Effects of very low-carbohydrate diets on the management of blood glucose levels in individuals with type 1 diabetes mellitus

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Executive overview

Poor glycaemic control plays an important role in the aetiology of long-term complications in people with type 1 diabetes mellitus (T1DM). Despite the many advances in both technology and diabetes education, it is extremely challenging for individuals with T1DM to maintain their blood glucose levels (BGL) within the normal physiological range (4–8 mmol/L). Hence, it is not uncommon for these individuals to restrict their carbohydrate (CHO) intake to reduce the magnitude of their glycaemic excursions. This approach raises the issue of whether the adoption of very low CHO ketogenic diets provides an effective strategy to manage blood glucose levels in T1DM. The purpose of this thesis was to examine the effect of ketogenic diets (< 55g CHO/day) on glycaemic control, glycaemic variability, time spent in range, blood lipid profile, and the risk of exercise-mediated ketoacidosis in individuals with T1DM.

The aim of the first study in this thesis (Chapter 2) was to examine the relationship between ketogenic diets and glycaemic control, along with the risk of dyslipidaemia in a sample of individuals with T1DM. In this observational study, 11 adults with T1DM who were consuming a ketogenic diet (< 55 g CHO/day) for 2.6 ± 3.3 yr had their blood sampled and analysed for glycated haemoglobin (HbA1c), blood lipid profile, as well as hepatic and renal function. They were also fitted with a blinded continuous glucose monitor for 7 days to measure their glycaemic variability. Mean HbA1c levels were 35 ± 4 mmol/mol (5.3 ± 0.4%), and participants spent 74 ± 20 and 3 ± 8% of their time in the euglycaemic (4-8 mmol/L) and hyperglycaemic (> 10 mmol/L) range, respectively, with little daily glycaemic variability (SD 1.5 ± 0.7 mmol/L; CV 26 ± 8%). Blood glucose levels were below 3.0 mmol/L for 3.6% of the time, and participants experienced 0.9 (0.0-2.0) daily episodes of hypoglycaemia. Total cholesterol, LDL cholesterol, total cholesterol/HDL ratio, and triglycerides levels were above the recommended range in 82, 82, 64 and 27% of participants, respectively. However, HDL levels were within the recommended range for all participants. Participants displayed no or little evidence of hepatic or renal dysfunction. This study provides evidence that although ketogenic diets in adults with T1DM are associated with optimal HbA1c levels and little glycaemic variability, they are also associated with marked dyslipidaemia and a high number of hypoglycaemic episodes.

Following from the findings in Chapter 2 that ketogenic diets in individuals with T1DM have optimal HbA1c levels and glycaemic variability, the aim of the next study of this thesis (Chapter 3) was to establish whether there is a relationship between daily CHO intake and HbA1c levels. In this observational study, 56 individuals with T1DM completed a 3-day CHO diary to estimate their average daily CHO intake and had their blood sampled after an overnight fast for analysis of HbA1c levels. This study showed that the relationship between HbA1c levels and daily CHO intake was best described by a sigmoidal curve, with a steep inflection point and an abrupt leap in HbA1c levels at a CHO intake of 99.5 g/day (1.25 g/kg body mass/day), with CHO intakes below 99.5 g/day being associated with lower mean HbA1c levels compared with daily CHO intake above this inflection point.

Many exercise guidelines for individuals with T1DM advocate the avoidance and termination of exercise when ketone body (KB) concentrations are elevated. However, one limitation with these guidelines is that they overlook the fact that moderately elevated KB concentrations can arise in response to the nutritional ketosis associated with ketogenic diets, thus raising the issue of whether it is safe for individuals with T1DM to exercise in a state of diet-induced ketosis. For this reason, the third study of this thesis (Chapter 4) investigated whether the risks of ketoacidosis and hypoglycaemia increase during and early after exercise in mildly ketotic adults with T1DM on a ketogenic diet. Eight adults with T1DM on such a diet performed the following exercise tasks on separate occasions; graded cycling to exhaustion, 60 min of aerobic cycling at 55% $\dot{V}O_{2peak}$, and a 30-s maximal sprint cycling effort. Immediately after exercise, ketone bodies (KB) concentrations decreased by 0.4 mmol/L ($p < 0.05$) in response to graded exercise, but remained stable in response to both aerobic exercise and sprinting ($p > 0.05$). After 60 min of recovery, KB concentrations remained below the baseline level for graded exercise ($p < 0.05$) and did not differ from the baseline levels for aerobic exercise and sprinting ($p > 0.05$). Plasma glucose levels did not fall in response to any of these three exercise modalities, with the rate of CHO oxidation accounting for only 17.0 ± 11.4% of the fuel oxidised during aerobic exercise. This study thus shows that, irrespective of exercise modality, KB concentrations do not increase and blood glucose levels do not fall during and early after exercise in insulin-treated adults with T1DM on a ketogenic diet when exercise is performed under basal insulin conditions. Such results suggest that these modes of exercise do not increase the risks of both

ketoacidosis and hypoglycaemia during and early after exercise, and are thus safe to perform under these conditions.

In summary, ketogenic diets are associated with near normal HbA1c levels and do not increase the risks of both exercise-mediated hypoglycaemia and ketoacidosis when exercise is performend under basal insulin conditions. However, ketogenic diets do appear to be associated with increased risk of dyslipideamia, the clinical significance of which remains to be established. More research is thus required to better understand the long-term benefits and risks associated with the use of these diets in the management of blood glucose levels in individuals with T1DM.

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List of abbreviations

β-HB	3-beta hydroxybutyrate
AcAc	Acetoacetate
AcAcCoA	Acetoacetyl-CoA
AcCoA	Acetyl-CoA
ACC	Acetyl-CoA carboxylase
AKA	Alcoholic ketoacidosis
AMPK	AMP dependent kinase
AS	Acetoacetyl-CoA synthetase
BGL	Blood glucose levels
CGI58	Comparative gene identification-58
CGM	Continuous glucose monitor
CHO	Carbohydrate
CNS	Central nervous system
DEXA	Dual-energy X-ray absorptionmetry
DKA	Diabetic ketoacidosis
FA-CoA	Fatty acyl-CoA
FACS	Fatty actyl CoA synthetase
Fatp2	Fatty acid transport protein 2
Fatp4	Fatty acid transport protein 4
FBP1	Fatty acid binding protein 1
FGF21	Fibroblast growth factor 21
GABA	Gamma-aminobutyric acid
HbA1c	Glycated haemoglobin
HDLc	High-density lipoprotein cholesterol
HL	HMG-CoA lyase
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HMGCS2	Hydroxymethylglutaryl CoA synthase 2
KB	Ketone bodies
KD	Ketogenic diet
LCAD	Long chain acyl dehydrogenase
LCHF	Low carbohydrate high fat
LDLc	Low-density lipoprotein cholesterol
MAT	methylacetoacetyl-CoA thiolase

MCT1	Monocarboxylate tranporter
mTORC1	Mammalian target of rapamycin complex 1
NAD	Nicotinamide-adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PGC-1 α	Peroxisome proliferator activated receptors γ co-activator α
PPARα	Peroxisome proliferator activated receptor alpha
ROS	Reactive oxygen species
SCOT	Succinyl-CoA:3-ketoacid CoA transferase
SD	Standard deviation
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

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Back in 2011, as a second year undergraduate student, I knew that I wanted to do my PhD in exercise physiology and I came to Australia with that mission: to attain my first class Honours and secure a PhD scholarship.

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Thesis declaration

I, Zac Leow, Certify that:

This thesis has been substantially accomplished during enrolment in the degree.

This thesis does not contain material which has been submitted for the award of any other degree or diploma in my name, in any university or other tertiary institution.

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The research involving human data reported in this thesis was assessed and approved by The University of Western Australia Human Research Ethics Committee. Approval #: RA/4/1/8103.

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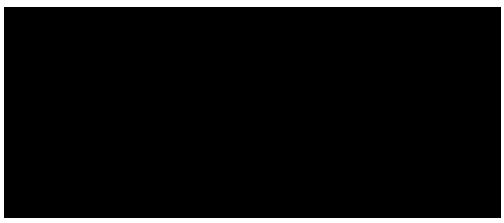
Date: 24 Feb 2020

I, Dr Fournier, certify that the student statements regarding contribution to each of the works presented in this thesis are correct

Signature: 

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Chapter 1

Literature review

Literature review

1.1 Brief overview of the aetiology of type 1 diabetes mellitus and the limitations of insulin therapy

Type 1 diabetes mellitus (T1DM) is a heterogeneous disorder that affects over 16 million people worldwide (Garvan Institute of Medical Research, 2017) and 140,000 people in Australia, accounting for approximately 10% of all cases of diabetes mellitus (Daneman, 2006). T1DM affects individuals of all ages, but, in most populations, the incidence is highest between birth and 14 years of age (ADA, 2008; Ekoé, 1989). Type 1 diabetes mellitus is also one of the most widespread chronic diseases of childhood (International Diabetes Federation, 2017), and the global incidence of T1DM in children and adolescents is rising, with an estimated overall annual increase of approximately 3% (Bonora & DeFronzo, 2018).

Type 1 diabetes mellitus results from the autoimmune destruction of the insulin-producing β-cells of the pancreas, causing a state of complete or near complete insulin deficiency (Bonora & DeFronzo, 2018; Concannon et al., 2009). In the absence of insulin in the blood, people with T1DM experience high blood glucose levels (BGL) and ketone body (KB) concentrations due to the absence of both insulin-mediated inhibition of hepatic glucose and KB production, and stimulation of peripheral glucose uptake (Rizza et al. 1981). If left untreated, these metabolic perturbations can lead to severe hyperglycaemia-mediated septicaemia and fatal diabetic ketoacidosis (DKA; English and Williams 2004; Rizza et al. 1981). This explains why individuals with newly diagnosed T1DM die within one to two years if left untreated (English & Williams, 2004).

Currently, there is no cure for T1DM; however, the discovery of insulin almost a century ago, and its widespread use in the treatment of T1DM, has considerably increased the life expectancy of people with T1DM. T1DM is typically treated through regular daily insulin administration to replace the endogenous insulin that would normally be produced by the pancreas (Rosenfeld, 2002). Insulin is generally administered either subcutaneously, using a syringe or insulin pen (Alemzadeh et al., 2004), or via an insulin pump that infuses insulin via a catheter inserted subcutaneously

into the abdomen (Pickup, 2012), with the rate of insulin delivery by the insulin infusion pump being adjusted to provide an insulin bolus with each meal to simulate the body's natural pattern of insulin secretion (Stephens & Riddle, 2003).

Although insulin therapy provides an effective means to prevent premature death in individuals with T1DM, one major limitation with such a therapy is that the body cannot alter the delivery rate of exogenous insulin to the blood and control blood insulin levels. Without such natural feedback mechanisms, plasma insulin levels cannot be regulated and respond acutely to changes in BGL. This is of concern because under conditions where the body receives too much insulin relative to the amount of ingested carbohydrate (CHO), the insulin-mediated stimulation of glucose uptake from the bloodstream, together with the insulin-mediated inhibition of hepatic glucose production, can cause BGL to fall below the healthy physiological range, a condition known as hypoglycaemia (< 53 md/dL or <3.0 mmol/L; < 70 mg/dL or < 3.9 mmol/L; International Hypoglycaemia Study Group, 2017; ADA, 2020). Hypoglycaemia is associated with symptoms such as trembling, sweating, palpitations, difficulty in concentrating, confusion, weakness, difficulties speaking and dizziness (Diabetes Canada Clinical Practice Guidelines Expert Committee, 2018). Given that blood glucose is the primary fuel used by the brain, it comes as no surprise that hypoglycaemia is also characterised by symptoms such as lowered reaction time (Herold et al., 1985; Holmes et al., 1983), reduction in fine motor performance (Beker & Ryan, 2000; Cryer et al., 2003; Fanelli et al., 1994; Schwartz et al., 1987), cognitive impairments (Beker & Ryan, 2000; Cryer et al., 2003; Fanelli et al., 1994; Schwartz et al., 1987; Stevens et al., 1989), and, in severe cases, hypoglycaemia can cause brain damage (Becker & Ryan, 2000; Cryer et al., 2003) or death (Becker & Ryan, 2000; Cryer 1997; Cryer et al., 2003; Laing et al., 1999).

Given the many adverse effects of hypoglycaemia, it is not uncommon for individuals with T1DM to maintain their BGL above the healthy physiological range (> 8 mmol/L). Even for individuals who do attempt to maintain their BGL within the physiological range, a significant proportion of their time may still be spent with BGL above physiological levels (DCCT, 1993; Garg et al., 2017; Rohlfing et al., 2002). This is of concern because years of chronic exposure to high BGL can have several detrimental effects on health. These long-term health complications are categorised broadly into microvascular and macrovascular complications. Macrovascular complications include,

for instance, coronary artery disease, peripheral arterial disease, and stroke (Fowler, 2008), whereas microvascular complications cover a range of nephropathic conditions, such as kidney failure; an increased likelihood of infections; diabetic retinopathy including cataracts and blindness; and poor blood circulation, particularly in the legs and feet, to list a few (DCCT, 1993; Williams et al., 2002). As a result of these long-term complications, combined with repeated exposures to hypoglycaemia, the life expectancies of insulin-treated individuals with T1DM is markedly reduced compared to the general population. For instance, insulin-treated individuals with T1DM in Australia have an average life expectancy that is approximately 10 years below that of the general population of Australians (Huo et al., 2016). For this reason, individuals with T1DM are strongly encouraged to maintain their BGL as close as possible to the physiological range.

1.2 Strategies to manage blood glucose levels and their limitations

The clinical tool of choice currently adopted to evaluate the extent to which individuals with T1DM keep their BGL within the physiological range consists of measuring their levels of glycated haemoglobin (HbA1c) in blood. HbA1c levels provide an effective indirect marker of long-term average BGL (e.g. previous 2 – 3 months; Saudek et al., 2006). This link between HbA1c levels and average long-term BGL is explained on the grounds that glucose, when in contact with haemoglobin, reacts slowly to form an irreversible covalent bond with haemoglobin during the life cycle of the circulating red blood cell (~120 days), slowly glycating haemoglobin in a BGL-dependent manner. HbA1c levels thus reflect average BGL over the preceding 2-3 months, weighted toward the most recent 4 weeks, thus providing a measure of glycaemic control (ADA, 2018; Rewers et al., 2014; Saudek et al., 2006). This close association between average BGL and HbA1c levels explains why this measure provides a strong predictor of long-term microvascular and macrovascular complications (DCCT, 1993, 1994).

Individuals with T1DM generally attempt to maintain their BGL within the physiological range through tightly balancing their self-administered dose of exogenous insulin, regular glucose monitoring, and careful control of their diet and physical activity levels. These individuals are educated to estimate the CHO content of their

meals and snacks because the amount of ingested CHO is the single most important determinant of the post-prandial glucose response (Bell et al., 2014; Gillespie et al., 1998; Rabasa-Lhoret et al., 1999; Slama et al., 1981; Wolever et al., 1999), and they are required to manage their pre-meal insulin bolus dose to maintain BGL within the physiological range after a meal (Harper et al., 2013; Wolever et al., 1999). Unfortunately, since it is not possible to predict how BGL and plasma insulin levels respond to CHO intake and insulin dose administration on an individual basis, it is not surprising that people with T1DM often misjudge their CHO intake (Bishop et al., 2009; Brazeau et al., 2013; Spiegel et al., 2012) and the amount of insulin required to keep peak post-prandial BGL (typically occurring within 30-90 min following a CHO based meal; Smart et al., 2012) within the physiological range. In addition, the type of food and macronutrient composition of meals can markedly affect the blood glucose response to a given CHO intake (Bell et al., 2015; Mohammed & Wolever, 2004). For instance, when protein, fibers and/or fat are added to CHO in a meal, there is an increase in late postprandial blood glucose excursion, thus highlighting the need to bolus appropriately for protein and fat (Smart et al., 2013).

Another major factor that makes managing BGL even more challenging is physical activity. This is because exercise can increase the risk of both hypo- and hyperglycaemia in individuals with T1DM. Hypoglycaemia risk is increased during (Rabasa-Lhoret et al., 2001; Tuominen et al., 1995) and after exercise (MacDonald, 1987) due, at least in part, to the exercise-mediated increase in subcutaneous blood flow and associated increase in the absorption of subcutaneously injected insulin (Zinman et al., 1977). Also, since blood glucose is one of the many fuels used by skeletal muscles, muscle contraction *per se* can stimulate a large increase in muscle glucose uptake (Wasserman et al., 2002). Furthermore, the inability of individuals with T1DM to decrease their plasma insulin levels during exercise after a bolus insulin injection can cause a state of relative hyperinsulinaemia that impairs hepatic glucose production and stimulate further peripheral glucose utilization, altogether increasing hypoglycaemia risk usually within 20 to 60 min after the onset of exercise, depending on the blood glucose concentration at the commencement of exercise (Riddell et al., 1999).

In contrast, there are other situations where exercise increases the risk of hyperglycaemia. For instance, exercising in an insulin deprived state causes a disproportionate increase in hepatic glucose production rates, resulting in

hyperglycaemia (Berger et al., 1997; Kemmer & Berger, 1986; Vranic et al., 1976; Zinman et al., 1977). High intensity exercise performed under basal insulinaemic conditions can also cause an increase in BGL that can last for several hours post-exercise (Marliss & Vranic, 2002). Such a rise in BGL has been explained on the basis that the elevated blood catecholamines levels associated with high intensity exercise result in a disproportionate increase in hepatic glucose output compared to the increase in muscle glucose utilisation rates (Marliss & Vranic, 2002). Other factors such as pre-exercise anxiety and excitement (MacKnight et al., 2009) can also increase BGL. Although an additional dose of insulin has been recommended for those individuals who regularly experience hyperglycaemia following intense exercise (Marliss & Vranic, 2002; Riddell & Perkins, 2006), these individuals may be unwilling to take additional insulin following exercise because this increases their risks of post-exercise hypoglycaemia in the following hours (Riddell & Perkins, 2006).

Given the complexity of balancing insulin dose with exercise and food intake, notable fluctuations in blood glucose levels and increased occurrence of hypoglycaemic events are common in individuals with T1DM, with many experiencing a fear of exercise-mediated hypoglycaemia (Boland et al., 2001; Cameron & Ambler, 2004; Cheyne & Kerr, 2002; Salardi et al., 2002). It is not surprising, therefore, that these individuals are less enthusiastic about physical activities (Ludvigsson et al. 1980), and are often discouraged from participating in sports and games by their parents, school staff or physicians (Fremion, 1987). For these reasons, and because of the complexity of matching insulin dose and CHO intake for the prevention of both hypo- and hyperglycemia (Brazeau et al., 2013), individuals with T1DM are, in general, less active and fit than the general population (Brazeau et al., 2013; Plotnikoff et al., 2006). This is unfortunate given the well-established benefits of regular exercise, including enhanced quality of life (Bize et al., 2007) and the prevention of chronic diseases such as cardiovascular disease, some form of cancers, obesity, and depression (Lee et al., 2003; Harriss et al., 2009; Huxley et al., 2009; Jeon et al., 2007, Warburton et al., 2006, Williams, 2001).

A number of glycaemia management strategies currently exist to decrease the frequency and magnitude of glycaemic excursions, improve average BGL, and reduce the risk of exercise-mediated hypoglycaemia. Ideally, insulin bolus injection should mimic the physiological insulin profile typical of that associated with the insulin released from the

pancreatic β-cells in healthy individuals. To this end, a range of insulin preparations offering different onset of action, peak of action, and duration of effect, are available for individuals on multiple daily insulin injection therapy (Iqbal et al., 2018). The use of insulin pumps also provides an effective means of insulin delivery, where both bolus and basal insulin dose can be adjusted on a daily basis. Unfortunately, due to the delay for the injected insulin to reach the circulation, current insulin formulations and modes of delivery do not fully reproduce the insulin profile normally associated with the insulin released from the β-cell.

Another approach to manage BGL is to 'CHO-count' so as to better match short-acting insulin bolus doses with CHO intake. Although this strategy has been shown to help improve glycaemic control, such matching is difficult to achieve, and often results in marked glycaemic excursions, particularly in response to a CHO-rich meal (Bell et al., 2014; Brazeau et al. 2013). This is not surprising given that a set amount of CHO available from different food sources can result in varying blood glucose responses (Mohammed & Wolever, 2004). Also, the efficacy of 'CHO counting' is limited by one's competence at accurately estimating the CHO content of food, with some studies reporting large variations in this regard (Ahola et al. 2010). Although these fluctuations can be reduced by increasing the intake of low-glycaemic index food, the impact of such a strategy is in general modest (Ajala et al. 2013; Nansel et al. 2016).

Considering the many challenges of accurately matching CHO intake with insulin bolus dose, self-monitoring of BGL is required to determine whether such a match is achieved, but many individuals with T1DM fail to monitor their blood glucose regularly (Nathan et al., 2009; Miller et al., 2015). The use of continuous glucose monitors and closed-loop systems offers significant promise for improving blood glucose management (Ly et al., 2013; O'Grady et al. 2012). However, their impact on glycaemic control is still suboptimal (Boughton & Hovorka, 2019; Elleri, et al., 2011; Emami et al., 2017; Franc et al., 2018; Garg et al., 2017; Kumareswaren et al., 2011; Pickup et al., 2002; Ruan etal., 2017; Thabit et al., 2015; Weisman et al., 2017). Furthermore, their accuracies are limited under conditions of rapid glycaemic excursions, such as in response to a CHO-rich meal or during exercise (Davey et al., 2010; Kumareswaran et al., 2013).

Since optimal glycaemic control is achieved by only a minority of individuals with T1DM, there is a need to not only improve, but also develop new BGL management strategies. In this regard, new tools have been tested to help improve BGL management. For instance, the benefits of administering metformin and SGLT2/SGLT1, two drugs used in the treatment of type 2 diabetes mellitus (T2DM), have been recently tested as potential therapeutic tools for improving glycaemic control in individuals with T1DM (Iqbal et al., 2018). Although both medications reduce the insulin requirements and improve HbA1c levels, the improved glycaemic control is not sustained in metformin users (Petrie et al., 2017), and SGLT2/SGLT1 inhibitors increase the risk of DKA (Peters et al., 2015; Dandona et al., 2017; Garg et al., 2017). Therefore, more research is needed in this area.

1.3 Low CHO diet: a potential strategy to improve glycaemic control

Despite the many advances in technology and diabetes education, it is still challenging for individuals with T1DM to match their CHO intake with their insulin dose to maintain their BGL within the normal physiological range (4-8 mmol/L). Given the difficulties of achieving such a match, it is not uncommon for people with T1DM to restrict their CHO intake to reduce the magnitude of their glycaemic excursions (Delahanty et al., 2008; Toeller et al., 1996), with individuals with T1DM consistently reporting low CHO intake ranging between 37 and 45% (Toeller et al 1996; Delahanty et al., 2009) compared to the typically recommended CHO intake of 45–65% of dietary energy intake for adults and children (Australia Government National Health and Medical Research Council, 2014; Smart et al., 2018). Since, in theory, any error at counting CHO or adjusting insulin dosage would be expected to have a lesser impact on glycaemia when CHO intake is reduced, this raises the issue of whether the adoption of a low CHO diet could provide a valid strategy for both improving glycaemic control and preventing unphysiological glycaemic excursions. In their mildest forms, these low CHO diets include non-ketogenic low-CHO high fat (LCHF) diets which are operationally defined as diets providing 50 -130 g CHO/day (or < 26% total energy) in adults (Feinman et al. 2015). In their most extreme forms, low CHO diets include very low CHO ketogenic diets, also known as ketogenic diets (KD), where CHO intake is restricted to less than 50 g/day (Feinman et al. 2015).

The potential benefit of LCHF non-ketogenic diets for the management of T1DM is indirectly supported by a number of studies in individuals with T2DM. These studies have shown that LCHF diets improve HbA1c levels (Dyson, 2008; Elhayany et al., 2010; Gannon & Nuttall, 2004; Garg et al., 1988, 1994; Grundy & Unger, 1992; Gutierrez et al., 1998; Sargrad et al., 2005; Tay et al., 2018), even without weight loss (Ganon et al., 2003; Guldbrand et al., 2012). These studies have also reported that LCHF diets reduce the levels of fasting blood glucose (Gannon et al., 2003), plasma triglyceride (Garg et al., 1988; Gerhard 2004; Garg et al., 1992; Gumbiner et al., 1998) and cholesterol (Gannon and Nuttall, 2004; Garg et al., 1988, 1992; Gumbiner et al., 1998) in individuals with T2DM. Although weight loss *per se* may have contributed to the aforementioned benefits of LCHF diets on HbA1c levels, further evidence that the benefits of LCHF diets on glycaemic control are not due to weight loss *per se* comes from a recent 2-year clinical trial in adults with T2DM randomised into either a low or high CHO intake group, where both groups achieved comparable weight loss, but with glycaemic control improving only in the low CHO group (Tay et al., 2018). Based on this growing body of research, Diabetes UK has recently proposed that low CHO diets may offer an effective treatment option for T2DM (Dyson et al., 2011). The American Diabetic Association has also provided some support with respect to the effectiveness of such diets for blood glucose and lipid management in T2DM (ADA, 2019). It is important to note, however, that some studies investigating the long-term effect of LCHF diets in people with T2DM have reported no improvement in glycaemic control (Wheeler et al 2012; Naude et al., 2014; Dyson, 2015), a rise in Low-density lipoprotein cholesterol (LDLc) level, and either no changes in blood lipid levels or plasma triglyceride levels (Dyson, 2015; Naude et al. 2014).

The aforementioned findings in people with T2DM suggest that the glycaemic control of individuals with T1DM may also benefit from the adoption of a LCHF diet. However, this prediction assumes that LCHF diets provide weight-loss independent benefits on glycaemic control, and important assumption since the primary purpose of LCHF diets in T1DM is to improve glycaemic control without any weight loss. In order to examine the benefits of LCHF diet on glycaemic control, a range of observational/prospective cohort studies have been performed to establish whether there is an association between these variables, an important, but inconclusive step, toward establishing causality. Within the range of moderate to high CHO diets (> 35% CHO as

energy source), where CHO intake is well above that associated with a LCHF diet (< 130 g CHO/day; Feinman et al., 2015), some studies (Delahanty et al., 2008; Nansel et al., 2016) have reported that glycaemic control deteriorates with decreasing CHO intake. In a 3-year longitudinal study, Nansel and colleagues (2016) showed that HbA1c levels were inversely associated with the proportion of energy derived from CHO ($p = 0.04$) and natural sugar ($p < 0.001$) in youth with T1DM. Similarly, Delahanty and colleagues (2008) reported an inverse relationship between HbA1c levels and the fraction of energy obtained from CHO at the end of their 5-year study ($p = 0.01$). In contrast, Meissner and colleagues (2014) found a positive association between CHO intake and HbA1c level in boys and girls ($p < 0.001$ and $p < 0.001$, respectively). Similarly, Mosso and colleagues (2015) reported HbA1c level to be significantly positiviely correlated with CHO intake ($r = 0.42$, $p < 0.02$). One limitation with the aforementioned studies, however, is that food data were estimated rather than obtained from a weighed food diary (Feinman et al. 2015). Another limitation is their adoption of diets with too much CHO to be classified as truly LCHF diets (Feinman et al. 2015), thus raising the question of the effect of LCHF diets with daily CHO intake below 130 g/day on glycaemic control and cardiovascular risk in individuals with T1DM.

Only a handful of studies have examined the effect of true non-ketogenic LCHF diets (55-130 g/day CHO) on glycaemic control in people with T1DM, and their findings suggest that LCHF diets can improve glycaemic control in adults with T1DM without causing any dyslipidaemia (Krebs et al., 2016; Nielsen et al., 2005, 2012). The most compelling evidence that LCHF diets may improve glycaemic control is found in an intervention study in which a single group of 19 individuals with T1DM was subjected to a LCHF diet (~80 g CHO, ~200 g fat/day) for a year (Nielsen et al., 2005). These researchers observed significant reductions in BGL fluctuation and in HbA1c levels (from 7.5 to 6.4% expressed relative to NGSP equivalent), lower meal insulin dosage (from 21.1 to 12.4 I.U./day), and decreased episodes of self-reported hypoglycaemia (from 2.9 to 0.5 cases/week). Following this one-year study, Nielsen and colleagues (2012) published a 4-year long study with a larger sample size and a design similar to that of their 2005 intervention study. They found that their LCHF diet (~75 g CHO/ day) resulted in a fall in HbA1c levels (from 8.8 to 7.0% expressed relative to NGSP equivalent in patients with excellence adherence), a significant increase in high-density lipoprotein cholesterol (HDLc) levels (from 1.5 ± 0.4 to 1.7 ± 0.4 mmol/l), and no changes in body mass, total cholesterol and triacylglycerol concentrations. Furthermore,

these researchers observed less blood glucose fluctuations, with a reduction in mean post-prandial BGL from 14.0 mmol/l (range: 5.9 to 23.1 mmol/l) to 6.4 mmol/l (range: 4.0 to 9.5 mmol/l). More recently, the findings of Nielsen and colleagues (2005, 2012) were corroborated in a 4-month trial performed on a small group of participants (n=5) subjected to a moderate LCHF diet (103 ± 22 g CHO/day). This study also reported a small significant fall in HbA1c levels (from 63 ± 10 to 55 ± 4 mmol/mol (8.9 to 8.2%); $p < 0.05$), and lower total daily insulin administration (from 64.4 to 44.2 units; $p < 0.05$; Krebs et al., 2016).

Of note, the findings of the two studies by Nielsen and colleagues (2005, 2012) should be interpreted with caution. Firstly, no control group was included in these studies. Secondly, intensive glucose monitoring (> 4 times daily) was required of the participants, thus raising the possibility that the improvements in average BGL could have resulted from the increased self-awareness of BGL, rather than the LCHF diet itself. Lastly, participants were instructed to refrain from snacking between meals. This would decrease the number of post-prandial fluctuations in BGL, and may have also contributed to better CHO counting. With respect to the study of Krebs and colleagues (2016), the main limitation is its small sample size, with only 5 individuals per group.

Another limitation with the studies that have examined the effect of low CHO diet (55-120 g CHO/day) on glycaemic control is that the improvements in HbA1c levels reported is not pronounced enough to normalise BGL to levels typical of healthy individuals without T1DM (Krebs et al., 2016; Nielsen et al., 2005, 2012). This raises the issue of whether the much lower CHO intake typical of ketogenic diets (KD, < 55 g/day) can not only improve glycaemic control without any ill health effects in individuals with T1DM, but also to an extent where average BGL are close to those of non-diabetic individuals - an important issue to address given the growing popularity of such diets in the general population. Also, since KD are associated, by definition, with low CHO intake and an increased production of ketone bodies (KB), one would expect that the resulting increased reliance of the body on KB as a fuel both at rest and during exercise might decrease the risk of hypogycaemia by sparing CHO, and minimise the magnitude of post-prandial hyperglycaemia in individuals with T1DM.

As discussed later, only a few studies have examined the effect of KD and nutritional ketosis on glycaemic control in people with T1DM (section 1.10). In contrast, a much

larger volume of basic and clinical research has focused on the use of KD for the treatment of a number of other clinical conditions such as epilepsy, cancer, obesity, and T2DM. In the following sections, a brief narrative review of the physiology of both KB metabolism and of the regulation of KB production and utilization will be presented. These sections will be followed by a narrative review of the role that KB play in nutritional ketosis in non-diabetic individuals, together with a brief overview of the pathophysiology of ketoacidosis and of the many clinical uses of KD. Finally, a detailed review of the use of KD as a modality to improve glycaemic control in T1DM will be discussed.

1.4 Ketone bodies: structure, function and regulation of ketogenesis and ketolysis

1.4.1 Structure and function of ketone bodies

Ketone bodies refer to a class of molecules including acetoacetate (AcAc) and two other molecules derived from AcAc, namely 3- β -hydroxybutyrate (β-HB) and acetone (Fig 1.1), with β-HB not being, strickly speaking, a ketone body because its structure does not contain any ketone moiety. As described in the next section, AcAc is derived mainly from fatty acid metabolism in the liver, β-HB is formed from the reduction of AcAc, and acetone is generated by the spontaneous decarboxylation of AcAc (Mitchell et al., 1995), with acetone being responsible for the sweet odor of the breath of individuals with elevated circulating KB concentration.

The concentration of KB in healthy CHO-fed individuals is typically 0.1 mmol/L or less and increases to approximately 0.3 mmol/L after an overnight fast (Rich, 1990). Plasma KB can attain much higher levels in response not only to very low CHO diets (< 50 g CHO/day), but also to starvation (5-7 mmol/L; Newsholme and Leech 1984). Abnormally large plasma concentrations of KB (e.g. ~25 mmol/L) are typically associated with pathological conditions such as diabetic ketoacidosis, alcoholic ketoacidosis, salicylate poisoning, and other rare conditions (Laffel, 1999).

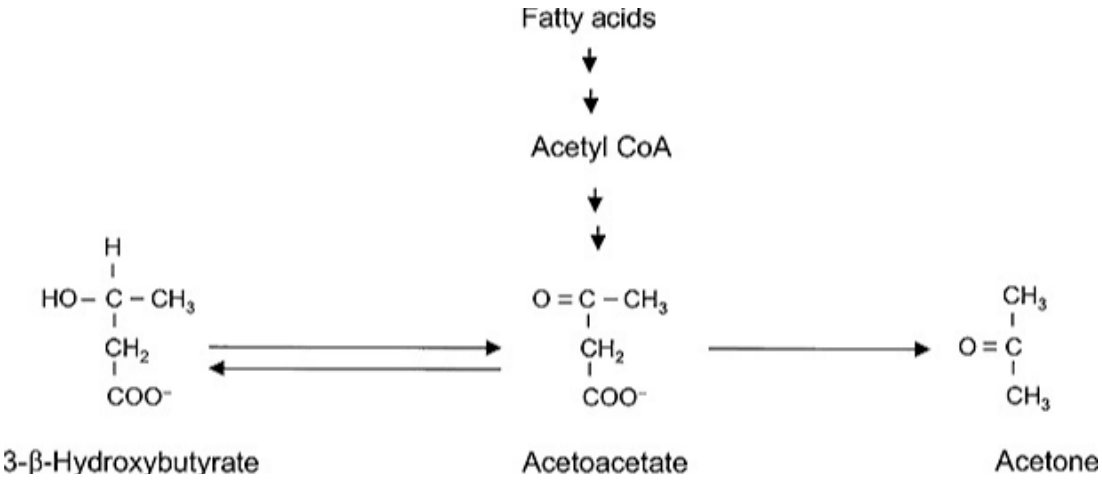

Figure 1.1 Structure of ketone bodies (3-β-hydroxybutyrate, acetoacetate, acetone)

Ketone bodies are produced primarily by the mitochondria of the liver in cells known as hepatocytes, but other sites such as astrocytes (Guzman and Blazquez, 2004), enterocytes (Clara et al., 2017) and the kidneys (Zhang et al., 2011) also have the capacity to produce KB, albeit to a much lesser extent. Almost all cells, excluding hepatocytes, have the capacity to oxidise KB via a process known as ketolysis. The primary function of the KB (acetoacetate and β-HB) produced by the liver is to act as an important alternative energy source for organs such as the brain, heart, kidney cortex and skeletal muscles when there is limited availability of CHO, or when CHO cannot be used effectively (Laffel, 1999). The availability of KB as alternative fuels is particularly important for the brain because, unlike most other tissues, the brain cannot oxidise long chain fatty acids (but can oxidise short chain and medium chain fat acids) to support its energy demands. During periods of restricted CHO intake, the body's increased reliance on KB as fuels promotes not only the sparing CHO (Francois et al., 1981), but also reduces the proteolysis normally occurring for the production of the amino acids used for the hepatic production of glucose (Nair et al., 1988).

Apart from the role KB play as fuels, there is compelling evidence that KB play an important role in cell signaling. β-HB, in particular, has been shown to activate a G-protein coupled receptor, known as hydroxycarboxylic acid receptor 2 (HCAR2; Ahmed et al., 2009) in adipose tissue, with it binding to this receptor playing an important role in the inhibition of lipolysis (Taggart et al., 2005). HCAR2 is also present in the brain and some immune cells (e.g. macrophage), with some evidence that HCAR2 plays some role in modulating inflammation and mediating the protective effect β-HB has against neurodegenerative diseases and stroke (Rahman et al., 2014). Other signaling effects of β-HB include the inhibition of the free fatty acid receptor R3 (FFAR3), another G-protein coupled receptor. The inhibition of these receptors oppose the increased metabolic rate associated with the activation of this receptor in the brain (Kimura et al., 2011). Finally, β-HB, at physiological levels, has been shown to inhibit the activity of a number of histone deacetylases (HDAC), thus favouring the hyperacetylation of histones and increasing gene transcription. In particular, the inhibition of one of these deacetylases, HDAC3, increases the expression of fibroblast growth factor 21 (FGF21) in the liver, a signaling protein which, as discussed later, plays an important role in the activation of both ketogenesis and fatty acids oxidation (Li et al., 2012). Overall, considering the many important roles played by KB, it is not surprising that the metabolism and regulation of KB synthesis (ketogenesis) and breakdown (ketolysis)

have been the object of much research effort as reviewed briefly in the following sections.

1.4.2 Ketogenic pathway

Ketogenesis occurs primarily in the mitochondria of hepatocytes, and involves the conversion of acetyl-CoA to KB. The acetyl-CoA used for KB synthesis originates mainly from the oxidation of the fatty acids taken from the circulation. Although ketogenic amino acids (e.g. leucine, isoleucine, lysine) also have the capacity to provide some acetyl-CoA for the synthesis of KB, they contribute little compared to fatty acids (Thomas et al., 1982). The transport of fatty acids through the plasma membrane of hepatocytes depends on their size. Fatty acids of eight or fewer carbon atoms can freely enter the hepatocytes and mitochondria where they are combined with coenzyme A (CoA) and readily converted to acetyl-CoA (Schönfeld & Wojtczak, 2016). Longer chain fatty acids, however, follow a different path of entry (Fig. 1.2). These fatty acids are transported inside the hepatocytes using either the fatty acid translocase, Cd36, or the fatty acid transporters 2 and 4 (Fatp2 and Fatp4; Kersten et al. 2014). Then, with the help of fatty acyl CoA synthase, the fatty acids translocated via Cd36 react with CoA to form fatty acyl-CoA. The fatty acids transported by Fatp2 and Fatp4 are combined with CoA by these transporters due to their constitutive fatty acyl-CoA synthetase activity. Because fatty acyl-CoA is too large to be transported inside mitochondria, the next reaction replaces the CoA moity of fatty acyl-CoA with carnitine to form a fatty acyl carnitine, a reaction catalysed by carnitine palmitoyltransferase 1a (CPT1a). Fatty acyl carnitine then moves through the inner membrane of the mitochondria with the help of a transporter known as translocase. Once inside the mitochondria, fatty acyl carnitine is converted back to fatty acyl-CoA by carnitine palmitoyltransferase 2 (CPT-2). The resulting fatty acyl-CoA is then oxidised to form acetyl-CoA via the metabolic pathway known as β-oxidation (Dhillon & Gupta, 2018; Kohlmeier, 2015; Laffel, 1999). The acetyl-CoA thus produced is either oxidised further through the Krebs cycle or converted to KB.

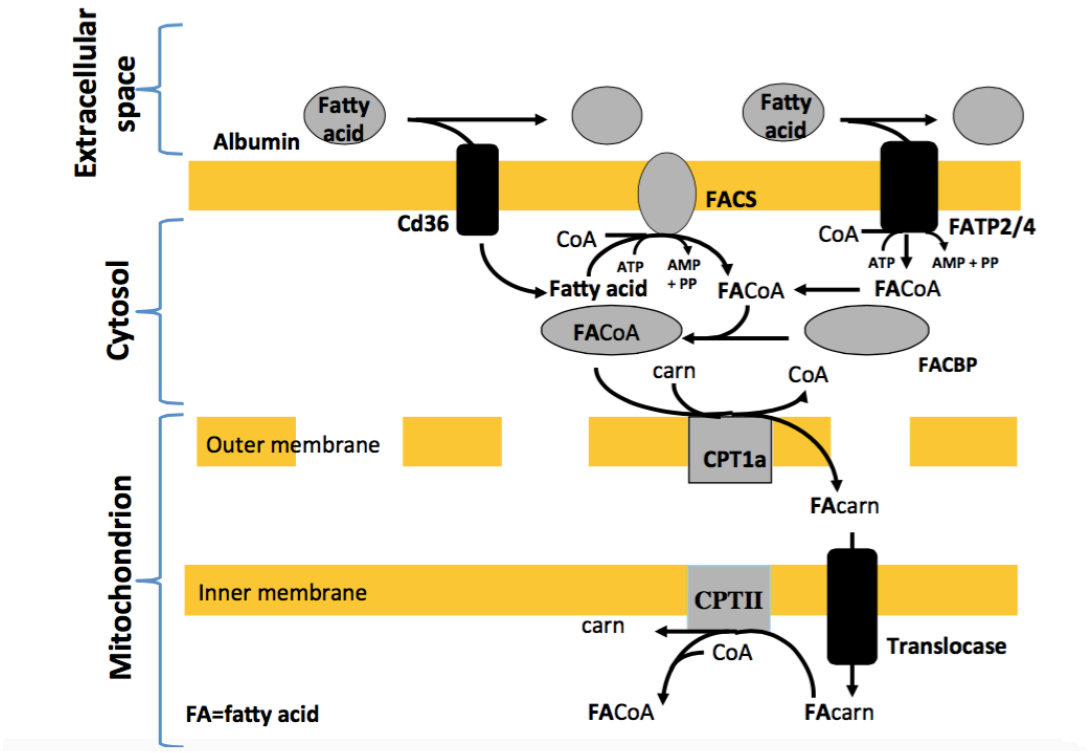

Figure 1.2 Fatty acid transport from the plasma to inside the mitochondrion. Carn (carnitine); CoA (coenzyme A); CPT (carnitine palmitoyltransferase); FA (fatty acyl); FACS (fatty acyl CoA synthetase; FATP (fatty acid transport protein);

Under conditions where ketogenesis is taking place, the production rate of acetyl-CoA from the β-oxidation of fatty acids exceeds the capacity of the Krebs cycle to metabolise acetyl-CoA. As a result, the excess acetyl-CoA molecules are converted to KB via the ketogenic pathway (Fig 1.3), also known as the hydroxymethylglutaryl-CoA (HMG-CoA) cycle. The first step of this pathway involves the combination of two molecules of acetyl-CoA to form acetoacetyl-CoA (Bismuth & Laffel, 2007; Fenselau & Wallis, 1975; McGarry & Foster, 1980; Veech, 2004) by acetoacetyl-CoA thiolase, also known as acetyl-CoA acetyltransferase. Then, another acetyl-CoA is attached to acetoacetyl-CoA by hydroxymethylglutaryl-CoA synthetase 2 (HMGCS2; Fig 1.3) to form hydroxymethylglutaryl-CoA (HMGCoA). Next, HMGCoA is converted by HMG-CoA lyase to acetyl-CoA and the ketone body, acetoacetate. As a result of the low NAD/NADH ratio inside mitochrondria, most of this acetoacetate is then reduced to β-HB by 3-hydroxybutyrate dehydrogenase, thus explaining why β-HB accounts for most

of the KB released by the liver. Another fraction of the acetoacetate undergoes spontaneous decarboxylation to form acetone. Overall, the rate of KB production can be as high as 150-185 g/day and can result in elevated plasma concentrations of KB, thus causing a state of ketosis (Fukao et al., 2004; Laffel, 1999; Veech, et al., 2001) if these rates are higher than the maximum rates at which KB are oxidised in the body (~129 g/day; Reichare et al., 1974).

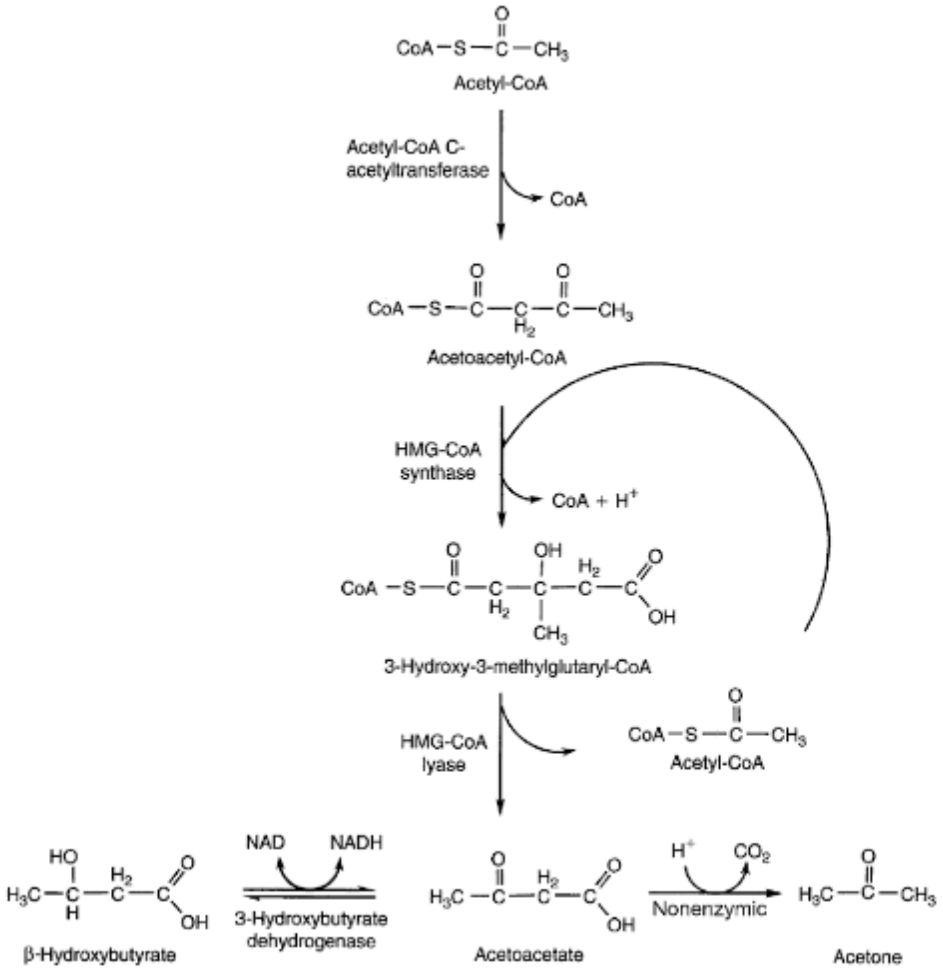

Figure 1.3 Ketogenesis from acetyl CoA (taken from Kohlmeier, 2015).

1.4.3 Acute regulation of hepatic ketogenesis

The acute activation of hepatic ketogenesis is mediated by rapid changes in the levels of key allosteric effectors and by changes in the state of phosphorylation, succinylation and acetylation of several key regulatory enzymes controlling the rate of fatty acid and KB metabolism. When the metabolic stresses (e.g. starvation, KD) at the origin of the increased demands for KB metabolism extend for several hours or days, a chronic increase in the gene expression of the enzymes along the ketogenic pathway plays an important role in the sustained activation of ketogenesis. In this section, the acute and chronic regulation of ketogenesis will be briefly examined.

In the CHO fed state characterised by high insulin to glucagon ratios, fatty acid metabolism in the liver is directed primarily toward triglyceride synthesis, and KB synthesis is inhibited. Insulin acts in two complementary manners: first, it inhibits lipolysis in the adipocytes, thus causing a fall in the blood supply of the fatty acids required for the synthesis of KB; secondly, insulin stimulates glucose uptake, storage, and oxidation by hepatocytes, a process that results in the generation of high levels of inhibitors of KB synthesis (Grabacka et al., 2016), as discussed later.

During a prolonged fast or other states of CHO deprivation (e.g. KD), there is a marked fall in plasma insulin levels, together with an increase in the levels of catabolic hormones, namely glucagon, cortisol, growth hormone, epinephrine and norepinephrine (McGarry & Foster, 1972; Schade & Eaton, 1975). High levels of catecholamines together with low plasma insulin levels stimulate lipolysis in adipose tissue, which in turn causes an increase in plasma fatty acids levels and their transport rate in hepatocytes. The ensuing activation of fatty acid oxidation and ketogenesis from these fatty acids is mediated, in part, by the high catecholamines levels and high glucagon-to-insulin ratios (Dhillion & Gupta, 2018). Of note, epinephrine and norepinephrine are potent activators of lipolysis, fatty acid oxidation and ketogenesis, regardless of insulin levels, whereas glucagon plays a more important role in the activation of ketogenesis (Bahnsen et al., 1984; Keller et al., 1989).

The regulation of lipolysis in adipose tissues, and of fatty acid oxidation and KB production in the liver, involves the control of the activity of several regulatory enzymes, namely hormone-sensitive lipase (HSL) and adipose tissue triglyceride lipase (ATGL) in the adipose tissue, as well as CPTI, long chain acyl dehydrogenase (LCAD),

and HMGCS2 in the liver. The mechanisms underlying the activation of these enzymes will be briefly examined in the following sections.

1.4.3.a Activation of lipolysis in adipocytes

The activation of triglyceride breakdown in the adipose tissue has been the object of much research effort as summarised in several recent reviews (Fruhbeck et al., 2014; Itabe et al., 2017; Kimmel and Sztalryd, 2016; Li and Sum, 2018; Wang et al., 2018). Briefly, under conditions favourable to KB synthesis (e.g. KD, prolonged fasting), plasma insulin levels are low and plasma levels of catecholamines, growth hormone, and cortisol are elevated, with catecholamines playing the most important role in the activation of lipolysis (Fruhbeck et al., 2014). The elevated levels of plasma catecholamines, the activation of the sympathetic nervous system, and low plasma insulin levels jointly stimulate HSL and ATGL (Fig 1.4), a process initiated by the binding of catecholamines to the adrenergic receptors at the surface of the adipocytes (Fruhbeck et al., 2014; Li and Sum, 2018). This binding results in the activation of cAMP production by adenylate cyclase, with the ensuing rise in the levels of cAMP and its binding to protein kinase A (PKA) causing its activation (Fig 1.4). This process is further facilitated by the low plasma insulin level-mediated inactivation of phophodiesterase 3B, an enzyme which when active catalyses the breakdown of cAMP (Fruhbeck et al., 2014; Li and Sum, 2018).

Protein kinase A, once activated, phosphorylates and activates HSL, a diglyceride lipase. PKA also phosphorylates both perilipin 1, a protein found at the interface between the cytosol and the lipid droplets (Fig 1.4; Fruhbeck et al., 2014; Li and Sum, 2018), and another protein known as comparative gene identification-58 (CGI58), a potent activator of ATGL that is normally attached to perilipin 1 when lipolysis is not stimulated. The phosphorylation of HSL favours its translocation from the cytosol toward the lipid droplet (Li and Sum 2018). In addition, the phosphorylation of perilipin 1 and HSL stimulates the docking of the phosphorylated HSL with the phosphorylated perilipin1, a process that brings HSL in immediate contact with the lipid droplet found in adipocytes, resulting in a further increase in the activity of HSL (Fruhbeck et al., 2014; Kimmel and Sztalryd, 2016). The phosphorylation of both perilipin1 and CGI58 also results in the release of the CGI58 normally bound to perilipin1. Upon its release, CGI58 binds to and activates ATGL (Fruhbeck et al., 2014; Granneman et al. 2009;

Itabe et al., 2017; Kimmel and Sztalryd, 2016; Lass et al. 2006; Li and Sum, 2018; Wang et al., 2018). Altogether, the activation of both ATGL and HSL results in the sequential activation of triglycerides and diglycerides breakdown, respectively, and a higher rate of release of fatty acids into the circulation, which in turn causes a rise in plasma fatty acid levels (Yonoug et al., 2013).

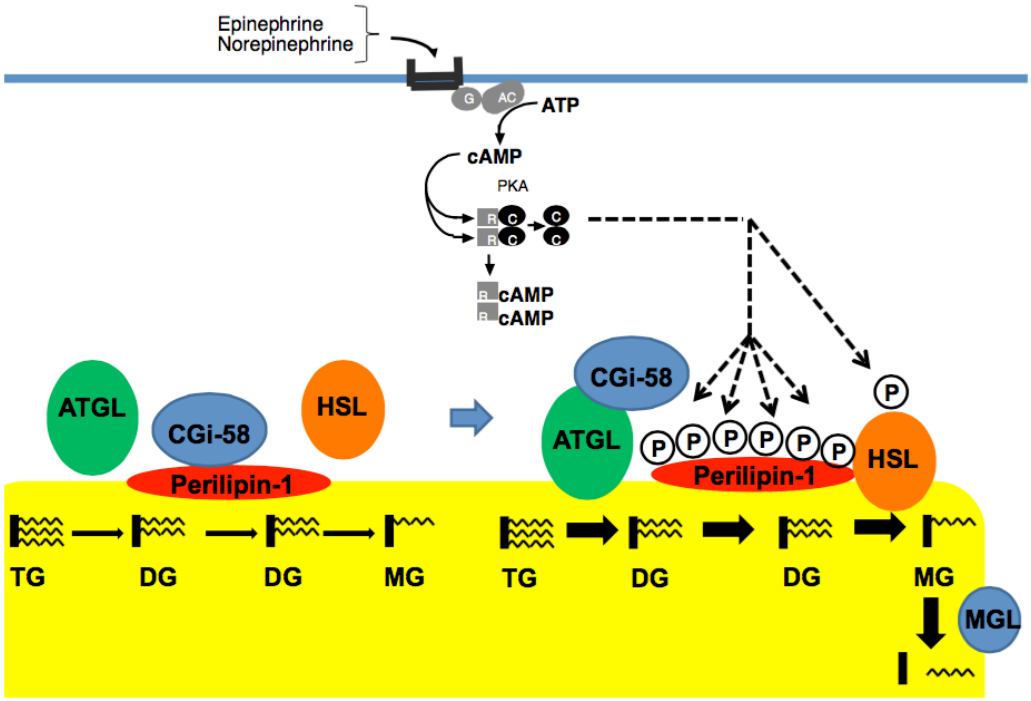

Figure 1.4 Activation of lipolysis in adipose tissue. AC (adenylate cyclase); ATGL (adipose triglyceride lipase); CGI58 (comparative gene identification-58); (DG (diglycerides); Gs (Gs protein); HSL (hormone sensitive lipase); MG (monoglycerides); MGL (monoglyceride lipase); PKA (protein kinase A); TG (triglycerides)

1.4.3.b Regulation of fatty acid oxidation

High levels of plasma fatty acids *per se* favour their increased uptake by the liver and, to some extent, their increased conversion to KB. However, for high rates of KB synthesis to take place, the rate of fatty acids transport into the mitochondria must be activated, a process that depends on the acute activation of CPT1a (hereforth referred to as CPT1; Schreurs et al., 2009; Fig 1.5). Under circumstances where CHO are not limiting, fatty acid metabolism in the liver is directed primarily toward triglyceride synthesis, with fatty acid oxidation being shut down because of the inhibition of CPT1

by high levels of malonyl CoA (McGarry et al, 1977; McGarry & Foster, 1980). In contrast, under conditions of CHO restriction, the levels of this inhibitor fall and activate CPT1, thus enabling higher rates of fatty acid oxidation and ketogenesis (McGarry et al., 1977).

Malonyl CoA is produced by acetylCoA carboxylase, with the activity of this enzyme being regulated by reversible phosphorylation and by a number of allosteric effectors (e.g. long chain fatty acyl-CoA and citrate). The liver contains two isoforms of this enzyme, acety-CoA carboxylase 1 and acety-CoA carboxylase 2, with the latter being closely attached to the outher mitochondrial membrane near CPT1 and playing the most important role in controlling fatty acid uptake by CPT1 (Schreurs et al., 2009). The phosphorylation state of acetyl CoA carboxylase in hepatocytes is under hormonal regulation and affected by the energy state of these cells. Under conditions of prolonged CHO restriction, there is a marked rise in the intracellular AMP/ATP ratio (Corton et al., 1994), and the levels of plasma insulin and catecholamines are low and high, respectively. As discussed above, such a low ratio of insulin to catecholamine levels is favourable to an increase in the levels of plasma long chain fatty acid and their entry into the liver cells. Upon their conversion to long chain fatty acyl-CoA, the activity of acetyl-CoA carboxylase is inhibited as these long chain fatty acyl-CoA are inhibitors of this enzyme (Brownsey et al., 2006). In addition, the high AMP/ATP ratio also inhibits acetylCoA carboxylase via the activation of AMP dependent kinase (AMPK, Carling et al., 1989; Schreurs et al., 2009), a kinase that catalyses the phosphorylation-mediated inhibition of acetylCoA carboxylase. Overall, the inhibition of acetyl-CoA carboxylase contributes, via a decline in malonyl CoA levels, to the activation of fatty acid transport into mitochondria (Fig 1.5, 1.6; Carling et al., 1989; Schreurs et al., 2009).

Of note, although acetyl-CoA carboxylase is also inhibited by high levels of catecholamines and high glucagon-to-insulin ratios, it is unlikely that the cAMP-mediated activation of PKA by these hormones acts directly by phosphorylating acetyl CoA carboxylase (Munday et al., 2002; Brownsey et al., 2006). Instead, there is evidence that cAMP and PKA act indirectly on AMPK (Munday et al., 2002; Brownsey et al., 2006). In this respect, the activation of acetyl-CoA carboxylase by catecholamines and high glucagon-to-insulin ratios occurs indirectly via their inhibitory effect on glycolysis and decerased production of citrate, a potent allosteric activator of acetyl-CoA carboxylase (Fig 1.6; Brownsey et al., 2006; Wakil et al., 1983). As a result

of the fall in citrate levels, acetyl-CoA carboxylase is no longer activated by citrate, and the fall in its activity results in a decrease in malonyl-CoA levels and thus in the activation of CPT1.

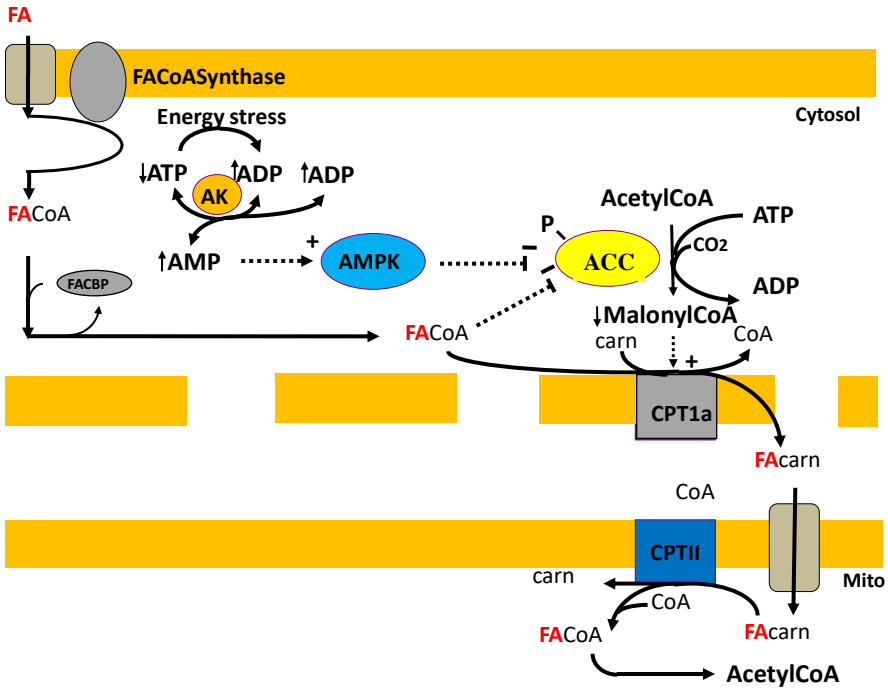

Figure 1.5 Acute activation of fatty acid transport in mitochondria. ACC (acetyl-CoA carboxylase); AK (adenylate kinase); AMPK (AMP dependent kinase); Carn (carnitine); CoA (coenzyme A); CPT (carnitine palmitoyltransferase); FA (fatty acyl); FACS (fatty acyl CoA synthetase).

1.4.3.c Acute regulation of ketogenesis

In order to stimulate KB production, it is not enough to activate CPT1 and fatty acid transport into the mitochondria alone – the ketogenic pathway must also be activated. The rate of ketogenesis is controlled primarily by hydroxymethylglutaryl CoA synthase 2 (HMGCS2), as well as by the long chain acyl dehydrogenase (LCAD), the latter being the rate limiting enzyme that controls the β-oxidation rate of fatty acids. The enzyme HMGCS2 is inhibited both allosterically by succinyl-CoA (Hegardt, 1999) and by the succinylation of some of its amino acids (Fig 1.6; Hegart, 1999; Hirshey et al 2010; Kostiuk et al., 2010). Similarly, the succinylation of LCAD inhibits its activity (Fig 1.6; Hirshey et al 2010).

Of note, although the acetylation-mediated inhibition of both HMGCS2 and LCAD has been proposed to play a role in the regulation of ketogenesis (Grabacka et al., 2016), the rise in acetyl-CoA levels associated with CHO restriction (Perry et al., 2017) and the increased acetylation level of these enzymes under condition of CHO restriction (Weinert et al., 2015) would be expected to inhibit rather than activate HMGCS2 and LCAD, and thus result in the inhibition rather than the activation of ketogenesis. Also, the very small stochiometry of acetylation of any of its target proteins in mitochondria brings into question the importance that this mechanism may have in the regulation of fatty acid oxidation and ketogenesis (Weinert et al., 2015). This explains why the acylation of mitochondrial proteins has been described as a metabolic lesion, with the Sirt3-mediated deacetylation of acetylated proteins having the function to repair rather than to regulate the activity of the acetylated proteins (Weinert et al., 2015)

Under conditions where CHO are plentiful, the rise in insulin levels stimulates glucose uptake and oxidation by hepatocytes, which, in turn, results in an increase in the intracellular levels of succinyl-CoA and citrate (Grabacka et al., 2016). Under these conditions, succinyl-CoA inhibits ketogenesis via the succinylation of LCAD and HMGCS2 (Hirschey et al 2010), and via the allosteric inhibition of HMGCS2. The inhibition of ketogenesis is further enhanced by the high levels of citrate that activate acetyl-CoA carboxylase (Fig 1.6A), with the resulting rise in the levels of malonyl-CoA inhibiting CPT1 and thus fatty acid transport and oxidation in mitochondria. There is also evidence that elevated levels of succinyl-CoA promote the succinylation-mediated inhibition of all the other enzymes of the ketogenic and fatty acid oxidation pathways (Rardin et al., 2013), thus inhibiting further ketogenesis.

Under conditions of CHO restriction, plasma insulin levels are low and glucagon and catecholamines levels are elevated, thus inhibiting glucose oxidation by hepatocytes and causing a fall in the levels of succinyl-CoA (Hegart, 1999). Ketogenesis is stimulated by the fall in the levels of succinyl-CoA because of a decrease in succinyl-CoA-mediated allosteric inhibition of HMGCS2 and the desuccinylation-mediated activation of both HMGCS2 (Hirshey et al., 2010) and LCAD by the mitochondrial desuccinylase, Sirt 5 (Rardin et al., 2013).

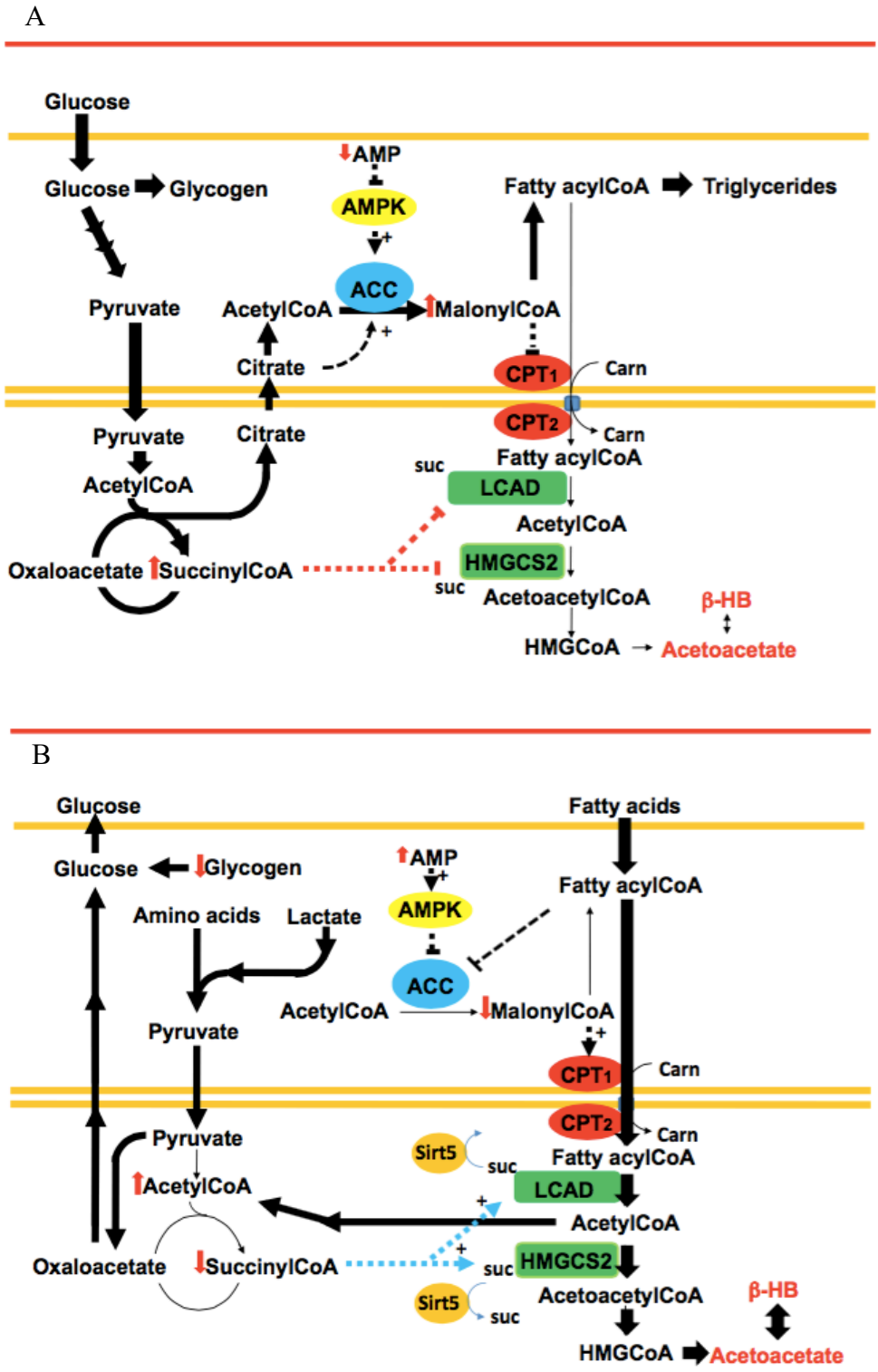

Figure 1.6 Inhibition (A) and activation of hepatic ketogenesis (B). ACC (acetyl-CoA carboxylase); AK (adenylate kinase); AMPK (AMP dependent kinase); β-HB (beta hydroxybutyrate); Carn (carnitine); CoA (coenzyme A); CPT (carnitine palmitoyltransferase); FA (fatty acid); FACS (fatty acyl-CoA synthetase); HMGCoA (hydroxymethylglutaryl-CoA); HMGCS2 (HMGCoA synthetase 2); LCAD (long chain acyl dehydrogenase); suc (succinylated). ⊣ Inhibition, ⊸ Activation.

1.4.4 Chronic regulation of ketogenesis

As mentioned above, when the CHO restriction state at the origin of the increased demands for KB synthesis lasts for several days, the body progressively increases its capacity to produce and oxidise fatty acids and generate KB. This is best exemplified by the progressive rise in plasma KB concentration which, over a period of a week, can increase by more than 50 fold (Fazeli et al., 2015). For these changes to occur, the gene expression of the enzymes involved in fatty acid transport, fatty acid oxidation, ketogenesis and ketolysis must increase to enhance and sustain the activation of these processes.

The increased gene expression of the enzymes involved in the transport and oxidation of fatty acids in hepatocytes, and the conversion of fatty acids to KB, is affected by the levels of circulating fatty acids and their derivatives, the degree of CHO deprivation state and associated intracellular energy stress, and the plasma glucagon/insulin ratio. All of these factors target, to a large extent, a common "master switch"; the nuclear transcription factor known as peroxisome proliferator activated receptors α (PPARα; Kersten et al., 1999,2000; Qi et al., 2000; Schoonjans et al, 1996). When activated, PPARα binds, in combination with other transcription factors and coactivators (Pawlak et al., 2015), to specific DNA sites found in the promoters of the genes coding for the proteins involved in the translocation of fatty acid across the plasma membrane (e.g. Cd36, Fatp2, Fatp4) and the cytosol (e.g. FABP1), the thio-exterification of fatty acids to form fatty acyl CoA (e.g. acyl-CoA synthetase 1 and 5), their intramitochondrial uptake (e.g. CPT1a, CPT2), oxidation (e.g. LCD), and their conversion to KB (e.g. acetyl-CoA transferase 1, HMGCS2, HMGCL, β-hydroxybutyrate dehydrogenase 1; Kersten, 2014; Pawlak et al., 2015). As a result of the binding of PPARα to the promoter region of each of these genes, the expression of these genes is activated. A brief overview of the mechanisms underlying the activation of PPARα by fatty acids, energy stress, and endocrine factors will be presented in the following lines (Fig 1.7).

Chronic high levels of plasma fatty acids, particularly the polyunsaturated fatty acids, associated with conditions of chronic CHO deprivation (e.g. fasting and KD) contribute to the activation of PPARα (Fig 1.7; Kersten et al., 2000; Varga et al., 2011). Following the transport of fatty acids and their conversion to fatty acyl CoA in the cytosol of

hepatocytes, these molecules (fatty acids and fatty acyl CoA) enter the nucleus where they bind to PPARα. The resulting fatty acid-bound PPARα has an increased affinity for retinoic X receptor (RXR), with which it forms a complex that binds to other co-activators and then to the promoter of the genes coding for the proteins involved in the transport and oxidation of fatty acids and their conversion to KB (Kersten 2014). Fatty acids can also stimulate PPARα following another route whereby the binding of fatty acids to fatty acid binding protein 1 (FBP1) results in the binding of this protein with PPARα and the activation of PPARα-dependent genes (Worfrum et al., 2001). Finally, high intracellular levels of fatty acyl-CoA promote the spontaneous non-enzymatically mediated palmitoylation of HMGCS2. Some of the resulting pamitoylated HMGCS2 then bind to PPARα to form a complex with other transcription factors that stimulates HMGCS2 own transcription, thus further increasing the liver's capacity to synthesise KB (Kostiuk et al., 2010).

Of note, it has been proposed that acetyl CoA may be involved in the activation of PPARα because PPARα is one of the many proteins inhibited when acetylated, with low acetyl CoA levels together with the deacylation of PPARα activating PPARα (Purushotham et al., 2009; Rodgers et al., 2005). As discussed above, the importance of this mechanism is challenged by the observation that the elevated levels of acetyl CoA associated with CHO restrictions would be expected to inhibit rather than activate PPARα.

Chronic CHO restriction and accompanying high AMP-to-ATP ratios also have the capacity to activate PPARα (Corton et al., 1994; Grabacka et al., 2016). The activation of AMPK by a high AMP/ATP ratio activates ketogenesis not only acutely, as discussed above, but also via the activation of PPARα. AMPK acts, in part, by phosphorylating peroxisome proliferator activated receptors γ co-activator α (PGC-1α; Vega et al, 2000). Once phosphorylated, PGC-1α binds to and activates PPARα (Rhee et al., 2013). Of note, the activation of PPARα, in combination with other transcription factors (e.g. cAMP responsive element binding H, CREBH; Nakagawa and Shimano, 2018) also upregulates the expression, in hepatocytes, of a growth factor in hepatocytes known as fibroblast growth factor 21 (FGF21; Badman et al., 2007). This growth factor plays an important role in the activation of hepatic lipolysis and ketogenesis in mice, but not as important in humans (Fazeli et al., 2015; Galman et al., 2008; Inagaki et al., 2007). FGF21 acts, in part, by increasing the expression of PGC-1α (Potthoff et al., 2009), thus

amplifying further the activation of PPARα by PGC-1α. AMPK also acts by inhibiting the activity of mammalian target of rapamycin complex 1 (mTORC1; Gwinn et al., 2008; Inoki et al., 2003), a complex of signaling proteins that play an important role in increasing the expression of proteins (e.g. nuclear receptor corepressor 1, nCoR1) involved in the stimulation of lipid synthesis and inhibition of fatty acid oxidation and ketogenesis. The mTORC1, once activated, impairs PPARα activity by promoting the accumulation of nCoR1, a corepressor and negative regulator of PPARα (Kim et al., 2012). This explains why the inhibition of mTORC1 is required to increase the liver's capacity to synthesis KB (Sengupta et al, 2010). The AMPK-mediated inhibition of mTORC1 occurs directly via the phosphorylation of one of its protein components, Raptor (Gwinn et al., 2008), and indirectly via the phosphorylation-mediated activation of tuberous sclerosis complex 2 (TSC2), which once activated leads to the inhibition of mTORC1 (Sato et al., 2009).

High catecholamines levels and high glucagon-to-insulin ratios also have the potential to activate PPARα and thus increase the liver's capacity to oxidise fatty acids and produce KB. Since insulin stimulates the Akt-mediated phosphorylation and activation of mTORC1, low plasma insulin levels oppose this effect and increase the liver's ketogenic capacity via the inhibition of mTORC1. Also, given that insulin inhibits the gene expression of CPT1a, low insulin levels and the associated rise in CPT1a level provide an environment favourable to increased fatty acid oxidation (Park et al., 1995). Glucagon, via its activation of CREBH, can also increase the expression of FGF21 which, in turn, increases the expression of PGC1α, thus promoting a rise in the expression of the genes controlling both fatty acid oxidation and ketogenesis (Fig 1.7, Potthoff et al, 2009; Habegger et al., 2013; Nakagawa et al., 2018).

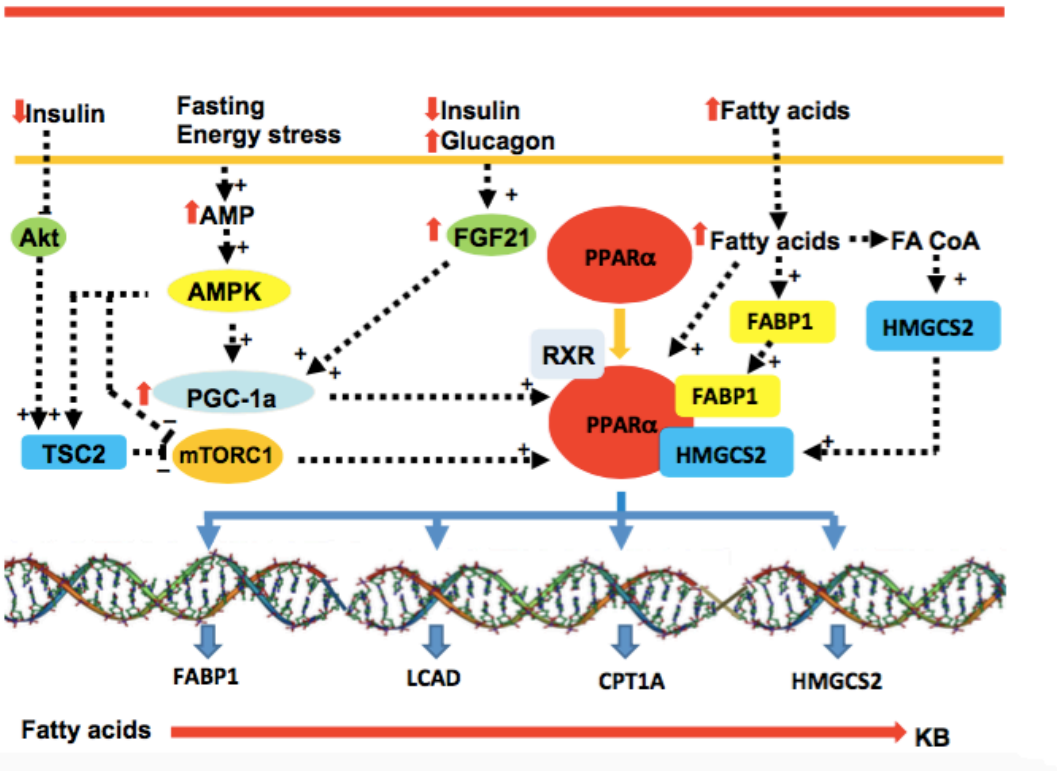

Fig 1.7 Chronic activation of hepatic ketogenesis. Akt (Akt serine/threonine kinase); AMPK (AMP dependent kinase); β-HB (β-hydroxybutyrate); CPT1a (carnitine palmitoyl transferase 1a); FABP1 (fatty acid binding protein 1); FACoA (Fatty acyl-CoA); FGF21 (fibroblast growth factor 21); HMGCS2 (hydroxymethylglutaryl-CoA synthetase 2); LCAD (long chain acyl dehydrogenase); mTORC1 (mammalian target of rapamycin complex 1); PPARα (peroxysome proliferator activated receptor alpha); PGC1α (peroxisome roliferator ctivated receptors γ co-activator α); RXR (retinoid X receptor); TSC2 (tuberous sclerosis complex2)

1.4.5 Acute and chronic regulation of ketolysis

Most organs in humans, including the brain (Achant & Rae, 2017; Courchesne-Loyer et al., 2017), skeletal muscle (Balasse & Fery, 1989; Fukao et al., 1997; Laffel, 1999; Mikkelsen et al. 2015; Robinson & Williamson, 1980), but not the hepatocytes (Fukao et al., 1997), have the capacity to oxidise KB via a metabolic pathway known as ketolysis. Ketolysis occurs in mitochondria, and involves the metabolism of β-HB and acetoacetate. Briefly, upon the transport of β-HB and acetoacetate inside the cell by the monocarboxylate tranporter 1 (MCT1), β-HB is oxidised to acetoacetate by β-hydroxybuturate dehydrogenase. Next, the mitochondrial enzyme, succinyl-CoA-dependent transferase (SCOT), converts acetoacetate to acetoacetyl-CoA (the absence of SCOT in hepatocytes explains why they lack the capacity to oxidise KB). Acetoacetyl-CoA is then converted by acetoacetyl-CoA thiolase 1 into two molecules of acetyl-CoA, which are then oxidised by the Krebs cycle for ATP production (Fig 1.8; Evan et al., 2017; Fukao et al., 1997). In contrast, most of the acetone is eliminated in urine or via the respiratory tract since acetone is a highly volatile molecule.

The levels of SCOT, which catalyses the rate-limiting step of ketolysis, are highest in the heart and kidney, followed by the central nervous system and skeletal muscles (Polin & Fox, 1998). However, due to the sheer mass of skeletal muscles, they account for the highest fraction of total ketone body metabolism in the resting state (Balassee & Fery, 1989). In the long-term, the expression of MCT1 and SCOT in peripheral tissues increases in states of energy shortage or CHO restriction, which allows increased uptake and oxidation of KB (Schonfeld et al., 2013). The expression of both MCT1 and SCOT is mediated to a large extent by the activation PPARα, a process favoured, as discussed above, by the activation of AMPK (Konig et al., 2008; Wentz et al., 2010). Of note, SCOT levels are down-regulated by high (> 5 mM) intracellular levels of AcAC, thus limiting KB oxidation (Jencks, 1973; Weitz et al., 2010). This phenomenon is responsible, in part, for the observed increase in circulating levels of KB during the early phases (3 days to 2 weeks) of starvation, despite relatively constant rates of hepatic ketogenesis during this period.

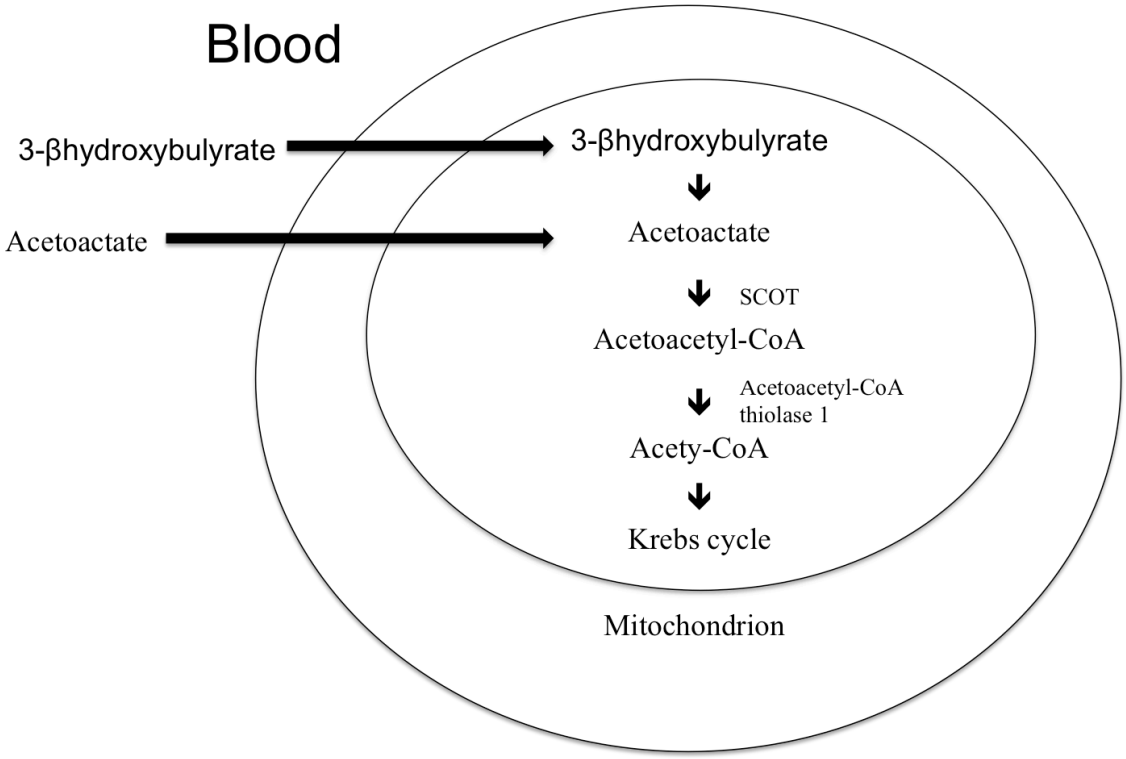

Figure 1.8 Ketolysis and the production of acetyl-CoA

1.5 Nutritional ketosis

Under conditions of nutritional plenty, the body relies mainly on CHO and fat to support its energy demands, with blood glucose being the sole fuel metabolised by organs and tissues such as the brain, kidney medulla, and red blood cells (Newsholme and Leech, 1984). However, when food availability is restricted, the limited amount of glycogen, mainly in liver and skeletal muscles, can, in theory, support no more than a day worth of energy demands in an individual at rest (Cahill et al., 1976), and be nearly completely depleted in response to a only few hours of submaximal intensity exercise (Hargreaves and Spriet, 2006). During the early stages of fasting, the body meets its glucose demands via the breakdown of its hepatic glycogen stores and the activation of hepatic and renal gluconeogenesis, primarily from the amino acids derived from body proteins (Exton 1972). In addition, ketogenesis is activated so as to provide an alternative fuel to glucose (Owen et al., 1967; Ruderman et al., 1972). Under these conditions, the brain and other organs (e.g. skeletal muscles, heart) rely increasingly on

KB as a fuel source (Laffel, 1999). This transition in fuel preference helps to spare the limited stores of muscle glycogen (Francois et al., 1981), thus allowing the body to maintain its capacity to engage in flight or fight responses. The increased reliance on KB also spares proteins, which is physiologically important as no proteins in the body serves the sole purpose of storing amino acids, and all proteins play important functional and structural roles (Nair et al., 1988). Given that even lean individuals carry a significant amount of fat (e.g. Fleck, 1983), an increased reliance on this fuel, and the KB derived from fat, bring the body in a state of ketosis and provides a strategy of choice to spare both CHO and proteins.

The most extreme natural forms of nutritional ketosis are starvation (Westmam et al., 2003), severe undernourishment, with the latter condition afflicting more than 10% of the world population (Food and Argiculture Organization of the United Nations, 2019). and ketogenic diets characterised by a very low daily intake of CHO (< 55 g/day), moderate protein intake, and high fat intake (Bravata et al., 2003; Bueno et al., 2013; Paoli et al., 2013; Westman et al., 2003). As mentioned earlier, low concentrations of acetoacetate and β-HB (< 0.5 mmol/l) are usually found in the blood of healthy adults after an overnight fast (Laffel, 1999). However, in the absence of food, KB increase progressively to reach concentrations of 3-4 mM after 3 days of fasting, and close to 8 mM after a week or more of starvation (Cahill 2006).

In order to fully appreciate the adaptive value underlying the human body's capacity to engage in nutritional ketosis, one must realise that since the origin of our species, approximately 200,000-300,000 years ago (Conroy and Pontzer, 2012; McDuugall et al., 2005), humans have subsisted as hunter-gatherers until the advent of agriculture (10,000-11,000 years ago; Bar-Yosef, 1998; Godding and Kramer, 2016), with hunter-gathering being a lifestyle that still persists in some areas of the world in the 21st century (Godding and and Kramer, 2016; Kelly , 2013). This lifestyle relies extensively on hunted/fished game as the primary source of proteins and plant-derived products (e.g. tubers, roots, honey, fruits, seeds, nuts; Cordain et al., 2000; Hardy et al., 2015) as a source of CHO, with cooking, introduced most likely prior to the origin of our species (~250,000-400,000 years ago and likely earlier; Carmody and Wrangham, 2009; Roebroeks and Villa, 2011; Shahack-Gross et al., 2014), and other forms of food processing markedly increasing the bioavailability of CHO and other nutrients (Carmody et al., 2011; Wrangham et al., 2003; Zink and Lieverman, 2016).

Despite the many strategies developed by hunter-gatherers to optimise the bioavailabity of their food sources, the main constraint that they face relates to the fact that the availability of plant and animal foods is affected by geography and local ecology, with dietary diversity being typical for hunter-gatherers (Cordain et al., 2000; Hardy et al., 2015). In particular, there are conditions where the availability of plant foods, and thus of CHO, can be highly restricted. For instance, the proportion of plant foods decreases markedly above 40^{o} absolute latitude (Cordain et al., 2000), which corresponds to temperate, sub-arctic and arctic habitats. Moreover, the availability of animal and plant food sources (e.g. fruits) is highly seasonal and depends on climate. This is best illustrated by the restricted access to plant foods, and thus to CHO, during winter at high latitude that may lead to seasonal shortage in CHO, thus forcing the body into a state of obligatory nutritional ketosis.

In industrialised and rich societies, cases of obligatory nutritional ketosis are seldom found given the almost unlimited supplies of CHO these societies provide. Despite this, a proportion of the population in these opulent societies engages in facultative CHO-restricted KD (Finney et al., 2008; Jallinoja et al., 2014), primarily for clinical or health-related purposes. The literature abounds with a growing popular and scientific literature describing different KD to better suit clinical purposes as describe in Section 1.7 of this thesis.

1.6 Dysregulation of ketone bodies metabolism

Considering the important role that KB play as a fuel for survival under extreme nutritional and energy stresses, it is not surprising that both ketogenesis and ketolysis are tightly regulated (outlined in Section 1.4). This raises the issue of the effect that extreme unphysiological dysregulation of KB metabolism may have on general health. In this regard, a number of clinicial conditions are associated with elevated KB concentrations, such as inborn errors in ketolysis (Sass 2012), alcoholism (see Section 1.8.2), some cases of type 2 diabetes treated with sodium glucose transporter 2 inhibitors (SGLT2; Barski et al., 2019), and insulin-untreated T1DM (Nyenwe and Kitabochi, 2016), with the latter being of particular concern given the ketoacidosis associated with this condition.

1.6.1 Dysregulation of ketone bodies metabolism: diabetic ketoacidosis

The increase in blood KB concentration in insulin-untreated individuals with T1DM results primarily from the complete or near complete absence of insulin combined with high levels of glucagon and other counterregulatory hormones. The resulting high glucagon-to-insulin ratio and high levels of catecholamines markedly activate KB production (Alberti et al., 1973; Exton, 1987; Kitabchi, 2003; Kitabchi et al., 2009; McGarry et al., 1989; Nyenwe and Kitabochi, 2016), with the levels of KB attained being dependent on the severity of the insulin deficiency and on the level of physical activity since exercise is normally associated with an increase in plasma glucagon and catecholamines levels (ADA, 2003). The increase in KB concentration, which can reach supraphysiological levels as high as 20-25 mM (Adrogué et al., 1982; Cahill, 2006), is to be expected given the stimulatory effect that high catecholamines levels and glucagon-to-insulin ratios have on lipolysis, plasma fatty acid levels, fatty acids uptake by hepatocytes, fatty acid oxidation and ketogenesis (Section 1.4; English & Williams, 2004; Kitabchi et al., 2009; Laffel, 1999; McGarry et al., 1989). In addition, high blood KB concentrations also result from a fall in ketone clearance due to low plasma insulin levels (Hall et al., 1984; Sherwin et al., 1976), and decreased activities of SCOT and β-hydroxybuturate dehydrogenase (Grinblat et al., 1986; Turko et al., 2001). Also, since β-HB and acetoacetate are weak acids, excessive levels of these KB may lead to a state of metabolic acidosis (blood pH < 7.3; Savage et al., 2011), with the resulting clinical condition being referred to as diabetic ketoacidosis (DKA; English & Williams, 2004; Laffel, 1999; Meas et al., 2005).

DKA is a life-threatening complication of T1DM (Kanikarla-Marie and Jain, 2016) that occurs frequently in individuals with newly diagnosed T1DM, or patients who interrupt their insulin therapy (Lawrence et al., 2005). Because DKA has a significant mortality rate (Wolfsdorf et al., 2009), hospitalisation is required, thus incurring high healthcare costs (Laffel et al., 2006; Wolfsdorf et al., 2009). DKA can cause brain damage and neurocognitive impairments (Cameron et al., 2014; Wooton-Corges et al., 2007), with the brain oedema associated with DKA believed to be the major cause of mortality associated with this condition (Long and Koyfman, 2017).

The mechanisms underlying the DKA-mediated brain oedema and damage are still poorly understood (Long and Koyfman, 2017). There is evidence that the systemic inflammation associated with DKA may contribute to brain oedema (Close et al., 2013;

Stamatovic et al., 2006), with cerebral oedema and hemorrhagic stroke being implicated in the aetiology of neuronal damage (Foster et al., 2011; Long and Koyfman, 2017; Wolfsdorf et al., 2006). Indeed, DKA has pro-inflammatory effects (Close et al., 2013), including macrophage and lymphocyte activation and cytokine release (Hofrman et al, 2003), which in turn have the capacity to cause damage to the endothelial cells (Karavanaki et al., 2011). Ketoacidosis also increases systemic oxidative stress (Close et al., 2013; Jain et al., 1999), as well as oxidative stress in the brain (Hoffman et al., 2011). Of note, the relative role that acidosis and ketosis play in DKA-induced brain damage appears to favour the latter. Indeed, an important role for acidosis *per se* is challenged by the observation that DKA does not produce a very marked acidification of brain cells, with intracellular pH being far from neuronal death thresholds (Pekun et al., 2013). In contrast, there is evidence, based on in vitro experiments, that KB *per se* can induce oxidative stress (Jain et al., 1999,1998; Kanikarla-Marie et al., 2015).

1.6.2 Dysregulation of KB metabolism associated with alcohol ketoacidosis

A marked dysregulation of KB metabolism is also associated with a condition known as alcohol ketoacidosis (AKA). AKA is more prevalent in patients with a history of long-term alcohol intake and liver disease, with AKA typically arising after a period of binge drinking and minimal food intake (Kraut and Kurtz, 2008). AKA presents itself as a syndrome of abdominal pain, nausea, vomiting, and dehydration (Duffens & Marx, 1987; Fulop et al., 1986; Umpierrez et al., 2000). In contrast to diabetic individuals with DKA, people with AKA are usually alert despite the severity of their acidosis and marked ketonaemia (McGuire et al., 2006). The mortality rate associated with AKA is generally low (Wrenn et al., 1991).

The increase in KB production associated with AKA results primarily from the metabolism of alcohol. Alcohol (ethanol) is oxidised in the liver to acetaldehyde via three distinct oxidative pathways, namely: the cytosolic alcohol dehydrogenase pathway, the microsomal ethanol oxidising system, and the peroxisomal catalase pathway. Of these pathways, the alcohol dehydrogenase pathway is the main one, and involves the reduction of NAD to NADH (Fig 1.9; McGuire et al., 2006). The acetaldehyde thus produced form alcohol dehydrogenase is then further oxidised to acetic acid prior to being converted to acetyl-CoA. Due to the high glucagon to insulin ratio and elevated levels of catacholamines and cortisol associated with the low food

intake of people in a pre-AKA state (Laffel, 1999), ketogenesis from acetyl CoA is activated to form acetoacetate (Laffel, 1999). Also, as a result of the conversion of acetic acid to acetyl-CoA, mitochondrial NAD is reduced to NADH (Palmer, 1983), with this mitochondrial NADH accumulating and increasing the NADH/NAD ratio. This higher NADH/NAD ratio, in turn, favours the conversion of acetoacetate into β-HB (Umpierrez et al., 2000), with the accumulation of these KB (Laffel, 1999; Levy et al., 1973) playing an important role in the development of ketoacidosis (Palmer, 1983; McGuire et al., 2006).

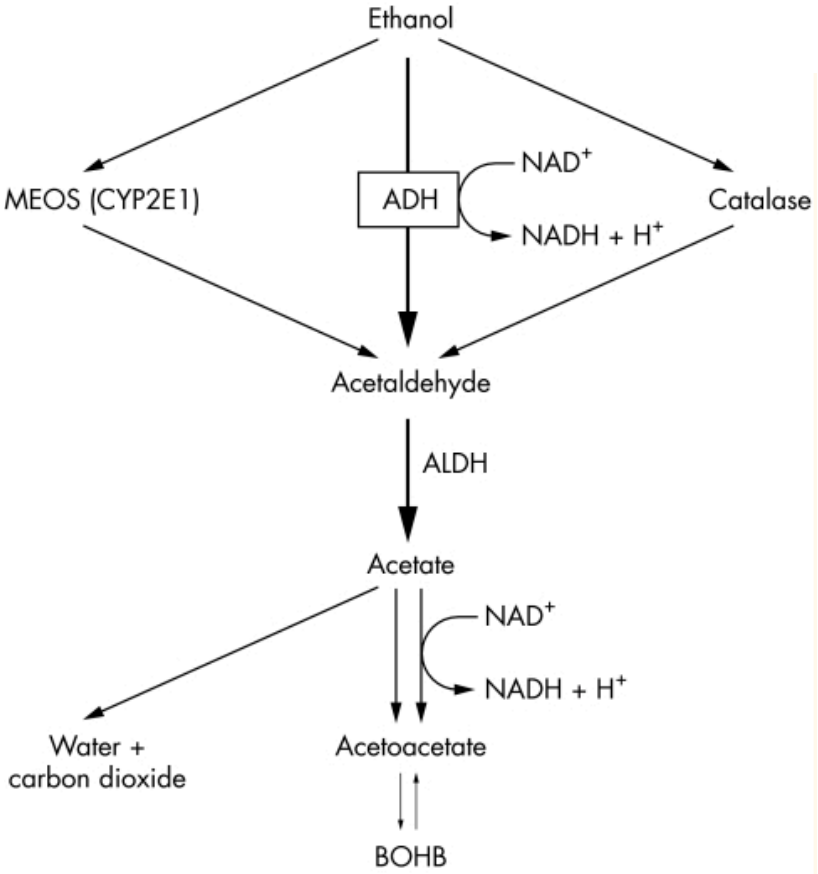

Figure 1.9 Metabolism of ethanol. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; MEOS, microsomal ethanol oxidising; CYP2E1, cytochrome p450 isoform (McGuire, Cruickshank, & Munro, 2006).

1.7 Exercise-induced ketosis

In addition to the many physiological ketosis-prone conditions discussed above, exercise can also increase blood KB levels, particularly during recovery from exhaustive exercise or exercise performed under CHO-restricted conditions (Johnson et al., 1969; Koeslag, 1982). This increase in plasma KB concentration is typically of the order of 1-2 mM, and is referred to as post-exercise ketosis (Koeslag, 1982). This is a condition that can last for several hours after exercise in individuals without (Adams et al., 1987; Johnson et al., 1969; Koeslag, 1982,1985) and with T1DM (Wahren et al., 1984).

During exercise performed under CHO-restricted conditions, there is an increase in both hepatic KB production and peripheral KB utilization rates by skeletal muscles (Fery & Balasse 1986, 1988), with exercise also stimulating the metabolic clearance of KB (Balasse et al., 1978; Fery & Balassee, 1986; Dohm et al., 1986). The progressive fall in muscle glycogen content during exhaustive exercises is compensated for by an increase in fatty acid and KB oxidation rates (Brooks & Mercier, 1994).

The metabolism of KB during exercise is influenced by a variety of factors including metabolic status (Fery & Balasse, 1986; Wahren et al. 1984), training status (Beattie & Winder, 1985; Johnson & Walton, 1972; Rennie et al. 1974), and the intensity of exercise (Cox et al. 2016). However, one of the most important determinants of KB metabolism during exercise is the degree of ketonaemia (Evans et al., 2017). During exercise, KB kinetics and oxidation are affected by blood KB concentration which in turn is determined by the duration of fasting (Fery & Balasse, 1984). There is a curvilinear relationship between KB oxidation rate and blood KB concentration, with KB contribution to fuel oxidation in skeletal muscle during exercise rising by ~10% after an overnight fast (Hagenfeldt & Wahren, 1968; Owen & Reichard, 1971), with the relative contribution of KB ranging from 2 to 10% (Balasse et al. 1978; Fery & Balasse, 1983; Wahren et al. 1984). Such a small contribution of KB is consistent with the majority of the energy provision in working muscle being from CHO and fat (van Loon et al. 2001). After 72 h of fasting, the contribution of KB increases by 20-50% (Elia et al. 1990; Owen & Reichard, 1971), but declines after 24 days of starvation (Owen & Reichard, 1971). Consistent with these findings, the metabolic clearance rate of KB increases by 50 to 75% during prolonged exercise of low-to-moderate intensity

performed after an overnight fast (Fery & Balasse, 1983, 1986). However, when ketonaemia exceeds 2.5 mM, such as following a fast lasting more than 72 h, the exercise-induced rise in the metabolic clearance rate of KB is abolished (Fery & Balasse, 1986), with only a negligible contribution of KB oxidation to energy provision (Fery & Balasse, 1986; Hagenfeldt & Wahren, 1971). Therefore, there is progressive attenuation of the oxidation of KB with rising ketonaemia, thus indicating that the mobilisation of KBs is not the factor limiting KB oxidation in skeletal muscle.

The attenuation of the exercise-stimulated metabolic clearance of KB is consistent with the existence of a KB concentration above which the oxidation of KB is either saturated or inhibited by hyperketonaemia itself (Balasse & Fery, 1989). As discussed earlier (Section 1.4.5), there is evidence that the hyperketonaemia-mediated inhibition of KB oxidation results from the inhibition of SCOT by elevated AcAc and/or FFA-mediated inhibition of ketolysis (Robinson & Williamson, 1980). As mentioned in section 1.4.5, the inhibition of KB oxidation by high circulating KB levels is believed to be a critical feature of the starvation response so that the capacity of the liver to produce KB closely matches the requirements of the brain's KB demands as an energy source (Robinson & Williamson, 1980). In this respect, excessive KB oxidation by working muscles would be expected to threaten survival, whereas the inhibition of KB oxidation spares circulating KB for the brain (Fery & Balasse, 1983; Hagenfeldt & Wahren, 1971).

During recovery from exercise, there is a rapid decrease in KB utilisation rate (Johnson & Walton, 1972; Koeslag, 1982; Standl et al., 1976) while KB production rate remains elevated, thus explaining the resulting state of post-exercise ketosis. The levels of plasma hormones associated with ketogenesis (e.g. glucagon) are unrelated to the presence or degree of post-exercise ketosis (Adam and Koeslag 1988; Bloom et al. 1976; Koeslag & Noakes, 1980; Rennie & Johnson, 1974). However, there is an inverse relationship between blood KB concentrations and liver glycogen levels post-exercise (Adam & Koeslag, 1988), implying a role for low hepatic glycogen stores in mediating the activation of liver ketogenesis post-exercise.

A number of factors affect the level of post-exercise ketosis, including exercise duration and intensity (Adams et al., 1987; Johnson et al., 1969; Koeslag, 1982,1985), the length of fasting (Balasse et al. 1978; Elia et al. 1990; Fery & Balasse, 1983, 1986; Hagebfeldt, 1979; Hagenfeldt & Wahren, 1968, 1971; Knapik et al., 1988; Minuk et al., 1980; Owen

& Reichard, 1971), diet (Askew et al. 1975; Koeslag, 1982; Koselag et al., 1980; Langfort et al., 1996, 1997; O'Malley et al., 2017; Rennie & Johnson, 1974b), training status (Adams & Koeslag, 1988, 1989; Beattie & Winder, 1984, 1985; Johnson et al. 1969; Johnson & Walton, 1972; Koeslag, 1982; Ohmori et al. 1990; Rennie et al. 1974; Rennie & Johnson, 1974. 1974a; Winder et al. 1975), level of ketosis prior to exercise (Fery & Balasse, 1984; Wahren et al., 1975, 1984) and environmental temperature (Passmore & Johnson, 1958), with greater ketonuria being observed in cool than in warm environment after prolonged exercise (Passmore & Johnson, 1958). The impact of some of these factors on post-exercise ketosis will be discussed in the following paragraphs.

It is well documented that exercise type, duration and/or intensity are contributing factors to post-exercise ketosis (Adams et al., 1987; Johnson et al., 1969; Koeslag, 1982; Koeslag et al., 1985). In particular, post-exercise ketosis is generally observed after prolonged (Adams et al., 1987; Koeslag et al., 1985) and exhaustive exercise (Johnson et al., 1969; Koeslag, 1982), but not after graded or sprint exercise in healthy individuals who are on a KD (Langfort et al., 1996, 1997).

As mentioned above, post-exercise ketosis occurs mostly in individuals who are on a CHO-restricted diet, and rarely in those on a high CHO diet (Koeslag, 1982). In CHO-restricted individuals, blood KB levels can increase by 1-2 mM following exhaustive exercise (Johnson et al., 1969; Impey et al. 2016; Koeslag, 1982), whereas a ketosis of only ~0.1 mM is found post-exercise in post-absorptive individuals (Johnson et al., 1969). High CHO feeding prior to exercise attenuates post-exercise ketosis regardless of training status (Askew et al. 1975; Koeslag et al. 1980; Rennie & Johnson, 1974b). In contrast, higher blood KB concentrations are also found immediately after exercise in healthy individuals on a ketogenic diet, a severe form of CHO restriction (Langfort et al., 1996, 1997; O'Malley et al., 2017).

The magnitude of the post-exercise ketosis can be dampened by the ingestion of food during recovery from exercise. For instance, glucose ingestion at 2 h into recovery (Carlin et al. 1987; Koeslag et al. 1982) as well as alanine intake during that time (Carlin et al. 1987; Koeslag et al. 1980, 1985) attenuates the level of post-exercise ketosis, but not if glucose is ingested immediately after exercise. The effects of alanine and glucose ingestion have been explained as follows. Alanine or glucose ingestion

increases mitochondrial oxaloacetate levels in the liver, thereby allowing the increased condensation of this oxaloacetate with the acetyl CoA derived from fatty acid oxidation, thus diverting acetyl CoA away from ketogenesis (Evans et al., 2017). Also, the rise in plasma insulin levels associated with glucose or alanine ingestion is also likely to dampen post-exercise ketosis due to the inhibitory effect of insulin on ketogenesis.

The training status of an individual is another factor that affects the level of post-exercise ketosis. The metabolism of KB during and after exercise differs between trained and untrained individuals (Johnson et al. 1969; Johnson & Walton, 1972; Rennie et al. 1974; Rennie & Johnson, 1974), with numerous studies reporting post-exercise ketosis to be lesser in athletes as compared to non-athletes (Johnson et al., 1969; Johnson & Walton, 1971, 1972; Rennie & Johnson, 1974) or after a period of exercise training (Adams & Koeslag, 1988, 1989; Beattie & Winder, 1984, 1985; Ohmori et al. 1990; Rennie & Johnson, 1974a). Past studies have also shown that in the absence of food restriction, non-athletes are more prone than athletes to develop post-exercise ketosis (Koeslag, 1982; Winder et al., 1982). Similar observations have also been made in rats, with KB concentrations within 60-90 min post-exercise being significantly higher in untrained animals as opposed to trained animals (Adam & Koeslag (1988). The enhanced ketolytic capacity associated with training may explain these findings. Indeed, KB utilisation rate is higher in exercise-trained skeletal muscle (Winder et al. 1975), and exercise training increases β-HB clearance due to the enhanced oxidative capacity of skeletal muscles, which in turn results from the over-expression of PGC-1α (Svensson et al. 2016). There is also evidence that differences in the levels of liver glycogen may explain the dampening effect of training on post-exercise ketosis (Adams & Koeslag, 1988), with both the higher levels of resting liver glycogen and attenuated rates of liver glycogen depletion during exercise in exercise-trained individuals opposing ketogenesis (Baldwin et al. 1975).

The effect of exercise on KB levels has also been investigated in individuals with T1DM. It is well established that exercising in a relatively insulin-deprived state results in both an increase in glycaemia and ketonaemia in individuals with T1DM (Berger et al., 1977; Fery et al., 1987; Koivisto et al., 1974; Zinman & Vranic, 1985). These findings have been explained on the grounds that exercise increases the rate of ketogenesis in insulin-deprived individuals with T1DM (Berger et al., 1977; Wahren et al., 1975). In this respect, Wahren and colleagues (1975, 1984) found that the increase

in ketogenesis induced by prolonged exercise is exaggerated in insulin-deprived patients with T1DM who received their last insulin injection 24-26 hours before exercise as compared to control overnight fasted subjects. Also, they reported a higher ketogenic response in ketotic than in non-ketotic individuals with T1DM (Wahren et al., 1975, 1984). Of note, a rise in arterial KB levels is not observed in these individuals until after exercise, during which time KB increase to levels of 1.8-1.9 mmol/L and splanchnic ketone production is sustained, whereas KB production in non-diabetic fasted individuals rapidly declines after peaking at 10-20 min of recovery.

1.8 Potential risks associated with ketogenic diets

Given that KD are associated with a high intake of protein and fat, and low intake of plant materials, some concerns have been raised about the impact that such diets may have on health for the general population, including individuals with T1DM (Sekold et al., 2018). One of these concerns relates to the possibility that a diet high in saturated fat and low in unsaturated fat may increase the risk of coronary heart disease and stroke as indicated by studies performed in the general population (Mozaffarian et al., 2010; Siri-Tarino et al., 2010) and in individuals with T1DM (Seckold et al., 2018). Consistent with their high fat content, isoenergetic KD have been found in some studies to be associated with adverse effects such as increased mean levels of total cholesterol in individuals with epilepsy (Kossoff et al., 2006, 2008) or T1DM (Lennerz et al., 2018), higher levels of low density lipoproteins cholesterol (LDLc) and triglycerides, and decreased levels of high density lipoproteins cholesterol (HDLc) in people with epilepsy (Coppola et al., 2002; Kossoff et al., 2006; Kwiterovich et al., 2003; Sirven et al., 1999). Moreover, Wycherley et al. (2010) found that endothelial function in overweight and obese adults was worse after 12 months on a very low CHO high saturated fat diet despite falls in weight, blood pressure and fasting blood glucose. Of note, however, despite the initial deterioration in blood lipid profile, the blood lipid values observed at the end of some of the studies performed in people with diabetes were still within the average risk ranges (Gluckman et al., 2004). Also, in studies that lasted for more than 2 years in people with epilepsy, LDLc and HDLc levels may stabilise (Kwiterovich et al., 2003), and the lipid profiles of epileptic children on ketogenic diet for > 6 years appear to return toward baseline (Groesbeck et al., 2006), with evidence that the deterioration in lipid profile and arterial function observed during the first year of a ketogenic diet is

greatly diminished or no longer present after two years of exposure to such a diet (Kapetanakis et al., 2014). Finally, there is evidence that very low-CHO diets in overweight and obese individuals may also cause a shift from more harmful small, dense LDLc particles, to less-harmful large, low density LDLc levels (Volek et al., 2005), with the association between LDLc and cardiovascular risk in these individuals being dependent on particle size (Austin et al., 1988; Davidson et al., 2011). Since very low-CHO KD tend to increase LDLc particle size (Gerber and Berneis, 2012), this implies that the increase in total LDLc found in some studies may not necessarily be accompanied by increased cardiovascular risk. It is also noteworthy that although some studies in obese individuals have reported that KD rich in saturated fat elevates LDLc despite weight loss (e.g. Brinkworth et al., 2009; Mansoor et al., 2016; Nordmann et al., 2006), there is evidence that diets rich in unsaturated fat do not have this effect (Miller et al., 2009). However, concerns have been expressed about the benefits of replacing saturated fats with dietary polyunsaturated fats given that some recent studies suggest that such substitution may be harmful (Ramsden et al., 2016; Shapira and Israeli, 2007).

Of note, since, as discussed above, the high-dietary fat content typical of KD diets, but not of the less extreme LCHF diets (Dong et al, 2020), leads to increased postprandial triglycerides levels,), it has been proposed that this may increase cardiovascular disease risk maybe by increasing postprandial inflammation (de Vries et al., 2014). This view is not supported by the studies in the general population reporting that saturated fats and other dietary fats, except for trans-fats, are not associated with all-cause mortality and coronary heart disease as had been previously thought (Astrup et al., 2010; Mozaffarian et al., 2010; De Souza et al., 2015). However, a Cochrane review (Hooper et al, 2015) recently reported that a small, but potentially important, reduction in cardiovascular risk can be achieved when reducing saturated fat intake, with the consensus still recommending the replacement of saturated fats with unsaturated fats due to superiority of the latter in promoting cardiovascular health (NIHCE, 2016).

Concerns have also been raised with respect to the potential for KD to provide toxic amount of proteins (Hardy et al. 2015). Indeed, there is evidence, based on a paucity of clinical data, that excessive consumption of dietary protein (> 35-40% of energy intake) in the face of a diet poor in fat may lead to a condition referred to by the early American explorers as “rabbit starvation” (rabbit meat is very lean), which initially results in nausea, followed by diarrhea, and then death (Bilsborough and Mann, 2006; Speth

1983). This condition has been attributed to the limited capacity of the liver to upregulate the enzymes involved in urea synthesis in the face of increasing dietary protein intake, thus limiting the liver's capacity to synthesis KB from amino acids when the diet is poor in fat as a result of consuming very lean meat. Also, toxic hyperammonemia and hyperaminoacidemia may occur when protein intake exceeds the liver's capacity to produce urea (Rudman et al., 1973). It must be stressed, however, that current KD typically avoid such complications by recommending protein intake way below 35-40% of energy intake. Still, even with lower protein intake, KD may not be suitable for individuals with already impaired renal function, as it is recommended that their daily protein intake should be kept low (Giugliano et al., 2016).

The impact that KD or other forms of CHO-restricted diets may have on micronutrient intake in individuals with or without T1DM has also been the object of some concerns, particularly when these diets are used for losing weight without nutrient supplementation (Churuangsuk et al., 2019; Seckold et al., 2018). The concern here is that the restriction on the intake of fruits, vegetables, wholegrains, and cereals might decrease the consumption of minerals, vitamins, and antioxidant nutrients if the diet is not well designed nutritionally, thus potentially risking micronutrient insufficiencies (Bowman and Spence, 2002; Calton, 2002; Churuangsuk et al., 2019). In a recent systematic review of the literature, Churuangsuk and colleagues (2019) found some consistent patterns of reduced micronutrient intakes and status in individuals kept on a ketogenic diet. In particular, the intakes of folate, thiamine, magnesium, calcium iron and iodine were lower than normal in response to all of the CHO-restricted diets examined in their review (Churuangsuk et al., 2019). This finding is not surprising given that CHO-rich foods are a good source of most of the aforementioned micronutrients (excluding iodine). For instance, the shortage of some micronutrients in response to CHO-restricted diets is affected by the amount of fruits and vegetables intake allowed in these diets, with a fall in carotenoid and vitamin C intake reported in some diets (e.g. Atkins diet), but not in other diets (e.g. paleolithic diet; Churuangsuk et al., 2019). These findings highlight the importance of providing professional dietary advice and counselling to people undertaking a ketogenic diet or any other CHO-restricted diet to meet recommended micronutrient intake and avoid micronutrient inadequacy (Zinn, Rush & Johnson, 2018).

A suboptimal intake of dietary fibre may also arise as a result of adopting a KD. By restricting dietary CHO intake to less than 55 g/day, the likelihood of ingesting enough fibre is reduced. This is concerning because diets rich in dietary fibre are associated with lower incidences of cardiovascular disease, coronary events, stroke, and colorectal cancer. Therefore, not reaching recommended targets could increase the likelihood of the onset of these conditions (SACN, 2015). However, with proper dietary advice and use of supplements, it may be feasible to achieve fibre targets while following a low-CHO diet (Spritzler, 2015)

The possibility that CHO-restricted diets may compromise fetal growth and infant development in individuals with or without T1DM is another important issue to address (de Bock et al., 2017; Seckold et al., 2018). Consistent with the fact that glucose is the primary fuel for fetal growth, there is evidence that low glucose availability can compromise fetal survival (Herrera, 2000). Moreover, in healthy pregnant women, fetal growth is directly related to maternal glucose concentration in the blood (Metzger et al., 2009), with evidence of a link between gestational ketonemia and a lower child intelligence quotient (Rizzo et al., 1991). Of note, however, since the micronutrient status of the pregnant women investigated in the aforementioned studies was not assessed as a possible confounding variable, it is unclear whether micronutrient deficiencies *per se,* rather than CHO restriction and ketonemia, were responsible for the aforementioned complications associated with CHO-restricted diet. For instance, the inadequate intake of folate and iodine associated with CHO-restricted diets in women may increase the risk of fetal maldevelopment (Gernand et al., 2016).

Other adverse effects associated with KD such as constipation, vomiting, lack of energy, hunger (Klein et al., 2010; Neal et al., 2008) and menstrual dysfunction (Mady et al., 2003) have been observed in some individuals when first introduced to KD, but some of these effects appear to be short-lived. Indeed, during the early stages of adapting to a ketogenic diet (the first 4 or 5 days), individuals may complain of lethargy (Lefevre & Aronson, 2000; Vining et al., 1998). This effect, however, passes rapidly, and people on such diets subsequently report an improved condition (Brinkworth et al., 2009; Paoli et al., 2010; Yancy et al., 2009).

Of note, since no study has investigated the long-term effect (e.g. >10 years) of KD in healthy and clinical populations, there is no evidence-based data to set a time limit on

the continuous use of KD. It is also important to note that our knowledge of the effect of several years of exposure to KD on general health originates from work performed in young individuals with refractory epilepsy, thus implying that the translatability of these findings to the population of adults with metabolic diseases such as T1DM, T2DM and obesity should be performed with caution. Future research is required to assess the long-term implications of adopting such a diet, particularly in the case of individuals with refractory epilepsy (Freeman et al., 2007) or other clinical cases (e.g. weight loss, diabetes management).

1.9 Clinical use of ketogenic diet

Although there is a lack of information about the long-term health effects of KD, a number of studies have explored the clinical use of these diets for the treatment of several illnesses and diseases such as epilepsy, cancer, insulin resistance and obesity, with such clinical uses of KD first introduced almost a century ago for the treatment of epilepsy. A brief overview of the clinical use of KD will be presented in the following sections before discussing its potential use in the management of T1DM.

1.9.1 Ketogenic diets and the treatment of epilepsy

Almost a century ago, Dr Wilder proposed that the consumption of a high-fat/low-CHO diet designed to mimick the rise in blood KB concentration associated with fasting may have an anti-seizure effect (Freeman et al., 1998). The first attempt to test this hypothesis was performed using the extremely low CHO diet introduced in 1920s by the paediatricians at Johns Hopkins University (Lefevre & Aronson, 2000). Their intention was to restrict CHO intake severely enough to produce ketosis and reduce the incidence of epileptic seizure (Freeman et al., 2006). As such, less than 10 g of CHO were consumed/day together with 1 g protein per kg of body weight, with most of the energy (~90%) being obtained from unsaturated fat. This study, and subsequent ones, showed that this ketogenic diet was associated with seizure reduction (Freeman et al., 2006). However, adherence to such an extremely low CHO diet was a challenge due to its low palatability (Peterman, 1925; Wilder, 1921). For this reason, a less restrictive low-CHO diet was introduced in the 1960s at John Hopkins University (Hopkins & Lynch, 1970). This very low-CHO high fat ketogenic diet was more palatable as it allowed adults to consume up to 30 g of CHO/day, with ~65% of the energy originating from fat sources (Kossoff & Dorward, 2008), thus providing a better adherence to the diet compared to previous diets (Coppola et al., 2002; Klein et al., 2010; Kossoff & Dorward, 2008; Kossoff et al., 2006). Another variation of the KD relies on the intake of medium-chain triglycerides as a primary source of fat (Huttenlocher et al., 1971).

Although KD have been used for the treatment of epilepsy since the 1920s, it was only in the mid 2000s that randomised control trials were conducted to explore the efficacy of these diets (Neal et al., 2008, 2009; Seo et al., 2007). Neal and colleagues (2008), in particular, conducted the largest study involving 145 children with epilepsy. Their randomised controlled trial showed that after 3 months on a KD, the mean percentage of

baseline seizures was significantly lower than in the controls (62.0% *vs* 136.9%), thus supporting the findings of many earlier prospective (Coppola et al., 2002; Hosain et al., 2005; Kossoff et al., 2008; Vining et al., 1998) and retrospective studies (DiMario & Holland, 2002; Hemingway et al., 2001; Kang et al., 2005; Mackay et al., 2005). Despite the effectiveness of KD for the treatment of epilepsy, the high attrition rate and side effects during the initiation phase are some the main concerns raised in most studies.

The mechanisms underlying the anti-seizure effect of KD have been the object of much research effort as evidenced by the many recent reviews of literature recently published on this topic (e.g. Gano et al., 2014; Lima et al., 2014; McNally et al., 2012; Ryan et al., 2017; Simeone et al., 2018)). The anti-seizure mechanisms of these diets include, for instance: (1) reduced glycolytic activity and increased oxidation of free fatty acids and KB; (2) decreased glucose-mediated protein acetylation (e.g. histones H3); (3) altered synthesis and/or release and/or clearance of the neurotransmitters glutamate and inhibitory neurotransmitter gamma-aminobutyric acid (GABA) from the synaptic cleft, resulting in increased levels of GABA relative to glutamate levels (Bough, 2008; Juge et al., 2010); (4) increased mitochondrial biogenesis (e.g. hippocampus), leading to enhanced ATP production and stores (Bough et al., 2006); (5) increased antioxidant activity, lesser production of reactive oxygen species, and decreased ROS-mediated damage (McDonald and Cervenka, 2018); (6) reduced neuronal excitability via enhanced open probability of K_{ATP} channels (Ma et al., 2007); and (7) activation of PPARα and inhibition of mTOR. Of note, the role and relative importance of the aforementioned mechanisms still remain to be established, with some controversies with regard to their importance.

1.9.2 Ketogenic diets and the treatment of cancer

Given that cancer cells preferentially use glucose for survival and metastasis (Branco et al., 2015; Vander et al, 2009), and that KB inhibit glycolysis and enhance oxidative metabolism in tumours (Allen et al., 2014; Poff et al, 2019), it has been proposed that KD may represent a viable clinical approach to limit tumour growth and increase survival time. Several studies in a range of animal models of colon cancer, gastric cancer, prostate cancer and gliomas have shown that KD not only oppose tumour growth and increase survival (Allen et al., 2014; Poff et al, 2019), but also potentiate the effects of radiotherapy and chemotherapy in some cancer models (Allen et al., 2014).

Indeed, 72% of animal studies provide evidence in support of the anti-tumour effect of KD as a result of either slower tumour growth or longer survival time (Klement, 2017). Case reports in human patients with malignant astrocytoma or glioblastoma multiforme have shown that KD are associated with a reduction in tumour size (Varshneya et al., 2015). Also, a recent review of the literature revealed that 42% of studies in humans provide some evidence for an anti-tumour effect of KD (Klement, 2017). It is important to note, however, that most of the literature on this topic (Chung and Park, 2017; Erickson et al., 2017; Klement, 2017; Oliveira et al., 2018) is based on case reports, with only one randomised control trial and mixed results in terms of the outcomes of efficacy of KD in cancer therapy (Klement, 2017, 2019). Clearly, larger cohort studies are required to show more conclusively whether KD can improve patient prognosis.

The mechanisms underlying the anti-cancer effect of KD have been reviewed extensively in recent years, and are related, in part, to the increased glucose metabolism typical of cancer cells (Allen et al., 2014; Branco et al., 2016; Poff et al, 2019). Cancer cells rely extensively on both the anaerobic glucose conversion to lactate, and glucose metabolism via the pentose phosphate pathway to support their energy needs, with both metabolic pathways resulting in an increased production of both pyruvate and NADPH. This anaerobic metabolism displayed by cancer cells renders them hypoxia-resistant, whereas the production of both pyruvate, which has the potential to act as an antioxidant (McPherson and McEneny, 2012), and NADPH helps to support the antioxidant defence of these cells against the oxidative stress they typically experience as a result of their dysfunctional mitochondria (Allen et al., 2014; Branco et al., 2016; Poff et al, 2019).

Ketogenic diets and their high fat content may oppose tumour growth by increasing oxidative stress inside cancer cells (Poff et al., 2019). The notion that KD increase oxidative stress is supported both clinically and in animal models (Allen et al, 2014; Branco et al., 2015). The inhibition of glucose utilisation rate and its metabolism via the pentose phosphate pathway, as a result of the increased oxidation of KB and fat, results in reduced production of reducing equivalent to oppose the production of reactive oxygen species (ROS), thus contributing to increasing oxidative stress (Allen et al, 2014; Branco et al., 2015). In addition, the provision of KB and fat is expected to increase the reliance of cancer cells on the aerobic metabolism carried by their

dysfunctional mitochondria, increasing further oxidative stress (Allen et al., 2014; Branco et al,. 2016; Poff et al, 2019).

Of note, although glucose deprivation was the rationale for introducing KD for cancer treatment, there are a number of other mechanisms whereby KD may help oppose cancer (reviewed in Branco et al., 2015; Poff et al., 2019), namely: (1) decrease in BGL and associated fall in tumour glucose uptake; (2) low insulin levels consecutive to low plasma glucose levels opposing the tumourigenic and angiogenic effect of high insulin levels; (3) inhibition of inflammation; (5) increase in anti-tumour immunity; (6) fall in the expression of the genes involved in hypoxia response, angiogenesis and vascular remodeling; (7) increased histone deacetylation; and (8) sensitisation of tumours to standard therapy (e.g. chemotherapy and radiation therapy; Branco et al., 2015; Poff et al., 2019).

Given the limited number of studies on the anti-cancer effect of KD and their different designs (eg sample size, type of cancer, cancer stage, treatment duration), the quality of evidence in humans is poor as opposed to what has been learned from animal models (Erickson et al., 2017; Klement 2019). However, given the evidence that KD may be beneficial for the treatment of cancer, it is the general concensus that there is a need (Chung and Park, 2017; Erickson et al., 2017; Klement, 2017; Oliveira et al., 2018) for well-designed prolonged randomised controlled trials examining the anti-cancer benefits of these types of diets alone or in combination with other treatment modalities.

1.9.3 Ketogenic diets for weight loss

With the global rise in obesity has come an intensive search for effective weight-loss strategies. The use of hypocaloric KD became popular in the 1970s for weight loss, especially following the publication of the 'Dr Atkins Diet Revolution' monograph (Atkins, 1972). Since then, the effectiveness of these diets has been extensively investigated, with several randomised controlled (ad-libitum and calorie-restricted) studies reporting increased weight loss in response to KD as compared with energy-restricted low fat diets (Brehm et al., 2003; Buono et al., 2013; Gardner et al., 2007; Shai et al., 2008). However, it is important to stress that some studies have shown no difference in weight loss between low fat high CHO diets and low CHO high fat diets (Dansinger et al., 2005; Golay et al., 1996). Also, a recent review of the literature (Noakes et al., 2017) concluded that many tested diets are effective at causing at least

short-term weight loss, but are usually followed by some weight regain due to a fall in adherence (Foster et al., 2003)

Energy restricted KD are not only effective at promoting weight loss, they may also provide other health benefits such as the lowering of blood pressure (Pérez-Guisado et al., 2008; Sumithran et al., 2013; Yancy et al., 2004), blood glucose levels (Brehm et al., 2003; Dashti et al., 2006, 2007; Johnstone et al., 2008; Paoli, Cenci, & Grimaldi, 2011; Paoli et al., 2013; Pérez-Guisado, et al., 2008; Sondike et al., 2003; Sumithran et al., 2013), as well as decreasing the levels of blood LDLc, total cholesterol, and triglycerides (Brehm et al., 2003; Dashti et al., 2006, 2007; Johnstone et al., 2008; Paoli et al., 2011, 2013; Pérez-Guisado et al., 2008; Sondike et al., 2003; Yancy et al., 2004), while increasing HDLc levels (Dashti et al., 2006, 2007; Paoli et al., 2011; Pérez-Guisado et al., 2008) and insulin sensitivity (Fuehrlein et al., 2004), with this latter response being more pronounced in response to the consumption of a diet rich in polyunsaturated fat as opposed to a diet high in saturated fat (Fuehrlein et al., 2004). Low CHO diets have also been reported to reduce the proportion of small, dense LDLc particles, while increasing the number of large, non-atherogenic LDLc particles (Noakes et al., 2017). It is noteworthy, however, that not all studies have reported these aforementioned health benefits associated with energy restricted KD (Sharman et al., 2002; Westman et al., 2006).

A number of mechanisms have been proposed to explain the weight loss associated with KD despite the consumption of an increased proportion of energy-dense fat-rich foods. The rapid weight loss observed during the first week following the start of hypocaloric KD has been attributed to the loss of water due to increased diuresis (Yang et al., 1976; Pogozelski et al., 2005; Hall et al., 2016). However, Dual-energy X-ray absorptionmetry (DEXA) analysis clearly indicates that long-term weight loss is predominantly the result of the loss of fat mass combined with some loss of fat-free mass. Also, weight losses of 10 kg or more, as observed in some trials, cannot be due to water loss alone. The efficacy of low energy KD with ad libitum food intake may also result, at least in part, from the great limitation in food choice imposed by those CHO-restricted diets (Bravata et al., 2003). A few studies have explained the weight-loss benefits of hypoclaroic KD on the basis of the improved satiety and metabolic advantages provided by these types of diets as briefly explained in the

following paragraphs.

Energy-restrited diets are typically associated with a compensatory increase in hunger, a feature not shared to a similar extent by KD, as these diets are associated with a lesser rise in appetite (Gibson et al., 2015). The mechanisms whereby hypoclaroic KD inhibit hunger compared to high CHO low fat diets still remain to be established (Gibson et al., 2015; Paoli et al., 2015). There is evidence that the reduction in appetite, and thus of energy intake, associated with KD could be due to the direct appetite-suppressant action of KB (Gibson et al, 2015; Johnstone et al., 2008), the satiety effect of high protein intake (Paddon-Jones et al., 2008; Soenen et al, 2012; Veldhorst et al., 2008; Weigle et al., 2005; Westerterp-Plantenga et al., 2009), the high blood fatty acid levels which may act by decreasing the expression of the orexogenic neuropeptide, NPY (Obici et al, 2003), or other effects on appetite control hormones (Sumithran et al., 2013). In this respect, it is noteworthy that the increases in blood ghrelin levels and appetite are less pronounced in response to ketogenic compared to other hypocaloric diets (Sumitharn et al., 2013). Also, the lesser fall in the levels of the satiety-promoting peptide YY from baseline in response to a year-long low CHO non-ketogenic diet compared to more traditional low-fat diets (Hu et al. 2016) raises the issue of whether this may also play a role in mediating the lesser increase in appetite associated with KD, thus suggesting that satiety may be better maintained on a low-CHO diet as compared to a higher CHO/low fat diet.

A number of metabolic factors have also been proposed to explain the positive effect of KD on weight loss. One of these is the increased loss of KB in sweat and urine and acetone loss in expiratory air (Paoli, 2014). Also, resting energy expenditure has been shown to decrease by more than 1200 kJ/d on a low-fat diet, while there was little decline in metabolic rate on a very low-CHO diet (Ebbeling et al., 2012), suggesting that the relatively higher resting energy expenditure associated with hypocaloric KD may contribute to the effectiveness of these diets on weigh loss. Another metabolic factor relates to the thermic effect of proteins (Feinman and Fine, 2007; Fine and Feinman, 2004) and the increased metabolic costs of gluconeogenesis from the amino acids derived from dietary proteins for those who ingest extra proteins (Feinman & Fine, 2007; Halton & Hu, 2004; Paoli et al., 2012). Regardless, future research is needed to determine whether KD are indeed superior to traditional diets, and the precise mechanism by which this is achieved.

1.9.4 Ketogenic diets for the treatment of T2DM

Given that the total daily amount of CHO consumed has the biggest effect on postprandial blood glucose levels (Diabetes UK, 2011; Accurso et al., 2008; Sheard et al., 2004), it is not suprising that the use of low CHO diets, including KD, have been the object of much research effort to test their effectiveness in the treatment of type 2 diabetes mellitus (T2DM), a disease with a global prevalence at 8.5% among adults in 2014 (World Health Organization, 2016). As discussed in Section 1.3, there is evidence that low CHO diet improves glycaemic control in individuals with T2DM, with some (Sainsburry et al 2018; Snorgaard et al., 2017) but not all studies (McArdle et al 2019) showing the largest fall in HbA1c levels being associated with the lowest intake in CHO, thus raising the issue of whether KD is associated with very low or even normal HbA1c levels.

There is a strong body of evidence that the use of KD over a few weeks-months can improve glycaemic control in individuals with T2DM (Boden et al., 2005; Goday et al., 2016; Halberg et al., 2018; Hussain et al., 2012; Lim et al., 2010; McKenzie et al., 2017; Saslow et al., 2017; Tay et al., 2015, 2018; Westman et al., 2008; Yancy et al., 2009). Boden and colleagues (2005) in a non-randomised control study lacking a strict control group reported lower plasma fasting glucose and HbA1c levels in a group of 10 obese individuals with T2DM after 14 days on an energy restricted KD (21 g CHO/day; 9054 kJ/day) compared with baseline. In another nonrandomised, parallel arm, outpatient intervention study, but this time involving a 10-week exposure to a KD in 262 adults with T2DM fed *ad libitum*, HbA1c levels fell by 1% while significantly decreasing medication use (McKenzie et al., 2017). These findings support those of Dyson and colleagues (2007) who found in their nonrandomised study with no strict control group that 3 months of a KD (<40 g CHO/d) resulted in a significant fall in HbA1c levels in a small group of obese individuals with T2DM fed *ad libitum*. Similarly, in their single arm diet intervention trial, Yancy and colleagues (2005) reported an improvement in HbA1c levels in response to ~4 months on a KD providing only 20 g of CHO/day in a group of 28 participants with T2DM fed *ad libitum*. Comparable findings arose from a4-month long prospective, open-label, multi-centric randomised clinical trial in 45 obese men and women with T2DM exposed to an energy-restricted KD (< 50 g CHO/day) resulting in a 0.9% fall in their HbA1c levels (Goday et al., 2016).

The glycaemic benefit of the aforementieond short-duration interventions also extends to KD lasting 6 months or more. In a randomised trial, but with no strict-control group, Westman and colleagues (2008) tested the efficacy of a low CHO non energy-restricted diet (< 20 g CHO/day) in 49 obese individuals with T2DM over a 24-week period, and observed a significant 1.5% reduction in HbA1c levels. Of note, the use of diabetes medications were also reduced or eliminated in 95% of participants at the end of this study. These findings were subsequently corroborated in a randomised trial with no strict control group that was performed by Tay and colleagues (2008) who found that 24 weeks of energy-restricted KD was associated with a fall in fasting blood glucose levels. Also, Hussain and colleagues (2012) found in their non-randomised trial with no strict control group that 24 weeks of a low-energy KD resulted in a significant fall in average blood glucose, which reached levels approaching those of non diabetic individuals. Their diet also resulted in a marked decrease in HbA1c, reaching levels of ~6.2% (Hussain et al., 2012). More recently, in a randomised trial with no strict control group, 57 adults with T2DM were exposed to an energy-restricted KD (< 50 g CHO/day) rich in unsaturated fat and limited in saturated fat (Tay et al., 2018), and achieved significant falls in both HbA1c and fasting blood glucose levels after both one (Tay et al., 2015) and two years (Tay et al., 2018). Saslow and colleagues (2017) also showed in their randomised trial with no strict control group that 12 months on a non-energy restricted KD in 16 overweight adults with T2DM or prediabetes was associated with a significant fall in HbA1c levels and diabetes-related medication use (Saslow et al 2017). Finally, Haldberg and colleagues (2018) performed an open-label non-randomised controlled study in a group of 349 participants with T2DM, and reported that a KD combined with remote monitoring of ketone levels to assess adherence and personalise the diet led to improved glycaemic controla reduction in diabetes medication use, and substantial average weight loss. Of note, not all studies have reported that KD, in the long-term, results in a fall in HbA1c levels (Iqbal et al., 2018). Indeed, Iqbal and colleagues (2018) performed a randomised trial with no strict-control group, and reported no significant fall in HbA1c levels after 12 and 24 months of non-energy restricted KD.

The benefits of KD for the treatment of T2DM extend to the many cardiovascular risk factors associated with this disease (Dong et al., 2020). Despite the increased proportion of fat intake associated with these diets, it is generally the case that KD in T2DM result

in a fall in body mass (Boden et al, 2005; Dyson 2007; Dashti et al., 2007; Goday et al., 2016; Hussain et al., 2012; Lim et al., 2010; McKenzie et al., 2017; Saslow et al 2017; Tay et al., 2015, 2018; Westman et al., 2008; Yancy et al., 2005). Also, some studies have reported that KD are associated with a rise in HDLc levels (Dashti et al., 2007; Hussain et al., 2012; Tay et al., 2015, 2018; Westman et al., 2008), and a decrease in systolic blood pressure (Tay et al., 2015, 2018), diastolic blood pressure (Tay et al., 2015, 2018), and in blood triglycerides levels (Boden et al, 2005; Dashti et al., 2007; Dong et al., 2020; Goday et al., 2016; Hussain et al., 2012; Tay et al., 2015, 2018; Westman et al., 2008; Yancy et al., 2005), total cholesterol (Boden et al., 2005) and LDLc (Dashti et al., 2007; Hussain et al., 2012; Tay et al., 2015). Although a recent study reported that KD increased LDLc levels in patients with T2DM, this increase was limited to the large non-atherogenic LDLc subfraction, which increased in size (Bhanpuri et al., 2018).

Of note, some studies have found that KD do not improve the levels of HbA1c, as mentioned above (Iqbal et al., 2010), total cholesterol (Dyson et al., 2007; Goday et al., 2016), HDLc (Boden et al., 2005; Goday et al., 2016), LDLc (Boden et al, 2005; Dyson et al., 2007; Goday et al., 2016; Yancy et al., 2005) and triglycerides levels (Dyson et al., 2007) in individulas with T2DM. It must be stressed, however, that in the case of the study of Dyson and colleagues (2007), their negative findings are not surprising given the small sample size of their study, with only six participants being involved (< 40 g CHO/day). Another study with negative findings is that of Davis and colleagues (2009) who found that one year of a KD initiated with an intake of 20 g CHO/day in a group of 105 overweight adults with T2DM resulted in a fall in body mass and HbA1c level after 3 months, but not after one year. At this point in time, there was also no change in blood pressure, systolic and diastolic blood pressure, total cholesterol, LDLc, and triglycerides levels compared with baseline. These negative findings could be explained on the basis that for most of the duration of the trial, the prescribed diet was not ketotic, as the CHO intake of their participants increased at a prescribed rate of 5 g/week (Davis et al., 2009).

Importantly, the design of most of the aforementioned studies did not take into account the possibility that the quality of the dietary fat and CHO they replaced, as well as the amount of protein ingested during exposure to a KD, may be important modifiers of cardiovascular risk (Siri-Tarino et al., 2015). Distinguishing the specific effects of

ketosis from the effects of other components of the KD (e.g. low CHOs, high fat intake) is challenging. People placed on a KD may not only change the macronutrient composition of their diets, but may also improve the quality of the food they eat in favour of healthier foods (e.g. lean meats, nuts). Unfortunately, detailed dietary data are generally not provided, thus making it challenging to distinguish the effects of ketosis, CHO quantity, CHO quality and other macronutrients. That such omissions may affect the outcome of a study is best illustrated by the recent findings that a low-CHO diet rich in saturated fat was shown to abolish any improvement in LDLc particle size distribution in contrast to a low-CHO diet with equal total fat, but rich in polyunsaturated fatty acids in people with an atherogenic LDLc pattern (Chiu et al., 2017). The amount of protein ingested may also influence the effect of KD, since high protein diets, as opposed to low protein diets, have a positive effect on triglyceride reduction in patients with T2DM, but have limited effect on weight loss, glycaemic control, blood pressure, total cholesterol, LDLc, and HDLc levels (Zhao et al., 2018). Of note, however, others have reported that a high protein intake can enhance weight loss, and improve glycaemic control and cardiovascular disease risk factors in this population (Wing et al., 2011).

It also remains to be determined whether the short-term improvement in glycaemic control and a range of cardiovascular risk factors in response to KD is a consequence of weight loss, or a direct result of ketosis or CHO restriction *per se,* and/or the consequence of redistributing the proportion of energy provided by the other macronutrients (fat and proteins). This is an important issue given that low fat mass *per se* can significantly improve several markers of cardiovascular risk such as blood pressure, HDLc, triglycerides and glycaemic control (Horton et al., 2010; Wing et al., 2011).

It is important to stress that most of the aforementioned studies are likely to have underestimated the effect of KD on glycaemic control and risk factors for cardiovascular diseases. This is because adherence to KD is problematic mainly because of the low palatability and restricted food choice associated with KD, the appeal that many individuals have for CHO-rich food, and the peer pressure from people not on KD (Korsmo-Haugen 2019), with a recent meta-analysis reporting that only one (Westman et al., 2008) out of the six KD trials it examined actually achieved its low CHO intake target (Huntries et al., 2018). In fact, self-selected CHO intake by people prescribed a

KD is typically higher than recommended by the diet (e.g. Sacks et al., 2009; Lim et al., 2010). Such a failure to comply with KD would be expected to result in an underestimation of the effects of KD on health. It is also regrettable that the ketotic state of the participants on KD is rarely monitored, as one advantage of KD is that they allow one to objectively monitor compliance by measuring blood ketone levels. This precaution was adopted in a recent trial performed in patients with T2DM who were asked to monitor their blood KB concentration regularly to ensure compliance (Hallbert et al., 2018). The resulting impressive 13% average weight loss, improvement in glycaemic control, and reduction in diabetes medication use may have been due, at least in part, to the ability of the participants and experimenters to accurately track diet adherence in real-time by measuring blood KB concentration (Hallbert et al., 2018). Another limitation conducive to underestimating the benefits of KD relates to the decrease in diabetes medication being a common outcome of these types of dietary intervention (Huntries et al, 2018). Because of the fall in medication use reported in studies on KD, it is highly challenging to assess the "true" efficacy of these dietary interventions (Van Wyke et al., 2015). Had medications not been reduced, greater reductions in HbA1c and improvement in cardiometabolic variables may have been found (Davis et al., 2009; Tay et al, 2014).

The challenge of evaluating the benefits of KD for the treatment of T2DM is also compounded by the lack of data examining the long-term efficacy (> 2 years), safety and health benefits of KD. This is an important issue to address given that most chronic diseases such as diabetes, cardiovascular diseases, and other chronic conditions have a development period of 10–20 years or more (Brouns et al., 2018). Finally, on methodological grounds, all published KD trials carry with them a high risk of bias due to participants and experimenters not being blinded to the study protocol. Also, most weight loss intervention studies have adopted subjective methods to assess dietary intake that are known to be inaccurate, with patients often erroneously reporting continued diet adherence over the long-term despite objective measurements to the contrary (Freedhoff et al., 2016).

1.10 Ketogenic diets for the management of blood glucose levels in T1DM

Poor glycaemic control, recurrent hypoglycaemia, and possibly marked glycaemic excursions, all play important roles in the aetiology of long-term complications in people with T1DM. Given the difficulty of matching CHO intake with insulin dose, it is not uncommon for adults with T1DM to restrict their CHO intake to reduce the magnitude of their glycaemic excursions and improve glycaemic control (Delahanty et al., 2009), but the proportion of adults with T1DM on a KD is unknown. Since KD can improve the glycaemic control of individuals with T2DM without causing any dyslipidaemia (Section 1.9.4), this raises the issue of whether KD can not only improve glycaemic control without any ill health effects in T1DM adults, but also to an extent where average BGL is close to those of non-diabetic individuals (reviewed in Seckold et al., 2018; Turton et al., 2018). Of note, such a prediction based on studies showing the benefits of KD on T2DM assumes that KD can provide weight-loss independent benefits on glycaemic control in people with T1DM, and important assumption since the primary purpose of LCHF diets in T1DM is to improve glycaemic control without any weight loss as opposed to the weight loss KD causes in people with T2DM.

Unfortunately, only two studies have examined the benefits of KD on HbA1c levels in individuals with T1DM, namely one retrospective case study (O'Neill et al., 2003) and one observational study (Lennerz et al., 2018), with no randomized control trial performed to this date. For this reason, we are providing a systematic review of the strengths and limations of these two studies. The notion that KD may be beneficial is suggested by the retrospective case series analysis of O'Neill and colleagues (2003) which showed that very low prescribed CHO intake (~30 g/day) in individuals with T1DM resulted in HbA1c levels of 5.5% without causing any dyslipidaemia. However, no dietary analyses or blood KB assays were conducted in this study to show that the actual CHO intake was as low as prescribed (O'Neill et al., 2003), and that the diet was effectively ketogenic. These are important limitations given that compliance with low CHO diets can be less than optimal (Krebs et al., 2016; Nielsen et al., 2005).

Recently, Lennerz and colleagues (2018) conducted a study based on self-reported observational surveys to evaluate glycaemic control among 316 individuals with T1DM who consumed a very low CHO diet (< 51 g CHO/day). Although this study reported optimal mean HbA1c levels of 5.67 ± 0.66%, 62% of the participants were classified as dyslipidaemic. Also, there were some important limitations to this study. Firstly, participants were predominantly recruited from a single social media group, potentially

creating group bias. Secondly, only 137 (42.4%) and 101 (32.0%) participants provided their CGM data and lipid profiles, respectively. It is possible that participants self-selected which data to report, and/or chose not to report their less-than-desirable data. Finally, no blood KB analyses were performed to confirm that the participants were compliant with their diet and effectively in a ketotic state. Given these limitations, it is still unclear to what extend KD can improve glycaemic control in individuals with T1DM.

Another area of concern relates to the issue of whether individuals with T1DM on a ketogenic diet are at an increased risk of ketoacidosis during and after exercise. This concern arises from the observation that exercise in insulin-deprived mildly ketotic individuals with T1DM is not recommended as the rise in catecholamines and glucagon levels during exercise could markedly increase their risk of exercise-mediated diabetic ketoacidosis (DKA), a leading cause of hospitalisation, morbidity and death in T1DM (Veech, 2004). To the best of our knowledge, it is still unknown whether exercise increases the risk of DKA in T1DM people consuming a ketogenic diet.

1.11 Statement of the problem and hypotheses

In summary, there is some evidence, albeit inconclusive, that the restriction of dietary CHO may help individuals with T1DM improve their glycaemic control and participate in physical activities without experiencing severe cases of hyper- or hypoglycaemia. Since at the time this thesis was initiated, the effect of KD on glycaemic control had never been examined before, this thesis proposed to meet the following aims.

Aim 1: To test the hypothesis that individuals already on a ketogenic diet (< 50 g CHO/day) display optimal glycaemic control approaching that of non-diabetic healthy individuals, low levels of glycaemic excursion, and reduced hypoglycemia risk, with no significant dyslipidaemia or other detrimental health effects.

Aim 2: To characterise the relationship between daily CHO intake and HbA1c over a broad range of CHO intake, including very low CHO intake.

Aim 3: To test the hypothesis that individuals already on a ketogenic diet (< 55 g CHO/day) do not experience any increase in blood KB concentration during and early after different types of exercise, including graded exercise to volitional exhaustion, prolonged aerobic exercise, and a maximal sprint effort.

Chapter 2

The glycaemic benefits of a very low carbohydrate ketogenic diet in adults with type 1 diabetes mellitus may be opposed by increased hypoglycaemia risk and dyslipidaemia

Based on a paper reviewed and published in Diabetic Medicine:

Leow, Z. Z. X., Guelfi, K. J., Davis, E. A., Jones, T. W., & Fournier, P. A. (2018). The glycaemic benefits of a very-low-carbohydrate ketogenic diet in adults with Type 1 diabetes mellitus may be opposed by increased hypoglycaemia risk and dyslipidaemia. *Diabetic Medicine*, *35*(9), 1258-1263.

2.1 Abstract

Aims Low-carbohydrate high-fat diets are known to improve glycaemic control without causing any ill health effect in adults with T1DM. However, it is unknown if this is also the case with the more extreme very low-CHO high fat diets typical of KD.

Methods In this observational study, 11 adults with T1DM (7 men, 4 women; age 36.1 ± 6.8 y; duration of diabetes 12.8 ± 10.3 y) on KD (< 50 g CHO/day) for 2.6 ± 3.3 y (β-hydroxybutyrate 1.6 ± 1.3 mmol/L) had fasting blood sampled and analysed, and were fitted with a blinded continuous glucose monitor for 7 days to measure glycaemic variability.

Results Mean HbA1c levels were 35 ± 4 mmol/mol (5.3 ± 0.4%), and participants spent 74 ± 20 and 3 ± 8% of their time in the euglycaemic (4-8 mmol/L) and hyperglycaemic (>10 mmol/L) range, respectively, with little daily glycaemic variability (SD 1.5 ± 0.7 mmol/L; CV 26 ± 8%). Blood glucose levels were below 3.0 mmol/L for 3.6% of their time, and participants experienced 0.9(0.0-2.0) daily episodes of hypoglycaemia. Total cholesterol, LDL cholesterol, total cholesterol/HDL ratio, and triglycerides were above the recommended range in 82, 82, 64 and 27% of participants, respectively. However, HDL levels were within recommended range for all participants. Participants displayed no or little evidence of hepatic or renal dysfunction.

Conclusion This study provides the first evidence that although KD in adults with T1DM may be associated with optimal HbA1c levels and little glycaemic variability, they may also be associated with marked dyslipidaemia and a high number of hypoglycaemic episodes.

2.2 Introduction

Poor glycaemic control, recurrent hypoglycaemia, and possibly marked glycaemic excursions play important roles in the aetiology of long-term complications in people with type 1 diabetes (T1DM). Given the difficulty of matching carbohydrate (CHO) intake with insulin dose, it is not uncommon for adults with T1DM to restrict their CHO intake to reduce the magnitude of their glycaemic excursions and improve glycaemic control (Delahanty et al., 2009).

Several studies have shown that non-ketogenic low CHO high fat (LCHF; < 100g CHO/day) diets improve glycaemic control, with significant reduction of glycated haemoglobin (HbA1c) in adults with T1DM without causing any dyslipidaemia or increased hypoglycaemia risk (Krebs et al., 2015; Nielsen et al., 2005, 2012; O'Neill et al., 2003). At the time of this study, it was unclear, however, whether ketogenic diets (KD), typically characterised by much lower CHO intake (< 50 g/day), also improve glycaemic control without any ill health effects in T1DM adults, an important issue to address given the growing popularity of such diets and the recent findings that low CHO diets may be detrimental to the growth of T1DM children (de Bock et al., 2017). Before undertaking any randomised controlled trial to examine this timely clinical issue, we undertook this observational study to determine whether the optimal glycaemic control predicted to occur in response to KD is associated with any detrimental health effects in adults with T1DM already consuming such diets.

2.3 Participants and methods

Participants were recruited between June 2016 - June2017 with the help of local endocrinologists, diabetes health centers, diabetes support groups as well as through social media (facebook, instagram, twitter), local diabetes communities and organisations, and via word of mouth targeting past and current participants mainly from metropolitan Perth, Western Australia. Eligibility criteria were ingestion of < 50 g CHO/day for > 6 months, fasting blood β-hydroxybutyrate levels ≥ 0.4 mmol/L, duration of diagnosed T1DM ≥ 2 yr, C-peptide < 0.05 nmol/L, and not taking any prescribed medication other than insulin. Twenty individuals expressed interest, of which eleven were eligible for inclusion (7 men and 4 women; mean±SD; age, 36.1 ±

6.8 yr; height, 174 ± 9 cm; body mass, 71.3 ± 13.1 kg; median (range) duration of ketogenic diet, 1.5 (0.6-3.0) yr. Some potential participants had to be excluced on the grounds that their average daily CHO intake was above our cut-off of 50 g/day. All 11 participants were on a multiple-insulin injection regimen and self-selected their ketogenic diet, with no professional (e.g. dietitian) advice given to participants. Recruitment was challenging given the small pool of individuals on such a diet in Perth, Western Australia. The study protocol was approved by the University of Western Australia Ethics Committee (RA/4/1/6360), and informed consent was obtained from all participants.

Given the high costs of performing a randomised controlled trial (RCT) to examine the long-term effects (>1 year) of KD on glycaemic control and lipid profile as well as the challenges of recruiting T1DM participants willing to be involved in such a study, we undertook to carry out an observational study targetting individuals already on a KD. It was our view that the information thus obtained from this observational study would help inform the design of future RCT on the long-term benefits and risks of KD in T1DM. Each participant attended our laboratory on three separate occasions. On one occasion, blood was taken by a certified nurse after an overnight fast to measure HbA1c, c-peptide, β-hydroxybutyrate levels, lipoprotein profile, and markers of liver and kidney function. All assays were performed by a certified independent laboratory (PathWest, Osborne Park, Western Australia). On another visit, height, body mass, blood pressure and haemoglobin levels (HemoCue Hb201+ system, Sweden) were measured. Height was measured using a stadiometer (Stadi-o-meter, Novel Products Inc, Addison, IL, USA), with shoes removed, feet placed together, and head positioned in the Frankfort plane. Body mass was recorded using an electronic scale (Sauter, Gmbh D-7470, Albstadt, Ebingen, Germany). Blood pressure was measured using an electronic sphygmomanometer (Omron, HEM 7120, Healthcare Co, Kyoto, Japan; accuracy 3 mm Hg). To measure blood pressure, each participant rested for 15 minutes while in a seated position, with one of the arms flexed so that the elbow was level with the heart, and the lowest of three consecutive blood pressure measurements was taken as the participant's blood pressure. In addition, body composition was assessed using dual energy x-ray absorptiometry (Prodigy, General Electric Company, United States), and, after proper training, participants were provided with a blinded continuous glucose monitor (CGM; Dexcom-G4, USA) and a glucometer (OneTouch VeiroIQ, LifeScan Europe, Switzerland) to be used for a 7-day period. Before leaving the laboratory,

participants were given a diary and a portable weighing scale (Homemaker 9753, Tooronga, Vic) to record all cases of hypoglycaemia, insulin therapy regimen, food and drink intake. Participants were then sent home and asked to follow their usual diet and activity routine for 7 days. After this, they returned to the laboratory and data from the CGM and food diaries were collected.

Data analyses

The data collected from the CGM was downloaded and analysed using Dexcom STUDIO (Dexcom Inc, USA). Food and drink records were analysed via Foodworks-7 (Xyris Software, Australia). The average blood glucose level (BGL) for each participant was defined as the sum of total blood glucose values divided by the total number of measurements across the 7-day period. Daily blood glucose variability was expressed using standard deviation (SD) and coefficient of variance. Postprandial blood glucose excursion was defined as the difference between peak BGL (within 90 min of eating) and pre-meal BGL. The primary outcome measure for this study was HbA1c levels. Despite the absence of any prior study to inform our sample size calculation, we calculated that a sample size of 8 participants should provide enough statistical power (0.8) to detect significant differences at $p<0.05$. All results are expressed as means ± SD or median (range) as appropriate.

2.4 Results

2.4.1 Blood glucose management and dietary profiles of participants

Participants monitored their BGL 14.3 ± 6.9 times/day, with no relationship between HbA1c levels and the number of blood glucose tests ($r = 0.16$). On average, participants performed 6.0 ± 2.9 insulin injections every day, for a daily total of 18.4 ± 11.2 units, with long acting insulin accounting for 62.4% of total daily insulin administered. Total daily energy intake, together with the contributions of CHO, fat and protein to intake are shown in Table 2.1. Of note, saturated fat accounted for 39 ± 11% of daily fat intake, and on average (range) 5.5 (0.8-13.0), 9.3 (1.5-21.5), 12.0 (3.4-29.6) and 4.7 (0.3-12.1) g CHO were ingested during breakfast, lunch, dinner and snacks, respectively.

Table 2.1. Descriptive statistics.

	Total
Age (yrs)	36.1 ± 6.8
Duration of diabetes (yrs)	12.8 ± 10.3
Duration on LCHF diet (yrs)	2.6 ± 3.3
Height (cm)	174.± 9
Weight (kg)	71.3 ± 13.1
Body mass index (kg/m^2)	23.4 ± 3.1
Systolic blood pressure (mmHg)	119 ± 12
Diastolic blood pressure (mmHg)	72 ± 11
Wait-to-hip ratio	0.8 ± 0.1
Body fat (%)	18.4 ± 8.1
Lean muscle (%)	81.6 ± 8.1
Total energy intake (kcal)	1966 ± 803
Carbohydrate (g)	32 ± 18
Carbohydrate (%)	6 ± 3
Fat (g)	148 ± 79
Fat (%)	65 ± 10
Protein (g)	131 ± 157
Protein (%)	24 ± 8

Data are expressed as mean ± SD (n = 11).

Table 2.1. Lipid profile (total cholesterol, triglyceride, LDL, HDL and total cholesterol/HDL), HbA1c, kidney function (blood creatinine, eGFR, urine albumin, urine creatinine and urine albumin/creatinine), liver function (total protein, albumin, globulins, bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP] and Gamma-glutamyl transferase [GGT]), and haemoglobin status (Hb) of participants with T1DM on a ketogenic diet.

Variables	Participants	Reference range	No. of participants out of reference range
HbA1c (mmol/mol)	34.5±3.8	<42	0
Lipids	7.9 ± 1.9*	< 5.5	9
Total cholesterol (mmol/L)			
Triglyceride (mmol/L)	1.2 ± 0.9	< 1.7	3
LDL (mmol/L)	5.5 ± 1.7*	< 3.0	9
HDL (mmol/L)	2.0 ± 0.9	> 1.0	0
Total cholesterol/HDL	4.8 ± 2.1*	< 3.5	7
Kidney function			
Blood Creatinine (µmol/L)	72.3 ± 15.1	60 – 110	1
eGFR ($mLmin^{-1}1.73m^{-2}$)	89.3 ± 1.6	> 60	1
Urine albumin (mg/L)	4.6 ± 0.7	-	0
Urine creatinine (mmol/L)	5.9 ± 4.7	-	0
Urine albumin/creatinine (mg/mmol)	1.2 ± 0.7	<3.5 (women); <2.5 (men)	0 woman; 0 man
Liver function			
Total protein (g/L)	69.7 ± 2.4	60 - 80	1
Albumin (g/L)	43.2 ± 1.5	35 – 50	0
Globulins (g/L)	26.7 ± 1.8	25 – 42	1
Bilirubin (µmol/L)	10.8 ± 4.1	< 20	0
ALT (U/L)	28.7 ± 8.4	< 40	1
AST (U/L)	30.2 ± 6.5	< 45	0
ALP (U/L)	61.7 ± 12.0	30 – 110	0
GGT (U/L)	20.8 ± 11.4	< 60	0
Ketone bodies			
β-hydroxybutyrate (mmol/L)	1.6 ± 1.3*	< 0.4	11
Haemoglobin status			
Hb (g/L)	122.7 ± 20.8	>120 (women); >135 (men)	0 woman; 3 man

Data are expressed as mean±SD. * indicates values that are not within the recommended range.

2.4.2 Glycaemic profile

The mean and median HbA1c level of our participants was 35 ± 4 mmol/mol (5.3 ± 0.4%) and 36 (28-41) mmol/mol (5.4, 4.7-5.9%), respectively, and their average and median BGL determined from their CGM were 5.8 ± 1.2 and 5.5 (3.1-8.4) mmol/L, respectively. Average and median BGL obtained from personal glucometers were 5.1 ± 1.5 and 4.8 (4.4-6.5) mmol/L, respectively. Daily blood glucose variability expressed as SD and CV were 1.5 ± 0.7 mmol/L and 26.4 ± 8.0%, respectively. The mean and median magnitude of postprandial blood glucose excursions were 0.8 ± 1.5 and 0.5 (0-2.2) mmol/L, respectively. Participants spent 73.7 ± 20.1% (median 76.8, 52.8-90.3) of their time within a blood glucose range of 4-8 mmol/L, and 10.3 ± 15.6% (median 6.6, 0.0-39.3%) and 3.0 ± 7.6% (median 0.0, 0.0-18.8%) of their time above 8 and 10 mmol/L, respectively (Fig. 2.1). Although participants self-reported a median of 0.0 (0.0-2.0) and a mean of 0.4 ± 0.7 episodes of hypoglycaemia per week, they spent approximately 3.6% of their time below 3.0 mmol/L, and experienced 0.9 (0.0-2.0) episodes of hypoglycaemia/day (defined as BGL < 3.0 mmol/L; International Hypoglycaemia Study Group, 2017).

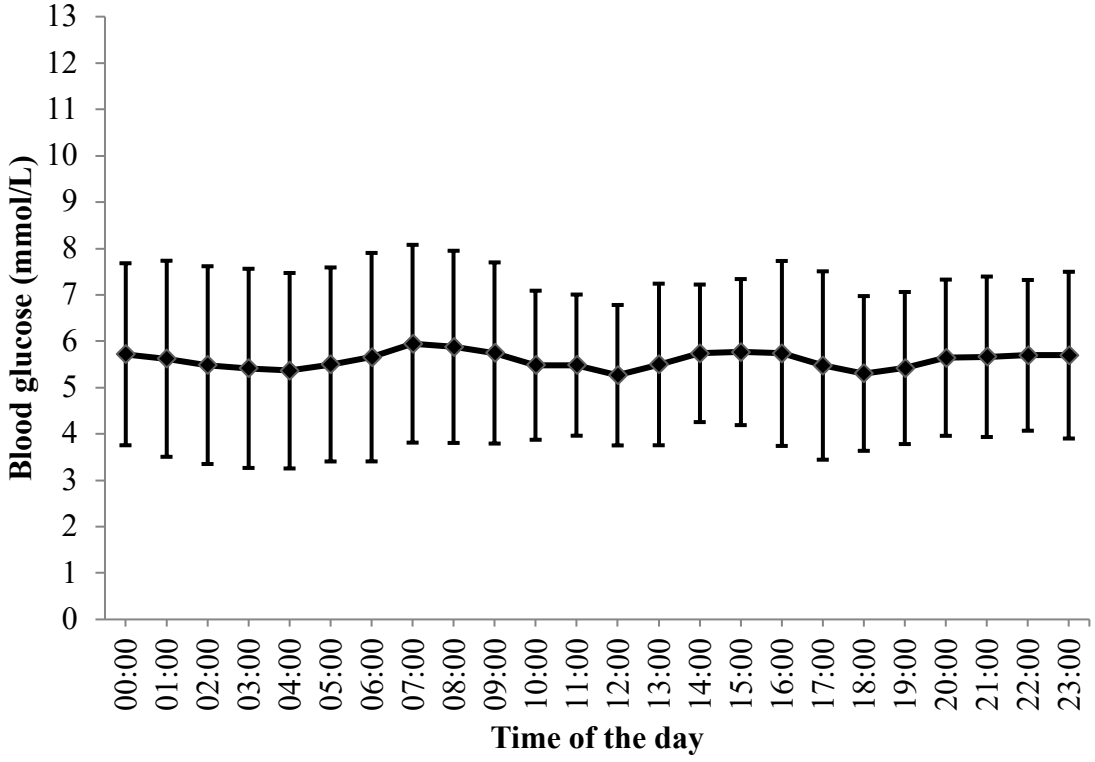

Figure 2.1 Average blood glucose levels (mmol/L) over 24-hr in adults with T1DM on a ketogenic diet. Data are expressed as mean ± SD. (n = 11).

2.4.3 Lipid profile, kidney and liver function

Markers for kidney and liver function were within the recommended range (Table 2.2). Total cholesterol, LDL cholesterol, total cholesterol/HDL ratios, and triglycerides were above recommended range in 82, 82, 64 and 27% of participants, but HDL levels were within the recommended range for all participants, and two male participants had low haemoglobin levels (Table 2.2).

2.5 Discussion

This study provides evidence that although KD in adults with T1DM may be associated with both optimal HbA1c levels and little glycaemic variability, they may also be associated with a high risk of dyslipidaemia and a high frequency of hypoglycaemic episodes.

The evidence provided here that KD may normalise HbA1c levels in adults with T1DM is consistent with the findings of others who showed that moderate LCHF diets are associated with low HbA1c levels in adults with T1DM (Krebs et al., 2015; Nielsen et al., 2005, 2012; O'Neill et al., 2003), but with higher HbA1c than reported here. Our findings also corroborate those of Lennerz and colleagues (2018) who published their findings at nearly the same time as ours (Leow et al., 2018). As discussed in Chapter 1, Lennerz and colleagues (2018) conducted a study based on self-reported observational surveys to evaluate glycaemic control among 316 individuals with T1DM who consumed a very low CHO diet (< 51 g CHO/day). Although this study reported optimal mean HbA1c levels of 5.67 ± 0.66%, 62% of the participants were classified as dyslipidaemic. This study has been criticized not only by us (Chapter 1), but also by others (Mayer-Davis et al., 2018), as there were some important limitations with its design. Firstly, participants were predominantly recruited from a single social media group, potentially creating group bias. Secondly, only 137 (42.4%) and 101 (32.0%) participants provided their CGM data and lipid profiles, respectively. It is possible that participants self-selected which data to report, and/or chose not to report their less-than-desirable data. Finally, no blood KB analyses were performed to confirm that the participants were compliant with their diet and effectively in a ketotic state. Of note, the outstanding HbA1c levels reported in the present study were achieved using conventional insulin therapy, an important finding given that none of the current diabetes therapies, including insulin pump therapy, multiple daily blood glucose

measurements, CHO counting, and daily use of continuous glucose monitors, achieves HbA1c levels comparable to those of our participants (Benkhadra et al., 2017; Miller et al., 2013; Schmidt et al., 2014; Yeh et al., 2012).

The optimal HbA1c levels achieved by our participants are unlikely to result from the intensive self-monitoring of their BGL. Indeed, there was no relationship between average HbA1c levels and average daily number of blood glucose tests (r=0.16). Moreover, despite the finding by others that HbA1c levels improve with ≥10 daily blood glucose tests (Miller et al., 2013), the HbA1c levels attained (HbA1c ~61 mmol/mol; 7.7%) are much higher than those reported here.

The glycaemic variability experienced by our participants was very low. Unfortunately, no detailed analyses of glycaemic variability were performed in earlier studies on LCHF diets in T1DM (Nielsen et al., 2005, 2012), thus making it difficult to compare our findings with others. This low glycaemic variability and normalisation in BGL are likely related to our participants' low CHO intake causing only a small increase in BGL per meal.

Of concern, however, is the high frequency and duration of hypoglycaemic episodes experienced by our participants (6.3 episodes/week) as compared with the literature (1-2 episodes/week; Frier, 2014). It is remarkable that this number of recorded hypoglycaemic episodes is considerably higher than that self-reported by our participants and comparable to those by Nielsen and colleagues (2005). This discrepancy might have to do, at least in part, with hypoglycaemia unawareness and/or a lowering of the hypoglycaemia threshold for neuroglycopenic and neurogenic symptoms due to the mild ketotic state of our participants. This latter interpretation is supported by the findings of Amiel and colleagues (1991) who showed that ketone infusion reduces cognitive impairment during induced hypoglycaemia.

There is no or little evidence that KD impair renal and hepatic function. However, one area of concern is the dyslipidaemia experienced by most participants. These findings differ markedly from all previous studies that have examined the effect of LCHF diets on adults with T1DM (Krebs et al., 2015; Nielsen et al., 2005, 2012; O'Neill et al., 2003), with these studies reporting normal lipid profiles. It is unclear whether this discrepancy is related to the much lower fat intake in these studies (Krebs et al., 2015;

Nielsen et al., 2005, 2012; O'Neill et al., 2003), or the high level of saturated fat of the self-selected diet of our participants, as high saturated fat intake may be a risk factor for dyslipidaemia (Jenkins et al., 1993). Another potential concern is the low haemoglobin levels of some of our participants, a finding consistent with the observation that a high-fat diet has been shown to be associated with iron deficiency (Sonnweber et al., 2012).

Given the observational nature of this study, its lack of a control group, and its small sample size, our findings have to be interpreted with caution and require corroboration from future randomised control trials. In particular, it is unclear whether similar findings would have arisen had participants been recruited from other parts of Australia or other countries. Also, our findings do not exclude the possibility that the personality type of the T1DM people who engage in KD may have contributed to their glycaemic control. What is required is a randomised-control intervention trial that examines the lontg-term effect of KD on HbA1c levels and other health variables (e.g. lipid profile) in people with T1DM. Of note, our main findings cannot be explained on the basis of a few outliers skewing the results, since all participants had low HbA1c levels and low glycaemic variability, and most were dyslipidaemic and experienced high rates of hypoglycaemia. For these reasons, future studies should consider designing and testing a ketogenic diet that minimises these aforementioned risks, rather than relying on diets self-selected by participants.

In conclusion, this study suggests that adults with T1DM on a ketogenic diet may have near normal HbA1c levels and experience little glycaemic variability while using traditional glycaemic management techniques. However, these individuals may be at a high risk of dyslipidaemia and hypoglycaemia.

Chapter 3

The relationship between daily carbohydrate intake and HbA1c levels in adults with type 1 diabetes mellitus

Based on a manuscript under preparation:

Leow, Z. Z. X., Guelfi, K. J., & Fournier, P. A. The relationship between daily carbohydrate intake and HbA1c levels in adults with type 1 diabetes mellitus. Prepared for submission to Diabetes Care.

3.1 Abstract

Aims This study aimed to examine the relationship between daily carbohydrate (CHO) intake and HbA1c levels over a broad range of CHO intakes in individuals with type 1 diabetes mellitus (T1DM).

Methods In this observational study, 56 individuals with T1DM (24 men and 32 women; age 25.6 ± 10.2 yr, body mass index 23.9 ± 3.7 kg/m^2; mean ± SD) had their blood sampled after an overnight fast, and each completed a 3-day CHO-intake diary.

Results Mean CHO intake was 141 ± 88 g/day. Mean HbA1c was 51.0 ± 12.6 mmol/mol (6.8 ± 1.2%), with no significant differences between men and women, or between insulin therapy modes ($p > 0.05$). The relationship between daily CHO intake and HbA1c levels was best described by a sigmoidal relationship, with a steep inflection point and an abrupt leap in HbA1c levels at a CHO intake of 99.5 g/day (1.25 g/kg/day). Above or below this point, there was no longer any relationship between daily CHO intake and HbA1c levels ($p > 0.05$). Daily CHO intakes below 99.5 g/day were associated with mean HbA1c levels of 38.3 ± 9.4 mmol/mol (5.7 ± 0.9%), as opposed to HbA1c levels of 57.5 ± 8.4 mmol/mol (7.4 ± 0.8%) for daily CHO intakes above this inflection point.

Conclusion The relationship between daily CHO intake and HbA1c levels is best described by a sigmoidal curve, with an abrupt inflection point in HbA1c levels at a CHO intake approaching 99.5 g/day.

3.2 Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that destroys the β cells of the pancreas, thus impairing its ability to produce insulin (ADA, 2008; Greenhouse & Lardinois, 1996; Riddell & Perkins, 2006; Rizza et al., 1981). In the absence of the inhibitory effect of insulin on the hepatic production of glucose and ketone bodies (KB), untreated individuals with T1DM are at a high risk of premature death from hyperglycaemia-mediated septicaemia and ketoacidosis (Rizza et al., 1981). Although insulin therapy provides an effective means to prevent early death in these individuals, their life expectancies are still below that of the general population (Huo et al., 2016). This is, in part, because blood glucose must be tightly managed so as to remain as close to physiological levels as possible, with severe hypoglycaemia and chronic hyperglycaemia being associated with the development of several short-term and long-term diabetes complications, respectively (DCCT, 1993). Despite the importance of good glycaemic control, balancing insulin dose with exercise and food intake to keep blood glucose levels within the physiological range is not an easy task to achieve, and notable fluctuations in blood glucose levels are common in individuals with T1DM (Bishop et al., 2009; Brazeau et al., 2013; Spiegel et al., 2012).

Since the total carbohydrate (CHO) content of meals and snacks is a major determinant of the post-prandial blood glucose response to a meal (Gillespie et al., 1998; Rabasa-Lhoret et al., 1999; Slama et al., 1981; Wolever et al., 1999), it is not uncommon for individuals with T1DM to restrict their CHO intake to reduce the magnitude of their glycaemic excursions and attempt to improve their glycaemic control (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications, 2009; Leow et al., 2018; Lennerz et al., 2018; Toeller et al., 1996). Within the range of moderate to high CHO diets (> 35% CHO as energy source), where CHO intake is well above the amount associated with low CHO diets (Feinman et al., 2015), some (Delahanty et al., 2008; Nansel et al., 2016), but not all (Meissner et al., 2014; Buyken et al., 2000; Mosso et al., 2015), studies have reported that glycaemic control deteriorates with decreasing CHO intake. In contrast, low CHO diets (~100 g CHO/day) are associated with improved glycaemic control, as shown by reduced HbA1c levels in individuals with T1DM (Krebs et al., 2016; Meissner et al., 2014; Nielsen et al., 2005, 2012; Schmidt et al., 2019). More recently, very low CHO KD (< 55 g CHO/day) for > 3 months have been associated with optimal glycaemic control, with HbA1c levels

being comparable to those of non-diabetic individuals (Lennerz et al., 2018; Leow et al., 2018).

The aforementioned studies raise the issue of whether there is a linear relationship between daily CHO intake and HbA1c. Some previous studies have found no relationship between CHO intake and HA1c level (Balk et al., 2016; Powers et al., 2018; Wolever et al., 1999), while others have reported a positive (Buyken et al., 2000; Meissner et al., 2014) or negative relationship (Delahanty et al., 2008) between CHO intake and HbA1c level. Importantly, there is no evidence or mention in these studies that they recruited individuals on very low CHO KD. Given the much lower HbA1c levels associated with these types of diets (Leow et al., 2018; Lennerz et al., 2018; Leow et al., 2018), this raises the issue of whether the relationship between daily CHO intake and HbA1c levels over a broad range of daily CHO intake that covers the intake typical of individuals on KD is associated with an abrupt leap or a progressive change between these variables, particularly between individuals on very low daily CHO intake and those with typical or high daily CHO intake.

Determining whether there is an abrupt leap between CHO intake and HbA1c levels may be a challenge given the limited resolution of food diaries at precisely estimating CHO intake. Indeed, although food diaries are commonly used for dietary analysis because of their non-invasive nature and ease of administration (Burke et al., 2012; Eakin etal., 2007; Hunter et al., 2008), it is common for people using these diaries to under-report food intake (Adams, 1998; Macdiarmid & Blundell, 1997, 1998; Poppitt et al., 1998; Salle et al., 2006), or to report a healthier option instead of the actual food being consumed (Macdiarmid & Blundell, 1998). However, since individuals with T1DM are trained and encouraged to count the CHO content of their food, the use of a 3-day food diary may be more suitable (Crawford et al., 1994) and reproducible for this population (Seckold et al., 2019). Accordingly, the purposes of this pilot observational study were to investigate the association between daily CHO intake and HbA1c levels over a range of daily CHO intake encompassing very low CHO intake, and to examine the reproducibility of 3-day CHO-intake diaries at estimating CHO intake in individuals with T1DM. The results thus obtained are intended to inform the design of similar, but much larger future studies.

3.3 Methods

3.3.1 Participants

Participants were recruited with the help of local endocrinologists, diabetes health centers, diabetes support groups as well as through social media (facebook, instagram, twitter), local diabetes communities and organisations, and via word of mouth targeting past and current participants mainly from metropolitan Perth, Western Australia. Fifty six adults (24 men and 32 women; mean ± SD; age, 25.6 ± 10.2 yr; height, 1.7 ± 0.1 m; body mass, 70.7 ± 13.4 kg; body mass index 23.9 ± 3.7 kg/m^2) with T1DM volunteered to participate in this cross-sectional study and provided written consent to participate. Eligibility criteria were; duration of T1DM diagnosis > 2 yr (12.1 ± 9.4 yr), no change in diet and physical activity in the last 3 months, currently not on a weight-loss regime, free of complications, and not taking any prescribed medication other than insulin. Twenty participants (8 men, 12 women) were on an insulin pump regimen. A subset of 35 of these participants (18 men and 17 women; mean ± SD; age, 24.9 ± 9.6 yr; height, 1.7 ± 0.1 m; body mass, 70.2 ± 13.5 kg; body mass index 23.9 ± 3.9 kg/m^2) provided written consent to participate in the component of the study addressing the reproducibility of the daily CHO intake provided by food diaries. Of those participants, 15 participants were on insulin pump therapy (7 men and 8 women) while the other 20 participants were on a multiple daily injection therapy (MDI; 9 men and 11 women). The University of Western Australia Ethics Committee approved the study protocol (RA/4/1/6360).

3.3.2 Experimental design

Given the high costs and impracticality of performing a randomised controlled trial examining the long-term effects (>1 year) of diets providing different daily amounts CHO on glycaemic control and lipid profile as well as the challenges of recruiting T1DM participants willing to be involved in such a long-term study, we undertook to carry out an observational study targetting individuals with different daily intake of CHO. We reasoned that the information thus obtained from this observational study could help us explore the relationship between daily CHO intake and health-related variables (e.g. glycaemic control and lipid profile), and help us inform future studies of the same kind but with a much larger sample size. Each participant attended the laboratory on one or two separate occasions. On the first visit, participants provided

information on their age, diabetes duration, insulin therapy regimen and medical history. Then, body mass and height were measured as described in Chapter 2. In addition, participants had their HbA1c levels measured and were given a blood collection form and specific instructions to visit a certified independent laboratory (PathWest, Osborne Park, Western Australia) after an overnight fast to measure their lipid profile. Before leaving the laboratory, each participant was given a diary and a portable weighing scale (Homemaker 9753, Tooronga, Vic) to record daily food intake. Participants were then sent home and asked to follow their usual diet and activity routine for 7 days. During this 7-day period, participants recorded a minimum of 3 days' worth of CHO intake (2 weekdays and 1 weekend day). Then, food diaries were collected. For those participants that volunteered to assess the reproducibility of the data collected in the food diaries, they were asked to complete a second 3-day diary (week 2) on a separate occasion.

3.3.3 Data analyses

The primary outcome measure for this study was HbA1c levels. Because of the absence of any prior study to inform our sample size calculation to support our regression analyses, no sample size calculation was performed for this study. Because of the small sample size of this study (n=56), proper multiple regression analysis with appropriate adjustments for potential confounders such as sex, age, socioeconomical status, body composition, physical activity levels, alcohol consumption, animal protein intake, vegetable protein intake, dietary fibre intake, and insulin treatment regimen (Balk et al., 2016; Buyken et al, 2000) could not be performed. Notwithstanding the aforementioned limitations, Pearson's correlation coefficients were used to calculate the relationship between HbA1c (%, mmol/mol) and absolute (g/day) and relative daily CHO intake (g/kg body mass/day). Sequential polynomial regression analysis was also adopted to investigate the nature of the relationship between daily CHO intake and HbA1c. After performing a linear model analysis, each additional step involved entering the next highest power of the predictor (daily CHO intake). This continued until the addition of the next highest power increased the fit of the model to the data by an insignificant or otherwise trivial amount. Further regression analyses were also performed on the data using GraphPad Prism Software (San Diego, CA) to examine the extent to which the data fit a sigmoidal relationship.

For those participants who completed two consecutive food-diaries, only the results from the first diary were used for the main study examining the relationship between daily CHO intake and HbA1c levels. Food and drink records were analysed via Foodworks 7 (Xyris Software, Australia). Daily CHO intake was derived from the average CHO intake and expressed as g/day. Relative daily CHO intake was reported as the average CHO intake (g) per unit of body mass (g/kg body mass/day). In order to examine the relationship between reproducibility and CHO intake range, the average CHO intake between both diaries was used to determine the CHO intake range for each individual participant. Statistical analyses were performed using SPSS Version 16.0 for Windows (SPSS, Inc., Chicago, IL), with comparisons between HbA1c scores or reproducibility data across CHO intake ranges performed using ANOVA followed by LSD post-hoc test, and with statistical significance accepted at $p < 0.05$. All results are expressed as means ± SD or median (interquartile range) for skewed data, and as mean ± standard error (SE) in figures unless otherwise stated.

3.4 Results

3.4.1 Relationship between daily CHO intake and HbA1c levels

Participants had an average HbA1c level of 51.0 ± 12.6 mmol/mol (6.8 ± 1.2%), with no significant differences between men (50.2 ± 14.1 mmol/mol; 6.7 ± 1.3%) and women participants (51.6 ± 11.5 mmol/mol; 6.9 ± 1.1%; $p = 0.69$). The participants' mean and median CHO intake were 133 ± 81 and 135 (45-194) g/day, respectively, with no significant differences between men and women for mean (138.8 ± 89.4 and 126.3 ± 72.2; $p = 0.51$) and median (138.5 [51.9-218.6] and 132.9 [44.7-185.8]) daily CHO intake, respectively. Relative to body mass, mean and median CHO intakes were 2.0 ± 1.3 and 2.0 (0.6-2.7) g/kg body mass/day, respectively, with no significant differences between men and women for both mean (1.9 ± 1.4 and 2.0 ± 1.3; $p = 0.92$) and median [1.7 (0.7-2.8) and 2.0 (0.6-2.6)] relative daily CHO intake, respectively. The number of participants for each CHO intake category (0-49, 50-99, 100-149, 150-199, 200-249 and >249 g/day) was not evenly distributed (Fig 3.1). Of note, since many participants did not report their intake of CHO-free food (e.g. oil, butter, meat), we do not have the data to report the macronutrient intake of our participants.

There was a positive moderate correlation between daily CHO intake and HbA1c level (r^2 = 0.38, p < 0.01; Fig 3.2). Daily CHO intake relative to body mass was also moderately positively correlated with HbA1c (r^2 = 0.30, p < 0.01; Fig 3.3). Further to performing a sequential polynomial regression analysis of our data, adding a quadratic component to the model produced a significant increase in fit, but adding a cubic component did not. Accordingly, the quadratic model was adopted, with [HbA1c] = 27.74 + 0.36 [CHO intake] – 0.01 [CHO intake]^2, $F(2,53) = 26.0$, p < 0.001 and R^2 = 0.495 (Table 3.1). Taking the same approach, the quadratic model was adopted for relative daily CHO intake, with [HbA1c] = 31.19 + 17.65 [relative CHO intake] – 2.60 [relative CHO intake]^2, $F(2,53) = 27.3$, p < 0.001 and R^2 = 0.508 (Table 3.2). However, further regression analysis using Prism Graphpad revealed that the relationship between daily CHO intake and HbA1c level was marginally better described by a sigmoidal relationship (r^2 = 0.545), with an inflection point at a CHO intake of 99.5 g/day. There were no significant relationships between daily CHO intake and HbA1c level both below (r = 0.2, p = 0.43) or above (r = -0.1, p = 0.75) this inflection point. The relationship between daily CHO intake relative to body mass and HbA1c level was also best described by a sigmoidal plot (r^2 = 0.527) with an inflection point at a CHO intake of 1.25 g/kg/day. There were no relationships found between HbAIc level and relative daily CHO intake both below (r = 0.03, p = 0.91) and above (r = -0.06, p= 0.72) 1.25 g/kg/day. HbA1c levels for CHO intake within the 0-99.5 g/day range were significantly lower than any of the upper CHO intake ranges (Fig 3.4; p = 0.04), with no difference between the other CHO intake ranges examined here (p > 0.05). HbA1c levels for relative CHO intake within the 0.0-1.25 g/kg/day range were also significantly lower than for any of the upper CHO intake ranges (Fig 3.5; p < 0.01), with no difference between the other relative daily CHO intake ranges examined here (p > 0.05). HbA1c levels did not differ between individuals using multiple daily injection (MDI) and those on insulin pump, irrespective of their CHO intake (Fig 3.6).

Table 3.1. Sequential polynomial regression analysis to best predict HbA1c from daily absolute CHO intake.

Step	ΔR^2	F for ΔR^2	df	p
Linear	0.375	32.336	1,54	0.000
Quadratic	0.121	12.676	2,53	0.001
Cubic	0.002	0.249	3,52	0.620

Table 3.2. Sequential polynomial regression analysis to best predict HbA1c from relative daily CHO intake.

Step	ΔR^2	F for ΔR^2	df	p
Linear	0.296	22.746	1,54	0.000
Quadratic	0.211	22.777	2,53	0.000
Cubic	0.005	0.522	3,52	0.000

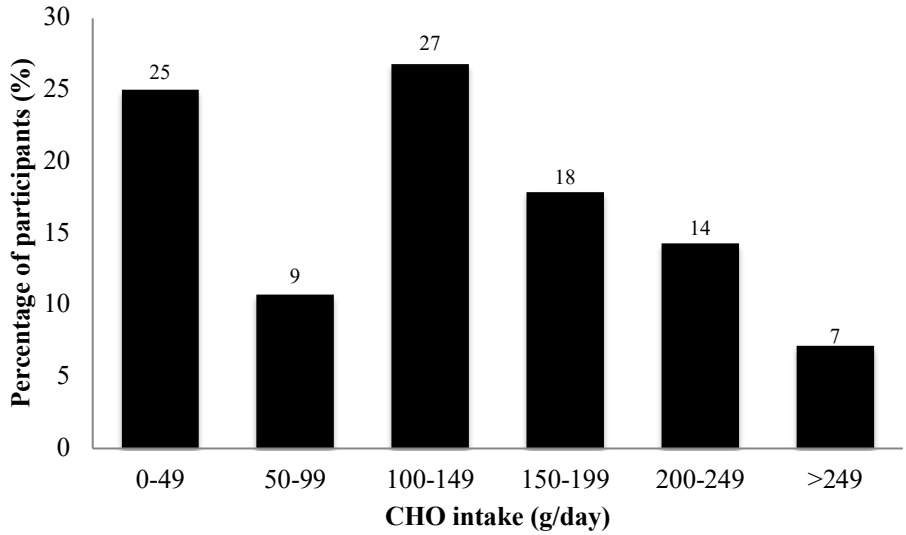

Figure 3.1. Proportion of participants in the different daily CHO intake groups (0-49, 50-99, 100-149, 150-199, 200-249 and >249 g/day). (n=56).

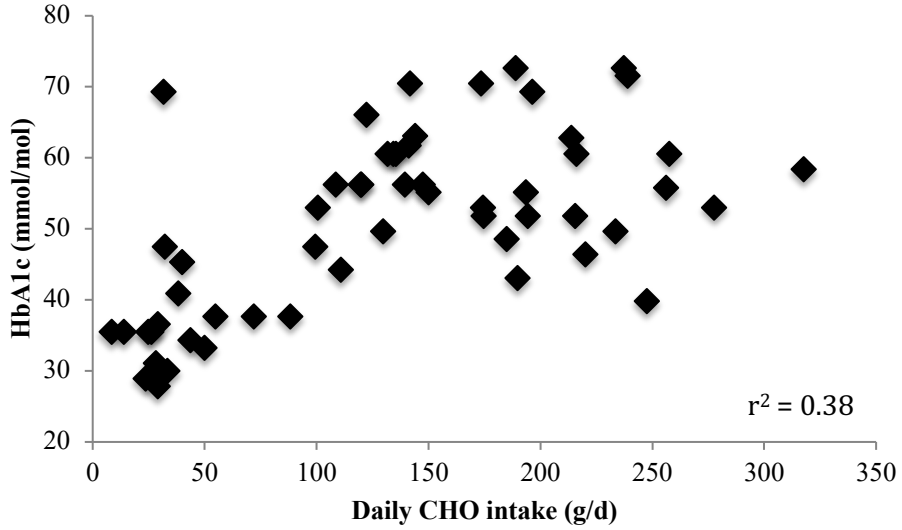

Figure 3.2. Relationship between daily CHO intake (g/day) and HbA1c levels (mmol/mol). (n = 56).

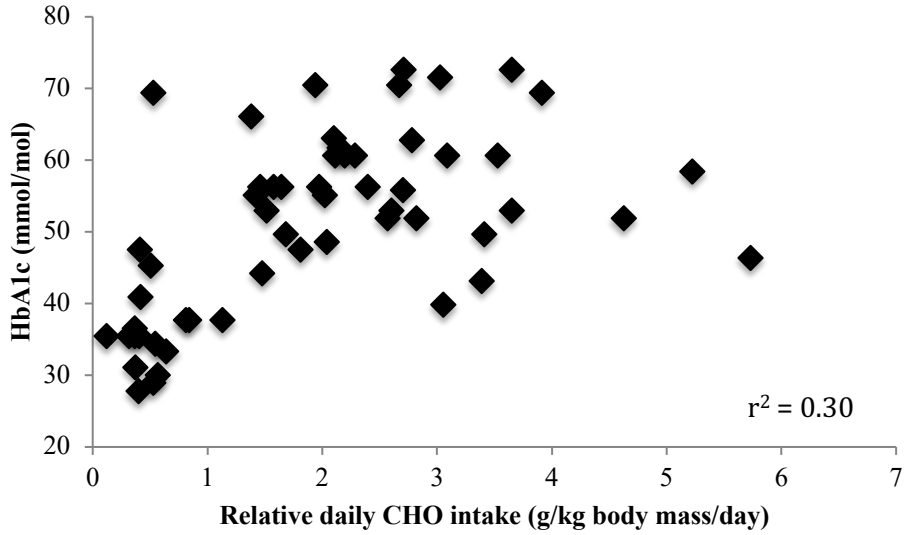

Figure 3.3. Relationship between daily CHO intake relative to body mass (g/kg body mass/day) and HbA1c levels (mmol/mol). (n = 56).

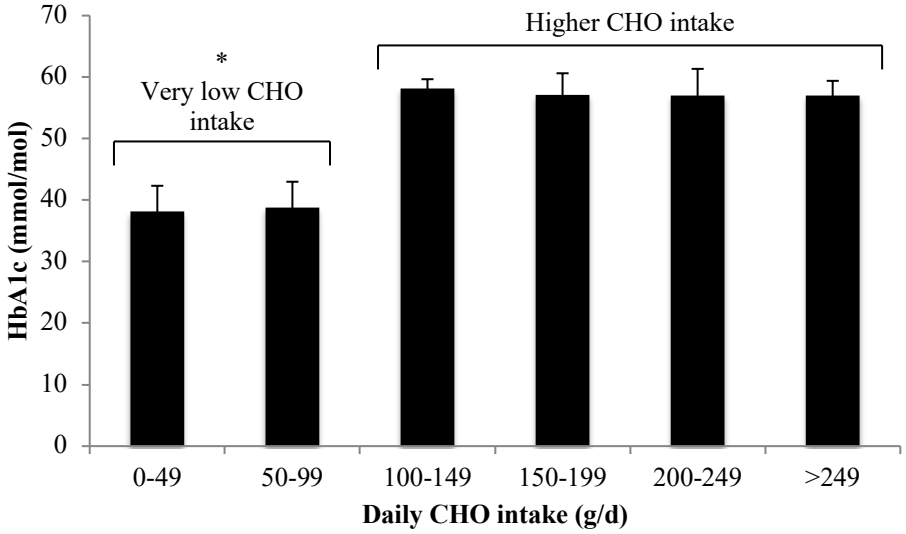

Figure 3.4 Effect of different daily CHO intake ranges (0-49, 50-99, 100-149, 150-199, 200-249, and >249 g/day) on HbA1c levels (mmol/mol) in individuals with T1DM. *Significantly different ($p<0.05$) from the higher CHO intake groups. Data are expressed as mean ± SE, (n = 56).

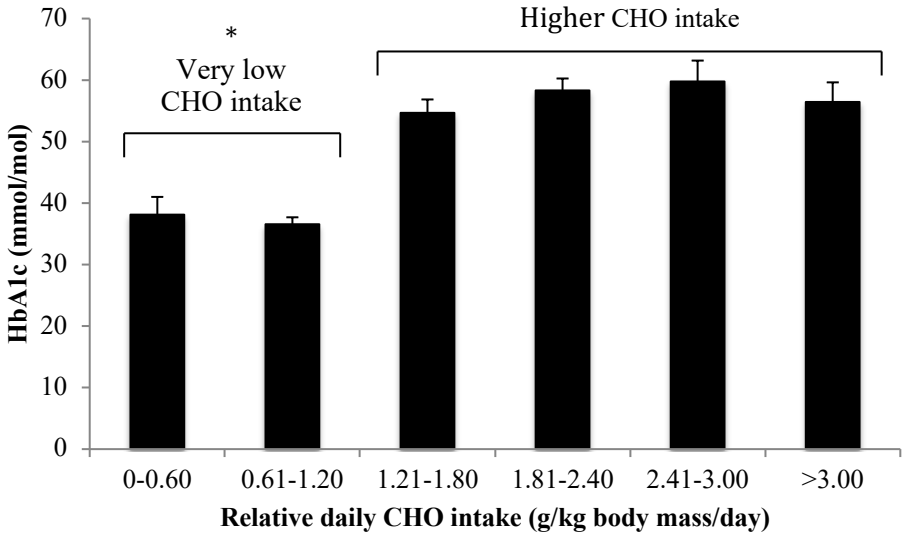

Figure 3.5 Effect of different daily relative CHO intake ranges (0-0.60, 0.61-1.20, 1.21-1.80, 1.81-2.40, 2.41-3.00, >3.00 g/ kg body mass/day) on HbA1c levels (mmol/mol) in individuals with T1DM. *Significantly different ($p<0.05$) from the higher CHO intake groups. Data are expressed as mean ± SE (n = 56).

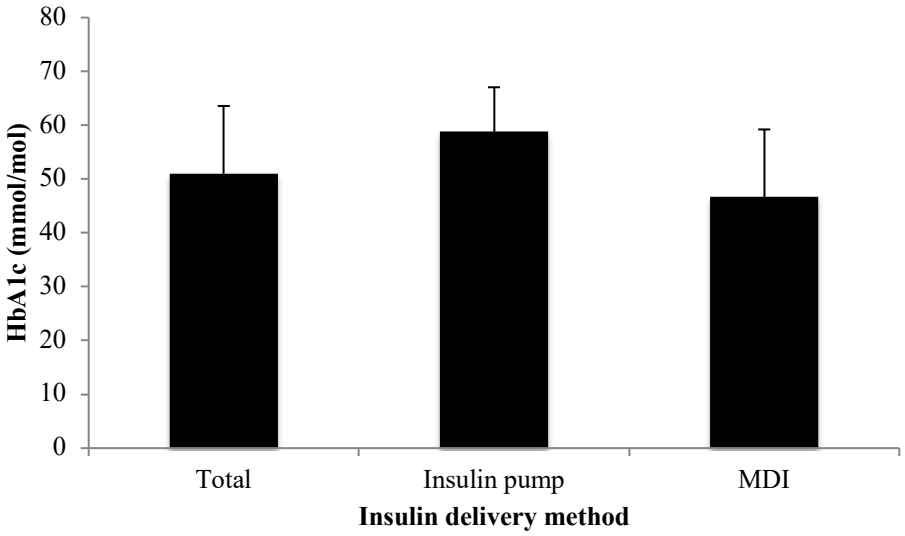

Figure 3.6. **HbA1c profile of all individuals, individuals using insulin pump therapy and individuals using multiple daily injection (MDI).** Data are expressed as mean ± SD (n =56).

3.4.2 Reproducibility of 3-day CHO intake diary

Daily CHO intake did not differ significantly between week 1 (136 ± 93 g/day) and week 2 (148 ± 85 g/day; p = 0.115), with a median CHO intake of 135 (30-200) and 149 (70-206) g/day for week 1 and 2, respectively. Mean CHO intake did not differ between week 1 and 2 for men (114 ± 84 vs 123 ± 79 g/day; p= 0.36) and women (160 ± 99 vs 175 ± 85 g/day; p= 0.21). CHO intake relative to body mass did not differ between week 1 (2.0 ± 1.5 g/kg body mass/day) and 2 (2.2 ± 1.5 g/kg body mass/day; p = 0.08). Mean CHO intake relative to body mass did not differ between week 1 and 2 for men (2.5 ± 1.7 vs 2.7 ± 1.4 g/kg body mass/day; p = 0.22) and women (1.6 ± 1.3 vs 1.8 ± 1.4 g/kg body mass/day; p = 0.21).

For individuals consuming across both week 1 and 2 an average CHO intake of 0-75, 76-150, 151-225 and > 225 g/day, CHO intake within the 0-75 g/day range was significantly lower at week 1 compared with week 2 ($p < 0.01$), but did not differ between these two weeks for the other CHO intake ranges (Fig 3.7 & 3.8). For individuals consuming across both week 1 and 2 an average CHO intake of 0-1.0, 1.1-2.0, 2.1-3.0, and > 3.1 g/kg body mass/day, CHO intake within the 0-1.0 g/kg/day range

was significantly lower on week 1 compared with week 2 ($p < 0.01$), but did not differ between these two weeks for the other CHO intake ranges (Fig 3.9 & 3.10).

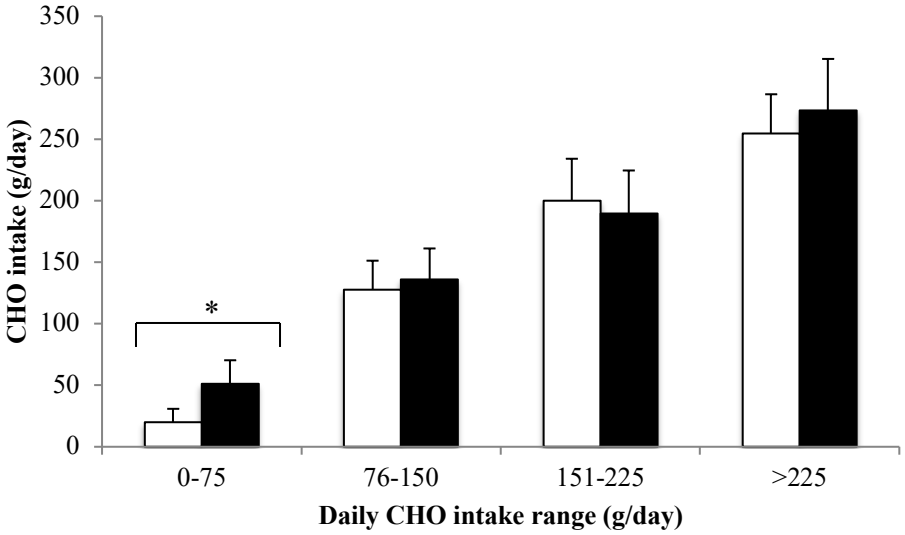

Figure 3.7 CHO intake during week 1 (□) compared with week 2 (■) for individuals consuming across both weeks an average of 0-75, 76-150, 151-225 and >226 g/day. *Significantly different ($p<0.05$) between week 1 and week 2. Data are expressed as mean ± SD (n = 35).

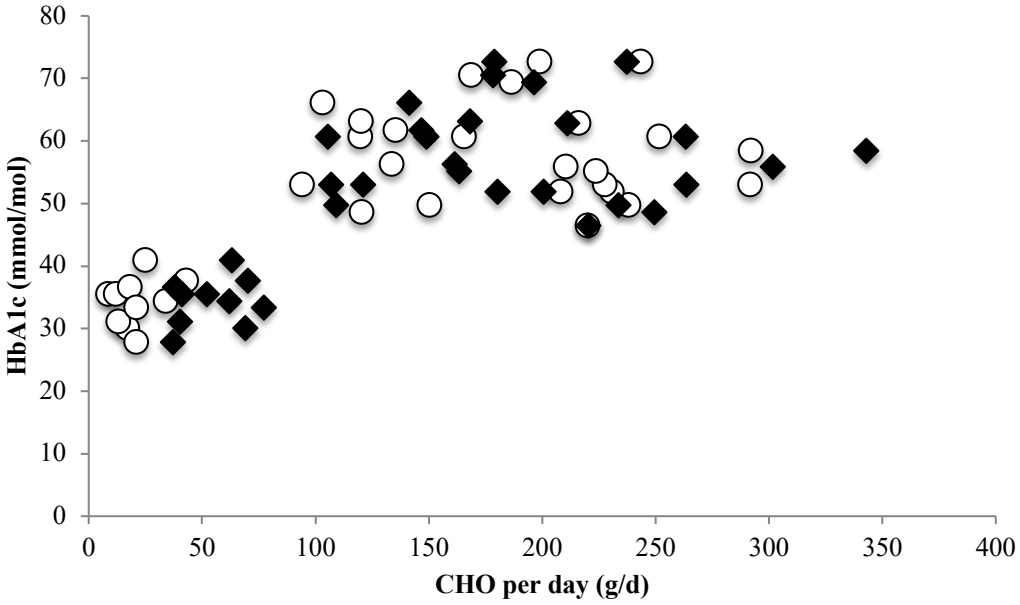

Figure 3.8. Relationship between HbA1c levels and average CHO intake during week 1 (○) and week 2 □◆). (n=35).

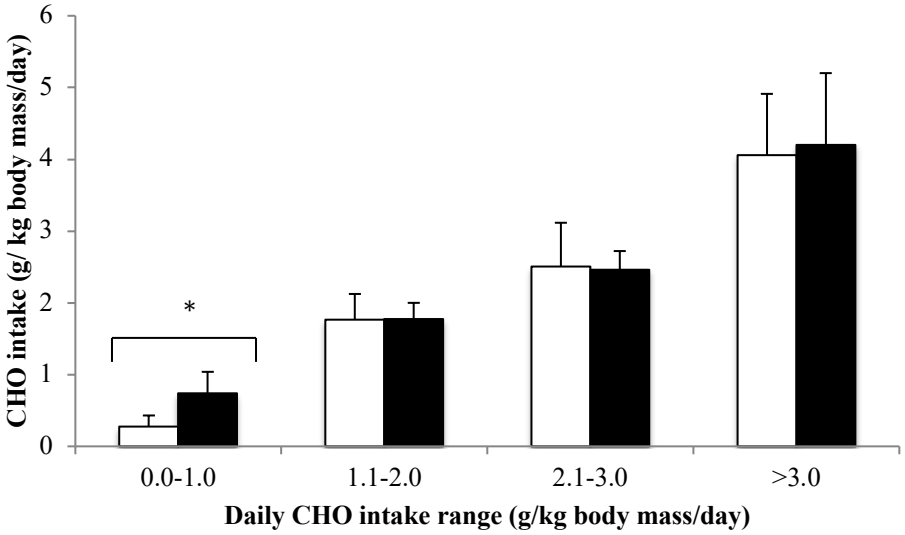

Figure 3.9. Daily CHO intake relative to body mass during week 1 (□) compared with week 2 (■) for individuals consuming across both weeks an average of 0.0-1.0, 1.1-2.0, 2.1-3.0, >3.0 g CHO/kg body mass/day. *Significantly different ($p<0.05$) between week 1 and week 2. Data are expressed as mean ± SD (n = 35).

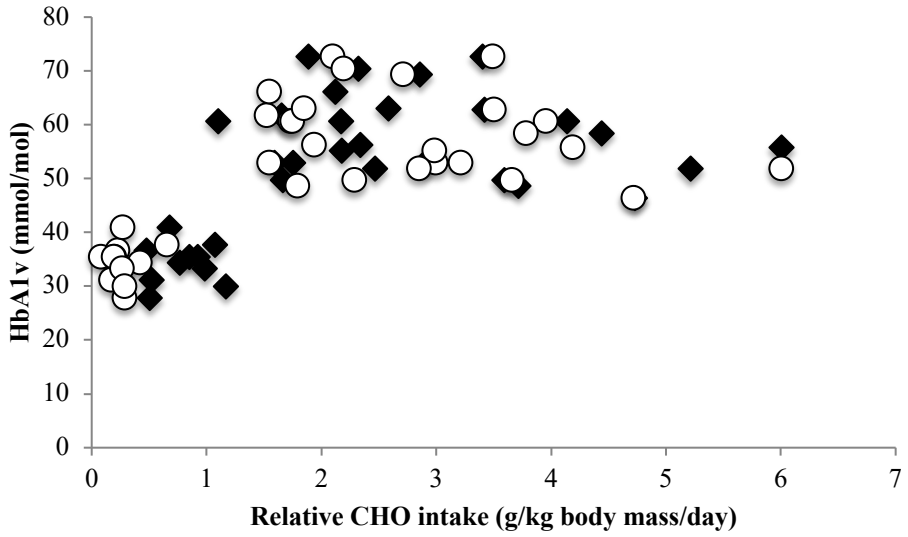

Figure 3.10. Relationship between HbA1c levels and average relative daily CHO intake expressed relative to body mass during week 1 (○) and week 2□□◆). (n=35).

3.5 Discussion

We, and others, have found that individuals with T1DM on a low CHO diet have low HbA1c levels (Chapter 2; Leow et al., 2018; Lennerz et al., 2018; Nielsen et al., 2005; 2012), with individuals on very low CHO KD having HbA1c levels comparable to those in healthy non-diabetic individuals (Lennerz et al., 2018; Leow et al., 2018). These findings raise the issue of whether there is a linear relationship between daily CHO intake and HbA1c levels, or whether there is an abrupt leap between these variables, particularly between individuals on a very low CHO KD diet and those on typical or high CHO diets. This study, aimed at informing the design of a future much larger study, suggests that the relationship between daily absolute and relative CHO intake and HbA1c levels is best described by a sigmoidal curve, with an abrupt inflection point in HbA1c levels at a CHO intake of approximately 99.5 g/day (1.25 g/kg body mass/day). However, the poor reproducibility of the CHO intake records obtained from our participants within the 0-75 g CHO intake/day range brings into question the accuracy of our estimate of this inflection point, and this limitation should be taken into account in the design of larger future prospective or cross-sectional multicentre studies.

Our findings corroborate, to some extent, those of Buyken and colleagues (2000), who reported higher HbA1c levels in individuals with higher CHO intake, but challenge those of others who did not report such a relationship (Balk et al., 2016; Powers et al., 2018; Wolever et al., 1999). Of these latter studies, that of Powers and colleagues (2018) was based on a 24-hour food recall instead of a 3-day food diary. As food recalls rely on an individual's memory, portion sizes can be difficult to evaluate (Guthrie, 1984), thus providing less accurate estimates of dietary intake (Biro et al., 2002). This may explain the aforementioned negative findings of Powers and colleagues (2018). In contrast, Buyken and colleagues (2000) administered a 3-day food diary (2 weekdays and 1 weekend day) to best reflect their participants' usual dietary habits, with the increased resolution provided by their approach maybe increasing the likelihood of detecting a relationship between daily CHO intake and HbA1c levels. However, Balk and colleagues (2016) did not uncover any such a relationship despite their use of 3-day food diaries. It is also important to note that our study is the first to suggest the existence of a sigmoidal relationship between daily CHO intake and HbA1c levels, a finding that most probably results from our recruitment of a higher proportion of participants on a low CHO diet. Had our study not specifically targeted these latter individuals, a much weaker or no relationnship may have been found, as reported by

others (Balk et al., 2016; Powers et al., 2018; Wolever et al., 1999). This is because our own results show no relationship between CHO intake and HbA1c levels for CHO intake above 100 g CHO/day. Conversely, had more participants in the aforementioned studies (Balk et al., 2016; Powers et al., 2018; Wolever et al., 1999) been on a low or very low CHO diet (< 100g CHO/day), maybe these studies would have also found a sigmoidal relationship between daily CHO intake and HbA1c levels.

The abrupt leap in HbA1c levels reported here as CHO intake approached the inflection point of ~99.5 g/day (1.25 g/kg body mass/day) most certainly explains the moderate positive linear relationship found here between daily CHO intake and HbA1c levels, as no such relationship was found between these variables for CHO intake below or above 99.5 g/day (1.25 g/kg body mass/day). Of note, since it is common for individuals completing food diaries to under-report their food intake (Adams, 1998; Macdiarmid & Blundell, 1997, 1998; Poppitt et al., 1998; Salle et al., 2006) and/or report healthier options instead of some actual foods consumed (Macdiarmid & Blundell, 1998), our findings do not exclude the possibility that the levels of daily CHO intake at the inflection point in HbA1c levels described here may be systematically underestimated.

The mechanism underlying the abrupt leap in HbA1c levels at a CHO intake of near 99.5 g/day (1.25 g/kg body mass/day) remains to be elucidated. It is important to note that the association between these variables does not imply a cause and effect relationship between them. These findings are unlikely to be explained on the basis of the participants within the 0-100 g CHO intake/day range using better glucose management technology, since all our participants on very low daily CHO intake (< 50 g/day) used conventional insulin and glucose monitoring therapies as opposed to the participants on higher daily CHO intake. It may be argued that any mismatch between insulin dose and CHO intake is likely to result in greater peaks in blood glucose levels in individuals on a high compared to a low CHO diet (Gillespie et al., 1998; Rabasa-Lhoret et al., 1999; Slama et al., 1981; Wolever et al., 1999) and cause higher HbA1c levels. Unfortunately, the design of our study does not allow us to determine whether this was the case, and it is unclear how this interpretation could explain the abrupt leap found here. Finally, since a proper multiple regression analyses of our results could not be performed due to the small sample size of our study, it is possible that some of our findings may be explained, at least in part, by potential uncontrolled confounders such as sex, age, socioeconomical status, body composition, physical activity levels, alcohol

consumption, animal protein intake, vegetable protein intake, dietary fibre intake, or insulin treatment regimen. Of note, however, these confounding variables taken individually have been shown in the literature to have no or too small an effect on HbA1c levels (e.g. Balk et al., 2016; Buyken et al., 2000) to account for the near marked 20 mmol/mol difference reported here between HbA1c levels below and above the 99.5 g CHO/day inflection point.

Although daily CHO intakes were comparable between almost all CHO intake ranges examined here, the food diary records were not reproducible for individuals on a low daily CHO intake (<100 g/day) since their CHO intake on week 2 was significantly higher than on week 1, with no participant on week 1 having CHO intake within the 50-100 g/day range. As a result, the data collected during week 1, but not week 2, was too incomplete to allow us to calculate precisely the inflection point in HbA1c level for week 1. It is unclear whether the significant rise in reported daily CHO intake between week 1 and 2 reflects an actual increase in daily CHO intake or a case of faulty CHO intake records (Adams, 1998; Macdiarmid & Blundell, 1997, 1998; Poppitt et al., 1998; Salle et al., 2006). Also, it is important to note that a single meal with a marginally higher CHO intake during week 2 is all that was required for average CHO intake to differ between weeks, particularly against a backgournd of very low CHO intake. If anything, these findings suggest that the CHO intake of our participants on KD oscillates weekly between the 0-55 and 50-100 g CHO intake/day ranges. Also, it is possible that our participants on KD were more stringent with their diet adherence when first surveyed on week 1, thus implying that their food diary on week 2 reflected better their usual CHO intake patterns.

Clearly, the poor reproducibility of the CHO intake data obtained from the 3-day food diaries completed by the participants within the 0-100 g CHO intake/day range brings into question the accuracy of the estimate of the inflection point uncovered in this study. This limitation will have to be not only resolved, maybe by subjecting any individual within this CHO intake range to a multitude of 3-day food diaries, but also taken into account in the design of any future studies aimed at examining more thoroughly the relationship between daily CHO intake and glycaemic control in people with T1DM. Another limitation relates to the issue of whether similar findings would have arisen had participants been recruited from other parts of Australia or other countries. Finally, future studies with a much larger sample size will have to address the challenges of

recruiting enough participants with consistent CHO intake of 50-100 g/day as our findings suggest that only a small proportion of individuals with T1DM belong to this CHO intake range. Such studies will enable us to determine whether there is a "real" cut-off point in HbA1c level at CHO intakes of approximately 100 g/day of whether there is a smoother transition

On clinical grounds, this study suggests that daily CHO intakes do not have to be within the extremely low CHO intake range (0-50 g/day) typical of KD to lead to optimal glycaemic control, as higher intakes of 50-100 g CHO/day appear just as effective. Of course, this interpretation has to be taken with caution given the small number of participants in our study whose CHO intakes fell within the 50-100 g CHO/day range, and the evidence that the CHO intake of individuals within the 0-50 g CHO intake/day can oscillate between this range and the 50-100g CHO intake/day range. Given that some individuals on KD (< 50 g CHO intake/day) have been reported in past studies to be dyslipidaemic (Chapter 2, Leow et al., 2018), our results raise the issue of whether this would also be the case for individuals with CHO intake ranging between 50-100 g/day, with an abrupt normalisation of their lipid profiles occurring at the same or lower/higher daily CHO intake as that associated with the inflection point uncovered here. Unfortunately, this issue could not be addressed as too few participants were willing to provide some blood for lipid analyses. Future studies are needed to address this issue.

In conclusion, this study suggests that the relationship between daily CHO intake and HbA1c levels is best described by a sigmoidal curve, with an abrupt inflection point in HbA1c levels at a CHO intake approaching 99.5 g/day (1.25 g/kg body mass/day). This study also suggests that CHO intakes do not have to be very low (< 50 g/day) for CHO restricted diets to be associated with low HbA1c levels, since comparable glycaemic controls were achieved with CHO intakes of 50-100 g/day. Of note, however, the poor reproducibility of the daily CHO intake records obtained from our participants within the 0-100 g CHO intake/day range and the lack of adjustments for potential confounders in our regression analysis bring into question the accuracy of our estimate of this inflection point. Overall, the findings of this study should help inform the design of future larger studies aimed at investigating further the relationship between daily CHO intake and variables such as HbA1c levels and lipid profile across a range of CHO intake encompassing very low daily CHO intake.

Chapter 4

The risk of ketoacidosis and hypoglycaemia is not increased in response to different exercise formats in adults with type 1 diabetes mellitus on a very low carbohydrate ketogenic diet

As based on a paper prepared for submission:

Leow, Z. Z. X., Guelfi, K. J., & Fournier, P. A. The risk of ketoacidosis and hypoglycaemia is not increased in response to different exercise formates in adults with type 1 diabetes mellitus on a very low carbohydrate ketogenic diet. Prepared for submission to Diabetes Care.

4.1 Abstract

Aims This study examined whether the risks of ketoacidosis and hypoglycaemia during and early after exercise performed under a near basal insulinaemic condition increase in mildly ketotic adults with T1DM on a ketogenic diet (KD).

Methods Eight adults (5 men, 3 women; age 34.8 ± 5.0 y; duration of T1DM 9.9 ± 7.2 y) on a KD (< 55 g CHO/day for 1.3 ± 0.9 y) in a basal insulinaemic state performed; graded cycling to exhaustion, 60 min of submaximal aerobic cycling at 55% $\dot{V}O_{2peak}$, and a 30-s sprint cycling on separate occasions. The circulating concentrations of ketone bodies (KB) and glucose were measured for 60 min after exercise.

Results KB concentrations decreased by 0.4 mmol/L ($p < 0.05$) during graded exercise, but remained stable during submaximal aerobic exercise and sprinting ($p > 0.05$). After 60 min of recovery, KB remained below baseline levels for graded exercise ($p < 0.05$) and did not differ from baseline for aerobic exercise or sprinting ($p > 0.05$). Plasma glucose levels did not fall in response to any of the three exercise modalities, with carbohydrates accounting for only 17 ± 11% (mean ± SD) of the fuel oxidised during aerobic exercise.

Conclusion Irrespective of exercise format (graded, submaximal aerobic and sprint exercise) performed in a basal insulinaemic state, KB concentrations did not increase and blood glucose levels did not decline in adults with T1DM on a ketogenic diet. These findings suggest that these types of exercise performed in a basal insulinaemic state do not increase the risks of both ketoacidosis and hypoglycaemia during and early after exercie, and are thus safe for people with T1DM on a ketogenic diet.

4.2 Introduction

Although individuals with type 1 diabetes mellitus (T1DM) can experience considerable health-related benefits from regular exercise (Yardley et al., 2014), a large proportion of these individuals are sedentary (Plotnikoff et al., 2006). This is related, at least in part, to the numerous barriers to exercise they face, including the complexity of managing blood glucose levels and associated risk of both hyperglycaemia and hypoglycaemia (Brazeau et al., 2013). Hypoglycaemia, in particular, can occur suddenly during (Rabasa-Lhoret et al., 2001; Tuominen et al., 1995) or after exercise (MacDonald, 1987), particularly in overinsulinised individuals. This is due to the inhibition of hepatic glucose production by high insulin levels, the stimulatory effect that hyperinsulinaemia and muscle contraction have on peripheral glucose disposal, and the exercise-mediated increase in subcutaneous blood flow and associated increase in the absorption of subcutaneously injected insulin (Zinman et al., 1977).

The prevention of exercise-mediated hypoglycaemia is possible through rigorous blood glucose monitoring, ingestion of carbohydrate (CHO) before, during and after exercise, as well as via a pre-exercise reduction in insulin dosage (Riddell et al., 2017); however, hypoglycaemia prevention is still an ongoing challenge for individuals with T1DM (Riddell et al., 2017). Furthermore, the practice of interrupting insulin infusion for people on an insulin pump or avoiding insulin injection so as to prevent hypoglycaemia during exercise may result in severe hyperglycaemia during and after exercise due to impaired rate of glucose utilisation and increased rate of hepatic glucose production (Chipkin et al., 2001; Kemmer & Berger, 1986; MacKnight et al., 2009; Wasserman & Zinman, 1994). Insulin deficiency can also results in ketosis during and after exercise (Berger et al., 1977), which in turn can lead to diabetic ketoacidosis (DKA), the leading cause of hospitalisation, morbidity and death in T1DM (Veech, 2004).

Given the serious implications of DKA, some current guidelines state that blood ketone body (KB) concentrations should be < 0.6 mmol/L at rest (Australian Diabetes Educators Association, 2014; Dhatariya, 2016), and that exercise should be avoided or ceased when KB concentration are > 0.5 mmol/L (Robertson et al., 2014; Colberg et al., 2016). However, one limitation with many T1DM exercise guidelines (Robertson et al., 2014; Colberg et al., 2016) is that they overlook the fact that moderately elevated KB concentrations are often associated with a low CHO high fat ketogenic diet (Fukao et al., 2004). There is also evidence that these diets may lead to levels of glycaemic

control comparable to those of healthy non-diabetic individuals (Lennerz et al., 2018; Leow et al., 2018). This raises the issue of whether it is safe for these individuals to exercise under a state of diet-induced ketosis given that exercise increases the levels of ketogenic hormones (Evans et al., 2017). Previous studies in non-diabetic individuals on KD have reported small increments in blood KB concentrations immediately after exercise followed by a steady decline post-exercise (Fery & Balasse, 1983; Langfort et al., 1997; O'Malley et al., 2017). To our knowledge, no published study has examined the effect of exercise on KB concentrations in individuals with T1DM on a ketogenic diet. For these reasons, the purpose of this study was to investigate whether the risk of ketoacidosis and hypoglycaemia is increased in response to a range of exercise types (including graded, submaximal aerobic and sprint exercise) performed under a near basal insulinaemic state in ketotic adults with T1DM that consume a low CHO ketogenic diet. Based on earlier findings in healthy non-diabetic individuals, we hypothesised that exercise does not increase the risk of ketoacidosis or hypoglycaemia in individuals with T1DM on KD.

4.3 Research design and methods

4.3.1 Participants

Eligibility criteria for participation in this study were ingestion of < 55 g CHO/day for > 6 months, fasting blood β-hydroxybutyrate levels ≥ 0.4 mmol/L, duration of diagnosed T1DM ≥ 2 yr, C-peptide < 0.05 nmol/L, and not taking any prescribed medication other than insulin. Recruitment was challenging given the small pool of individuals on a ketogenic diet in Perth, Western Australia. Ten individuals expressed interest, with eight volunteers being eligible for inclusion [5 men, 3 women; mean ± SD (median; range); age 34.8 ± 5.0 (33.5; 29.0-46.0) yr, body mass index 23.9 ± 3.4 (24.0; 17.4-29.6) $kg \cdot m^{-2}$, daily insulin dose 21.8 ± 10.1 (22.7; 1.5-45.0) mU, peak oxygen uptake ($\dot{V}O_{2peak}$) 47.3 ± 7.6 (47.3; 34.8-58.7) $ml \cdot kg^{-1} \cdot min^{-1}$, body fat percentage 19.3 ± 8.3% (19.1; 9.9-30.8); T1DM duration 9.9 ± 7.2 (10.5; 1.6-19.0) yr, ketogenic diet duration 1.3 ± 0.9 (1.0; 0.5-3.0) yr) and fasting HbA1c 34.9 ± 3.9 mmol/mol (5.3 ± 0.3%,). All participants were on a multiple-insulin injection regimen (6.3 ± 4.8 mU rapid acting, 2.7 ± 3.2 mU intermediate acting, and 12.8 ± 6.4 mU long acting). Of note, none of the participants had ever required medical care for DKA. Human Research Ethics

Committee at the University of Western Australia approved the study protocol (RA/4/1/6360), and informed consent was obtained from all participants.

4.3.2 Experimental design

Of note, the purpose of this study is not to compare the effect of exercise on blood KB levels between T1D people on KD and those on a "normal" diet. Instead, our purpose is to determine whether exercise increases the risk of ketoacidosis in individuals on KD, with our participants at rest acting as their own control, thus explaining the absence of a "comparator" group on a "normal" diet. All testing sessions were performed in the morning (start of the exercise between 0700 and 0900 h) and at the same time of the day to avoid any circadian effect. After a 10-hour overnight fast, participants visited the laboratory while under the influence of only basal insulin on four separate occasions, including a familiarisation session and three different exercise sessions; graded exercise to exhaustion, 60 min of submaximal aerobic exercise at 55% VO_{2peak}, and a maximal sprint effort, with all exercise formats performed on a customised air-braked cycle ergometer (Front Access Bike, School of Human Sciences, University of Western Australia, WA, Australia), and with at least one week between visits. During the familiarisation session, body composition was assessed using dual energy x-ray absorptiometry (Prodigy, General Electric Company, United States), and participants were given a diary and a portable weighing scale (Homemaker 9753, Tooronga, Vic) to record all food and drink intake, insulin doses, and cases of hypoglycaemia for 7 days. Participants also had their blood sampled to measure their HbA1c levels.

For each of the subsequent three sessions, participants were instructed to complete an additional food diary for the 24 h prior to each testing session (subsequently analysed using Foodworks-7 (Xyris Software, Australia), and to maintain consistent physical activity levels, but to avoid any vigorous activities. Testing was rescheduled if a participant experienced an episode of hypoglycaemia in the 48 h before testing. Female participants were tested during the follicular phase of the menstrual cycle only (days 4-12).

Upon arrival at the laboratory, baseline BGL and the concentration of the KB, β-HB, were measured (HemoCue 201+, HemoCue, Sweden; FreeStyle Optium, Abbott Diabetes Care, Australia respectively) before commencing the prescribed exercise

(graded exercise, 60-min aerobic endurance or 30-s sprint protocol). The graded exercise consisted of 3-min stages, with participants cycling at a set power output that increased by 25 W at each stage until volitional exhaustion, or until the participant was unable to maintain the prescribed power output. A respiratory exchange ratio (RER) >1.1 and leveling off of O_2 uptake (defined as an increase of no more than 2 $ml.kg^{-1}$ body $weight^{-1}.min^{-1}$) during the latter stages of the graded exercises test was taken to indicate that maximal $\dot{O}_2$ uptake had been attained. $\dot{V}O_{2peak}$ was calculated as the average oxygen uptake of the last 60 s of the test. Aerobic exercise required participants to cycle continuously for 60 min at a power output equivalent to 55% of their $\dot{V}O_{2peak}$, whereas the sprint cycling consisted of a 30-s maximal all-out sprint effort.

4.3.3 Indirect calorimetry

Indirect calorimetry was used to determine the rate of CHO and fat oxidation for aerobic exercise only. This is because the metabolic acidosis associated with both sprinting and graded exercise to exhaustion increases the CO_2 levels in expiratory gases, thereby invalidating the use of RER as a means to estimate CHO and fat oxidation rates, with RER overestimating CHO oxidation rate during high intensity exercise associated with lactic acidosis (Ferrannini 1988; Jeukendrup & Wallis, 2005) and underestimate CHO oxidation rate during recovery from this type of exercise (Henderson et al., 1985). During the aerobic exercise, participants breathed through a mouthpiece connected to a Hans Rudolph valve and tubing into a computerised gas-analysis system (Meta 2000, School of Human Sciences, University of Western Australia). This system consisted of a ventilometer (Universal ventilation meter, VacuMed, Ventura, California, USA) to measure the volume of inspired air, while expired oxygen and carbon dioxide concentrations were analysed using Ametek gas analysers (Applied Electrochemistry, SOV S-3A11 and COV CD-3A, Pittsburgh, PA, USA). The ventilometer was calibrated using a 1-L calibration syringe (Model 5540: Hans Rudolph, Kansas City, MO), while the O_2 and CO_2 analysers were calibrated against a beta gas containing a known physiological concentration of O_2 and CO_2 (BOC Gases, Chatswood, Australia). Rates of CHO and fat oxidation were calculated based on ventilation rates and the concentrations of O_2 and CO_2 in the expired gas as described in Jeukendrup and Gleeson (2018).

4.3.4 Statistical analyses

Despite the absence of any prior study to inform our sample size calculation, we calculated that a sample size of 8 participants should provide enough statistical power (0.8) to detect significant differences at $p<0.05$, a prediction corroborated by our findings. The effects of different exercise formats on KB concentrations and BGL were compared with baseline measurements using one-way repeated measures ANOVA followed by Fisher LSD post-hoc test, with statistical significance accepted at $p < 0.05$. Statistical analyses were performed using SPSS Version 16.0 for Windows (SPSS, Inc., Chicago, IL). All results are expressed as mean ± SD or median (IQR) in the text, and as mean ± standard error (SE) in figures.

4.4 Results

Dietary intake for the 24 hr period prior to each testing session was consistent between sessions ($p > 0.05$); with average total energy intake 9,114 (5,731-10,669) kJ, CHO 34 (21-43) g, protein 122 (89-141) g, fat 154 (90-203) g, and fibre 13 (7-16) g; ($p > 0.05$ between sessions). Baseline (fasting) KB concentrations were also similar between testing sessions (Fig 4.1, $p > 0.05$).

4.4.1 Effect of graded exercise to exhaustion on blood ketone body and glucose concentrations

Baseline blood KB concentrations were 1.2 ± 0.7 (0.9; 0.5-2.4) mmol/L (Fig 4.1) and decreased significantly in response to graded exercise to exhaustion ($p < 0.01$). KB concentrations remained significantly lower at 15 ($p = 0.01$), 45 ($p = 0.02$) and 60 min post-exercise ($p = 0.01$) compared with baseline (Fig 4.1; $p < 0.05$). At the end of the 60-min recovery period, blood KB concentrations were 1.0 ± 0.6 (0.9; 0.2-1.9) mmol/L.

Baseline BGL were 6.4 ± 1.1 (5.9; 5.0-7.9) mmol/L (Fig 4.2) and increased significantly at the end of graded exercise ($p = 0.02$). BGL peaked at 45 min of recovery (10.2 ± 3.7 mmol/L, $p = 0.02$; Fig 4.2, 4.3) and were statistically higher than baseline levels at 15 ($p = 0.01$) and 30-min post-exercise ($p < 0.01$). At the end of the 60-min recovery period, BGL remained significantly higher than baseline levels (10.1 ± 3.7 [11.3; 4.7-14.3] mmol/L; $p = 0.01$; Fig 4.2).

4.4.2 Effect of aerobic exercise on blood ketone body and glucose concentrations

Baseline blood KB concentrations were 1.0 ± 0.4 (1.1; 0.4-1.5) mmol/L (Fig 4.1) and did not change significantly after aerobic exercise (Fig 4.1, $p = 0.37$). Significant increases in KB concentrations were observed at 15 ($p = 0.02$) and 30 min ($p = 0.03$) post-aerobic exercise ($p < 0.05$), but decreased afterwards and were not significantly different at 45 ($p = 0.08$) and 60 min ($p = 0.08$) post-exercise compared with baseline. At the end of the 60-min recovery period, KB concentrations were 1.4 ± 1.0 (1.3; 0.4-2.7) mmol/L.

Baseline BGL were 5.6 ± 1.5 mmol/L (Fig 4.2, 4.3) and remained stable during exercise and throughout the 60-min recovery period post-aerobic exercise ($p < 0.05$; Fig 4.2, 4.3). At the end of recovery, BGL were 6.2 ± 2.0 (7.1; 3.3-8.9) mmol/L ($p = 0.14$).

4.4.3 Effect of sprint exercise on blood ketone body and glucose concentrations

Baseline blood KB concentrations were 1.3 ± 1.4 mmol/L (Fig 4.1) and did not change in immediate response to sprinting (Fig 4.1; $p = 0.10$). During recovery, KB concentrations were significantly lower at 15 ($p = 0.01$), 30 ($p = 0.02$) and 45 min post-sprint ($p = 0.04$). At the end of the 60-min recovery period, blood KB concentrations were not statistically significantly different from baseline concentrations (1.1 ± 1.5 (0.3; (0.2-4.4); $p = 0.08$).

Baseline BGL were 4.4 ± 1.1 mmol/L (Fig 4.2) and did not change during exercise ($p > 0.05$). During recovery, BGL was higher at 15 ($p < 0.01$; Fig 4.2), 30 ($p < 0.01$) and 45 min post-exercise ($p < 0.01$) compared with baseline. At the end of the 60-min recovery period, BGL remained significantly higher than baseline concentrations (6.7 ± 2.2 (6.3; (4.2-10.2) mmol/L; $p < 0.01$; Fig 4.2).

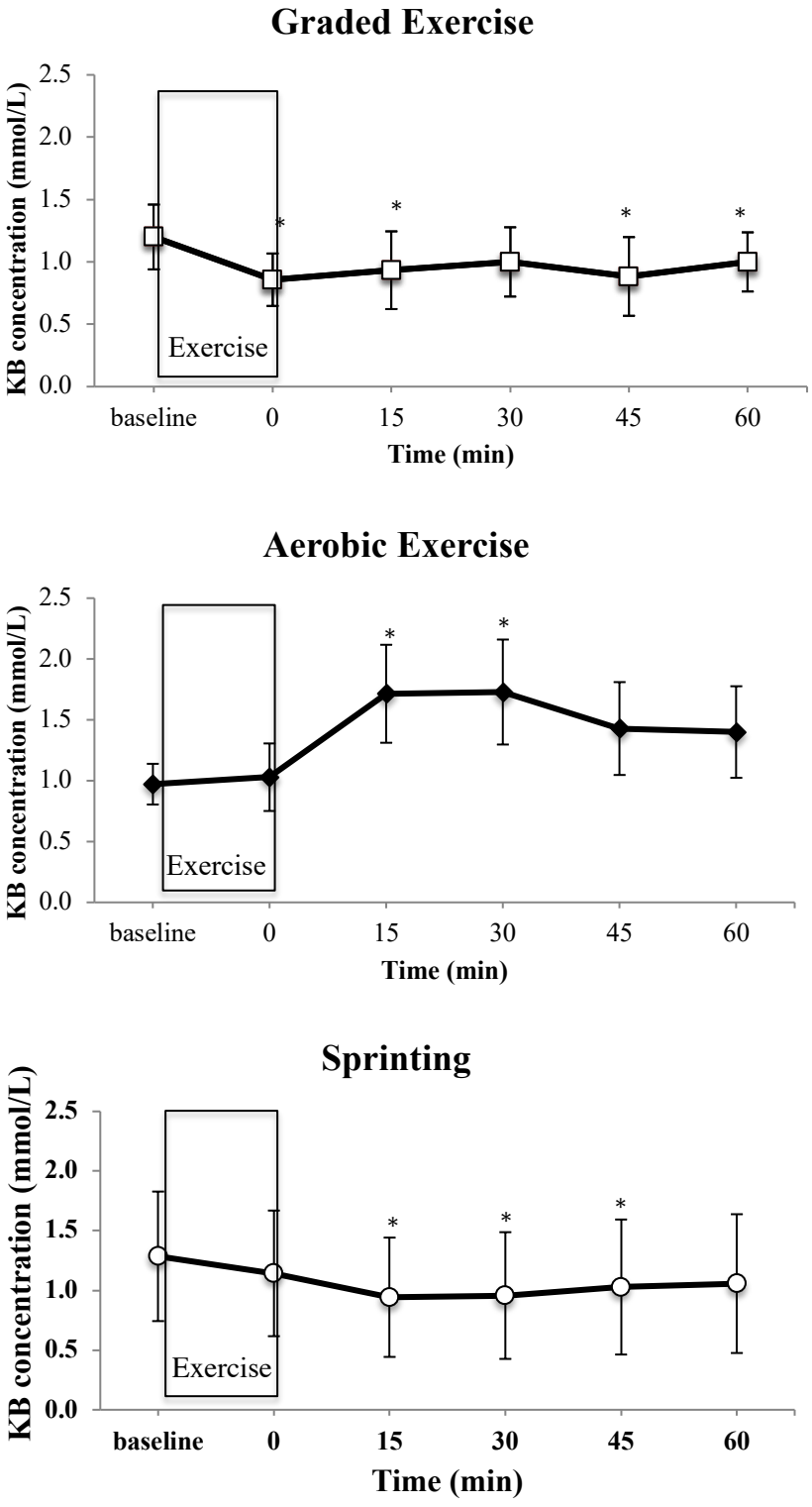

Figure 4.1 Effect of (□) graded exercise, (◆) aerobic exercise and (O) sprinting on blood ketone body concentrations. Results are expressed as mean ± SE. *Statistically significant difference ($p < 0.05$) from baseline levels.

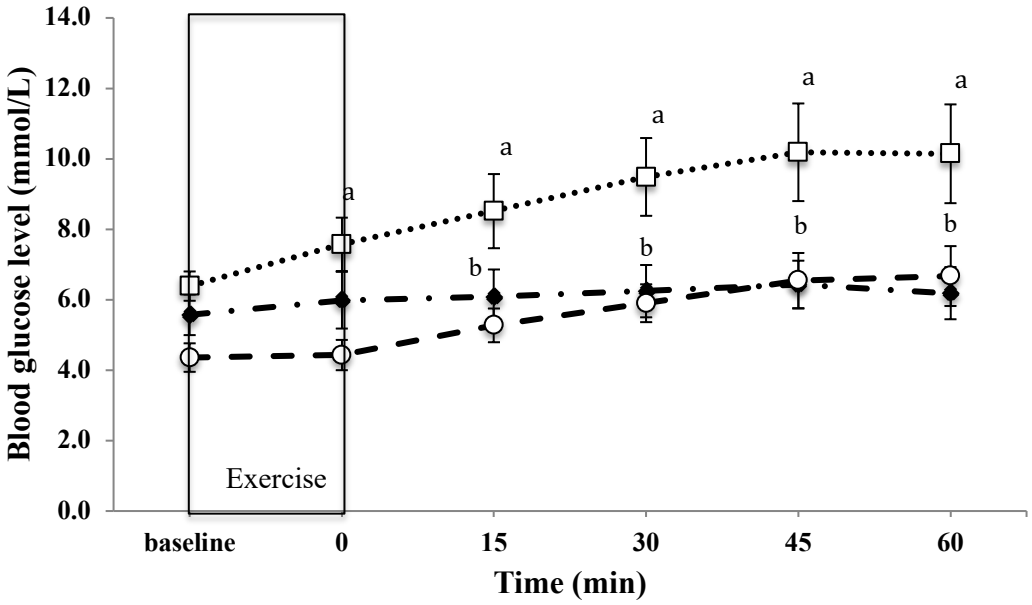

Figure 4.2 Effect of (□) graded exercise, (◆) aerobic exercise and (O) sprinting on blood glucose concentrations. Results are expressed as means ± SE. [a]Statistically significant difference ($p<0.05$) from baseline in response to graded exercise. [b]Statistically significant difference ($p<0.05$) from baseline in response to sprinting.

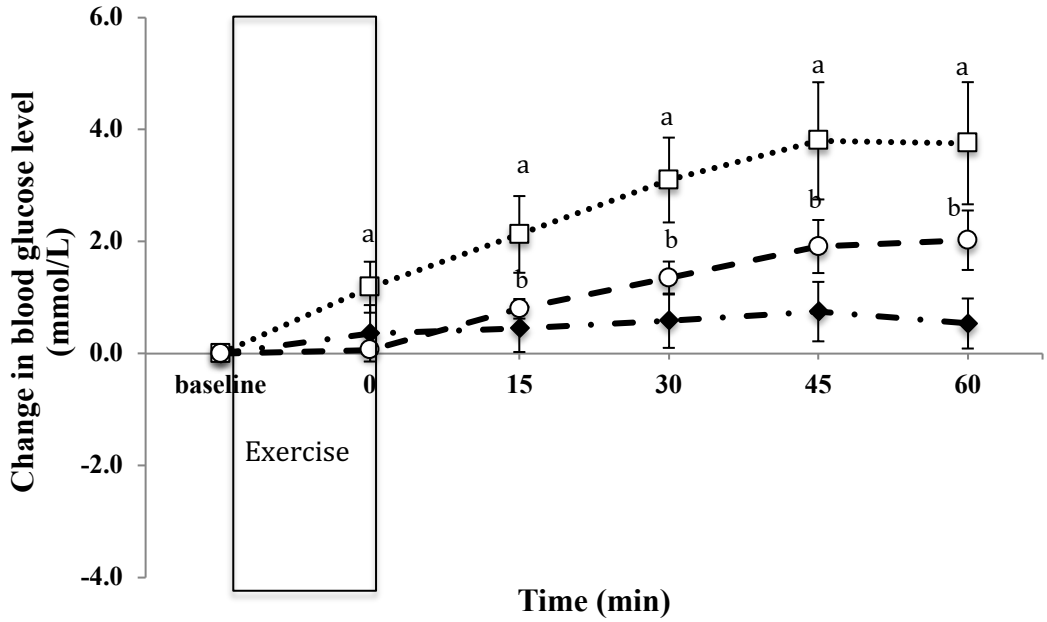

Figure 4.3 Effect of (□) graded exercise, (◆) aerobic exercise or (O) sprinting on the change in blood glucose levels. Results are expressed as means ± SE. [a]Statistically significant difference ($p < 0.05$) from baseline in response to graded exercise. [b]Statistically significant difference ($p < 0.05$) from baseline in response to sprint exercise.

4.5 Rate of fuel oxidation during aerobic exercise

During aerobic exercise, the rates of CHO and fat oxidation were 0.12 ± 0.10 and 0.23 ± 0.05 g/min respectively, with CHO accounting for 16.7 ± 11 [19; 0-37]% of the fuel being oxidised, and with the relative contributions of CHO and fat oxidation rates remaining unchanged for the duration of exercise ($p < 0.05$; Fig 4.4).

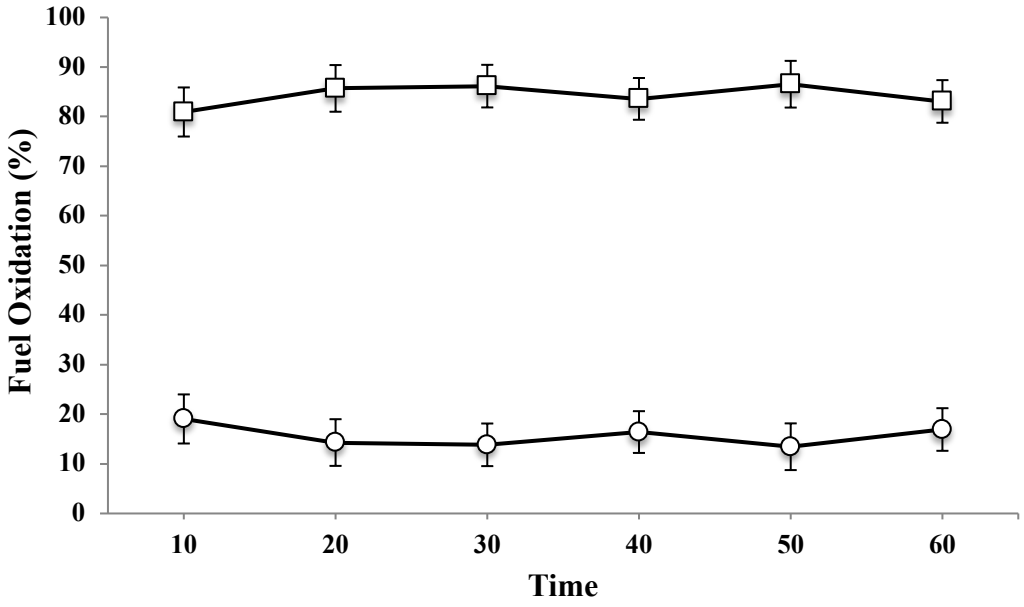

Figure 4.4 Percentage of (□) fat and (o) carbohydrate oxidation during 60 min of aerobic exercise performed at 55% of VO_{2peak}.

4.6 Discussion

Blood KB concentrations have been reported to increase immediately after exercise, before decreasing steadily during recovery in non-diabetic individuals on a KD (Langfort et al., 1997; O'Malley et al., 2017). To date, no study has examined the effect of exercise (graded, constant, or sprint) on blood KB concentration in individuals with T1DM on a KD. This leaves unanswered questions regarding the safety of exercising under the mild ketotic state induced by KD in individuals with T1DM. For this reason, the aim of this study was to examine whether exercise performed in a basal insulinaemic state increases the risks of DKA and hypoglycaemia during and early after exercise in individuals with T1DM on a low CHO ketogenic diet. This study provides the first evidence that regardless of exercise format (graded, continuous aerobic or sprint exercise), no significant sustained increment in blood KB concentration occurs in

response to exercise and at 60 min of recovery compared with baseline (pre-exercise) levels. Also, the absence of a fall in BGL in response to the three exercise protocols examined here suggests that these formats of exercise do not increase the risk of early post-exercise hypoglycaemia in individuals with T1DM on a low CHO ketogenic diet, when performed under the influence of basal insulin. These findings suggest that exercising under the mild ketosis brought about by KD is safe for individuals with T1DM.

Ketone body response to exercise

Our study shows that blood KB concentrations did not increase immediately after aerobic exercise in individuals with T1DM, a finding consistent with those of Wahren and colleagues (1984). However, it is important to note that blood KB concentrations increased progressively throughout recovery and peaked at 60 min in the study by Wahren and cowrokers (1984), as opposed to the transient short duration increase reported here. These different findings between studies can be explained on the basis that the participants in the study of Wahren and colleagues (1984) were not on a LCHF diet, and were in an insulin-deprived state as opposed to our participants. Indeed, our participants were under the influence of their basal insulin, whereas those involved in the study of Wharen and colleagues (1984) had their last insulin injected 24-26 hr before exercise. Given that the duration of action of the slow-release insulin preparation (morning dose 34.9 ± 13.2 IU; evening dose 17.6 ± 9.2 IU) used at the time of their study (1980s) is well below 24-hr (Plank et al., 2005; Starke et al., 1989), it is highly likely that the exercise in this study was performed in the almost complete absence of circulating insulin of exogenous origin, a condition highly favourable to hepatic ketogenesis and severe ketosis (McGarry et al., 1989; Zammit, 1994), and consistent with the elevated pre-exercise BGL (13.8 ± 1.1 mmol/L) and KB (β-HB 0.81 ± 0.17 mmol/L) levels reported in their study.

The absence of a sustained increase in KB concentration observed here in response to sustained aerobic exercise suggests that the contracting muscles oxidise KB at a rate similar to that of hepatic KB production rates. An abrupt fall in the rate of KB oxidation at the onset of recovery could explain the early rise in ketonemia (Fery & Balasse, 1983), particularly considering the findings of others that the enhanced rate of ketogenesis during aerobic exercise remains elevated for another 30 min after the end of exercise (Wasserman & Zinman, 1994). Finally, the delayed post-exercise fall in KB

concentration reported here is most likely the result of the rates of ketogenesis eventually falling below the rates of KB oxidation after 30 min of recovery (Laffel, 1999).

The small but significant decrease in blood KB concentrations at the termination of graded exercise followed by sustained low KB concentration until the end of the 60-min recovery period are findings consistent with those of Langfort and colleagues (1996) who examined the effect of graded exercise on KB concentration in non-diabetic individuals pre-exposed to a 3-day low CHO ketogenic diet. They attributed the early decrease in KB concentrations at the end of graded exercise to reduced hepatic KB output resulting from a diminished hepatic blood flow associated with high intensity exercise (Langfort et al., 1996). During the remainder of the recovery period, the stable, but reduced, KB concentration observed here, and in the study of Langfort and colleagues (1996), indicates that both the rates of KB utilisation and production were likely matched during that time period.

The small significant fall in KB concentration also observed here during early recovery from sprinting and subsequent stabilisation of KB at low levels for the remainder of the recovery period are findings consistent with those of Langfort and colleagues (1997). They reported a similar post-sprint pattern of change in blood KB concentrations in non-diabetic individuals exposed to a 3-day low CHO ketogenic diet prior to exercise. As mentioned earlier, a transient fall in hepatic blood flow during and early after sprinting might have reduced hepatic KB output (Langfort et al., 1996), which together with enhanced KB utilisation during early recovery might explain the early post-sprinting fall in KB concentration.

Blood glucose response to exercise

The absence of change in BGL between the start and the end of 60 min of aerobic exercise performed under near basal insulinaemic conditions, together with the maintenance of stable BGL during the whole 60-min recovery period, suggests that aerobic exercise of moderate intensity performed under a near basal insulinaemic state does not increase the risk of hypoglycaemia in individuals with T1DM on a ketogenic diet. These findings are in agreement with those of others in individuals with T1DM on a normal CHO diet, where exercise performed in a basal insulinaemic state has been shown to have little or no effect on BGL during and after moderate intensity exercise

(Biankin et al., 2013; Soon et al., 2019). Of note, none of our participants experienced a decrease in BGL during and after aerobic exercise, a finding that differs from others where individual falls in BGL post-exercise have been observed despite no average decrease in BGL (Soon et al., 2019). Overall, the absence of a fall in BGL following aerobic exercise performed under near basal insulinaemic conditions can be explained on the basis of the rates of both endogenous glucose production and peripheral glucose disposal being well matched during and after aerobic exercise (Shetty et al., 2016). Also, the much lower contribution of CHO oxidation rates observed here, compared with those found in the literature (Shetty et al., 2016), may have contributed to the sparing of CHO (Laffel, 1999; Phinney et al., 1983), thus contributing further to stabilising BGL during and after exercise. Of note, such a reduced contribution of CHO oxidation is consistent with previous studies where lowered RER after a high-fat diet intervention has been found in non-T1DM athletes (Lambert et al., 1994; Stepto et al., 2002).

Our findings that both graded exercise and sprinting result in a post-exercise increase in BGL in participants with T1DM on a ketogenic diet are consistent with those in T1DM individuals kept on a normal CHO diet. Indeed, a short duration sprint in individuals with T1DM on both a normal CHO diet and in a basal insulinaemic state has been shown to result in a sustained increase in BGL post-exercise, with this rise in BGL being attributed to a transient fall in the peripheral rate of blood glucose utilisation (Fahey et al. 2012). Similarly, intense aerobic exercise performed in individuals with T1DM in a basal insulinaemic state has also been reported to cause a long lasting increase in BGL post-exercise, but with this increase being the result of a disproportionate rise in endogenous glucose production rates relatives to peripheral glucose disposal rates (reviewed Marliss and Vranic, 2002).

Limitations

One of the main limitations of this study is that no isotope tracer methodology or hormones assays were used to explore the mechanisms underlying our findings. This is mainly because of the challenges of finding enough participants on KD willing to be recruited for such invasive studies. Another limitation relates to the use of three-day food records rather than records of longer duration. Given the observational nature of this study and its small sample size, our findings have to be interpreted with caution, and require further corroboration as only three exercise formats were tested here, thus

raising the issue of whether other types, duration and intensity of exercise would result is similar findings.

Conclusion

This study shows that no significant sustained increase in blood KB concentrations, or decline in blood glucose levels occur during or after exercise, irrespective of exercise format (aerobic, graded or sprint exercise), when performed in a basal insulinaemic state in insulin-treated T1DM adults on a ketogenic diet. These results thus suggest that these types of exercise do not increase the early risks of ketoacidosis and hypoglycaemia in individuals with T1DM. Our findings should assist in the development of improved guidelines that do not discourage mildly-ketotic individuals with T1DM on a ketogenic diet from enjoying the benefits of regular physical activity.

Chapter 5

General discussion

5.1 Overview of findings

Poor glycaemic control plays an important role in the aetiology of long-term complications in individuals with T1DM (see Chapter 1). Since CHO intake is one of the most important determinants of blood glucose level increments in insulin-treated individuals with T1DM, and given the difficulty of matching CHO intake with insulin dose, it is not uncommon for these individuals to restrict their CHO intake to reduce the magnitude of their glycaemic excursion and improve their glycaemic control (Delahanty et al., 2009; Toeller et al., 1996). As discussed in Chapter 1, only a handful of studies have examined the effect of low CHO high fat (LCHF; 50-130 g CHO/day) diets on glycaemic control in people with T1DM, and their findings suggest that these diets can improve glycaemic control (Krebs et al., 2016; Nielsen et al., 2005, 2012), reduce the level of blood glucose fluctuations, and lower total daily insulin dose without causing any dyslipidaemia and changes in body mass (Krebs et al., 2016; Nielsen et al., 2005, 2012; Schmidt et al., 2019). However, as pointed out earlier, the findings of these studies should be interpreted with caution due to both their lack of a control group and the concomitant introduction of intensive glucose monitoring (Nielsen et al., 2005, 2012) or their small sample size (n = 5, Krebs et al., 2016).

Given the evidence that LCHF diets may improve glycaemic control in T1DM, this raises the issue of whether more extreme diets that provide far less CHO, such as KD (CHO intake < 55 g/day), can further enhance these improvements without any ill health effects. That KD may be beneficial is suggested by the retrospective case series analysis of O'Neill and colleagues (2003) who reported that very low prescribed CHO intake (~30 g/day) in individuals with T1DM resulted in HbA1c levels of 5.5% without causing any dyslipidaemia. However, no dietary analyses and blood KB assays were conducted in this study to show that the CHO intakes were as low as prescribed, and that the diet was effectively ketogenic (O'Neill et al., 2003). As discussed in Chapter 1, these limitations were also shared by the study of Lennerz and colleagues (2018), which was based on the use of self-reported surveys of individuals with T1DM who consumed very low CHO diets (< 51 g CHO/day). Although this study reported optimal average HbA1c levels of 5.7 ± 0.7%, with 62% of the participants classified as being dyslipidaemic, there were some important limitations to this study. In particular, only 42.4% and 32.0% of their participants provided their CGM data and lipid profiles,

respectively, raising the possibility that these participants may have self-selected the data to report, and/or chose not to report their less than desirable data. Also, as was the case for the study of O'Neill et al. (2003), no blood KB analyses were performed to ascertain that participants were indeed compliant with their diets and effectively in a ketotic state. Given these limitations, the first aim of this thesis was to test the hypothesis that individuals already on a ketogenic diet (< 55 g CHO/day) display optimal glycaemic control approaching that of non-diabetic healthy individuals, low levels of glycaemic excursion, and reduced hypoglycaemia risk, with no significant dyslipidaemia or other detrimental health effects.

Although a positive association between daily CHO intake and HbA1c level has been found in the study of Meisser and colleagues (2014), the ranges of daily CHO intake covered in that study did not extend to the very low CHO intakes typical of KD. Given the evidence that very low CHO diets are associated with low HbA1c levels (O'Neill et al., 2003; Lennerz et al., 2018), this raises the issue of whether the relationship between daily CHO intake and HbA1c level over a broader range of daily CHO intake is associated with an abrupt leap or a progressive change between these variables, particularly between individuals on very low daily CHO intake and those on higher daily CHO intake. Hence, the second aim of this thesis was to investigate the relationship between daily CHO intake and HbA1c levels over a broad range of daily CHO intake encompassing very low CHO intake (< 55 g/d). As a secondary aim, this thesis also investigated how reproducible 3-day CHO-intake diaries are at estimating CHO intake over different CHO intake ranges in individuals with T1DM.

Another area of concern relates to the issue of whether individuals with T1DM on a KD are at an increased risk of ketoacidosis during and after exercise. This concern arose from the observation that exercise in non-diabetic individuals under a hypoinsulinaemic state (e.g. after a prolonged fast) results in mild post-exercise ketosis (Berger et al., 1977). Also, as described in Chapter 1, exercise in insulin-deprived ketotic individuals with T1DM is not recommended as the rise in catecholamines and glucagon levels during exercise have the potential to markedly increase the risks of exercise-mediated diabetic ketoacidosis (DKA), a leading cause of hospitalisation, morbidity and death in T1DM (Veech, 2004). Unfortunately, the issue of whether exercise can cause unphysiological increases in KB concentrations in individuals with T1DM on a ketogenic diet has never been examined before. For this reason, the third aim of this

thesis was to test the hypothesis that individuals with T1DM in a basal insulinaemic state and already on a ketogenic diet (< 55 g CHO/day) do not experience any unphysiological increase in blood KB concentration in response to different types of exercise including graded exercise to volitional exhaustion, prolonged aerobic exercise, and a maximal sprint effort.

In order to address the first aim of this thesis, 11 complication-free participants with T1DM on a very low CHO ketogenic diet (< 55 g CHO/day) were recruited for our study. All participants had their blood taken after an overnight fast to measure their levels of HbA1c, c-peptide, β-hydroxybutyrate, lipoproteins, and markers of liver and kidney function. In addition, height, body mass, body composition, blood pressure, and haemoglobin levels were measured. Each participant was provided with a diary, a portable weighing scale, a blinded continuous glucose monitor and a glucometer to be used for a 7-day period to record all cases of hypoglycaemia, insulin therapy regimen, food and drink intake. Our results, described in Chapter 2, showed that mean HbA1c levels were 35 ± 4 mmol/mol (5.3 ± 0.4%), and participants spent 74 ± 20 and 3 ± 8% of their time in the euglycaemic (4-8 mmol/L) and hyperglycaemic (> 10 mmol/L) range, respectively, with little daily glycaemic variability. Blood glucose levels were below 3.0 mmol/L for 3.6% of their time, and participants experienced 0.9(0.0-2.0) daily episodes of hypoglycaemia. These glycaemic outcomes were, however, opposed by the levels of total cholesterol, LDL cholesterol, total cholesterol/HDL ratio, and triglycerides being above the recommended range in 82, 82, 64 and 27% of the participants, respectively. However, HDL levels were within the recommended range for all participants.

It is noteworthy that the optimal HbA1c levels associated with KD observed here approached those of healthy individuals without diabetes, and were achieved using conventional insulin therapy, an important finding given that none of the current diabetes therapies, including insulin pump therapy, multiple daily blood glucose measurements, CHO counting, and daily use of continuous glucose monitors, achieves HbA1c levels comparable to those of our participants. Although our participants experienced a higher frequency and duration of hypoglycaemic episodes compared with those reported in the literature (1-2 episodes/week), the number of hypoglycaemic episodes self-reported by our participants was much lower, and comparable to those found by Nielsen and colleagues (Nielsen et al., 2005). Although these low levels of

self-reported hypoglycaemic episodes may be related to hypoglycaemia unawareness, we raised the possibility in Chapter 2 that they may also be due to the lowering of the hypoglycaemia threshold for neuroglycopaenic and neurogenic symptoms due to the protective effect that mild ketosis has against hypoglycaemia, an interpretation supported by the observation that ketone infusion reduces cognitive impairment during induced hypoglycaemia (Amiel et al., 1991).

The dyslipidaemia experienced by some, but not all, participants was also observed in the study of Lennerz and colleagues (2018), with 62% of their participants being dyslipidaemic. The underlying cause of this high rate of dyslipidaemia remains to be determined, but we tentatively speculate that this may have to do with the high levels of saturated fat in the diet of our participants, as high saturated fat intake may be a risk factor for dyslipidaemia (Jenkins et al., 1993). Of note, however, since the epidemiological research on the basis of which dyslipidaemia is defined is grounded largely on data collected from individuals on normal or high CHO diets, this raises the issue of what is considered a "normal physiological" lipid profile for individuals on KD, and whether such a profile is similar or not to that of the general population. Until, such a gap in the literature is addressed, the interpretation of our lipid data should be performed with caution.

In order to address the second aim of this thesis, 56 adults with T1DM were recruited, with special care taken to recruit individuals on a KD. Body mass, height, three-day food diaries and HbA1c levels were measured in all participants. Among these participants, a subset of 35 individuals agreed to complete a second 3-day diary (week 2) on a separate occasion to evaluate the reproducibility of the food diary data. As detailed in Chapter 3, this study showed a positive but weak correlation between daily absolute CHO intake and HbA1c levels, as well as between daily CHO intake relative to body mass and HbA1c levels. However, the relationship between daily CHO intake and HbA1c levels was best described by a sigmoidal relationship, with a steep inflection point and an abrupt leap in HbA1c levels at a daily CHO intake of 99.5 g/day. Above or below this point, there was no longer any relationship between daily CHO intake and HbA1c levels ($p>0.05$), with daily CHO intakes below 99.5 g/day being associated with lower mean HbA1c levels compared with daily CHO intake above this inflection point. We also found that the use of a 3-day CHO diary on different weeks provided, in general, reproducible findings, but not for individuals on a low daily CHO intake (< 75

g/day). As discussed in Chapter 3, the mechanisms underlying the abrupt leap in HbA1c levels at a daily CHO intake of ~99.5 g/day remain to be elucidated. Such an abrupt leap is unlikely to be explained on the basis of differences in the glucose management technology used between those on low and moderate/high CHO diets, since ~95% (18/19) of our participants in the low CHO group were using conventional insulin and glucose monitoring therapy as opposed to the participants on high daily CHO intake, with ~50% (18/37) of them relying on more advanced therapeutic tools to manage their blood glucose levels.

In order to address the third aim of this thesis, eight healthy adult participants with T1DM on a KD, and in a near basal insulinaemic state, were subjected on separate occasions to graded intensity cycling to volitional exhaustion, 60-min of cycling at 55% of their $\dot{V}O_{2peak}$, and a 30-s maximal sprint cycling effort. We found that KB concentration decreased by 0.4 mmol/L ($p < 0.05$) in response to graded exercise, but remained stable in immediate response to aerobic aerobic exercise and sprinting ($p > 0.05$). After 60 min of recovery, KB remained below baseline levels for the graded exercise to volitional exhaustion ($p < 0.05$) and did not differ from baseline for aerobic exercise and sprinting ($p > 0.05$). Of note, plasma glucose levels did not fall in response to any of the three exercise formats. Our findings thus provide the first evidence that, regardless of exercise format (graded, aerobic or sprint exercise), there is no significant sustained increment in blood KB concentration in response to exercise and the following 60 min of recovery compared with baseline (pre-exercise) levels, implying that exercise performed under near basal insulinaemic conditions does not increase the risk of post-exercise ketoacidosis in insulin-treated adults with T1DM on a ketogenic diet.

The absence of a fall in BGL in response to our three exercise protocols performed under near basal insulin levels suggests that exercise in T1DM individuals on a KD does not increase the risk of early post-exercise hypoglycemia. The absence of any decrease in blood glucose levels during and after moderate intensity exercise was explained in Chapter 4 on the grounds that CHO oxidation rates were much lower during and after exercise compared with those rates found in the literature (Shetty et al., 2016), thus contributing to the sparing of CHO (Laffel, 1999; Phinney et al., 1983) and stabilising BGL during and after exercise. In contrast, our findings that both graded exercise and sprinting resulted in a post-exercise increase in BGL are findings

consistent with those in individuals with T1DM in a basal insulinaemic state and kept on a normal diet, with short duration sprinting, as well as intense aerobic exercise, resulting in an increase in BGL in these individuals post-exercise (Fahey et al., 2004; Marlis & Vranic, 2002). On the basis of these findings, we tentatively conclude that, irrespective of exercise type (aerobic, graded and sprint exercise), BGL do not fall during and early after exercise performed in a basal insulinaemic state in insulin-treated T1DM adults on a ketogenic diet. These findings thus suggest that these types of exercise do not increase the risks of ketoacidosis and hypoglycaemia during and early after exercise, and are thus safe.

5.2 Limitations

Although the findings of this thesis may contribute to improving the glycaemic management of people with T1DM on KD, the limitations with the studies at the origin of our findings should be acknowledged. In the study described in Chapter 2, its small sample size, the recruitment of self-selected individuals, and observational nature imply that its findings have to be interpreted with caution, and require further corroboration from future randomised control trials with larger sample sizes. In particular, it is unclear how representative are our findings of the whole population of individuals on KD. Indeed, since the results in Chapter 2 were specific to young adults with a healthy BMI, it is unclear whether our findings can be generalised to individuals of different ages or BMI. Also, it is not known to what extent the self-selected participants involved in our study were representative of the general population. A larger sample size would also allow one to investigate whether the blood lipid profile of individuals on KD is affected by different types of KD and their macronutrients composition (e.g. proportion of saturated, monounsaturated and polyunsaturated fat). Another limitation relates to the absence of information about the long-term health effect of KD, as well as the lack of information defining what constitutes a healthy blood lipid profile in people on these diets. Future studies may also consider testing specific KD that minimise the aforementioned cardiovascular risks rather than relying on diets self-selected by participants. Another limitation of our study was the use of continuous glucose monitors as this technology measures interstitial glucose levels rather than blood glucose levels. The levels of sensor glucose readings recorded by these monitors have been found to lag behind blood glucose levels (Zaharieva et al., 2019), thus implying that the

glycaemic responses reported in this study would have probably been more accurate had blood glucose level been measured.

There were also some limitations with the study described in Chapter 3. In particular, the recruitment of participants was challenging, particularly the recruitment of participants with a daily CHO intake of 50-99 g/day. Future studies based on a randomzed control trial design with a larger sample size should consider adopting a broader multi-centre approach to improve recruitment across all CHO intake categories. Also, although all our participants were willing to provide small amounts of blood for the assay of their HbAIc levels, most of them were unwilling to have larger volumes of blood taken using more invasive intravenous blood sampling procedures for lipid assays. As a result, we were unable to perform any meaningful analysis of the relationship between CHO intake and blood lipid profile, thus explaining why no lipid data was included in Chapter 3.

The study described in Chapter 4 shares some of the same limitations as those of Chapter 2. For instance, the small sample size of this study implies that its findings have to be interpreted with caution as it is unclear how representative the findings are for the broader population. Furthermore, only three exercise formats were tested in this study, thus raising the issue of whether other types, modes, durations and intensities of exercise would result in similar findings. Also, no isotope tracer methodology or hormones assays were adopted in this study, thus preventing us from elucidating the mechanisms underlying our findings.

5.3 Clinical implications

Overall, the findings of this thesis may have some clinical implications for the development of future guidelines to assist adults with T1DM on KD to manage their blood glucose levels optimally and exercise safely. As stated earlier (Chapter 1 and 2), several studies have shown that non-ketogenic LCHF diets (~100 g CHO/day) improve glycaemic control without causing dyslipidaemia or increasing hypoglycaemia risk (Krebs et al., 2015; Nielsen et al., 2005, 2012; O'Neill et al., 2003). Here we show that individuals with T1DM on KD with much lower CHO intake (< 55 g/day) experience limited glycaemic variability and optimal glycaemic control, to such an extent that their glycaemic control approaches that of healthy non-diabetic individuals (Chapter 2). Our

findings also show that such optimal glycaemic control can be achieved while using traditional glycaemic management strategies (i.e. on a MDI regimen). Although these findings suggest that the adoption of KD may reduce the risk of long-term diabetes complications in individuals with T1DM, the dyslipidaemia found in many participants also suggests that these individuals may be at higher risk of cardiovascular disease. It is unclear to what extent the predicted reduced risk of cardiovascular diseases due to the optimal glycaemic control associated with KD is outweighted by the expected higher risk of cardiovascular disease associated with dyslipidaemia. Until evidence-based norms of what constitutes a healthy lipid profile for individuals on KD is provided, it will remain unclear to what extent individuals on KD and with "abnormal" lipid profiles are "truely dyslipidaemic". Finally, it remains to be determined whether the hypoglycaemic threshold is lower in individual on a KD, and whether these individuals are more resistant to hypoglycaemia due to the increased reliance of the brain on KB as a fuel. Until all of the aforementioned unresolved issues are addressed, the clinical implications of the findings presented in Chapter 2 have to be taken with caution.

On clinical grounds, the findings of Chapter 3 of this thesis suggest that daily CHO intakes do not have to be within the extremely low CHO intake range (0-50 g/day) typical of KD to achieve optimal glycaemic control, as higher intakes of 50-100 g CHO/day appear just as beneficial, but with an abrupt rise in HbA1c levels observed with daily CHO intake above ~99.5 g/day. This is potentially an important observation, particularly considering the findings of others that CHO intake within the 50-100 g/day range may not be associated with any dyslipidaemia (Krebs et al., 2016; Nielsen et al., 2005, 2012). Our interpretation has to be taken with caution given the small number of participants whose daily CHO intakes fell within this range. More research is thus required to determine whether a daily CHO intake within the range of 50-100 g/day is associated with a "healthy" blood lipid profile, as alluded to above, or whether dyslipidaemia is also found within that range, with an abrupt normalisation of lipid profiles taking place with a daily CHO intake above 100 g/day. In this respect, our findings that the use of a 3-day CHO diary on different weeks provides, in general, reproducible findings, but not for CHO intake below 100g/day should help inform the design and sample size of future larger epidemiological studies aimed at investigating further the relationship between daily CHO intake and HbA1c levels.

From a clinical perspective, the results of Chapter 4 imply that, irrespective of exercise format (aerobic, graded and sprint exercise), KB concentrations do not undergo a sustained increase, and blood glucose levels do not fall, during and after exercise performed in a basal insulinaemic state in insulin-treated T1DM adults on a KD. These findings suggest that these types of exercise do not increase the early risks of ketoacidosis and hypoglycaemia in T1DM individuals on a KD, and are thus safe for these individuals. Therefore, future diabetes management guidelines should not discourage mildly-ketotic individuals with T1DM on a KD from enjoying the benefits of regular physical activity.

5.4 Directions for future research

On the basis of the research presented in this thesis, and the work of others, there is some evidence that ketogenic and LCHF diets may provide an effective approach for managing T1DM. However, before advocating the widespread adoption of these diets for the management of T1DM, future studies should endeavour to develop dietary strategies to prevent the dyslipidaemia and micronutrient deficiency associated with KD, and to evaluate the extent to which such dyslipidaemia, in the long-term, increases the risks of cardiovascular disease. Also, future studies are required to evaluate whether these risks, in the long-term, outweigh the predicted cardiovascular benefits associated with the long-term optimal glycaemic control associated with KD. Given the small sample size of the study performed in Chapter 3, longer-term and larger-scale randomised control trials should be conducted on a larger population sample across genders, ages, and methods of insulin delivery to better understand the relationship between daily CHO intake and different health markers (HbA1c, HDL, LDL, blood pressure, triglyceride, fasting glucose), and to further investigate the long-term effectiveness of ketogenic and LCHF diets at improving diabetes management. Since compliance to the prolonged use of ketogenic diet may prove to be challenging, more research is required to determine whether non-KD with low CHO intake in the 50-100 g/day range could attract a higher level of compliance together with comparable glycaemic control. Additional research is also needed to elucidate the mechanisms underlying the abrupt threshold increase in HbA1c levels that occurs with CHO intake ~99.5 g/day.

As indicated above, the risk of hypoglycaemia and DKA should be evaluated against other exercise types, duration and intensity to corroborate further the evidence provided in this thesis that exercise is safe for people on KD. More in-depth studies using isotope tracer methodology are also required to explain the absence of increase in KB concentration and fall in BGL in response to exercise in individuals on KD. Overall, although there is now growing evidence that low CHO diets may help improve the glycaemic management of people with T1DM, more research is required to better understand the long-term benefits and risks associated with the use of these diets for the management of blood glucose levels, and participation in regular exercise, in individuals with type 1 diabetes.

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Appendices

Participant detail form (Chapter 2, 3 & 4)

Date:

Name:	**Age:**
Gender: Male / Female	
Mobile:	
Email:	
Duration of diabetes (years):	
Duration on current diet (years):	
Insulin therapy regimen:	
Smoking status: Current / Ex-smoker / Never	
Average alcoholic beverage per week:	
Average cup of coffee per week:	
Current medication(s):	
Occupation:	
Highest education level:	
Income (estimation):	

For experimenter to fill in:

HbA1C:	**HDL:**	**LDL:**	**KB:**
Hb:			
Body composition:			
Bone:	Muscle:	Fat:	
Basal metabolic rate:			
Seat height:			
VO2peak:		**55% VO2:**	

Food diary (Chapter 2, 3 & 4)

Daily Food Diary

Name: _____

Please record all food and drinks that you have for the next 7 days on the form provided. You should write the food and drinks down as soon as you have them. At a minimum you will need to fill in the form at the end of each day.

For all food and drinks that you eat, please record as many details as possible including;

- the **type** of food (i.e. full cream milk or hilo or skim)
- the **amount** (grams / mL / number of pieces) of each food/drink
- the **brand** name if possible
- the **way that the meal was cooked** (i.e. grilled versus deep fried)

Below is an example

Meal	Food Type (e.g. wholegrain bread, vegemite)	Brand (e.g. Mias, Kraft)	Cooking method (or other comments)	Amount (in grams or mL or number of pieces)
BREAKFAST	Wholegrain bread	Woolworths	Toasted	4 slices
	Orange Juice	Brownes Orange C		2 cups
	Vegemite spread	Vegemite		½ tablespoon
LUNCH	Multigrain roll	Local bakery		Medium size
	Ham	Deli item		3 thick cut slices
	Cheddar cheese	Kraft slices		2 thin slices
	Grated carrot			1 cup
	Beetroot			2 slices
	Choc milk	Masters		600 ml
DINNER	Steak	Coles	BBQ cooked	350 g
	potatoes		baked	2 baby/small
	Garden Salad + dressing	Homemade		2 x cups salad 1 tablespoon dressing
	Choc chip ice cream, low fat	Nestle		3 med. scoops
	Water			3 cups (750 mL)
SNACKS	Low fat milk	Brownes		1 cup (300 ml)
	Banana muffin	homemade		Medium size
	Mixed nuts			1 ½ cup

Food Diary Day 1 Date:_____

Meal	Food Type (e.g. wholegrain bread, vegemite)	Brand (e.g. Mias, Kraft)	Cooking method (or other comments)	Amount (in grams or mL or number of pieces)
BREAKFAST				
LUNCH				
DINNER				
SNACKS				

Insulin log (Chapter 2, 3 & 4)

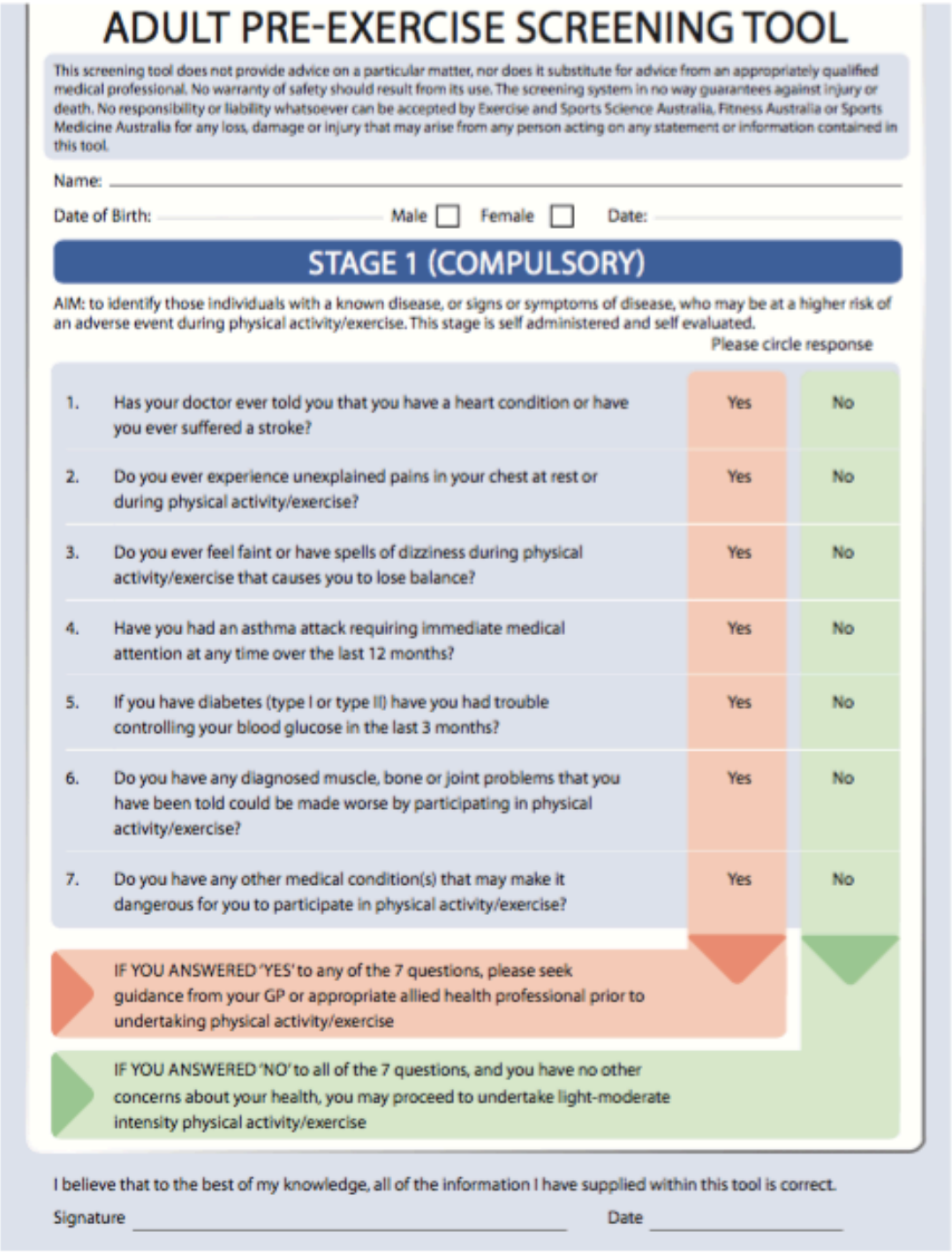
Insulin Therapy Log

Name:

Blood glucose units = mmol/L

Date	Time	Blood sugar	Insulin type	Units	Remarks
1-Jan-16	8am	5.4	0	0	wake up
	12pm	7	Intermediate	4.7	post lunch
	2pm	6.8	0	0	2 hr post lunch
	11pm	9	long acting	1.7	before bedtime

Adult pre-exercise screening tool (Chapter 4)



ADULT PRE-EXERCISE SCREENING TOOL

This screening tool does not provide advice on a particular matter, nor does it substitute for advice from an appropriately qualified medical professional. No warranty of safety should result from its use. The screening system in no way guarantees against injury or death. No responsibility or liability whatsoever can be accepted by Exercise and Sports Science Australia, Fitness Australia or Sports Medicine Australia for any loss, damage or injury that may arise from any person acting on any statement or information contained in this tool.

Name: _____

Date of Birth: _____ Male ☐ Female ☐ Date: _____

STAGE 1 (COMPULSORY)

AIM: to identify those individuals with a known disease, or signs or symptoms of disease, who may be at a higher risk of an adverse event during physical activity/exercise. This stage is self administered and self evaluated.

Please circle response

1.	Has your doctor ever told you that you have a heart condition or have you ever suffered a stroke?	Yes	No
2.	Do you ever experience unexplained pains in your chest at rest or during physical activity/exercise?	Yes	No
3.	Do you ever feel faint or have spells of dizziness during physical activity/exercise that causes you to lose balance?	Yes	No
4.	Have you had an asthma attack requiring immediate medical attention at any time over the last 12 months?	Yes	No
5.	If you have diabetes (type I or type II) have you had trouble controlling your blood glucose in the last 3 months?	Yes	No
6.	Do you have any diagnosed muscle, bone or joint problems that you have been told could be made worse by participating in physical activity/exercise?	Yes	No
7.	Do you have any other medical condition(s) that may make it dangerous for you to participate in physical activity/exercise?	Yes	No

IF YOU ANSWERED 'YES' to any of the 7 questions, please seek guidance from your GP or appropriate allied health professional prior to undertaking physical activity/exercise

IF YOU ANSWERED 'NO' to all of the 7 questions, and you have no other concerns about your health, you may proceed to undertake light-moderate intensity physical activity/exercise

I believe that to the best of my knowledge, all of the information I have supplied within this tool is correct.

Signature _____ Date _____

ESSA Fitness Australia SPORTS MEDICINE AUSTRALIA

30-s sprint data entry form (Chapter 4)

Name:

	10s	20s	30s
Peak power			
Mean power			

Ketone Bodies

Baseline	0-post	15-post	30-post	45-post	60-post

BG

Baseline	0-post	15-post	30-post	45-post	60-post

Name:

	10s	20s	30s
Peak power			
Mean power			

Ketone Bodies

Baseline	0-post	15-post	30-post	45-post	60-post

BG

Baseline	0-post	15-post	30-post	45-post	60-post

Name:

	10s	20s	30s
Peak power			
Mean power			

Ketone Bodies

Baseline	0-post	15-post	30-post	45-post	60-post

BG

Baseline	0-post	15-post	30-post	45-post	60-post

60-min cycle data entry form (Chapter 4)

Name: **Date:**

Pre-exercise

KB:	Glucose:	Lactate:	HR:

15 min

Glucose:	HR:	RPE:

30 min

Glucose:	HR:	RPE:

45 min

Glucose:	HR:	RPE:

60 min –end of exercise

HR:		RPE:
KB:	Lactate:	Glucose:

15 min post

KB:	Glucose:	HR:

30 min post

KB:	Glucose:	HR:

45 min post

KB:	Glucose:	HR:

60 min post

KB:	Glucose:	HR:

Humidity:

Temperature:

Time:

$\dot{V}O_{2max}$ testing data entry form (Chapter 4)

VO_2 Max DATA SHEET

Name : _____ Test Date: _____

Age : _____ Gender: _____ Time : _____

Height : _____m Tester : _____

Weight: _____kg Seat Height : _____

Humidity: _____ % Temperature: _____

	Time (min)	Target power	HR	VO2	RER	RPE	Peak power	Mean power
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

$VO_{2\ max/peak}$ = $ml.kg.min^{-1}$

Achieved during min: _____