combined with weight loss (218). The Look AHEAD trial also demonstrated that increased physical activity can improve fitness,

reduce depression symptoms and improve health- related quality of life in people with T2D (100). However, despite these beneficial effects of exercise, the recommended physical activity guidelines are not met by the majority of people with T2D (219), highlighting the urgency to identify effective strategies to raise physical activity levels in people with T2D.

Compared with physical activity levels needed to improve glycaemic control and CV health, a greater volume of exercise (about seven hours/ week at moderate to vigorous intensity) is required to achieve significant long-term weight loss maintenance (204, 220). Up to 60min/ day of exercise is necessary if the caloric deficit for weight loss is derived solely from physical activity (201). Physical activity (one hour of daily moderate aerobic exercise) has been shown to produce greater improvements in insulin sensitivity and at least similar reductions in body fat compared to isoenergetic calorie restriction (221, 222). Exercise also reduced abdominal adiposity in the absence of weight loss (221, 222). Supervised exercise training by qualified exercise professionals has been associated with better adherence and improvements in glycaemic control (206, 223). Taken together, these findings have important implications in the design of strategies that promote physical activity for successful weight and diabetes control.

1.4.2 Dietary management of type 2 diabetes

Nutrition therapy, defined as the treatment of a disease or condition through dietary modification of nutrient or whole-food intake, plays an integral role in diabetes management and reducing potential complications related to poor glycemic, lipid or blood pressure control (80, 224). Findings from RCTs and systematic reviews provide support for the effectiveness of nutrition therapy for improving glycemic control and CVD risk markers in T2D (80).

Leading health authorities now advocate an individualized approach to the dietary management of diabetes to achieve individualized glycemic, blood pressure and lipid goals, and delay or prevent diabetes complications (80, 196, 225). The diet choice should be personalized based on total calorie and metabolic goals, health status, individual and cultural preferences, health literacy, readiness to make behavioral changes, ability to adhere to the chosen diet, and any impediments to change (5, 80). All calorie restricted diets, regardless of macronutrient composition are effective for achieving weight loss (226-228) and foods with the greatest consensus for improving health are encouraged (80). Diet plans that are adjusted for the individual's baseline weight and which provide ~1200-1500 kcal / day for women and 1500- 1800 kcal/ day for men (or a calorie deficit of 500-750 kcal/day) are recommended for achieving weight loss (5).

1.5 Effects of dietary macronutrients on glycaemic control and cardiovascular disease risk in type 2 diabetes

Dietary manipulation of macronutrients is critical for optimising glycaemic control and dietary patterns that limit postprandial hyperglycaemia are advantageous for diabetes management.

1.5.1 Dietary fats and protein

In people with T2D, ingested protein can increase insulin response without increasing plasma glucose concentrations (5, 229). Furthermore, consumption of dietary fat or protein before a meal independently attenuates PPG excursions in people with T2D through insulin secretion and stimulating an incretin response. This incretin response delays gastric emptying by potentiating the secretion of gut hormones such as glucagon-like petptide-1 (GLP-1) and glucose- dependant insulinotrophic polypeptide (GIP) in the small intestine (230, 231). This suggests that a dietary pattern that is high in protein and fat may be beneficial for improving blood glucose control in people with T2D.

Dietary fat is significantly implicated in CVD aetiology although evidence indicates that fat quality rather than quantity per se may be the more important determinant when considering metabolic goals and CVD risk (232). An increased saturated fat intake adversely influences CVD risk profile by promoting insulin resistance (233), and elevating LDL-C levels (234) which is a primary therapeutic CVD risk target (235-237).

In contrast, clinical trials have shown that high unsaturated fat intakes favourably modify lipoprotein profiles and reduce the risk of MIs (236). These findings are consistent with epidemiologic data that show inverse associations between unsaturated fat intake and CVD

(238). Diets that replace saturated fats with unsaturated (mono- and polyunsaturated) fats improve insulin sensitivity(233) and blood lipid profiles by reducing total cholesterol, LDL-C and triglycerides, and increasing HDL-C, thereby reducing CVD risk (239, 240). These findings are reflected in current nutrition guidelines which recommend reducing saturated fat (<10% of calories), cholesterol and trans fat intake as well as increasing plant stanols/ sterols, omega- 3 fatty acids and soluble fibre to improve the lipid profile of people with diabetes (5, 237).

In people with diabetes, high monounsaturated fat (MUFA) diets akin to Mediterranean-style diets have demonstrated beneficial effects on glycemic control and lipoprotein profiles, without adversely affecting LDL-C (241-245) and its use is strongly supported in the literature for people with high TAG and very low density lipoprotein cholesterol (VLDL-C) levels (246-248). Besides being considered a more palatable alternative, there is also no evidence that such MUFA enriched diets induce weight gain in people with T2D provided energy intake is controlled (248). These diets have thus been proposed as an effective alternative to traditional low fat, high carbohydrate (HC) diets in recent guidelines (5).

Additionally, a high saturated fat diet adversely affects endothelial function as assessed by brachial artery flow-mediated dilatation (FMD) (249), an important prognostic predictor for future cardiac events (250), whereas high MUFA diets have been shown to improve FMD (251, 252). Endothelial dysfunction precedes the appearance of clinical CVD and is implicated in its pathogenesis (253).

1.5.2 Dietary carbohydrates

Of all macronutrients, the total amount of carbohydrates ingested is the greatest determinant of PPG levels (254) although carbohydrate type or source also influences glycaemia (255, 256). The overall response is in turn dependant on insulin production, insulin resistance and medication dose (257), therefore highlighting the importance of monitoring blood glucose response to dietary carbohydrate for improving PPG control in diabetes (5).

Studies suggest that HC diets may worsen insulin resistance by accentuating hyperinsulinemia and hyperglycaemia (258, 259), making such diets unfavourable for insulin- resistant T2D patients. HC diets have been found to reduce LDL-C (260, 261), and are associated with hypertriglyceridemia (262) and reduced HDL-C (239), which are CV risk factors shown to precede the onset of T2D (263, 264). While these undesirable effects on blood glucose, insulin and lipids may be circumvented by energy restriction and weight loss, HC diets have shown unsatisfactory long- term weight loss results (265). The long term consumption of HC diets have also not been shown to prevent diabetes or CVD (266, 267).

The relative proportion of fat and carbohydrate in a diet may be less important than dietary fat quality and energy restriction for lowering TAG (259). Isoenergetic replacement of saturated fatty acids with carbohydrates has been shown to increase total: HDL cholesterol, a more specific marker of CVD, justifying some caution in the application of HC diets for CVD prevention (240). Nevertheless, carbohydrate- rich foods that have a low glycaemic index or are rich in soluble fibre may mitigate the potential for adverse glycaemic control and hyperlipidaemia (268). Hence wholegrain foods, vegetables, fruits, legumes, dairy products and high- fibre foods are recommended in the current nutrition guidelines for

diabetes while foods high in refined grains and added sugars are discouraged, for good health (5).

Conversely, reducing total carbohydrate intake reduces glycaemic load (GL) and improves glycaemic control. In support of this, previous research has shown an absolute reduction in mean HbA_{1c} by 2.2% (from 9.8% to 7.6%) independent of weight loss, with carbohydrate restriction, during energy balance (269). A Cochrane review also reported HbA1c reductions of -0.2% to -0.5% with lower glycaemic load diets (256). In patients with IGT, PPG was similarly lowered when an LC or low glycaemic index diet was consumed (270). Foods lower in glycaemic load are thus recommended for people with T2D (5).

Collectively, the evidence presented in this section suggests that higher unsaturated fat (particularly MUFA) intakes, on the premise of a controlled energy intake with carbohydrate restriction and limited saturated fat content, may be advantageous for improving glycaemic control and reducing CVD risk in patients with T2D.

1.6 Very low carbohydrate diets

Despite the benefits attributed to the lifestyle management of T2D outlined in preceding sections, the type of dietary therapy that is most efficacious in the long term for patients with T2D remains equivocal and debated. Importantly, different dietary patterns may vary in their efficacy for improving glycaemic control and reducing CVD risk which are key considerations in identifying the optimal dietary approach for T2D management. The previous section 1.5.2 has highlighted the growing evidence supporting the benefits of carbohydrate restriction for improving markers of blood glucose control, and the recommendation of foods with lower GL for people with diabetes in current nutrition guidelines (5, 80). However, while these guidelines acknowledge that a variety of eating patterns including very low carbohydrate (LC) diets are modestly effective in managing diabetes, it has been highlighted that there is insufficient evidence in isocaloric comparison to recommend an ideal quantity of carbohydrates for people with diabetes (5, 80, 225).

The physiological premise for carbohydrate restriction resides in the body's adaptive responses to the lower exogenous glucose availability, which leads to changes in insulin and glucagon concentrations that favour fat oxidation as oppose to fat storage, and improves glucose regulation by decreasing postabsorptive glycogenolysis (271, 272). While there is currently no clear consensus on what defines an LC diet, the following review will examine studies that have investigated the metabolic effects of LC diets which limited carbohydrates to <20- 70g/ day (254, 272, 273). These diets are also known as low carbohydrate ketogenic diets because such restrictions typically lead to ketosis and the presence of measurable ketones in the urine, reflecting a metabolic shift from using glucose to fatty acids and ketone bodies as fuel sources (272, 273). LC diets are thought to confer metabolic advantages, although a continuous response to carbohydrate restriction has been noted beyond the apparent threshold response (272).

Energy restricted, HC diets have traditionally been prescribed for the dietary management of T2D and have been the predominant public health recommendation for weight management for the past several decades (196, 274-278). However, accumulating evidence in nutrition research indicate that carbohydrate restriction and higher intakes of protein and unsaturated fats, independent of weight loss, improve glycaemic control and reduce some CVD risk markers, potentially conferring greater benefits over traditional HC diets (254, 260, 279-282). A systematic review and meta- analysis of different dietary approaches for T2D management concluded that LC diets led to significantly greater improvements in glycaemic control ($HbA_{1c,}$ -0.12%), weight loss (-0.69kg), increase in HDL (0.08mmol/L) with no significant reduction in LDL, relative to control diets (276). LC, higher-protein diets may also have beneficial effects on the changes in weight and body composition during energy restriction for weight loss. The results from a meta-regression which compared dietary interventions that provided >1000kcal/day from 87 trials found that diets with a lower carbohydrate composition (≤35-41.4% energy from carbohydrate) were associated with a 1.7 kg greater weight loss, 0.7 kg greater loss of fat-free mass, and a 2 kg greater loss of fat mass compared to diets with a higher carbohydrate content, after controlling for energy intakes (283). Greater effects were also observed (6.6 kg weight loss, 1.7 kg fat- free mass loss and 5.6 kg fat loss) in studies >12 weeks in duration. Protein intakes of >1.05 g/kg were also found to be associated with 0.60 kg additional lean mass preservation compared with diets with protein intakes ≤1.05 g/kg, after controlling for energy intakes (283). This difference increased to 1.2 kg in studies conducted for >12 weeks. Such findings challenge the current best practice recommendation of HC diets and have led to increased public interest and use of LC diets.

In spite of these favourable findings, there are limitations in the designs of existing studies that weaken conclusions that can be drawn about LC diets. A Cochrane review found a dearth of high quality data, attributing this to the methodological flaws of existing studies

(198).These limitations include small sample sizes, high attrition rates, relatively short study durations, inadequate assessment of dietary intakes or poor dietary compliance, and the lack of an appropriate dietary control group or randomisation. CVD risk assessment was limited to the measurement of surrogate markers of CVD and there is a dearth of definitive evidence of the effectiveness of LC diets on long-term CVD clinical endpoints. Existing studies conducted in people with T2D have also limited assessment of glycaemic control to HbA1c (281, 284-286), frequently without objective quantification of diabetes medication changes or measurements of GV and PPG. These confines have precluded clear conclusions on the role of LC diets in T2D management. The effects of LC diets were also often studied in isolation without inclusion of a formalized, exercise program or emphasis on physical activity which is an important adjunct of any lifestyle modification strategy (241, 281, 284, 286, 287). Importantly, the independent effect of weight change on indices of glucose tolerance and other CVD risk markers was often not considered because energy intake, a key confounder, was generally not controlled for (241, 281, 284, 286-288). The dietary effects resulting from the manipulation of macronutrient content may thus be difficult to interpret or even misconstrued. This issue has particular relevance to the interpretation of the effects of LC diets administered *ad libitum* because these LC diets have often produced a short- term weight loss advantage compared to energy- restricted HC diets, although this difference was usually non- statistically significant after 12 months (260). The limitations identified in this section are discussed in greater detail in conjunction with the findings from individual studies in the ensuing sections (1.6.1 and 1.6.2).

1.6.1 Effect of very low carbohydrate diets on weight loss and cardiovascular disease risk in individuals without diabetes

Ad libitum LC diets have been found to confer a weight loss advantage over HC diets, at least over the short-term (260, 289-293). Several RCTs have shown that, compared to a conventional HC diet, an LC diet consumed for up to two years results in comparable or greater weight loss in overweight/obese persons without T2D (260, 279-282, 294). These studies also showed that LC diets have more favourable effects on improving dyslipidaemia by lowering TAG concentrations and increasing HDL-C levels, as well as producing similar reductions in blood pressure, insulin resistance and improvements in glucose homeostasis (241, 260, 261, 279-282, 293, 294).

Compared to an HC diet, weight loss with an LC diet also resulted in at least similar or if not better improvements in adhesion molecules and inflammatory markers including C-reactive protein, interleukin-6, tumour necrosis factor-α, intercellular cell-adhesion molecule-1 and P-selectin (293, 295-298).

However, despite these beneficial effects, meta-analyses of previous trials suggest LC diets promote less favourable LDL-C responses compared to traditional HC diets (260, 261, 299). Previous studies have shown increases in LDL-C in LC diet groups (279-282). And even in the absence of a statistically significant difference in LDL- C between LC and HC diets, some studies have reported a rise in LDL-C in certain individuals (289, 291). However, data from lipoprotein subfractions analysis demonstrate that the increase in LDL particle size, such that large, buoyant LDL particles predominate in LC diet groups, could diminish this apparent increase in LDL atherogenicity (245, 300-303).
Extending these findings, it has been shown that FMD decreased after 12- months on an LC diet (5.7% to 3.7%) but remained unchanged after a HC diet (5.9% to 5.5%) in a group of overweight and obese individuals without T2D (297). This 2% absolute reduction in

magnitude of FMD in the LC diet group has been associated with a clinically relevant elevation in CVD risk (250). Hence, in the absence of data on the effects of long- term consumption of LC diets on CVD events and clinical endpoints, the increase in surrogate markers of CVD risk including LDL-C and endothelial dysfunction, following the consumption of LC diets, have raised concerns about the potential atherogenicity of these diets. Consequently, these concerns have limited the acceptance of LC diets. Notwithstanding this, prior studies evaluating LC diets have concomitantly increased saturated fat intake with total fat content. It is therefore conceivable that the higher saturated fat intake associated with LC diets in previous studies was responsible for the detrimental effects observed on LDL-C and FMD (233, 234, 237, 249). This has led to the perception that LC diets may promote an adverse CVD risk profile (238, 304).

1.6.2 Effect of very low carbohydrate diets on glycaemic control and cardiovascular disease risk in individuals with type 2 diabetes

1.6.2.1 Uncontrolled, non- randomised intervention studies

Two single-arm intervention studies in obese patients with T2D showed that an ad libitum LC diet significantly reduced body weight, improved glycaemic control (decreased HbA1c and 24-hr glucose profiles) and insulin sensitivity, decreased plasma TAG and lowered diabetes medication usage (305, 306). However, the inferences that can be drawn from these studies are somewhat limited by the relatively short study durations (≤ 16 weeks), the lack of appropriate control groups and small sample sizes.

A 6 months, a non-randomised study in 31 obese patients with T2D showed that, compared to a HC control diet, an LC diet produced greater reductions in weight (-11 vs -2 kg), FBG (-3.4 vs -0.6 mmol/L) and HbA1c (-1.4 vs -0.6 %) (307). However, energy intake levels

were poorly controlled and it is possible that the differential effects on glycaemic control may be due to differences in weight loss between groups. Retrospective follow-up of the 16 participants on the LC diet at 22 months showed that 7 patients maintained or further reduced their body weight, and all but one had a lower bodyweight than at baseline; HbA1c also remained significantly lower compared to baseline (7.0 vs 8.0%) (308). After 44 months, mean body weight remained 7% lower than baseline and 10% lower in 43% of the patients. HbA1c remained stable and significantly lower than baseline (6.8% vs 8.0%), and diabetes medications were either eliminated or reduced (309). This study did not report LDL-C levels, but reported a significant increase in HDL-C from baseline, with no change in TAG and total cholesterol levels (308, 309). However, the absence of a comparison with a matched HC control diet group precludes further interpretation of the results.

1.6.2.2 Randomised, controlled trials

Statistical regression modelling in a meta-analysis of studies in T2D patients showed that a 10% increased carbohydrate intake was associated with a 3.2% and 7.6% increase in blood glucose and TAG respectively, and these relationships remained significant after including weight change in the model (310).

In a 5- week randomised, crossover feeding study, compared to an HC diet, an LC diet produced greater reductions in HbA1c, FBG, 24 hour insulin and glucose, despite similar weight loss. TAG were significantly reduced on the LC diet (-1.1mmol/L vs. -0.4 mmol/L) although no significant changes were observed for other lipid parameters (269).

Significantly greater weight loss and decrease in total: HDL-C ratio was observed on an LC compared to a HC diet in a 3 month RCT (311). However, the greater improvement in HbA1c on the LC diet did not reach statistical significance and concomitant reductions in medication usage during the study could have masked the effect.

In a 24-week RCT, compared to an HC (low glycaemic index) diet, an LC diet produced a 1% (absolute) greater reduction in HbA1c (-1.5% vs. -0.5%), greater weight loss (-11.1 vs -6.9 kg), improvement in HDL-C (0.14 vs 0 mmol/L), and discontinued or reduced diabetes medication with no significant deterioration in other lipid parameters, in individuals with a mean baseline HbA1c of ~8% (285). However, because the LC diet was administered *ad libitum* without a fixed caloric intake, the differences (or lack of) between the diets on glycaemic control and cardio-metabolic risk factors may have been confounded by differences in energy intake and weight loss. However, the absence of any relationship between the change in HbA1c and weight loss suggests the greater effects of the LC diet may have been secondary to differences in the macronutrient profiles between the diets.

Moreover, a 48- week study where 32% of the study population had T2D, an LC diet produced greater reductions in blood pressure compared to an HC diet combined with orlistat therapy, despite similar weight loss between groups (288). HbA1c, TAG and LDL-C decreased, while HDL-C increased, with no differences between groups. These results suggest that, as an effective weight management strategy, LC diets may confer additional benefits for blood pressure control compared to a traditional calorie- reduced HC diet even when the latter was administered with a weight loss medication like orlistat. In a subgroup analysis of 46 patients with T2D from this study, the LC diet led to greater improvements in HbA1c compared with HC diet plus orlistat (-0.7% vs 0.2%, p=0.045) despite similar weight loss and greater reductions in anti-hyperglycaemic medications (determined using a medication effect score (MES) that was calculated from the daily dose and potency of medications) in the LC group (284). 71% of patients in the LC diet group compared to 30% of patients in the HC diet plus orlistat group had reductions of ≥50% in their MES. This small sub-analysis of a larger study is one of the first to objectively quantify changes in anti-hyperglycaemic medications use on an LC diet which is both an important measure

of glycaemic control and potential confounding factor that influences the interpretation of the effects of LC diets on other glycaemic markers.

A one year study also showed that HbA1c improved to a greater degree with an LC diet compared to a traditional HC diet (-0.7% vs -0.1%) after adjustment for weight loss differences in a small sub-set of participants with T2D (54 of 132 total participants) (281), but changes in other outcomes specific to the group with T2D were not reported. Conversely, another study did not show any significant difference in HbA1c after one year on an LC diet compared to HC diet (286). While a sustained significant increase in HDL-C was observed on the LC diet, there was no significant difference in LDL-C between diet groups. Although high retention rates were maintained, weight loss was minimal (3.4% over one year) and carbohydrate restriction on the LC diet was modest (average reported intake of 135g carbohydrates/ day at 12 months) with increased intakes over time reported. These findings suggest poor adherence could have weakened the effect sizes observed. The lacklustre results observed would also have to be considered in the context of its study design whereby medications were reduced pre- randomisation to minimise hypoglycaemia risk.

A low intensity intervention comparing energy matched LC and HC diets in a small study of 61 people did not produce significant differences in the change in weight, HbA1c or lipids after two years (287). Maximum weight loss was achieved at 6 months (-4 kg) with no differences between groups throughout the study. A significant reduction in HbA1c occurred within the LC group (-0.4%) at 6 months which also coincided with the lowest reported carbohydrate intake in the LC group (25% total energy, 91g carbohydrate/ day). However carbohydrate intake increased subsequently and HbA1c levels gradually returned to baseline levels after 6 months. As contact was limited to four group sessions throughout the 24 months, this could have affected dietary adherence and the effectiveness of the interventions, possibly explaining the overall lack of differences observed between groups.

To date, most studies on LC diets have investigated the diet in the context of a high saturated fat composition. However, two RCTs of one year duration have compared the effects of a moderately low carbohydrate diet (moderate LC; carbohydrates contributing 30- 40% of total energy) that encouraged monounsaturated fats (MUFAs) and limited saturated fats, with HC diets in T2D populations. In the first study, a moderate LC Mediterranean diet rich in MUFAs produced a significantly greater increase in HDL- C, reductions in TAG and HbA1c (- 2% vs. -1.6%), compared to an isocaloric, traditional HC diet over one year (312). LDL-C decreased by an additional 8% on the moderate LC Mediterranean diet, relative to the traditional HC diet, potentially a manifestation of its high MUFA content. Although the HDL-C and LDL-C results for the moderate LC Mediterranean diet were not significantly different from that observed in the isocaloric, HC Mediterranean diet. Given that the actual carbohydrate content of the moderate LC diet was relatively high and comparable to both HC diets (232g/ day vs. 250g/ day), limited inferences can be drawn from these results.

While the moderate LC diet in the second study did not produce any significant changes in HbA1c compared to both high and low glycaemic index, HC diets, it produced a greater reduction in TAG and increase in HDL-C, albeit transiently over the first 6 months (313). No significant difference in LDL-C between dietary groups was observed although high retention rates were maintained. However, energy intakes were not specifically controlled and not all participants were on calorie- restricted diets. Participants were also allowed to vary in their physical activity levels. These differences could have weakened the diet effects between groups. Furthermore, this study targeted patients with optimally, diet-controlled T2D (HbA_{1c} 6.1% at baseline) which limits the generalisability of the results and the likelihood of observing any further treatment effects.

Given the acknowledgement of the benefits of Mediterranean diets in current nutrition guidelines (5), one study has compared the effects of an *ad libitum* moderate LC diet

(~150-189g carbohydrates/ day, 41% total energy) with a calorie restricted Mediterranean diet, and an HC diet for 2 years (241).

In this study, the moderate LC diet produced the greatest weight loss (moderate LC 4.7kg, Mediterranean 4.4kg, HC 2.9kg), increases in HDL-C, reductions in TAG and total cholesterol: HDL-C amongst the 3 diets (241). LDL- C did not change significantly in all diet groups. In a small subgroup of 36 participants with T2D, a significant decrease in HbA1c was observed only in the moderate LC diet group although the changes were not significant between groups (moderate LC -0.9%, Mediterranean -0.5%, HC -0.4%) (241). Greater reductions in FBG were also observed in the Mediterranean diet group. These results showing the more favourable lipid effects observed on the moderate LC diet add to the growing evidence supporting carbohydrate restriction as an effective dietary approach to other comparison diets. Based on these results, it is possible that further carbohydrate restrictions in insulin- resistant populations such as people with T2D may confer greater benefits on glycaemic control and studies are needed to investigate this under isoenergetic conditions.

Considered together, the studies presented in this section suggest that, compared to a traditional HC diet, an LC diet enriched in MUFAs and limited in saturated fat may provide greater efficacy for improving glycaemic control, and potentially confer salutary effects on blood lipids (TAG and HDL-C, in particular). These improvements are likely to be independent of differences in energy intake and weight changes, but the results need to be confirmed in better designed, well- controlled, longer- term trials.

1.7 Long term safety effects of very low carbohydrate diets

Despite the growing evidence supporting LC diets for the management of blood glucose control and CV risk, concerns remain regarding the long- term safety and efficacy of these diets and guidelines of governing health authorities have deemed the available evidence inconclusive to recommend LC (80, 225). The nutrition composition of a typical LC diet may contravene conventional dietary guidelines. As LC diets restrict consumption of fibre-rich, plant –based carbohydrate foods such as wholegrain cereals, legumes and fruit, there is concern that the long- term consumption of such diets may lack adequate nutrition for optimal health and predispose towards micronutrient deficiencies (314, 315). Observational cohort studies have found LC diets to be associated with a significantly higher risk of all-cause mortality and CVD, suggesting that the long- term consumption of these diets may be harmful (316, 317). However, while prospective cohort studies allow for the determination of composite endpoints and fatal outcomes, they are susceptible to incomplete adjustment of unknown confounders (318). To this effect, well- designed RCTs minimise confounding and bias, and provide the best framework to study associations.

Renal impairment and compromised cognitive performance are often cited safety concerns of LC diets, particularly in patients with T2D who are at increased risk of developing diabetic kidney disease (DKD) and dementia (310, 314, 315, 319, 320). These concerns have important implications on the long- term safety and acceptability of LC diets but have rarely been systematically studied and confirmed in long- term studies. The following sections (1.7.1 and 1.7.2) explore the available literature on these topics.

1.7.1 Renal health outcomes

The National Kidney Foundation classification of chronic kidney disease stages is based on an assessment of kidney function (estimated glomerular filtration rate, eGFR) and kidney

damage (albuminuria) (321). eGFR can be estimated using serum creatinine (SCr) in formulae such as the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (322). While albuminuria is defined by an increased urinary albumin creatinine ratio (ACR ≥30mg/g Cr) in a random spot urine or 24h collection. Albuminuria is also an established marker for increased CVD risk (38). Ongoing monitoring of these markers are used in trials to assess the response to treatment and progression of DKD.

There are concerns that the higher protein content of LC diets may result in increases in glomerular pressure and hyperfiltration with consequent worsening of renal function in people with T2D at increased risk of DKD (314, 323). This is based on observational cohort data that found that a high protein intake (particularly non- dairy animal protein) may accelerate renal function decline in individuals with mild renal insufficiency (324). Earlier meta- analyses have also reported a small but statistically significant reduction in GFR decline and incidence of renal failure or death from dietary protein restriction (325-327). However, only a small number of people with diabetes (predominantly T1D) and non-randomised trials were included in these analyses (325-327). In contrast, more recent prospective cohort studies of people with T2D and without DKD have found no association between protein intake and eGFR decline (328), or a negative association between protein intake and risk of DKD (329). A meta- analyses also found no clear benefit on eGFR from consuming a low protein compared with a normal protein diet (0.9g/kg/day cf. 1.3g/kg/day) in people with DKD (330). Data from an RCT also concluded that protein restriction was neither feasible nor efficacious for the longer term prevention or delay of renal damage in adults with T2D and moderately increased albuminuria or a diabetes duration >5 years (331).

For people with non- dialysis dependent DKD, the American Diabetes Association guidelines recommend that dietary protein intake be in line with the recommended daily allowance or 0.8g/kg body weight per day (5). However a common misconception that

should be clarified is that LC diets are not strictly high- protein diets but are generally higher in protein content when compared with the lower protein levels recommended in HC diets (273). Furthermore, people who adopt LC diets do not necessarily replace carbohydrate with either fat or protein but instead reduce their intake of refined carbohydrates and sugar (332). Therefore, the absolute amounts of fat and protein consumed do not increase, although the composition of fat and protein in the diet increases (272).

In the few available studies that have compared LC and HC diets on renal outcomes in healthy, obese adults with normal renal function and without T2D, weight loss produced similar changes in eGFR and creatinine clearance (CrCl) in both diets after 1-2 years (333, 334). In studies that have included people with T2D and pre-existing DKD, eGFR and albuminuria improved with weight loss with no differences between LC and HC diet groups after 1-2 years (335, 336). However no long- term, well- controlled study has examined the effects of LC diets in people with T2D and without DKD on renal function in the context of a comprehensive lifestyle intervention incorporating regular physical activity, where the clinical applicability of LC diets as a strategy to manage weight, diabetes and other comorbidities such as hypertension and dyslipidemia is most relevant.

1.7.2 Cognitive function

LC diets may entail carbohydrate restrictions below the 130g recommended dietary allowance (RDA), which corresponds to a level of intake that provides adequate glucose as a fuel for the central nervous system without reliance on glucose production from dietary fat or protein (337). Nevertheless, whilst brain fuel needs can be met on LC diets, the long term metabolic effects of this are uncertain (337). Therefore there are concerns that long-

term consumption of LC diets may adversely affect cognitive performance particularly in people with T2D who are at increased risk of brain atrophy, cognitive impairment and dementia (319, 320). However, no studies have systematically examined the cognitive effects of LC diets in people with T2D. In people without diabetes, available HC/LC dietary studies have assessed cognitive performance using a limited test battery with mixed results. Longer term studies showed no difference in cognitive effects (working memory and speed of processing) following the consumption of HC and LC weight- loss diets for 24- 52 weeks (338, 339). Whereas short- term studies (3- 8weeks) showed LC diet groups had lesser improvements in speed of processing (340), better attention and poorer memory (341), compared to HC diet groups.

Moreover, the development of cognitive deficits in people with T2D may be modulated and mediated by factors such as glycaemic control, microvascular complications, and comorbidities including hypertension and stroke although the relative contribution of each factor has not been established (342). Findings from mechanistic studies also suggest that vascular disease, inflammation, oxidative stress, energy imbalance and changes in neuronal gene expression, glucose, insulin, and amyloid metabolism underlie the pathophysiology (342-344). These findings suggest that cognitive benefit may be achievable with diet and lifestyle interventions that lead to weight loss, improve glucose regulation and facilitate blood pressure control. This hypothesis was partly examined in the Memory in Diabetes (MIND) study of the ACCORD trial where the intensive control group (target HbA1c <6%) had a smaller decrease in total brain volume measured by MRI compared to the standard glycaemic control group (target HbA1c 7-7.9%) after 40 months (345). However, both groups achieved similar cognitive outcomes assessed primarily using the Digit Symbol Substitution Test (DSST). It is possible that improvements in metabolic control may affect more cognitively demanding tasks that require engagement of multiple cerebral systems with higher glucose metabolism requirements for optimal performance in these

tasks (346). Increased task demand and sensitivity would also negate the likelihood of ceiling effects inherent in simpler tasks where performance cannot be further improved (347).

The information presented in this section therefore highlights the importance of assessing the long- term cognitive effects of a lifestyle modification strategy that combines LC diets with regular physical activity, in people with T2D, using a comprehensive neuropsychological test battery that assesses all major cognitive domains.

1.8 Specific aims of this thesis

Overweight and obesity as well as related non- communicable diseases such as T2D are largely preventable by lifestyle modification. The persistence of the obesity and T2D epidemics highlight the urgency to develop sustainable and effective lifestyle strategies to facilitate weight loss and diabetes management to delay or prevent the development of CVD and other diabetic sequelae. The treatment goals in T2D entail achieving and maintaining optimal blood glucose, lipid and blood pressure levels (5). Diet and physical activity are fundamental to T2D management and prevention because these lifestyle strategies help correct glucose, lipid and blood pressure anomalies, as well as assist with weight loss and maintenance. Notably, the available evidence suggest that carbohydrate restriction is a promising nutritional approach for managing T2D and its related comorbidities. Therefore, the results of the study reported in Chapters 2-6 of this thesis aim to address the following research question,

"*Does the long- term consumption of an LC diet result in greater improvements in glycaemic control and CVD risk markers, in the context of similar changes in weight, body composition, renal function and cognitive performance when compared to an energy-matched, traditional HC diet?*"

This hypothesis was tested in a large, long-term, well-controlled efficacy trial that was designed to address prior research limitations. The intervention was delivered as a holistic, multifaceted lifestyle approach that incorporated a formalised, physical activity program to facilitate a high degree of adherence. A complete analysis of all aspects of glycaemia was undertaken on this lifestyle strategy that incorporated an LC diet aimed at lowering HbA1c and minimising PPG and GV by attenuating glycaemic excursions. Based on the concern that high intakes of saturated fats raise LDL- C and augment CVD risk, an innovative approach involved the design and delivery of an LC diet with a modified fatty acid profile

that was high in unsaturated fat (predominantly MUFA) and low in saturated fat to confer cardioprotective benefits. This nutritionally adequate and palatable eating pattern incorporated a high composition of unsaturated fat from nuts, oils, seeds and lean protein foods rather than saturated fat sources such as cream, processed and fatty meats. In addition, the study presented a further opportunity, in a well-controlled intervention, to monitor the impact of an LC diet on renal and cognitive outcomes in people with T2D at increased risk of developing renal and cognitive abnormalities. The findings from this aspect of the trial will contribute to the overall assessment and understanding of the role and safety of an LC diet as a diabetes and weight management strategy.

This study aims to provide scientific evidence to inform dietary recommendations and guide clinical practice for optimal T2D management by offering a comprehensive lifestyle strategy that can be translated to practice in the primary care environment and community. As hypothesised, if the proposed LC diet demonstrates greater efficacy whilst being safe, this would have fundamental implications for T2D management with direct relevance to patient care and education.

CHAPTER 2. GLYCEMIC VARIABILITY: ASSESSING GLYCEMIA DIFFERENTLY AND THE IMPLICATIONS FOR DIETARY MANAGEMENT OF DIABETES

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2.1 Summary

Glycaemic variability (GV) is defined as the amplitude, frequency and duration of glycaemic fluctuations around mean blood glucose. This review summarises the growing evidence supporting GV as an independent risk factor for diabetes complications and emerging marker of glycaemic control. The various methods of quantifying GV and the advantages of harnessing CGMS technology to measure GV are discussed. Several dietary factors such as carbohydrate quantity and quality have been shown to affect GV. These factors may assist patients with improving their blood glucose control and have important implications for the dietary management of diabetes.

Statement of Authorship

Title of Paper	**Glycemic Variability: Assessing Glycemia Differently and the Implications for Dietary Management of Diabetes.**
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Name of Principal Author (Candidate)	Jeannie Tay		
Contribution to the Paper	Drafted the manuscript, revised the manuscript for intellectual content, reviewed and approved the final manuscript.		
Overall percentage (%)	85-90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	1 February 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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CHAPTER 3. A VERY LOW CARBOHYDRATE, LOW SATURATED FAT DIET FOR TYPE 2 DIABETES MANAGEMENT: A RANDOMISED TRIAL

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3.1 Summary

The aim of this chapter was to report the short- term (24 weeks) effects of consuming an energy- restricted high-unrefined carbohydrate, low fat diet (HC) compared to an isocaloric very low carbohydrate, high unsaturated/low saturated fat diet (LC), on glycaemic control and CVD risk factors in overweight/ obese adults with T2D. Both diets were delivered as part of a holistic lifestyle intervention that incorporated a structured exercise program.

While both diets achieved significant improvements for several clinical glycaemic control and cardiometabolic risk markers, the LC diet group achieved greater reductions in GV and diabetes medications requirements, compared to the HC diet group. The LC diet reduced TAG and increased HDL-C to a greater extent, with comparable reductions in LDL-C as the HC diet. These lipid results suggest that the LC diet was more effective at correcting the most prevalent pattern of dyslipidaemia in T2D. These effects were most apparent in participants with greater metabolic derangements suggesting an LC diet with high unsaturated/low saturated fat content may confer additional therapeutic potential for T2D management beyond traditional lifestyle management strategies and weight loss, if effects are sustained in the long- term.

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Name of Principal Author (Candidate)	Jeannie Tay		
Contribution to the Paper	Study concept and design, data collection and extraction (e.g. glycaemic variability, accelerometry, medication effect score), analysis and interpretation of data, drafting of the manuscript, read and approved the final manuscript.		
Overall percentage (%)	60-70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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CHAPTER 4. COMPARISON OF LOW- AND HIGH-CARBOHYDRATE DIETS FOR TYPE 2 DIABETES MANAGEMENT: A RANDOMISED TRIAL

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Am J Clin Nutr 2015; 102:780-790

4.1 Summary

The aim of Chapter 4 was to evaluate the long- term (52 weeks) efficacy of the novel LC diet pattern described in Chapter 3 that limited carbohydrates and saturated fat, and increased protein and unsaturated fat, as part of a holistic lifestyle program under well-controlled conditions.

The data presented here showed both the LC and the traditional HC dietary approaches achieved substantial weight loss, and reduced HbA1c and fasting glucose. However, the LC diet achieved and continued to sustain greater improvements in lipid profile (HDL-C and TAG), blood glucose stability and reductions in diabetes medication requirements, suggesting this dietary approach may be a more effective long- term strategy for T2D management, in comparison with the traditional HC dietary approach.

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Overall percentage (%)	60-70%		
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CHAPTER 5. LONG-TERM EFFECTS OF A VERY LOW CARBOHYDRATE COMPARED WITH A HIGH CARBOHYDRATE DIET ON RENAL FUNCTION IN INDIVIDUALS WITH TYPE 2 DIABETES: A RANDOMIZED TRIAL

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Medicine. 2015; 94: e2181

5.1 Summary

Chapter 5 reports on the secondary outcomes of the diet and lifestyle intervention described in Chapter 4. The long- term effects of consuming a very low carbohydrate, high-protein, low saturated fat (LC) diet are compared with an isocaloric, traditional high unrefined carbohydrate, low-fat (HC) diet on markers of renal function in obese adults with T2D but without overt kidney disease.

Compared to the HC diet group, the LC diet group reported a higher protein intake derived from both plant and animal protein sources. However, both diet groups had similar effects on clinical markers of renal function such as albuminuria, eGFR and CrCl over a 12 month period. These results suggest that consumption of an LC weight loss diet does not adversely affect renal function in obese adults with T2D and without DKD. These findings thus provide further support for the clinical utility of LC diets as a weight management strategy for individuals with T2D and comorbidities like hypertension and dyslipidaemia, to improve glycaemic control and reduce CVD and diabetes complications risk.

Longer term follow-up will help ascertain the sustainability of these renal effects during weight loss maintenance and determine if long- term consumption of the diets differentially affect the development and progression of DKD.

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Co-Author Contributions

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Long-Term Effects of a Very Low Carbohydrate Compared With a High Carbohydrate Diet on Renal Function in Individuals With Type 2 Diabetes

A Randomized Trial

Jeannie Tay, BNutr, Diet (Hons), Campbell H. Thompson, MD, DPhil, Natalie D. Luscombe-Marsh, PhD, Manny Noakes, PhD, Jonathan D. Buckley, PhD, Gary A. Wittert, MD, and Grant D. Brinkworth, PhD

Abstract: To compare the long-term effects of a very low carbohydrate, high-protein, low saturated fat (LC) diet with a traditional high unrefined carbohydrate, low-fat (HC) diet on markers of renal function in obese adults with type 2 diabetes (T2DM), but without overt kidney disease.

One hundred fifteen adults (BMI 34.6 ± 4.3 kg/m^2, age 58 ± 7 years, HbA1c $7.3 \pm 1.1\%$, 56 ± 12 mmol/mol, serum creatinine (SCr) 69 ± 15 μmol/L, glomerular filtration rate estimated by the Chronic Kidney Disease Epidemiology Collaboration formula (eGFR 94 ± 12 mL/min/1.73 m^2)) were randomized to consume either an LC (14% energy as carbohydrate [CHO < 50 g/day], 28% protein [PRO], 58% fat [<10% saturated fat]) or an HC (53% CHO, 17% PRO, 30% fat [<10% saturated fat]) energy-matched, weight-loss diet combined with supervised exercise training (60 min, 3 day/wk) for 12 months. Body weight, blood pressure, and renal function assessed by eGFR, estimated creatinine clearance (Cockcroft–Gault, Salazar–Corcoran) and albumin excretion rate (AER), were measured pre- and post-intervention.

Both groups achieved similar completion rates (LC 71%, HC 65%) and reductions in weight (mean [95% CI]; −9.3 [−10.6, −8.0] kg) and blood pressure (−6 [−9, −4]/−6[−8, −5] mmHg), $P \geq 0.18$. Protein intake calculated from 24 hours urinary urea was higher in the LC than HC group (LC 120.1 ± 38.2 g/day, 1.3 g/kg/day; HC 95.8 ± 27.8 g/day, 1 g/kg/day), $P < 0.001$ diet effect. Changes in SCr (LC 3 [1, 5], HC 1 [−1, 3] μmol/L) and eGFR (LC −4 [−6, −2], HC −2 [−3, 0] mL/min/1.73 m^2) did not differ between diets ($P = 0.25$). AER decreased independent of diet composition (LC −−2.4 [−6, 1.2], HC −1.8 [−5.4, 1.8] mg/24 h, $P = 0.24$); 6 participants (LC 3, HC 3) had moderately elevated AER at baseline (30–300 mg/24 h), which normalized in 4 participants (LC 2, HC 2) after 52 weeks.

Compared with a traditional HC weight loss diet, consumption of an LC high protein diet does not adversely affect clinical markers of renal function in obese adults with T2DM and no preexisting kidney disease.

(*Medicine* 94(47):e2181)

Abbreviations: AER = albumin excretion rate, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration, CrC = creatinine clearance, CVD = cardiovascular disease, DKD = diabetic kidney disease, eGFR = estimated glomerular filtration rate, FFM = fat free mass, HC diet = high carbohydrate, low-fat diet, LC diet = very low carbohydrate, high protein, low saturated fat diet, SCr = serum creatinine, T2DM = type 2 diabetes mellitus.

INTRODUCTION

Concerns exist about the safety of protein-rich diets for renal function, particularly in populations with type 2 diabetes mellitus (T2DM) who are at risk of nephropathy.[1] Despite this, very low carbohydrate, high protein, high fat (LC) diets are rising in popularity amongst individuals with T2DM based on growing evidence of their efficacy as a weight management strategy to improve glycemic control and reduce cardiovascular disease (CVD) risk.[2] Few well-controlled, long-term studies have systematically examined the effects of LC diets as part of a comprehensive lifestyle modification program on renal function in T2DM, limiting the applicability of LC diets as a diabetes management strategy. This study compares the effects, after 1 year of consumption of a novel energy-restricted LC diet that limits saturated fat, with an energy-matched traditional high unrefined carbohydrate, low-fat (HC) diet whilst undertaking a supervised exercise program, on renal function in obese adults with T2DM and without diabetic kidney disease (DKD).

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The authors have no conflicts of interest to disclose.

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MATERIALS AND METHODS

Participants and Study Design

Enrollment criteria, study design, and the primary study outcomes have been previously described.[2] Briefly, 115 adults with T2DM and without overt kidney disease (57% male (mean ± SD) BMI 34.6 ± 4.3 kg/m^2, age 58 ± 7 years, HbA1c 7.3 ± 1.1%, 56 ± 12 mmol/mol, serum creatinine (SCr) 69 ± 15 μmol/L, glomerular filtration rate estimated by the Chronic Kidney Disease Epidemiology Collaboration formula (eGFR-CKD-EPI) 94 ± 12 mL/min/1.73 m^2) were recruited by public advertisement. Exclusion criteria were type-1 diabetes; preexisting renal disease (eGFR < 60 mL/min/1.73 m^2, elevated SCr > 120 μmol/l, and/or albumin excretion rate (AER) > 300 mg/24 h); abnormal liver function; any significant endocrinopathy (other than stable treated thyroid disease); history of malignancy, respiratory, gastrointestinal, or CVD; pregnancy/lactation; history of/or current eating disorder or smoking. The study was approved by the Human Research Ethics Committees of the Commonwealth Scientific and Industrial Research Organisation and the Universities of Adelaide and South Australia. All participants gave written informed consent.

In a parallel design, participants were randomized to consume either a hypocaloric (500–1000 kcal/day deficit) LC diet (14% energy as carbohydrate [CHO < 50 g/day], 28% protein [PRO], 58% fat [<10% saturated fat]) or an energy-matched HC diet (53% CHO, 17% PRO, 30% fat [<10% saturated fat]); combined with supervised aerobic/resistance exercise (60 minutes, 3 day/wk) for 12 months. Detailed description of the diets, exercise program and the effects of the dietary interventions on body weight, glycemic control, and CVD risk factors have been reported elsewhere.[2]

Body Weight, Blood Pressure, HbA1c, Renal Function Measures, and Protein Intake

Body weight was measured using calibrated electronic scales (Mercury AMZ1, Tokyo, Japan) at baseline and monthly intervals. Other outcomes were assessed at weeks 0, 24, and 52. Seated blood pressure was measured by automated sphygmomanometry (SureSigns VS3; Phillips, Andover, MA). HbA1c was measured at a certified pathology laboratory (Institute of Medical and Veterinary Science Pathology, Adelaide, Australia). Outcomes were assessed in the clinic after an overnight fast, with water consumed as required to avoid dehydration.

SCr was measured on a clinical analyzer (Beckman AU480; Beckman Coulter, Inc., Brea, CA) using a standardized assay (Beckman kit #OSR6178). eGFR (mL/min/1.73 m^2) was calculated according to CKD-EPI Eq.[3] which has greater accuracy compared to the Modification of Diet in Renal Disease (MDRD) equation at higher GFR (>60 mL/min/1.73 m^2).[4] Creatinine clearance (CrCl, mL/min) was estimated using the Cockcroft–Gault equation[5] with fat free mass (FFM—whole-body dual-energy X-ray absorptiometry, Lunar Prodigy; General Electric Corporation, Madison, WI) adjustment to improve estimation in obesity,[6] and the Salazar–Corcoran Eq.[7] developed for use in obese individuals based on estimated FFM. AER, urinary albumin, and urea obtained from 24 hours urine samples were measured at a certified commercial laboratory (Institute of Medical and Veterinary Science Pathology, Adelaide, Australia).

Protein intake (g/day) was estimated from 24 hours urinary urea excretion at weeks 0, 24, and 52.[8,9] Dietary compliance was assessed randomly from 7 consecutive days of daily weighed food records for every 14-day period, analyzed using Foodworks Professional Edition Version 7 (Xyris Software 2012, Highgate Hill, Australia). Both dietary patterns achieved a high level of compliance as reported.[2]

Statistical Analyses

Baseline group differences were compared by independent *t* tests and χ^2 tests for continuous and categorical variables, respectively. AER and urinary albumin were reciprocally transformed to improve normality before analysis. Comparisons of changes over time between diets were analyzed by random-coefficient analysis, restricted maximum likelihood, mixed effects model using an unstructured covariance with data assumed to be missing at random. The model included all available data from participants who commenced the study and contained the following fixed effects: main effect for each time-point, diet group assignment, and diet group by time-point interaction. The effect of the use of renin-angiotensin system blocking agents including angiotensin-converting-enzyme inhibitors and angiotensin receptor antagonists on the renal outcomes investigated was examined in sensitivity analyses that included this as a covariate. Estimated marginal means (95% confidence intervals, CI) and change from weeks 0 to 52 are reported. Repeated measures ANOVA with diet as between-subjects factor and time as within-subject factor was used to assess changes in dietary protein intake. All analyses were performed using SPSS 20.0 for Windows (SPSS, Inc., Chicago, IL); statistical tests were 2-tailed with statistical significance at $P < 0.05$.

RESULTS

At baseline, there were no significant clinical or biochemical differences between groups (Table 1). SCr and eGFR were within the normal range (Table 2); 7 participants (LC:4, HC:3) had moderately increased AER (30–300 mg/24 h).[10] Antihypertensive therapy use was similar in both groups (LC:41, HC:35, $P = 0.29$) with 56% of participants using renin-angiotensin system blocking agents (LC:32, HC:32). Sixty-eight percent of participants completed the study with no difference between groups (LC:41/58, HC:37/57; $P = 0.51$).

Over the 52 weeks, reductions in weight and blood pressure were comparable between groups ($P \geq 0.18$; Table 2). Nine participants reduced (LC:4, HC:5) and 3 increased (LC:2, HC:1) use of renin-angiotensin system blocking agents.

Self-reported protein intake was higher in LC compared to HC (LC:106.1 ± 18.9 g/d (26% energy), 1.2 g/kg/d; HC:78.5 ± 14.8 g/d (18% energy), 0.9 g/kg/d), $P < 0.001$. Protein intakes estimated from 24 hours urinary urea reflected similar differences (weeks 0, 24, and 52; LC: 112.1 ± 34.3, 118.4 ± 33.8, 120.1 ± 38.2 g/d; 1.1 ± 0.3, 1.4 ± 0.4, 1.3 ± 0.4 g/kg/d; HC: 107.7 ± 28.4, 89.8 ± 18.4, 95.8 ± 27.8 g/d; 1.0 ± 0.3, 1.0 ± 0.2, 1.1 ± 0.3 g/kg/d; $P < 0.001$).

Over time, SCr increased, while eGFR, CrCl, and AER decreased, with no difference in the responses between groups (Table 2). Sensitivity analyses that adjusted for the use of renin-angiotensin system blocking agents did not alter the results of the primary model. At baseline, 7 participants (LC:4, HC:3) had pathological albuminuria (range 41–101 mg/24 h). After 52 weeks, 4 of these participants (LC:2, HC:2) became normoalbuminuric, 2 (LC:1, HC:1) remained pathologically albuminuric, and 1 LC participant withdrew before week 52. All participants who were normoalbuminuric at baseline remained so after 52 weeks.

TABLE 1. Baseline Participant Characteristics*

	LC Diet (n = 58)	HC Diet (n = 57)
Demographics		
Age	58 (7)	58 (7)
Sex [n (%)]		
Females	21 (36)	28 (49)
Males	37 (64)	29 (51)
Duration of diabetes (years)	7 (5)	9 (7)
Body weight and BMI		
Body weight (kg)	101.7 (14.4)	101.6 (15.8)
BMI (kg/m^2)	34.2 (4.5)	35.1 (4.1)
Glycemic control		
HbA1c (%)	7.3 (1.1)	7.4 (1.1)
CVD risk markers		
SBP (mm Hg)	130.4 (13.1)	132.6 (13.2)
DBP (mm Hg)	80.0 (8.9)	80.8 (10.1)
Fasting insulin (mU/L)†	16.3 (8.3)	15.9 (7.6)
HOMA2-IR†	2.3 (1.1)	2.2 (1.0)
HOMA2-%B†	75.5 (38.7)	67.7 (33.4)
Total Cholesterol (mmol/L)	4.5 (1.0)	4.3 (1.0)
LDL-C (mmol/L)	2.5 (0.9)	2.4 (0.9)
HDL-C (mmol/L)	1.2 (0.2)	1.3 (0.3)
TG (mmol/L)	1.6 (0.7)	1.4 (0.6)
Medications		
[0,1-3] Diabetes medications		
Insulin [n (%)]	6 (10)	6 (11)
Metformin [n (%)]	46 (79)	41 (72)
Sulfonylureas [n (%)]	20 (34)	16 (28)
Thiazolidinediones [n (%)]	3 (5)	3 (5)
GLP-1 agonists [n (%)]	1 (2)	1 (2)
DPP-4 inhibitors [n (%)]	1 (2)	2 (4)
Antihypertensive medications [n (%)]	41 (71)	35 (61)
ACE-inhibitors [n (%)]	20 (34)	19 (33)
Angiotensin-II receptor antagonists [n (%)]	14 (24)	13 (23)
Calcium channel blockers [n (%)]	18 (31)	12 (21)
Beta blockers [n (%)]	6 (10)	3 (5)
Lipid lowering medications [n (%)]	35 (60)	36 (63)

Data are means (SD), unless otherwise stated.

To convert mmol/L to mg/dL, multiply by 38.7 (for cholesterol) and 88.6 (for triglycerides).

ACE-inhibitors = angiotensin-converting-enzyme inhibitors; BMI = body mass index; DBP = diastolic blood pressure; DPP-4 inhibitors = dipeptidyl-peptidase-4 inhibitors; GLP-1 agonists = glucagon-like peptide-1 agonists; HC diet = high carbohydrate, low-fat diet; HDL-C = high density lipoprotein cholesterol; HOMA2-%B = homeostasis model of assessment index 2-β cell function; HOMA2-IR = homeostasis model of assessment index 2-insulin resistance; LC diet = very low carbohydrate, high protein/low saturated fat diet; LDL-C = low density lipoprotein cholesterol; SBP = systolic blood pressure; TG = triglycerides.

* Total analyzed n = 115 (LC:58, HC:57) for all data unless otherwise stated. All baseline characteristics were not significantly different between diet groups ($P > 0.05$) by independent samples *t* test (continuous variables) or χ^2 test (categorical variables).

† Total analyzed n = 103 (LC:52, HC:51) for insulin and HOMA2 data; 12 participants on insulin medication at baseline were excluded from analyses.

DISCUSSION

GFR and albuminuria are established indicators of the presence and progression of DKD.[10] This study showed these markers responded similarly following consumption of either energy-matched LC or HC weight-loss diets administered as part of a holistic lifestyle modification program incorporating regular exercise. The overall conclusion was not altered after controlling for antihypertensive treatment. Within the limits of a modest sample size, our results in obese adults with T2DM, but without overt DKD, confirm and extend the findings of previous investigations conducted in people without diabetes and individuals with preexisting DKD.[11–14] The literature supporting the utility of protein restriction in slowing GFR decline or delaying DKD progression is controversial. Acknowledging the limitations of observational studies (such as the possibility of unaccounted residual confounding), prospective cohort studies of people with T2DM and without DKD at baseline have found no association between protein intake and eGFR decline,[15] or a negative association between protein intake and risk of DKD.[16] Although earlier meta-analyses have reported a small but statistically significant reduction in GFR decline and incidence of renal failure or death from dietary protein restriction, only a

TABLE 2. Estimated Marginal Means and Changes (95% CI) in Weight, Blood Pressure, Albuminuria, Serum Creatinine, Estimated Glomerular Filtration Rate, and Creatinine Clearance at Baseline and After 52 weeks on a Very Low Carbohydrate, High Protein, Low Saturated Fat (LC) Diet or an Isocaloric High Carbohydrate, Low-Fat (HC) Diet*

	LC Diet			HC Diet			
	Week 0	Week 52	Change†	Week 0	Week 52	Change†	*P*-Value†
Body weight and blood pressure							
Body weight (kg)‡	101.7 (97.8 to 105.7)	92.0 (87.9 to 96.1)	−9.8 (−11.7 to −7.9)	101.6 (97.6 to 105.6)	91.5 (87.3 to 95.6)	−10.1 (−12.0 to −8.2)	0.18
SBP (mm Hg)‡	130.4 (126.9 to 133.8)	123.2 (119.5 to 127.0)	−7.1 (−10.6 to −3.7)	132.6 (129.1 to 136.0)	126.8 (122.9 to 130.6)	−5.8 (−9.4 to −2.2)	0.81
DBP (mm Hg)‡	80.0 (77.5 to 82.4)	73.8 (71.4 to 76.1)	−6.2 (−8.2 to −4.1)	80.8 (78.2 to 83.3)	74.4 (72.0 to 76.8)	−6.4 (−8.4 to −4.3)	0.38
Renal markers							
Serum creatinine (μmol/L)‡	69 (65 to 72)	72 (68 to 76)	3 (1 to 5)	71 (67 to 75)	72 (68 to 76)	1 (−1 to 3)	0.25
Urinary albumin (mg/L)‡,§	3.3 (1.1 to 5.4)	1.8 (0.7 to 2.9)	−1.4 (−3.1 to 0.2)	2.4 (0.3 to 4.5)	1.6 (0.5 to 2.7)	−0.8 (−2.4 to 0.9)	0.21
AER (mg/24 h)‡,§	6 (2 to 11)	4 (2 to 6)	−2 (−6 to 1)	5 (1 to 9)	3 (1 to 6)	−1.8 (−5 to 2)	0.24
eGFR-CKD-EPI‡,‖ (mL/min/1.73 m^2)	96 (93 to 99)	92 (89 to 95)	−4 (−6 to −2)	92 (88 to 94)	90 (87 to 93)	−2 (−3 to 0)	0.25
CrCl-Cockcroft–Gault‡,¶ (mL/min)	88 (83 to 93)	80 (76 to 85)	−8 (−10 to −5)	82 (77 to 87)	77 (72 to 81)	−5 (−8 to −3)	0.32
CrCl-Salazar–Corcoran‡,# (mL/min)	127 (119 to 134)	113 (107 to 120)	−13 (−17 to −10)	120 (112 to 127)	109 (103 to 115)	−11 (−14 to −7)	0.50

Data are estimated marginal means (95% confidence intervals) by linear mixed-effects model analysis.

AER = albumin excretion rate; CKD-EPI = chronic kidney disease epidemiology collaboration; CrCl = creatinine clearance; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HC diet = high carbohydrate, low-fat diet; LC diet = very low carbohydrate, high protein, low saturated fat diet; SBP = systolic blood pressure.

To convert μmol/L to mg/dL, multiply by 0.0113 (for serum creatinine).

* Total analyzed n = 115 (LC:58, HC:57) for all data unless otherwise stated.

† *P*-value refers to between group differences over time (diet × time interaction) by linear mixed-effects model analysis.

‡ $P < 0.05$ significant change from baseline to 52 weeks (time effect).

§ Total analyzed n = 113 (LC:56, HC:57) for urinary albumin and AER data; no urine analyses were available at baseline for 2 participants in the LC diet group.

‖ eGFR-CKD-EPI (mL/min/1.73 m^2)^3 = 141 × min (SCr (mg/dL)/κ, 1)$^{\alpha}$ × max(SCr(mg/dL)/κ, 1)$^{-1.209}$ × 0.993^{Age}[x1.018 if female] [x1.159 if black]. where κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, SCr is serum creatinine, min indicates the minimum of SCr/κ or 1 and max indicates the maximum of SCr/κ or 1.

¶ CrCl-Cockcroft–Gault (mL/min)^5 = [140-age(years)] × FFM(kg)/(SCr (mg/dL) × 72) [x0.85 if female], where FFM is fat free mass.

CrCl-Salazar–Corcoran (mL/min)^7 = [137-age(years)] × {[0.285 × weight(kg)] + [12.1 × height(m^2)]}A/[51 × SCr(mg/dL)] for males; [147-age(years)] × {[0.287 × weight(kg)] + [9.74 × height(m^2)]}/[60 × SCr(mg/dL)] for females

small number of people with diabetes (predominantly T1DM) were studied and nonrandomized trials were included.[17–19] Moreover a reduced risk of renal failure attributed to low protein diets might reflect a delayed initiation of dialysis due to amelioration of uremic symptoms rather than an actual retardation of renal function decline. A recent meta-analysis of randomized controlled trials showed that a low protein diet was not associated with a significant improvement in renal function in people with DKD when compared with a normal protein diet (0.9 g/kg/day cf. 1.3 g/kg/day).[20] The present study did not specifically examine a low protein diet (0.8 g/kg/day, 16–18% total energy) recommended by current clinical guidelines for individuals with DKD.[21] However, actual protein intakes reported in low protein intervention groups have typically ranged from 0.7 to 1.1 g/kg/d[19,20] which includes the mean protein intake range of the comparison HC diet group in this study (1–1.1 g/kg/d) that more likely reflects a typical protein intake. Collectively, these data suggest that compared to a traditional HC diet, consumption of an LC, high protein diet (1.1–1.4 g/kg/d) derived from both plant and animal protein sources, does not adversely affect renal function in individuals with T2DM. This raises the clinical relevance of LC diets as a tenable weight management strategy for individuals with T2DM and comorbidities like hypertension and dyslipidemia, to improve glycemic control and reduce CVD and diabetes complications risk.[2]

Irrespective of any differences between the diets, both groups experienced an overall reduction in eGFR and CrCl despite substantial weight loss (9.1%), improvement in glycemic control (HbA1c −1%, −10.9 mmol/mol), reductions in blood pressure (−6/−6 mm Hg) and albuminuria.[2] The 2% to 4% decrease in eGFR observed in the present study is compatible with the expected age-related change in eGFR in T2DM[22,23] and is unlikely to be clinically significant. Previous studies that reported declines in eGFR following weight loss have examined individuals without renal impairment,[11,14] compared to those that reported increases in eGFR that examined individuals with preexisting renal dysfunction.[13,14] This suggests that weight loss-induced increases in eGFR may more likely occur in populations with preexisting renal dysfunction, a phenomenon that has also been observed postbariatric surgery.[24] Moreover, the overall reduction in albuminuria over time and the normalization of levels in patients with albuminuria at baseline support the benefits of weight loss.[25]

A limitation of this study was that GFR was estimated rather than directly measured. Clearance of inulin and radioisotope-labeled filtration markers (iGFR) are considered ''gold standards'' for measuring GFR. Nevertheless, equations for eGFR and CrCl are used widely in clinical practice for drug dosing, screening, risk stratification and for monitoring DKD progression. The equations used in this study have been validated in obese populations, including individuals with diabetes and conferred greater accuracy compared with other equations against iGFR.[6] Hence, this methodological approach is considered appropriate for the purpose of comparing the long-term renal effects of differing diet regimens.

Variations in determinants of creatinine generation (eg, FFM, exercise, and diet) can also influence eGFR calculations. The combined effect of a similar FFM loss (−1.7 kg) and increase in moderate to vigorous exercise[2] could explain the increase in SCr[26] and consequent reductions in creatinine-based eGFR calculations observed in both groups, potentially masking any diet-induced differences in renal function. Changes in body composition and the nonsteady-state clearance of endogenous filtration markers expose the limitations of existing methods and the complexity of assessing renal function after weight loss. The use of combined creatinine-cystatin C equations may improve precision by minimizing the influence of any particular non-GFR determinant.[27,28]

In conclusion, a hypocaloric LC diet and an energy-matched traditional HC diet had similar effects on markers of renal function in people with T2DM without DKD over a 12-month period. These results corroborate evidence that consumption of an LC weight loss diet does not adversely affect renal function in such populations. Longer-term follow-up studies are required to determine whether these renal effects are sustained during long-term weight loss maintenance and to establish if long-term adherence to the diets results in differences in the development and progression of DKD. Examining the effects of LC diets in populations with preexisting vascular complications besides nephropathy would further advance our understanding of the utility of LC diets.

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CHAPTER 6. LONG-TERM EFFECTS OF A VERY LOW CARBOHYDRATE AND HIGH CARBOHYDRATE WEIGHT LOSS DIETS ON COGNITIVE PERFORMANCE IN OBESE ADULTS WITH TYPE 2 DIABETES: A RANDOMIZED CONTROLLED TRIAL

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6.1 Summary

The long-term effects of consuming a very low carbohydrate, high far diet (LC) compared with a high carbohydrate, low fat (HC) diet on cognitive performance in individuals with T2D, are described in Chapter 6.

In adults with obesity and T2D, both LC and HC weight-loss diets combined with exercise training had similar effects on cognitive performance assessed by a comprehensive neuropsychological test battery, after 52 weeks. These results advance the idea of LC diets as an effective long- term therapeutic strategy for T2D management to improve glycemic control and reduce CVD and diabetes complication risks, with no deleterious effects on cognitive performance. Vascular risk factors such as hypertension, dyslipidaemia and obesity increase the risk of cognitive dysfunction. These associations therefore underscore the advantage of a lifestyle modification strategy in T2D that includes LC diets. Such a combined diet and lifestyle intervention would be able to target these risk factors concomitantly to potentially ameliorate cognitive function or attenuate/ prevent it's decline.

Further research is required to increase the generalisability of the findings reported in Chapter 6. This entails investigating the cognitive effects of LC diets in older adults with T2D, as well as in individuals with pre-existing diabetes complications, poorer cognitive function and glycaemic control, who are at greater risk of cognitive decline.

Statement of Authorship

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Contribution to the Paper	Study concept and design, data collection and extraction (e.g. cognitive tests), analysis and interpretation of data, drafting of the manuscript, read and approved the final manuscript.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Long-term effects of a very low carbohydrate and high carbohydrate weight loss diets on cognitive performance in obese adults with type 2 diabetes: a randomised controlled trial

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ABSTRACT

Background and Objective

This study examined the long-term effects of a very low carbohydrate, high far diet with a high carbohydrate, low fat diet on cognitive performance in individuals with type 2 diabetes (T2D).

Subjects/Methods

115 obese adults with T2D (66 males, BMI:34.6±4.3kg/m^2, age:58±7yrs, HbA1c:7.3±1.1%, diabetes duration:8±6yrs) were randomised to consume either a hypocaloric, very low carbohydrate, low saturated fat (LC) diet (14% energy as carbohydrate [CHO<50g/day], 28%protein [PRO], 58%fat [<10%saturated fat]) or an energy-matched high unrefined carbohydrate, low GI, low fat (HC) diet (53%CHO, 17%PRO, 30%fat[<10% saturated fat]); with supervised aerobic/resistance exercise (60mins,3d/wk) for 52 weeks. Body weight, HbA1c and cognitive performance assessing perceptual speed, reasoning speed, reasoning ability, working memory, verbal fluency, processing speed, short-term memory, inhibition and memory scanning speed were assessed pre- and post-intervention.

Results

Overall weight loss was (mean[95%CI]; -9.3[-10.6,-8.0]kg) and improvement in HbA1c was (-1[-1.2,-0.8]%); no difference between groups (P≥0.18). Scores for memory scanning speed, perceptual speed and reasoning speed improved (P≤0.03 time), and verbal fluency and short-term memory declined (P≤0.005 time), with no difference between diets (P≥0.24 time x diet). All other cognitive scores remained unchanged (P≥0.07). HbA1c reductions correlated with improvements in some cognitive abilities.

Conclusions

In obese adults with T2D, both LC and HC weight-loss diets combined with exercise training had similar effects on cognitive performance. This suggests an LC diet integrated

within a lifestyle modification program can be used as a strategy for weight and diabetes management without the concern of negatively affecting cognitive function.

INTRODUCTION

The growing prevalence of obesity and type 2 diabetes (T2D) has fuelled interest in very low carbohydrate diets (LC: 20-70g carbohydrates/day). This is supported by current dietary guidelines advocating personalised management (1), and increasing evidence demonstrating the long-term efficacy of LC diets for improving glycemic control and cardiometabolic outcomes compared to traditional high carbohydrate (HC) diets (2-4).

T2D is an independent risk factor for cognitive impairment and dementia (5, 6). Psychomotor efficiency, memory, learning, executive function and speed of information processing are often most affected (5, 7). This underscores the importance of considering the impact of diabetes management strategies on cognitive function. A concern with LC diets is that carbohydrate restriction below the recommended daily allowance of 130g/day, corresponding to the average minimum amount utilised by the brain (8), could impair cognitive function by lowering dietary glucose availability. Consequently, there are concerns that LC diets may adversely affect cognitive function (9).

Few studies have examined the long-term effects of LC diets on cognitive function. To date, only two longer-term (24-52 week) studies have been conducted, and these reported no differences in cognitive performance between participants consuming either an LC or HC diet (10, 11). However, these studies only examined a limited range of cognitive domains in individuals without T2D. It is therefore critical to examine the long-term effects of LC diets on cognitive performance on a more comprehensive range of cognitive domains in individuals with T2D. This study compares the long- term effects of a hypocaloric LC diet to an energy-matched HC diet, as part of a holistic lifestyle modification program, on cognitive performance in obese individuals with T2D.

METHODS

Design Overview, Setting and Participants

The study was conducted at the CSIRO Clinical Research Unit (Adelaide, Australia) between May 2012 and September 2013. The participants and study design have been described elsewhere (4). Briefly, 115 overweight/ obese adults (49 females/66 males) with T2D [mean±SD; age:58±7yrs, BMI:34.6±4.3kg/m^2, HbA1c:7.3±1.1% (56±12mmol/mol), diabetes duration:8±6yrs, highest education level (15% high school graduate, 35% diploma/vocational training, 50% university/higher degree)] were recruited by public advertisement. Exclusion criteria were type-1 diabetes; abnormal liver or renal function; any significant endocrinopathy (other than stable treated thyroid disease); history of malignancy, respiratory, gastrointestinal, cerebrovascular, peripheral or cardiovascular disease; pregnancy/lactation; severe depression; history of/or current eating disorder or smoking. The Human Research Ethics Committees of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Universities of Adelaide and South Australia approved the protocol. All participants provided written informed consent before study commencement.

In a 52-week parallel design, participants were block-matched for age, gender, BMI, HbA1c and diabetes medication before random computer generated assignment to consume either a hypocaloric (500-1000kcal/day deficit) LC, high unsaturated/low saturated fat diet (n=57; 14% energy as carbohydrate [CHO <50 g/day], 28% protein [PRO], 58% fat [<10% saturated fat]) or an energy-matched HC diet (n=58; 53% CHO, 17% PRO, 30% fat [<10% saturated fat]) that reflected conventional dietary guidelines (12), in a 1:1 ratio. Randomisation procedures (sequence generation and allocation concealment) were performed by research associates not involved in outcome assessments and intervention delivery. To achieve the targeted macronutrient profile, specific foods

were listed in a quantitative food record that participants completed daily. Diet plans were individualised and participants met individually with a dietician every 2 weeks for 12 weeks and monthly thereafter. To facilitate compliance, key foods (~30% total energy) representative of the assigned diet profiles were provided for the first 12 weeks, with key foods or a $50AUD voucher provided on alternate months thereafter. In addition, all participants undertook the same supervised moderate intensity aerobic/resistance exercise sessions (60mins, 3d/wk), consistent with diabetes management guidelines (13).

Detailed description of the diets, exercise program and the effects of the interventions on body weight, glycemic and cardiometabolic outcomes have been reported elsewhere (13). Both groups achieved high dietary compliance and had similar increases in physical activity levels (4). Compared to HC participants, LC participants reported a lower mean carbohydrate intake (LC 54-74g/day, HC 202-218g/day).

Outcome measures

Outcomes were assessed at weeks 0, 24 and 52. Body weight (assessed monthly) was measured using calibrated electronic scales (Mercury AMZ1, Tokyo, Japan). Height was measured using a stadiometer (SECA, Hamburg, Germany). HbA1c was measured at a certified pathology laboratory (SA Pathology; Adelaide, Australia). The test battery used to assess cognitive performance (Table 1), comprised several tasks measuring a broad variety of cognitive domains. Tests were selected because they assess cognitive domains most consistently affected by diabetes and have been shown to be sensitive to diet (6, 14-16). Specifications of each test have been fully described elsewhere (17).

Cognitive assessments were conducted at CSIRO's (Adelaide, Australia) cognitive laboratory after overnight fasting and water consumed as required. No hypoglycemic incidents were reported. Tests were administered in a consistent order across test sessions and scored by trained research personnel blinded to participants' treatment assignment.

Each session included computer (Inquisit v.2, Millisecond software) and paper tasks lasting approximately 1h. To reduce the likelihood of fatigue, speed and accuracy-based tasks were interspersed. Each task was preceded by practice items and queries were addressed before test commencement. Parallel versions of word memory and word endings tests were used to control for material- specific learning effects. Quality control included regular audit of test sessions, independent dual-scoring and regular review of session notes.

Depression, a potential confounder and frequent comorbidity in T2D was assessed by the Beck Depression Inventory-II (BDI-II)(18), to control for possible effects of mood disturbances or affective disorders. Participants' BDI-II scores were in the subclinical range (mean±SD; LC 6.3±6.2, LC 5.2±4.7, p=0.29). 8 LC participants reported taking antidepressant medications (ADM) and 2 HC participants began taking ADM during the study. None of the participants were on dementia medications.

Power & Statistical Analyses

Power analyses were conducted *a-priori* to determine the necessary number of participants using G*Power software (19). For a univariate repeated measures model it was determined a minimum combined sample of n=82 was sufficient to detect a small (*f*=.15) Time x Diet interaction effect with sufficient 95% power, assuming α=.05. This number of participants was also sufficient to determine time effects, indicating the number of participants recruited for this study exceeds this minimum requirement.

Baseline demographic and clinical characteristics between groups were compared using independent t-tests and χ^2 tests for continuous and categorical variables, respectively. Mean response latencies for speed tasks were converted to work rates (1000/mean latency) in order to normalise the distributions. Work rates represent the average number of items completed per second of measured response time, and higher scores indicate better performance. The effect of the diet intervention on change in cognitive function over time

was analyzed by random-coefficient analysis, restricted maximum likelihood, mixed effects models using an unstructured covariance that assumed data to be missing at random. Participants with a cognitive assessment at baseline and at least 24 or 52 weeks follow-up were included in the final analyses (n=114 for word memory, 2-choice reaction time and odd-man-out letter (OMO); n=113 for digit symbol substitution (DSST), word endings, number memory scanning (MScan) and colour stroop; n=112 for letter sets and operation span tasks). Four (LC:3, HC:1) participants were excluded from various cognitive function analyses due to non- compliance to the test protocol, accuracy score <50%, language difficulty and time constraints precluding completion of tests. The following fixed effects were included in the model: main effect for each time-point, diet group assignment, and diet group by time-point interaction. Estimated marginal means (95% confidence intervals, CI) and change from Week 0 to 52 are reported. Analyses with adjustment for age, gender, depression, and education status were conducted for sensitivity analyses. The magnitudes of effect size (d) for time effects were calculated as the mean difference in performance from week 0 to week 52 divided by the pooled SD of the difference (20). Linear regression analysis was used to assess the association between the change in anthropometric markers (weight and BMI) and metabolic control outcomes (HbA1c), with changes in cognitive function. Where significant univariate effects were found, multivariate models were used to adjust for the impact of sex and age. The sample size of the study was determined based of the primary outcome of HbA1c that has been previously reported [4]. All analyses were performed using SPSS 20.0 for Windows (SPSS Inc.; Chicago, IL, USA); statistical tests were two-tailed with statistical significance at $p<0.05$.

RESULTS

As reported elsewhere (4), both groups had comparable completion rates for the intervention (LC:71%, HC:65%, P=0.51) (**Figure 1**), reductions in weight (mean[95%CI]; LC -9.8[-11.7,-7.9], HC -10.1[-12.0,-8.2]kg, p=0.18) and HbA1c (LC -1.0[-1.2,-0.7], HC -1.0[-1.3,-0.8]%, p=0.65).

Cognitive outcomes based on estimated marginal means from primary mixed-models analyses are shown in **Table 2**. There were no statistically significant differences in cognitive test performance scores between the diet groups for any of the cognitive outcomes assessed (p≥ 0.24 time x diet). However, there were some main effects of time. Specifically, word endings (Cohen's d = 1.01**,** p<0.001) and word memory scores (Cohen's d = 0.31**,** p=0.005) decreased over time, whereas performance on DSST (Cohen's d = **-**0.28**,** p=0.03), MScan (Cohen's d = **-**0.34**,** p= p<0.001) and OMO performance (Cohen's d = **-**0.49**,** p= p<0.001) improved over time. Fully adjusted models controlling for covariates associated with cognitive function (age, gender, education and depression) did not alter the results for diet groups, although the time effect for word memory was no longer statistically significant (p=0.20) because education appeared to account for the difference in performance across time (p=0.02).

Linear regressions showed a higher % change in body weight (corresponding to a greater reduction) significantly predicted change (increase) in DSST performance (β=0.25, p=0.03). This effect remained significant after controlling for age and sex (β=0.23, p=0.03). Change in HbA1c significantly predicted change in operation span, a measure of working memory (β=-0.25, p=.03), and color stroop (β=-.28, p=.01). For operation span, reductions in HbA1c corresponded to a greater increase in the number of words recalled correctly during the working memory measure. For stroop, improvements (reductions) in HbA1c were associated with faster responses to incongruent stroop trials, suggesting better

inhibitory control. Neither of these effects changed notably after adjusting for age and sex (β=-.24, p=0.04 for operation span, and β=-.27, p=0.02 for color stroop).

DISCUSSION

This study showed after 52 weeks participation in a lifestyle intervention that consumption of either an LC or HC diet had comparable effects on cognitive performance in overweight and obese individuals with T2DM as assessed by a comprehensive neuropsychological test battery. These results are consistent with previous longer-term studies (24-52 weeks) conducted in populations without T2D that showed no difference in cognitive effects following consumption of LC and HC weight loss diets on tasks that assessed working memory and speed of processing (10, 11); attention and reaction time, short- term memory and problem solving (11). Results from smaller, shorter-term studies (3- 8weeks), however, do suggest some differences between HC and LC diets for speed of processing, attention and memory performance (21, 22). Whilst these studies suggest that differences in cognitive performance may occur between HC and LC diets over the short-term, these effects are unlikely to be sustained over the long term, as demonstrated by the present results.

Despite the absence of any differential effect of diet type on cognitive outcomes, significant improvements in performance on the DSST, MScan and OMO tasks were sustained over 52 weeks in both groups. It is possible these improvements over time reflect practice effects (23, 24) and may not be due to the effects of the intervention. Such responses are relatively common for cognitive testing (25). Alternatively, the improvements observed for these cognitive domains may be attributed to the increase in physical activity following participation in the planned exercise sessions that is associated with improved cognitive outcomes (26, 27), combined with the sustained weight loss and metabolic improvements resulting from the intervention. The significant association found between change in % body weight and DSST provide some support for this. A study by Witte et al. (28), for example, showed that cognitive performance improved in overweight individuals following 3-months of calorie restriction and weight loss, with no improvement

in the non-dieting control group. This study also showed the improvements in cognitive performance correlated with improvements in metabolic control, which supports the suggestion that improvements in physical health may underpin these effects. Moreover, the Look AHEAD study showed that participation in an intensive lifestyle, weight loss intervention improved cognitive function in overweight individuals with T2D (29). Collectively these data highlight the importance of weight loss and lifestyle modification for improving cognitive function in T2D that could be used as an effective strategy to reduce the increased risk of cognitive impairment and dementia in this patient population (5, 6).

It is important to consider the clinical implications of the potential positive effects of diet and lifestyle interventions on cognitive function in T2D. It has been shown that the average rate of change in cognitive function reflecting normal ageing is approximately 0.25 standard deviations per life-decade from age twenty onwards (30). The average effect size across DSST, memory scanning and OMO tasks herein was 0.37 standard deviations. Thus, it could be considered that the improvements observed in the present study are analogous to reversing the normal cognitive ageing process by around ten-to-fifteen years. These gains are likely to be particularly beneficial in populations such as T2D who are already at increased risk of cognitive impairment. However, the true magnitude of the effect is difficult to judge, since practice effects cannot be entirely excluded as a potential cause of changes observed.

An interesting finding in the present study was the significant decline in performance on the word endings test. Parallel forms of this test were used and they were designed to be of equivalent difficulty. However, these scores may have decreased at follow-up assessments due to increased test complexity. Evidence of this is apparent in the magnitude of this effect; performance decreased by one standard deviation which is a very large effect. Whilst other prospective studies have shown a decline in verbal fluency test performance

in adults with T2D, the effect sizes (for time) were comparatively smaller (Cohen's *d*=0.2) (24); mean annual decline of 0.4 points or 0.1±1SD units (31), and these effects emerged after 4 to 6 years. Therefore, given the 12-month time frame of the present study and magnitude of the effect observed, it is possible that the decline was due to increasing test difficulty, and future studies should be cautious of these methodological issues.

A limitation of the present study was the relatively modest sample size examined. However, previous shorter-term studies with smaller sample sizes have been sufficient to demonstrate differences in cognitive performance between HC and LC diets (21, 22), and the total number of participants recruited exceeded those determined by a-priori power analyses. This study was also conducted in a middle-aged, T2D population with relatively good metabolic control and minimal cognitive deficits based on age-specific normative scores (32). However, clinically-relevant cognitive decrements in T2D occur mainly in those with diabetes-related vascular complications and older adults >65years of age (7). Therefore, while our participants represent an age group at a critical window of opportunity for targeted interventions that may slow diabetes complication progression, further studies that examine the effects of these dietary patterns in older individuals with diabetes, pre-existing diabetes complications, poorer cognitive function and/or metabolic control are required to better understand the wider generalizability of the current findings. Additionally, it is possible that the tasks used to assess changes in cognitive performance lacked sufficient sensitivity to detect any differences between the diets examined. However, the tests comprising the large battery of cognitive tasks used in this study were selected based on their known sensitivity to dietary manipulation (15), and assessed different cognitive domains sensitive to T2D (6). This suggests the current battery would have been suitable to identify any diet differences.

In summary, in overweight and obese adults with T2D, both LC and HC weight-loss diets combined with exercise training had similar effects on cognitive performance. This

suggests both dietary patterns can be used as a strategy for diabetes and weight management without concerns for long-term negative impacts on cognitive function. In fact, the cognitive improvements observed over time in both diet groups and the modest relationship between weight loss and improvements in metabolic markers with cognitive performance demonstrate the potential benefits of combined diet and lifestyle interventions in T2D. Further studies should be undertaken to evaluate the cognitive effects of these diets in older adults with T2D, pre- existing diabetes complications, poorer cognitive function and glycemic control, who are at greater risk of cognitive decline.

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Author Contributions

A/Prof Grant Brinkworth is the guarantor of this work and, as such had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: All authors. Analysis and interpretation of data: All authors. Drafting of the manuscript: JT, ITZ and GDB. Critical revision the manuscript for intellectual content: NLM, CHT, VD, MN, JDB and GAW. Read and approved the final manuscript: All authors. Obtained funding: GDB, MN, JDB, NLM, and CHT. Study supervision: GDB, NLM, CHT, MN and JDB.

Conflict of Interest Disclosures: None declared

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Table 1. Cognitive test battery*

Task	Cognitive domain	Task description	Outcome measure
Digit-Symbol Substitution Test (DSST)	Perceptual speed	In this task a coding key is presented at the top of a page showing the numbers 1-9, each paired with a unique symbol. Participants copy as many symbols as possible corresponding to a random array of numbers (33).	Number of items completed correctly in 90 seconds
Odd-Man-Out Letter Task (OMO)	Reasoning speed	Participants decide as quickly as possible the whereabouts of the 'odd man out' in a set of letters.	Average work rate (1000/ mean response latency)
Two-Choice Reaction Time	Processing speed	Participants respond as quickly as possible to on-screen stimuli (the numbers '1' or '2') by pressing the left or right arrows on the numeric keypad of the keyboard.	Average work rate (1000/ mean response latency)
Number Memory Scanning (MScan)	Speed of memory scanning	Participants are presented with a string of numbers presented one at a time. After the presentation string, a target number is shown on screen. Participants indicate as quickly as possible	Average work rate (1000/ mean response latency)

		whether the number was in the sequence presented immediately before.	
Colour Stroop	Inhibition	Participants press the right shift key on the keyboard if the word is coloured blue, and the left shift key if it is coloured yellow. Words are presented in congruent (word matches the printed colour), incongruent (word does not match the printed colour), or neutral format (neutral words presented).	Difference between work rates (1000/mean response latency): Incongruent trial work rate minus mean work rate across congruent and neutral trials (30 each).
Operation Span	Working memory	Participants remember words whilst simultaneously completing arithmetic problems. There are different block lengths (from two words through to six words).	The number of words recalled correctly in the correct position within corresponding blocks
Letter Sets	Reasoning ability	In this task participants analyse five sets of four-letters in order to determine the common pattern amongst the sets, and to decide which set does not match the recurring pattern (34).	Number of items out of 15 answered correctly
Word Endings	Verbal fluency	In this task participants are given 2 minutes to freely recall as many words as possible which end with a prescribed set of	Number of freely recalled words in 2-minutes with a specified ending

		letters (e.g. '-ate') (34).	
Word Memory	Short- term memory	Fifteen words (nouns) are presented one at a time on the computer screen. After presentation of all words, participants write down as many as they can recall, in any order.	Number of words correctly recalled from presented list (maximum score of 15)

* All tasks adapted from (17).

Table 2. Estimated marginal means derived using linear mixed- effects model analysis and mean confidence intervals (95% CI) for cognitive performance across 52 weeks for low carbohydrate (LC) diet and high carbohydrate (HC) diet groups

	LC Diet				HC Diet				Difference in mean changes between groups	Diet x Time P Value
	Week 0	Week 24	Week 52	Change over 52 weeks	Week 0	Week 24	Week 52	Change over 52 weeks		
DSST score	51.2 (48.3, 54.1)	51.1 (47.9, 54.3)	53.5 (50.7, 56.3)	2.30 (0.39, 4.21)	53.0 (50.2, 55.9)	53.8 (50.7, 57.0)	54.1 (51.3, 56.9)	1.10 (-0.81, 3.0)	1.20 (-1.5, 3.9)	0.28
Letter sets score	8.55 (7.74, 9.37)	8.79 (7.84, 9.75)	9.10 (8.21, 10.0)	0.55 (-0.09, 1.19)	9.65 (8.83, 10.5)	9.96 (9.02, 10.9)	9.74 (8.84, 10.6)	0.10 (-0.55, 0.74)	0.45 (-0.45, 1.36)	0.48
Operation span score	29.6 (27.6, 31.7)	29.8 (27.3, 32.4)	31.1 (28.8, 33.3)	1.44 (-0.28, 3.16)	30.7 (28.7, 32.7)	29.4 (26.9, 31.9)	30.3 (28.0, 32.6)	-0.45 (-2.24, 1.35)	1.88 (-0.60, 4.37)	0.24
Word endings score	13.5 (12.0, 15.0)	12.3 (11.0, 13.5)	8.35 (6.88, 9.82)	-5.19 (-6.54, -3.84)	12.8 (11.3, 14.3)	11.8 (10.6, 13.1)	9.09 (7.60, 10.6)	-3.72 (-50.9, -2.34)	-1.47 (-3.40, 0.45)	0.27

Word memory score	6.89 (6.39, 7.40)	6.57 (6.00, 7.14)	6.39 (5.82, 6.96)	-0.51 (-1.05, 0.03)	7.14 (6.63, 7.65)	6.39 (5.83, 6.95)	6.51 (5.93, 7.09)	-0.63 (-1.18, -0.08)	0.12 (-0.65, 0.89)	0.53
2-Choice reaction time score	1.41 (1.33, 1.49)	1.40 (1.31, 1.48)	1.47 (1.39, 1.55)	0.06 (-0.007, 0.13)	1.44 (1.36, 1.52)	1.43 (1.34, 1.51)	1.45 (1.36, 1.53)	0.004 (-0.07, 0.08)	0.06 (-0.04, 0.16)	0.41
OMO score	0.57 (0.51, 0.62)	0.59 (0.54, 0.64)	0.65 (0.59, 0.71)	0.08 (0.04, 0.13)	0.60 (0.54, 0.66)	0.60 (0.55. 0.65)	0.67 (0.61, 0.73)	0.07 (0.02, 0.11)	0.01 (-0.05, 0.08)	0.83
MScan score	0.97 (0.91, 1.02)	0.90 (0.84, 0.96)	1.01 (0.95, 1.07)	0.04 (0.004, 0.08)	0.95 (0.90, 1.01)	0.91 (0.85, 0.97)	0.99 (0.93, 1.05)	0.03 (-0.005, 0.07)	0.008 (-0.05, 0.06)	0.65
Colour stroop score	-0.20 (-0.23, -0.17)	-0.19 (-0.23, -0.14)	-0.17 (-0.21, -0.13)	0.03 (-0.01, 0.07)	-0.18 (-0.21, -0.14)	-0.17 (-0.21, -0.13)	-0.14 (-0.18, -0.10)	0.04 (-0.004, 0.08)	-0.01 (-0.07, 0.05)	0.71

Abbreviation: DSST, Digit symbol substitution test; HC diet, High carbohydrate, low fat diet; LC diet, Very low carbohydrate, high unsaturated/ low saturated fat diet; MScan, Number memory scanning test; OMO, Odd- man- out letter test.

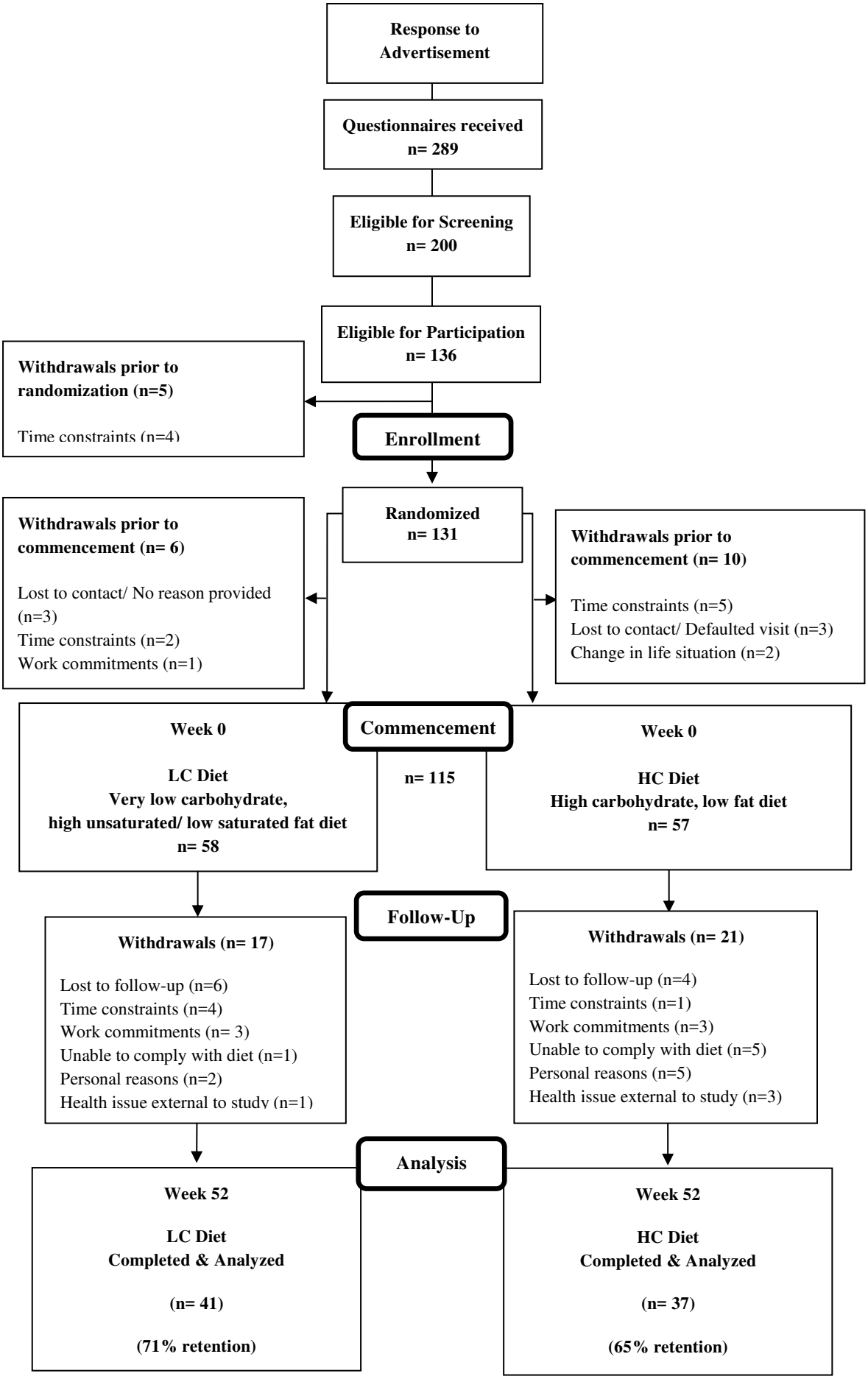

Figure 1. Study flow diagram

CHAPTER 7. DISCUSSION AND CONCLUSIONS

7.1 Overall significance of the work

The incidence and prevalence of diabetes have continued to surge worldwide despite therapeutic advances. The gravity of the problem highlights the urgent need for more efficient and effective treatment strategies. The research findings described in this dissertation have further clarified the long- term effects of LC diets in people with T2D. The papers published in Chapters 2 and 4 provide evidence for the long term clinical effectiveness of LC diets for improving overall glycaemic control, reducing CVD risk and achieving weight loss compared to an energy- matched HC diet, in the setting of a well-controlled RCT. These salutary effects conferred by a lifestyle intervention strategy that incorporated an LC diet and regular physical activity were maintained over 1 year and provide support for the long- term benefits of carbohydrate restriction for T2D management. Chapter 5 and 6 reported similar changes in renal markers and a comprehensive range of cognitive measures in participants who consumed either an LC or a traditional HC diet, suggesting that concerns regarding the long- term safety of LC diets are conjectural. There were no ostensible adverse effects on renal health and cognitive performance from the long- term consumption of LC diets in people with T2D without overt DKD, or any known cognitive impairment at baseline. The results presented support a treatment paradigm shift in which a lifestyle modification strategy that combines an LC diet with structured exercise is recommended for people with T2D. Considered together, these findings suggest that LC diets may offer greater benefits to people with T2D.

The findings from the lifestyle intervention strategy reported in Chapters 2 and 4 that incorporated an LC diet and structured physical activity provide credible evidence to guide public policy and clinical practice. The knowledge, and insights gained from working on the study described in this thesis will be crucial in determining the strategies and approaches needed to ensure that the results of the trial are well applied in real-world practice settings for obesity, prediabetes, and T2D. The successful replication of the findings in clinical management and public health practice hinges on several factors. Ongoing efforts to promote self-management education and support are required to ensure the sustained benefits of lifestyle interventions are realized and the risk of developing long-term diabetes- related complications is reduced. In addition to a focus on developing self-efficacy, efforts to increase the adoption and maintenance of diet interventions and PA require fostering social support from family, friends and healthcare providers (348-350).This accentuates the potential benefits of a community delivery of the lifestyle intervention model described in this thesis where community resources are harnessed to support individuals with diabetes in their efforts to make lifestyle improvements and ensure continuity of care. Other strategies that facilitate behavioural modification to reap continued benefits from LC diets are discussed in Section 7.3.

Often considered a fad diet, there is a palpable bias against LC dietary approaches due in part from the lack of understanding of the long- term effects of these diets (351). This misconception poses a significant barrier to the widespread adoption of LC diets. It is therefore crucial that healthcare providers are well-informed about nutrition therapy principles and keep abreast with emerging evidence on the long- term effects of different dietary patterns. This enables healthcare providers to be knowledgeable and supportive when patients are interested in trying different diets, such as with LC diets where there is growing evidence supporting their utility particularly in people with prediabetes and T2D.

7.2 Contribution to knowledge

Medical Management

A key feature of the study was its medical management plan where participants continued to receive their diabetes and general health care from external providers. To minimise the risk of hypoglycaemia especially during the first 6 months of the study when caloric restriction was first implemented and weight loss was expected to be greatest, all participants monitored their FBG daily and these readings were used by the study's medical staff to recommend adjustment of diabetes medications to participants' health care providers according to a pre-set algorithm. In addition, the study sought to facilitate effective medical management by providing participants and their physicians with periodic health reports of clinical data detailing participants' diabetes control and CV risk factors throughout the study, as well as ongoing communication with participants' physicians on any recommendations for the titration of medications. Changes in anti-hyperglycaemic medications during the study were also assessed objectively using the MES which provided an indication of glycaemic control.

Glycaemic variability

Chapter 3 outlined the development of GV which measures a different aspect of blood glucose regulation from conventional markers such as HbA1c and FBG, as an emerging marker of glycaemic control in diabetes. Recent GV research in human endothelial cells showed that transient hyperglycaemic spikes above a critical threshold level activates a multicomponent feedback loop which increases reactive oxygen species (ROS) production that persists for days during subsequent periods of normal glycaemia (352). These hyperglycaemic episodes that are too transient to affect HbA1c are thought to determine up

to 89% of diabetes complications risk not explained by HbA1c (352). Novel therapeutic agents for the prevention and treatment of diabetes complications such as loop disrupting peptides that inhibit various components of the feedback loop are being developed. The GLP-1 cleavage product, GLP-1(9-36)(amide) is one such substance that has been shown to normalise the persistent post-hyperglycaemic overproduction in ROS and its pathophysiologic effects (352). Chapters 2 and 4 reported the findings of the first RCT that assessed long term changes in GV in people with T2D who consumed an LC diet compared to a traditional HC diet. The LC diet was found to reduce GV parameters to a greater extent compared to the HC diet. These greater reductions persisted at 1 year in several GV parameters. These publications are the first to report on a diet strategy that produced greater improvements in GV and provides further support for the therapeutic potential for LC diets for improving glycaemic control in people with T2D. Future studies could focus on GV parameters that assess hyperglycaemic peaks above a certain threshold as a basis for comparing the efficacy of LC diets with these new therapeutic agents being developed.

Saturated fats and LDL-C

A significant barrier to widespread acceptance of LC diets has been concern about saturated fat, which might be substituted for the carbohydrate that is removed (351). A key focus of the dietary recommendations for CVD prevention and treatment has been limiting saturated fat intake, chiefly as a means of reducing LDL-C (353). RCT data suggest that atherosclerosis progression and CV events are minimized when LDL-C is lowered to <1.8 mmol/L. (354). Mendelian randomisation analyses demonstrated a 54% reduction in CVD risk for every 1 mmol/L reduction in LDL-C over a lifetime (355). A meta- analysis of

RCTS on statin therapy also showed an association between lower levels of these atherogenic lipoproteins achieved and a lower CVD risk (356).

Chapters 2 and 4 have reported the short and long- term effects of consuming an LC diet with a low saturated fat content, in an RCT. The quality of fats and oils in a diet have an influence on CVD risk (240) and different intakes of saturated fats in both LC and HC diet comparison groups in previous studies could explain some of the inconsistencies in blood lipid results observed. This potential confounding factor was addressed in the present study where both the LC and HC diets were limited in saturated fat. Importantly, this study showed that LDL-C did not increase in participants who had consumed an LC diet for 1 year and who were no longer in active weight loss.

Notwithstanding this, it should be acknowledged that the single grouping of saturated fats based merely on the absence of double bonds belies the considerable metabolic and biological diversity exhibited by individual saturated fats (357). Palmitic acid (C16:0) has displayed adverse metabolic effects in vitro while very long chain saturated fats (C20:0-24:0) have been associated with favourable blood lipid profiles and a lower CVD risk (358, 359). In contrast to carbohydrates, palmitic acid increased LDL-C but had no influence on apolipoprotein B, considered a superior indicator of atherogenic risk than LDL-C (240, 360). Endogenous de novo lipogenesis of myristic acid (C14:0) and C16:0, often produced from dietary carbohydrate also correlate more strongly with dietary intakes of refined carbohydrates than with meat and dairy consumption (359, 361). These findings which support the absence of significant harms from saturated fat intakes form the basis for recommending an unrestricted saturated fat intake in LC diets (273). However, dietary saturated fats are also derived from different foods that contain other nutrients which may influence the health effects of these foods. For example, flavoured/ sugar- sweetened skim milk may be lower in saturated fat compared to full- cream milk but contains a higher sugar content. Additionally, while some foods that contain saturated fats such as cheese,

yoghurt and nuts may be beneficial for health, excessive consumption of others such as red meat and especially processed meats may have deleterious effects for T2D risk and weight gain (362, 363). These data highlight the futility and limitations of solely focusing on modifying saturated fat intake as a single nutrient group. The LC diet studied herein differed from previous studies in that the dietary restriction of carbohydrates and saturated fat was achieved with the consumption of alternative foods that comprised dietary components that were consistent with good health and the prevention of nutrition- related chronic diseases such as CVD, dyslipidaemia, hypertension, cancer and obesity (e.g. MUFAs in nuts and oils, cruciferous and green leafy vegetables, low fat dairy). Strong evidence and general consensus exists for such a food- based dietary approach and the results from this study should serve to inform dietary recommendations (357).

7.3 Future directions

Greater use of behavioural modification strategies in long- term interventions

To facilitate the development of behaviours that support weight loss and long- term weight maintenance as well as to sustain clinically significant outcomes, future long- term interventions investigating LC diets should incorporate greater use of behavioural change strategies such as cognitive behavioural therapy (CBT) in the delivery of nutrition therapy (364). This will facilitate dietary modification and promote adherence to lifestyle changes to improve targeted diet- related outcomes such as weight, CV and diabetes risk factors (364, 365). Motivational interviewing, a highly effective counselling strategy associated with improved adherence and outcomes should be an added component of a CBT- based lifestyle modification program (366, 367). While self- monitoring, goal setting, problem solving, contingency management, stimulus control and social support were some of the effective strategies implemented in the current study, greater emphasis on cognitive

restructuring, stress management and relapse prevention may be useful in longer- term interventions (368, 369). Structured meal plans were used in this study to help participants streamline food choices and facilitate compliance to recommended energy intakes. However as mentioned in Section 1.2.5.1, meal replacements are another successful strategy that could be implemented. By reducing the time spent on food selection and preparation, meal replacements assist in limiting participants' exposure to foods that they may overconsume and help participants focus on portion control as they modify their dietary habits (370). Conversely, financial incentive strategies have been shown to be less effective and should not be used (371). Additional research is needed to investigate the efficacy of these behaviour modification strategies in the delivery of lifestyle interventions that incorporate LC diets.

Determining the effects of the quality and source of carbohydrates and protein within a carbohydrate restricted diet

Prospective cohort data from the Nurses' Health study and Health Professionals' follow-up study showed that LC diets that included plant sources of fat and protein reduced the risk of coronary heart disease and lowered all-cause and CVD mortality rates over at least 20 years of follow- up (372, 373). In contrast, an LC diet based on animal sources of fat and protein was associated with higher all-cause mortality (373). Among sources of animal protein, a high intake of processed meat has been linked consistently with an increased CVD and T2D risk (374-376). While some studies have shown that high consumption of red meat is associated with an increased risk of developing T2D as well as total, CVD, and cancer mortality, the association is weaker relative to processed meats(375-377). Substitutions of red meat with other protein foods such as fish, poultry, nuts, legumes and low-fat dairy has been shown to lower CV and mortality risk (377, 378). With regards to

carbohydrates, wholegrains have been shown to be advantageous for the T2D prevention, stroke, CVD and all-cause mortality (379-381). However high intakes of refined grains have not shown a protective effect on CV events and mortality rates (381, 382). Higher glycaemic load diets marked by increased consumption of foods rich in rapidly digestible, low fibre carbohydrates have also been associated with greater long- term weight gain (362).

The LC dietary pattern prescribed in the study and described in Chapters 2 and 4 of this thesis consisted of both plant and animal sources of protein. It was also limited in refined grains and designed to provide adequate fibre from non- starchy vegetables and some wholegrains. The epidemiology data presented above suggests that specific food choices can be incorporated or restricted in an LC diet because of their potential effects on CV health and mortality although this was not formally tested in this study. It is nevertheless conceivable that differences in the source and quality of carbohydrate and protein in an LC diet may yield different health effects. However, the effect of residual confounding in observational data cannot be excluded and future studies should confirm these findings in RCTs.

Examining the glycaemic and cardiometabolic effects of carbohydrate restricted diets in people with type 2 diabetes and pre-existing cardiovascular disease

Given the greater improvements in glycaemic control and CVD risk factors observed in the LC diet group reported in Chapters 2 and 4, an extension of the trial would be to investigate the long term effects of consuming an LC diet in people with T2D and a history of CVD. This would increase the generalisability of LC diets as diabetes has a significant impact on CV morbidity and mortality and a significant proportion of adults with T2D (~1/3) have a history of CVD (383). Moreover, lifestyle interventions incorporating diet

and exercise have been shown to be effective in people with CVD and have successfully reduced CVD rates in these individuals (384).

Evaluating the effects of carbohydrate restricted diets on diabetes complications

Further evaluation of the effects on hard clinical endpoints such as CV events and diabetes complications will provide greater understanding of the therapeutic potential of LC diets. Some evidence posits that the severity and progression of diabetic retinopathy are determinants of incident CV outcomes (385). Diabetic retinopathy may thus serve as a reliable surrogate for major CV events but with an earlier response to interventions (93). Moreover, in patients with severely increased albuminuria (albumin creatinine ratio, ACR ≥300mg/ g Cr), the occurrence of diabetic retinopathy strongly suggests DKD, another microvascular complication of diabetes (5). Hence future trials should consider the development/ progression of diabetic retinopathy as a possible outcome measure.

With regards to the long- term renal outcomes of LC diets reported in Chapter 5, while patients with persistent and severely increased albuminuria (ACR≥300mg/ g Cr) have a high likelihood of developing ESRD, spontaneous remission of albuminuria (ACR 30-299mg/ g Cr) has been reported in up to 40% of T1D patients (38, 386). Moreover, factors such as exercise within 24h, infection, markedly elevated blood glucose and blood pressure may increase ACR without kidney damage, and not all people with diabetes, kidney disease and reduced eGFR present with albuminuria (5). eGFR calculated with formula using SCr may also be influenced by factors that affect SCr such as age, ethnicity and muscle mass (387). Therefore the assessment of other renal markers beyond albuminuria such as electrolyte levels (e.g. potassium), renal ultrasound and eGFR calculated using combined creatinine-cystatin C equations would provide a clearer indication of DKD development/ progression as a result of the intervention (388, 389).

Investigating the cost effectiveness of implementing intensive lifestyle interventions that incorporate carbohydrate restricted diets

Another question worth investigating is the cost effectiveness of LC diets. Given the greater reductions in anti- hyperglycaemic medications observed amongst participants that consumed the LC diet (as reported in Chapter 2 and 4), an economic evaluation of the intervention will help inform healthcare spending and resource allocation. Such an analysis is indispensable for LC diets to be considered as an effective lifestyle treatment for preventing diabetes complications in a health- system reform that seeks to minimise the long-term economic burden of the growing diabetes epidemic. The delivery of intensive lifestyle interventions in the community does necessitate having wide access to resources, trained staff and space. However, keeping someone healthy has exponential long- term cost savings and such lifestyle programs have generally been found to be cost- effective, yielding important health benefits at reasonable cost (390, 391).

Studying the long- term effects of carbohydrate restricted diets in Asian populations as a lifestyle strategy for type 2 diabetes management

Based on the current findings presented in this thesis, an extension of the work would involve examining the efficacy of an LC diet as a lifestyle modification strategy for T2D management in diverse populations and in particular, Asian populations. The prevalence of T2D in Asia has increased rapidly in recent decades, fuelled by rising rates of overweight and obesity driven by socioeconomic development, nutrition transition, and increasingly sedentary lifestyles (12). However, there are important features of the T2D epidemic in

Asia that suggest LC diets may have specific relevance as a therapeutic lifestyle strategy for the prevention and treatment of T2D in Asia.

Rapid nutrition transitions in many Asian countries have led to certain changes in diet and lifestyle that predispose Asians to an increased risk of developing T2D (392). Socioeconomic development and global trade liberalisation have led to changes in food supply that promote overconsumption and positive energy balance (9). Higher intakes of trans and saturated fats from palm oil and animal/ vegetable ghee (clarified butter) commonly used in cooking have been reported in Asian populations (393). While rice is a staple of many Asian diets, there has been a large shift from the consumption of wholegrains to polished white rice and refined wheat (9). The consumption of sugar-sweetened beverages and added sugar have increased (394). Consequently, high intakes of these refined carbohydrates constitute a large percentage of daily energy intakes, contribute to excess calories consumed and increase the GL of diets. An increased consumption of sugary beverages has been associated with an increased risk of developing T2D (395). The consumption of white rice in high GL diets has also been associated with a two-fold increase in the risk of T2D (396). This increase in T2D risk may be mediated by the increase in demand for insulin from consuming high GL diets, leading to pancreatic β-cell exhaustion in the long- term (397), especially in overweight or obese individuals who are more likely to be insulin resistant (398). An inadequate compensatory β- cell response to increasing insulin resistance has been blamed for increasing T2D risk in Asians (12, 399). The resultant deterioration in glucose tolerance has been shown to occur even with minimal weight gain (400). This decrease in insulin secretory reserves in Asian populations at all stages of glucose tolerance suggests a specific role of lifestyle interventions that incorporate LC diets. The physiology of LC diets targets insulin resistance, a known pathophysiological defect associated with T2D. LC diets can compensate for decreases in insulin secretion by reducing glucose availability to insulin-

resistant tissue to achieve durable glycaemic control (272). Furthermore, the dietary changes in Asian populations have been accompanied by concurrent reductions in physical activity from the adoption of more technologically- driven lifestyles characterised by greater automation and declining energy expenditures. It has therefore been suggested that the underlying insulin resistance caused by obesity from excessive energy intakes and reduced energy expenditures may compound the deleterious cardiometabolic effects of HC diets in Asians (9, 398). Collectively, these data suggest that an LC, low glycaemic load eating pattern that is limited in refined carbohydrates and saturated fats may be a particularly useful lifestyle strategy for managing and preventing T2D in Asians. Future studies should study the effects of LC diets combined with regular physical activity in Asians with T2D.

Furthermore, Asians are at a higher risk of developing T2D compared to people of European descent (12) and the risk of T2D begins at a lower BMI for Asians (401). Although overweight and obesity rates are still relatively lower in Asian compared to Western populations (2), several Asian countries have a disproportionately higher prevalence of diabetes than Western countries (402). The “metabolically obese phenotype” is ubiquitous in Asian populations and describes a propensity towards increased abdominal and visceral adiposity with lower muscle mass (9, 403). The high prevalence of central obesity among Asians increases the likelihood of insulin resistance in apparently normal-weight individuals according to conventional BMI criteria and is an important risk factor in the development of diabetes and CVD in Asian populations (9, 403, 404). Moreover, visceral adiposity (including mesenteric fat) has been significantly associated with subclinical atherosclerosis (increased carotid intima- media thickness) in Asian populations (405). Taken together, these evidence suggest that abdominal and visceral adiposity are important diabetes and CVD risk factors especially pertinent to Asian populations. Future studies examining the effectiveness of LC diets in Asian populations should therefore

include measures of central obesity such as waist circumference and visceral adiposity (rather than weight or BMI) in the selection criteria and as target outcome measures.

7.4 Conclusions

The severity and relentless growth of the diabetes epidemic merits careful reappraisal of the dietary guidelines that form the basis of the lifestyle treatment strategy recommended for T2D management. While current guidelines acknowledge that a variety of approaches may be useful for inducing weight loss and improving metabolic control (5), evidence such as that presented in this thesis continue to accumulate. These evidence demonstrate that while any calorie is energetically equivalent for short- term weight loss, the metabolism of an ingested calorie may vary depending on the individual's underlying physiologic, metabolic and genetic status (278). The long- term metabolic effects and obesogenicity of a particular diet may be affected by the multifarious effects of its individual dietary components on glucose- insulin response, satiety, hepatic lipogenesis, adipocyte function, brain craving, the microbiome, metabolic expenditure and even maternal-foetal influences (406, 407). Consequently, some dietary approaches may elicit physiological compensatory mechanisms that favour long- term energy balance and advantages on glycaemic control or certain metabolic health measures, while others may confer neutral or even deleterious effects (357). In the study reported in this thesis, isoenergetic LC and HC diets produced similar long- term weight loss in people with T2D. However the LC diet demonstrated greater effectiveness for improving glycaemic control and reducing CVD risk factors with minimal risk and good compliance. These findings suggest that adults who are insulin resistant such as those with T2D are more successful at improving overall diabetes and CVD risk control on an LC compared to HC diet. Hyperglycaemia is a salient characteristic of T2D and dietary carbohydrates raise blood glucose. Carbohydrate

restriction to alleviate hyperglycaemia is thus an intuitive concept that is easily grasped by patients (273). The findings reported in this thesis therefore provide further support for the use of LC diets as a therapeutic lifestyle strategy for T2D management.

Finally, translating these research findings into clinical practice requires fundamental shifts in public policies and healthcare systems. Non- communicable diseases such as obesity and T2D are also known as "social and environmental" diseases (3) because the development of these conditions result largely from a confluence of individual responsibility (unfavourable diet and physical activity choices), and environmental factors such as public policy and marketing by food industry that promote overconsumption and sedentary lifestyles (408). Consequently, the successful implementation of any lifestyle intervention strategy for T2D necessitates simultaneous social, policy and environmental change. The macroeconomic and political milieu determines the resources available for individuals to make lifestyle choices that confer benefits for T2D management. Hence a multifaceted government policy is essential to facilitate a supportive economic and legal environment which promotes the availability, affordability and acceptability of diets such as LC diets that are effective for T2D management and restrains the marketing and consumption of foods such as those rich in refined carbohydrates that are detrimental for long- term health. Concurrent efforts should also be made to influence community design, urban planning and transport infrastructure to promote physical activity. These efforts require a coordinated, interdisciplinary approach involving the cooperation of key stakeholders including governments, food industry, healthcare providers and academia, and underpinned by reliable evidence from scientifically rigorous studies.

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