The Role of Residual Beta-cell Function on Glycaemic and Vascular Outcomes at Rest and Post Exercise in People with Type 1 Diabetes

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Abstract

Within patients with type 1 diabetes (T1D), residual β-cell function and endogenous insulin secretion occurs in a substantial number of individuals. The role this β-cell function plays in glycaemic control in individuals with T1D is currently not fully understood. This is especially true around exercise, where maintaining glycaemic control is challenging and a large inter-individual variation exists. Micro amounts of endogenous insulin secretion appears to offer some protection against vascular damage and diabetes complications. Endothelial progenitor cells (EPCs) are important for the repair and growth of blood vessels, with circulating numbers increased by exercise. However, the count of EPCs appear to be reduced in individuals with T1D. It is unknown whether residual β-cell function in individuals with T1D influences these circulating cells. Therefore, this thesis aimed to investigate the effects of residual β-cell function in individuals with T1D and its influence on glycaemic control under free-living conditions and after an acute bout of aerobic exercise, as well as on the count of circulating EPCs at rest and after exercise mobilisation.

Chapter 3 demonstrated that under free-living conditions, increased endogenous insulin secretion was associated with improved continuous glucose monitoring measures, including increased time spent in euglycaemia. The results of Chapter 4 revealed that within a cohort who had comparable glycaemic control under free-living conditions, individuals with higher residual β-cell function displayed a substantially greater amount of time spent in euglycaemia in the hours following a bout of moderate intensity exercise compared to those with undetectable or lower endogenous insulin secretion. Lastly, Chapter 5 assessed the number of circulating EPCs at rest and after mobilisation with a bout of moderate intensity exercise. In comparison to matched non-diabetes controls, all markers of EPCs were lower in the T1D group, with some markers having attenuated mobilisation with exercise. Despite comparable resting counts, only the high residual β-cell function group had mobilisation of these cells with exercise within the T1D participants.

In conclusion, the findings of this thesis demonstrate that residual β-cell function impacts upon individuals with longer duration T1D. Increased C-peptide secretion is associated with improved glycaemic control under free-living conditions and after an acute bout of exercise, as well as increasing the count of circulating EPCs that are mobilised with exercise.

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Taylor, G.S., Ross, M.D., Okwose, N.C., Capper, T., Bashir, A., Shaw, J.A., Stevenson, E.J. and West, D.J., 2019, *March. Plasma volume changes with exercise in the postprandial state and its influence on the count of rare circulating angiogenic cells in individuals with type 1 diabetes.* Abstracts of the Diabetes UK Professional Conference 2019, Liverpool, 6–8 March 2019. In Diabetic Medicine (Vol. 36, Pp. 72-72). 111 River St, Hoboken 07030-5774, NJ USA: Wiley.

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Introduction

Type 1 diabetes (T1D) is characterised by an autoimmune destruction of the insulin secreting β-cells. At present, the disease is irreversible and results in individuals being dependent upon exogenous insulin to manage their blood glucose throughout their lifetime (American Diabetes, 2010).

However, the immunological event and presentation of T1D is not homogenous (Leete et al., 2020), with residual β-cells still functional in a significant percentage of the population (Oram et al., 2015), even decades after clinical diagnosis (Keenan et al., 2010). In individuals with functioning β-cells, micro amounts of endogenous insulin and C-peptide are secreted in equimolar amounts into circulation in response to increasing blood glucose. Additionally, residual β-cells function potentially protects other cells within the islet of Langerhans including α-cell function (Rickels et al., 2020).

This residual β-cell function appears to offer some protection against the increased vascular damage seen in individuals with T1D. This protection includes both microvascular damage to the kidneys, eyes and nerves, and macrovascular damage, which increases the risk of cardiovascular disease (Steffes et al., 2003a, Lachin et al., 2014, Kuhtreiber et al., 2015). It is unclear if this protection is from improved glycaemic control - resulting in reduced vascular damage and improved repair, or another improved risk factor such as C-peptide having a protective impact upon the vasculature. The Diabetes Control and Complications Trial (DCCT) first demonstrated that individuals with T1D who have a C-peptide of >200 pmol/L had a reduced risk of retinopathy and neuropathy, lower HbA1c and a lower risk of episodes of severe hypoglycaemia (Steffes et al., 2003a, Lachin et al., 2014). It has since been established that even much lower levels of endogenous C-peptide (>10 pmol/L) can reduce the risk of microvascular damage (Kuhtreiber et al., 2015). The evidence of residual β-cells influence upon glycaemic control is currently lacking in individuals with long duration T1D, with initial research suggesting that low levels (C-peptide >10 pmol/L) may influence hypoglycaemia (Gibb et al., 2020), while higher levels (>200 pmol/L) influence time spent in euglycaemia and hyperglycaemia (Rickels et al., 2020).

Regular physical activity and exercise have a range of health and wellbeing benefits for individuals with T1D by improving a range of cardiovascular risk factors (Colberg et al., 2016, Chimen et al., 2012b). Active individuals with T1D have reduced rates of

both micro and macrovascular complications compared to more sedentary individuals (Bohn et al., 2015, LaPorte et al., 1986). Despite this, numbers of people with T1D who regularly partake in exercise are low, with fear of hypoglycaemia commonly cited as one of main barriers to completing regular exercise (Lascar et al., 2014). Insulin dose adjustments and carbohydrate recommendations incorporating both aerobic and anaerobic exercises are in place advising individuals with T1D how to maintain euglycaemia (Riddell et al., 2017). However a high inter-individual variability in the blood glucose response to exercises in individuals with T1D is a major obstacle to providing exercise management support (Riddell et al., 2017) and results in most active individuals having to use a trial and error approach (Dizon et al., 2019). This likely contributes to the lack of a clear benefit of physical activity on HbA1c in T1D (Yardley et al., 2014a, Kennedy et al., 2013, Tonoli et al., 2012). Research into inter-individual variability in glucose during and after exercise is an emerging area (Riddell et al., 2019b, Brockman et al., 2020, Al Khalifah et al., 2016), however no previous research has explored if residual β-cell function has an influence.

One mechanism by which exercise improves cardiovascular risk is the release of endothelial progenitor cells (EPCs) into circulation (Van Craenenbroeck et al., 2008, Ross et al., 2014). These cells have a role in vascular repair, either by differentiating into new endothelial cells and being incorporated into the vascular wall or by secreting pro-angiogenic cytokines (Asahara et al., 1997). It appears that the number and function of EPCs are reduced at rest in most populations with T1D (Sibal et al., 2009). However a cohort of individuals who had a duration of T1D ≥50 years and where an increased proportion retained β-cell function compared to the wider T1D population, were reported to have a count of circulating EPCs similar to age-matched controls without diabetes (Hernandez et al., 2014). Two previous studies have found a blunted exercise response in individuals with T1D, although as they did not find the expected mobilisation in non-diabetic controls it is hard to draw conclusion from these studies (West et al., 2015, Waclawovsky et al., 2015). No previous research has explored if residual β-cell function impacts upon the circulating resting count of these cell or if this play a role in mobilisation with exercise.

The overarching aim of this thesis is to examine the effects of residual β-cell function in individuals with T1D, specifically focussing on its influence on glycaemic control and vascular outcomes in both free-living and laboratory controlled exercise environments.

The thesis is presented in six subsequent chapters. These chapters are detailed as follows:

Chapter 1: This chapter reviews the current literature on the role residual β-cell function plays in T1D, focusing on glycaemic control, exercise and endothelial progenitor cells.

Chapter 2: This chapter details the materials and methods used to collect the data presented in the subsequent studies.

Chapter 3: This chapter presents the study investigating how residual β-cell function impacts free-living glycaemia in individuals with T1D.

Chapter 4: This chapter presents the study investigating how residual β-cell function impacts post-exercise glycaemic control in individuals with T1D.

Chapter 5: This chapter presents the study investigating EPCs and HPCs counts at rest and after acute submaximal exercise in individuals with T1D compared to non-diabetes controls. Individuals with T1D were further grouped and compared by undetectable, low and high C-peptide.

Chapter 6: This chapter discusses the findings presented in this thesis. Conclusions, limitations and future studies are discussed.

Chapter 1. Literature review

1. Chapter 1. Literature review

The following literature review will cover the main research in type 1 diabetes (T1D), glycaemic control at rest and around exercise in T1D, and the cardiovascular protective role that endothelial progenitor cells (EPC) play in T1D. Specifically, it will explore how residual β-cell function influences these aforementioned topics in individuals with T1D.

1.1. Type 1 Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic diseases, that are characterised by glycaemic dysregulation resulting from either defects in insulin secretion, insulin action, or both (American Diabetes, 2010). T1D is an immune-mediated disease originating from the selective destruction of pancreatic, insulin producing β-cells following the infiltration of the islets of Langerhans by immune cells (Anderson and Bluestone, 2005, Bluestone et al., 2010, Willcox et al., 2009). The loss of the β-cells and resulting dysregulation of glucose elicits symptoms of marked hyperglycaemia including polydipsia, polyuria, weight loss and polyphagia, and blurred vision. Acute hyperglycaemia resulting from a serve lack of insulin can also have life threatening consequences if a metabolic state known as ketoacidosis occurs (American Diabetes, 2010). The lack of insulin production results in the immediate need for exogenous insulin replacement to control blood glucose concentration, a therapeutic practice that currently lasts a lifetime to manage the disorder.

1.1.1. Pathogenesis of T1D

The exact pathology of T1D is hard to study as most of the immunological events have occurred before diagnosis (Wållberg and Cooke, 2013). However, efforts to identify individuals at high risk of developing T1D, post-mortems of recent onset patients and animal models have allowed researchers to identify pathways underlying how T1D develops (Campbell-Thompson et al., 2012, Ridgway et al., 2008, Vehik et al., 2013). Firstly, an immune response is elicited against β-cells antigens which develop a strong pro-inflammatory environment. Autoreactive T cells play a dominant role in the initiation and progression of T1D (Figure 1. A-E) (Bluestone et al., 2010). In normal circumstances T cells (T lymphocytes) play a central role in cell mediated immunity, producing cytokines, destroying or helping to produce antibodies to eliminate antigens and autoantigens (Roep, 2003). However in T1D, $CD8^+$ and $CD4^+$ T cells and macrophages become activated in the pancreatic draining lymph nodes and start

surrounding the islet (Piya, 2012), causing chronic inflammation (Bardsley and Want, 2004) (Figure 1. B-C). Once a subsequent trigger occurs the T cells become activated and start destroying the β-cells (Figure 1. C-D) (Pearl-Yafe et al., 2007, Yoon and Jun, 2001). In the insulitis lesion the destruction occurs by a macrophage engulfing and presenting the β-cell antigens that triggers the activation of β-cell cytotoxic $CD8^+$ T cells and autoreactive $CD4^+$ effector T cells (Yoon and Jun, 2001). This leads to the gradual deterioration of the β-cells and a reduction in insulin (Figure 1. E), resulting in glucose dysregulation and the need for exogenous insulin therapy to control blood glucose concentrations (Atkinson and Eisenbarth, 2001).

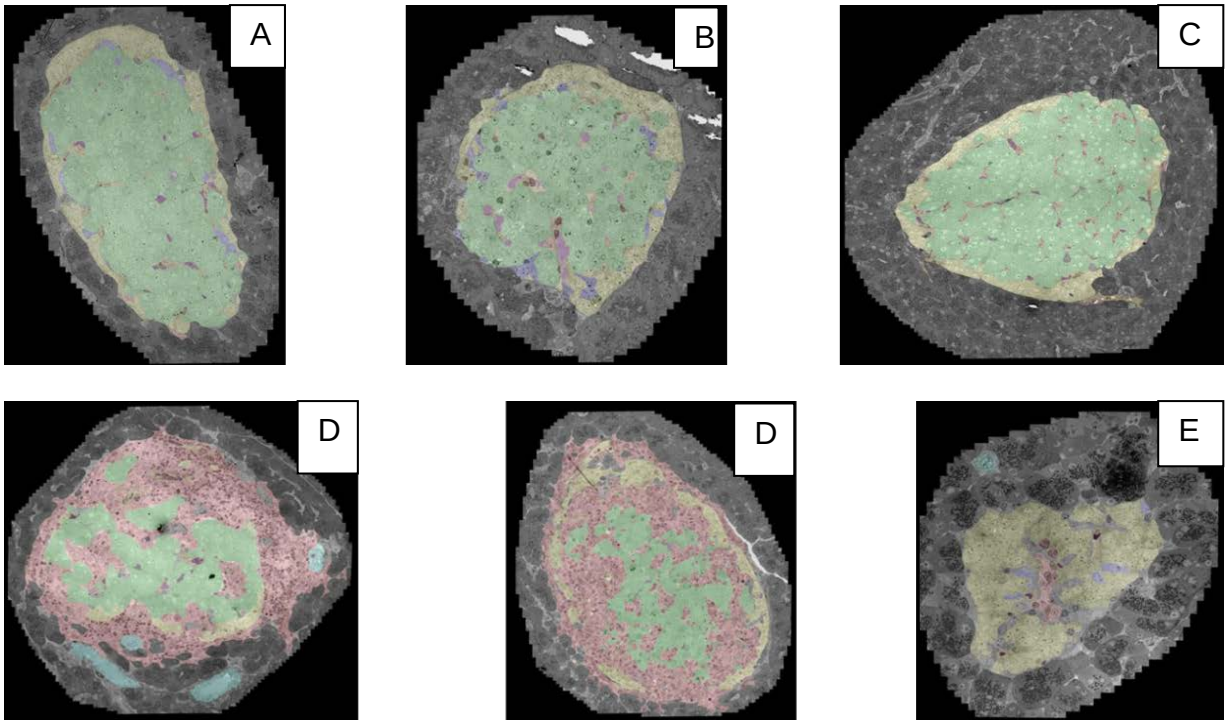

Figure 1.1 Images of the destruction of the tissue, cells and organelles in the islets of Langerhans of Type 1 Diabetic Rats Presented at Macromolecular Resolution. Individual cells are morphologically characterized and false-coloured according to cell-type: beta cell (green), delta cell (blue), alpha cell (yellow), leukocytes (red), vasculature (orange), erythrocyte (magenta) and duct (cyan). ***A*** *- Healthy β-cells with abundant insulin content.* ***B -*** *Healthy β-cells as well as exhausted β-cells with empty granules.* ***C*** *- Infiltration of leukocytes and β-cell phagocytosis.* ***D*** *- Mass endocrine extravasation is present with the majority of β-cells showing compromised morphology.* ***E*** *- End stage diabetes consists of primarily of α-cells, whilst reduced leukocyte count. Images reproduced with permission from Ravelli et al. (2013)*

1.1.2. Aetiology

T1D develops through a complex interaction of strong genetic and environmental influences (Kozhakhmetova and Gillespie, 2015). In the general population, the risk for developing T1D is approximately 0.4% (Jahromi and Eisenbarth, 2006), however for individuals with a relative with T1D the risk is much higher. In studies following

identical twins with evidence of autoimmunity, concordance rates have been estimated from 30 to 50% compared to 10% in dizygotic twins (Olmos et al., 1988, Kaprio et al., 1992, Verge et al., 1995, Hyttinen et al., 2003, Redondo et al., 2001). The risk of a child developing T1D is estimated to be 20% if the child has two first degree relatives with the disease, 8% if they have an affected sibling, 5% and 3 % if the father or mother has T1D respectively (Schenker et al., 1999). This increased risk emphasizes the significance of genetics in T1D.

The effort to determine the genetic mechanisms underlying T1D is challenging and has been ongoing since the 1970s (Cudworth et al., 1979, Cudworth and Woodrow, 1975). The Human Leucocyte Antigen (HLA) region on the short arm of chromosome 6 has been identified as the predominant genetic susceptibility factor for T1D (Ziegler and Nepom, 2010). The HLA region encodes for multiple roles including T cell selection, immune responses and antigen presentation, giving many different ways the HLA can influence the onset and progression of T1D (Kozhakhmetova and Gillespie, 2015). Specifically, the greatest risk of developing T1D is associated with the HLA class II haplotypes DRB1*0301-DQA1*0501-DQ*B10201 and DRB1*0401-DQA1*0301-DQB1*0301, which are linked to approximately 50% of disease heritability in white people (Lambert et al., 2004). As well as increasing risk, there are HLA genotypes that are extremely protective and dramatically reduce the risk of a child developing T1D even when first degree relatives have the disease. Though approximately 50% of heritability for T1D has been accounted for by loci within the HLA, meta-analyses and genome-wide association studies indicate that over 50 additional non-HLA loci are associated (Lee and Hwang, 2019)

The proportion of children with the highest-risk HLA genotype for T1D appears to be dropping. Two studies found that the high risk HLA DR and DQ class II loci was significant lower in children diagnosed between 1985 and 2002 and those diagnosed between 1920 and 1965 in the UK and Finland (Gillespie et al., 2004, Hermann et al., 2003). However, as both studies compared recently diagnosed subjects to previous eras when life expectancy for an individual with T1D was significantly lower than it is today, it relies on the HLA profiles of the older cohorts to represent the whole population of children with diabetes in the past. Interestingly the Gillespie et al. (2004) study found the proportion of lower risk genotypes to have increased.

The decreasing frequency of high risk HLA haplotypes combined with the increasing incidences of T1D (see 1.5), suggests that the environment is playing an increased role in the development of T1D (Gillespie et al., 2004, Fourlanos et al., 2008). A host of environmental factors (Knip and Simell, 2012) have been proposed to involved in triggering and increasing the incidences of T1D. These include; enterovirus infections and/or the increased immune responsiveness to a enterovirus infection (Yeung et al., 2011, Kostraba et al., 1993), diet (consumption of cow milk (Kostraba et al., 1993), early and late exposure to cereals (Norris et al., 2003, Ziegler et al., 2003), low vitamin D (Eva, 1999), the gut microbiome (Wen et al., 2008, Sysi-Aho et al., 2011), the hygiene hypothesis (Bach and Chatenoud, 2012), and seasonal changes (Karvonen et al., 2000, Knip et al., 2005).

1.1.3. Epidemiology

Within the UK, T1D affects around 10% of the estimated 4.5 million individuals with diabetes (Primary Care Domain, 2016). It is the most common endocrine disorder in children with 24.5 per 100,000 children aged 0 to 14 within the UK diagnosed with the condition every year (International Diabetes Federation, 2015). Around the world, the incidences of T1D differentiate dramatically, with the highest rate of 40 diagnoses per 100,000 children in Finland compared to 0.1 per thousand in China (Karvonen et al., 2000). While originally called juvenile diabetes and thought mostly to occur in individuals below the age of 20 (Daneman, 2006), it can occur at any age. Bruno et al. (2005) demonstrated that 13% of new cases of T1D were in individuals aged between 30-50, while Thomas et al. (2018) recently suggested that using a novel genomic method over 42% of T1D incidences occur after age 30. Diagnosis of T1D in adults can be challenging, and it's estimated that 5-15% of adults diagnosed with type 2 diabetes may actually have T1D with islet autoantibodies present (Turner et al., 1997, Tuomi, 2005). Thomas et al. (2019) demonstrated that a large proportion (38%) of patients with T1D diagnosed after 30 years and severe endogenous insulin deficiency are frequently diagnosed and treated as having type 2 diabetes without commencement of insulin at diagnosis. Thus, the number of individuals with T1D may be vastly underestimated.

The incidence of T1D has dramatically risen worldwide in recent decades with a current increase of around 3% a year. The increase in incidence rates has not occurred equally across all age groups, with the largest increase being in children under 5 years

old (Patterson *et al*., 2009). If current trends continue there will be a doubling of new cases in under 5 year olds in Europe between 2005 and 2020 and a 70% increase in under 15 year olds (Patterson et al., 2009). Interestingly, Sweden is the only country not to show this trend with incidence rates having plateaued in recent years (Berhan et al., 2011).

Unlike most other autoimmune diseases where females have a greater risk of developing, T1D does not have female bias with girls and boys equally affected by T1D in younger age groups (Soltesz et al., 2007). However in regions with a high incidence of T1D (European populations), greater number of males develop the disease compared to females, whereas in regions with low incidences this trend is reversed (Green et al., 1992, Karvonen et al., 1997).

1.1.4. Diagnosis of T1D

Diagnosis of diabetes is based upon a fasting blood glucose concentration >7.0 mmol/L, a random blood glucose concentration >11.1 mmol/L with symptoms, or an abnormal oral glucose tolerance test. A diagnosis can also be made upon a glycated haemoglobin (HbA1c) concentration above 48 mmol/mol, however as T1D often has a rapid onset, HbA1c can be less sensitive for the correct diagnosis (American Diabetes Association, 2018).

Common symptoms around diagnosis include polyuria (excessive urine production), polydipsia (excessive thirst), and weight loss (DiMeglio et al., 2018). Approximately a third of patients present with diabetic ketoacidosis (Dabelea et al., 2014), a serious and life-threatening medical condition in which the concentrations of the ketone bodies are markedly higher than normal, which is accompanied by acidosis (fall in blood pH) (Green and Bishop, 2019). The presentation of symptoms is more varied in adults diagnosed with T1D, showing deviation from the classical phenotype without weight loss, thirst and polyuria, likely due to a slower progression towards insulin dependence (Merger et al., 2013).

As no single clinical feature can distinguish T1D from other classifications of diabetes at diagnosis, other clinical features such as time to insulin treatment initiation, BMI and age of diagnosis, and biomarkers such as C-peptide and pancreatic autoantibodies, are needed to accurately diagnose individuals (Shields et al., 2015)

1.1.5. Autoantibodies of T1D

During the progression of T1D the first detectable sign is of an ongoing autoimmune attack is the appearance of autoantibodies. Autoantibodies are antibodies that react with self-molecules within an individual (Elkon and Casali, 2008), that within individuals developing T1D mark the destruction of the β-cells within the pancreas (Taplin and Barker, 2008). The main autoantibodies identified in T1D are; glutamate decarboxylase (GADA), insulinoma-associated antigen-2 (IA2A), antibodies to insulin (IAA), and zinc transporter 8 (ZnT8) (Wenzlau et al., 2007). Roughly 90% of newly diagnosed patients have at least one islet autoantibody (Verge et al., 1996b, Taplin and Barker, 2008), compared to around 1-2% of general non diabetes population (Sørgjerd et al., 2015). The development of T1D autoantibodies usually precede the clinical diagnosis of diabetes by years and can appear as early as the first year of life (Barker et al., 2004). The greater number of autoantibodies an individual has, the greater the likelihood of developing T1D (Orban et al., 2009), with the islet cell antigens appearing sequentially rather than simultaneously (Yu et al., 1996). While the appearance of multiple autoantibodies plays a role in the rate of development of T1D (Verge et al., 1996a, Bingley et al., 1994), it cannot completely explain the variation seen in the varying rates from autoimmunity to the diagnosis of clinical diabetes (Achenbach et al., 2013) .

1.1.6. Treatment of T1D with exogenous insulin and technology

Insulin treatment is the mainstay of therapy for individuals with T1D, and is designed to mimic normal insulin secretion from a healthy pancreas (Bolli, 2006). Optimal glycaemic control requires an insulin regiment that mimics physiological release by providing a basal long lasting background insulin that works overnight and in pre-prandial periods, and a bolus rapid-acting insulin to control post-prandial glucose events, carbohydrate intake and hyperglycaemia (DiMeglio et al., 2018). Currently insulin therapy is administrated through two methods, multiple daily injections (MDI) where basal and bolus insulin is injected with an insulin pen at different points throughout a day, or with continuous subcutaneous insulin infusion (CSII), where an insulin pump device delivers a constant continuous infusion of a short-acting insulin (Pańkowska et al., 2009).

The basal insulin analogue (such as insulin glargine and determir) is designed to provide a continuous and stable amount of insulin into the blood stream with no peak,

and have a reproducible absorption and little day to day variation in blood glucose lowering ability (Barnett, 2003). The constant circulation of insulin in post-prandial and overnight fasting periods is there to supress excessive postprandial hepatic gluconeogenesis and glycogenolysis and promote glycogenesis (Aronoff et al., 2004), as well as suppress excessive lipolysis and the development of ketogenisis (Owens et al., 2014). Postprandial glucose excursions are controlled by bolus rapid acting insulin analogue such as Aspart, Glulisine or Lispro. They are quickly absorbed and peak within 30-90 minutes following injection (Abiola et al., 2016).

With the publication of the Diabetes Control and Complication Trial in 1993 (Nathan and Group, 1993) and the observational follow up studies (Epidemiology of Diabetes Interventions and Complications (EDIC)) (Nathan and Group, 2014), it became clear that achieving and maintaining tight glucose concentrations, as close as possible to those seen in non-diabetic individual, leads to reductions in diabetes complications and cardiovascular disease (see 1.2 for further details). As nicely detailed in the blog post “42 Factors That Affect Blood Glucose?!” (Brown), managing blood glucose, especially in a tightly controlled manner, is a highly complex procedure. Variables as diverse as food, physical activity, illness, medication and sleep all individually influence blood glucose concentration. Appropriate insulin intake to return glucose to euglycaemia without inducing hypoglycaemia is required whenever food is consumed, with patients having to calculate the dose of rapid acting insulin on the amount of carbohydrates consumed and their sensitivity to the insulin (Mansell, 2012). Even with frequent blood glucose measurements most individuals with T1D fail to meet the recommended HbA1c (Coster et al., 2000), with only 29.9% of individuals with T1D in England and Wales meeting the old NICE recommendations of a HbA1c ≤58 mmol/mol in 2017 to 2018 (NHS Digital, 2019).

Over the last decade, minimally invasive continuous glucose monitoring (CGM) devices that allow examination of glucose concentration patterns over time have been developed. CGMs have a similar accuracy to capillary blood glucose monitors (Bailey et al., 2015), and the ability to warn users on the basis of current or projected glucose concentrations, with use associated with reducing risk of hypoglycaemia and hyperglycaemia, glycaemic variability and improved quality of life (Rodbard, 2017). The accuracy of CGM devices has allowed the development of hybrid closed-loop insulin-pump systems that automatically regulate basal infusion rates and has the

ability to suspend insulin infusion when hypoglycaemia is detected, although pre-prandial insulin dosing is still required (Bally et al., 2017). A true artificial pancreas system that does not require active adjustment of insulin dosing is the next step with several different systems currently being tested (Hanazaki et al., 2016). Despite challenges, including CGM accuracy and lag time especially around exercise, the artificial pancreas and novel β-cell replacement therapies are seen as promising to delivering optimal glycaemic control for individuals with T1D in the future (Nijhoff and de Koning, 2018).

1.2. Complications of T1D

With the discovery of insulin in 1922 by Banting and Best (Karamitsos, 2011), T1D progressed from being a terminal disease to one that could be treated and managed. Despite the vast technological and educational advances over the following century, T1D has array of complications include acute potentially life-threatening complications and damage to small and large blood vessels. While intensive glycaemic control reduces the risk of developing micro and macrovascular diabetes complications in the long term, it increases the likelihood of acute hypoglycaemia (Nathan and Group, 1993). Acute complications such as severe hypoglycaemia and ketoacidosis, can result in loss of consciousness or seizures that require medical assistance and hospital visits, and account for 4 to 10% and 13 to 19% of T1D related mortality, respectively (DiMeglio et al., 2018, Skrivarhaug et al., 2006, Patterson et al., 2007, Feltbower et al., 2008).

1.2.1. Vascular damage

Several risk factors are implicated in the pathogenesis of vascular damage resulting in diabetes complications, including modifiable factors such as; glycaemic control, lipid levels, blood pressure, diet, physical activity and smoking, and non-modifiable factors including; duration of diabetes, age at diagnosis and genetics (Chiarelli and Marcovecchio, 2013). Hyperglycaemia is the initiating and primary cause of diabetic tissue damage, with both acute and chronic hyperglycaemia having detrimental effects (Marcovecchio et al., 2011). Several mechanisms link hyperglycaemia to the development of vascular damage including; activation of protein kinase C, increased production of advanced glycation end-products, synthesis of growth factors, cytokines and angiotensin II, increased oxidative stress, and activation of the polyol and hexosamine pathways (Brownlee, 2005). These mechanisms induce endothelial

dysfunction and contribute to the development of micro- and macrovascular complications and damage to organs (Domingueti et al., 2016, Forbes and Cooper, 2013). As such, a reduction in HbA1c results in reduced vascular diabetes complications (Nathan and Group, 1993).

Glucose fluctuations, also known as glycaemic variation (GV), also appear to be an important risk factor for developing vascular damage (Zhang et al., 2019) and may be more harmful than a constant state of hyperglycaemia (Ceriello and Kilpatrick, 2013). GV appears to cause excessive oxidative stress and inflammation, activating downstream signalling pathways, including AKT, PKC, and MAPK, which damage vascular tissue and increase the likelihood of complications (Cavalot, 2013). There is also limited evidence that hypoglycaemia is associated with endothelial dysfunction, oxidative stress and inflammation (Ceriello et al., 2012).

In well controlled T1D, lipid levels are similar to non-diabetic individuals (Group, 1992), but worse glycaemic control in associated with a more atherogenic cholesterol distribution (Pérez et al., 2000, Maahs et al., 2010). Additionally, dysglycaemia can lead to increased apolipoprotein B levels and oxidative stress, leading to increased vascular damage (Shamir et al., 2008). Metabolic and hormonal changes also be dysfunctional and play a role in the pathophysiology of atherosclerosis (Ladeia et al., 2014). Decreased insulin deficiency reduces production of nitric oxide, resulting in increased oxidative stress and a consequent decrease in the ability to promote vessel dilation (Baron, 1994, Scherrer et al., 1994).

Individuals with diabetes are at a high risk of developing atherosclerosis (Stehouwer et al., 1992). The atherosclerosis plaque in individuals with T1D may be softer, more fibrous and in different locations compared to individuals with type 2 diabetes, resulting in an overall lower atherosclerotic burden (Djaberi et al., 2009, Burke et al., 2004). However compared to non-diabetes age and symptom matched healthy controls, individuals with T1D were significantly more likely to have severe narrowing in all three major coronary arteries (Valsania et al., 1991).

1.2.2. Microvascular damage

Retinopathy, neuropathy and nephropathy are the three major manifestations of microvascular disease, although any microvasculature vessel can sustain damage and result in complications. Large cross-sectional and longitudinal studies in Europe

and the UK demonstrate that at a duration of 15 years early renal disease had a prevalence of 30.6%, retinopathy 46% and impaired autonomic tests 19%, while 37% adults aged 28–37 years and diagnosed with T1D during childhood had serious microvascular complications (Bryden et al., 2003, Stephenson et al., 1994).

Damage to the microvasculature of the kidney is a common complication affecting around 15 to 40% of patients with T1D, peaking in incidences 15 to 20 years after diagnosis, and is the leading cause of end-stage renal disease and cardiovascular morbidity and mortality (Gross et al., 2005). Individuals with both T1D and diabetic nephropathy have a greater than 10-fold risk of CVD than individuals with T1D alone (Tuomilehto et al., 1998).

Vasculature damage in the eye, retinopathy, begins with non-proliferative retinal abnormalities where vascular obstruction, haemorrhages, exudates and infarction of retinal nerves cause cotton wool spots. As damage progresses retinopathy becomes proliferative, with ischemia leading to the development of new vessels (neovascularization) on the surface of the retina and optic disc which can threaten vision by causing retinal detachment or vitreous haemorrhage (Gardner et al., 2002). Nearly all individuals with T1D will have some degree of diabetes retinopathy after 20 years duration (Fong et al., 2004), with diabetes being the most prevalent cause of sight loss in 20 to 74 year olds (Control and Prevention, 2011).

Diabetic neuropathy is the damage of peripheral small sensory and large motor nerve fibres, resulting in symptoms such as a burning, deep-aching pain and impaired vibration, light touch and joint position senses (Boulton et al., 2005). Some degree of nerve damage affects approximately 60–70% of adult T1D patients (Trotta et al., 2004), although up to 50% of diabetic neuropathy may be asymptomatic increasing the risk of injuries to the feet (Boulton et al., 2005).

Poor glycaemic control is the major risk factor for the development of microvascular complications (Schrijvers et al., 2004). The DCCT and EDIC established that intensive glycaemic control reduced the risk of developing and progression of retinopathy, neuropathy and nephropathy (Nathan and Group, 1993, Steffes et al., 2003b). The intensive insulin therapy group had a 76% reduced risk of developing retinopathy and slowed the rate by 54%, reduced the occurrence of nephropathy by 39% and clinical

neuropathy by 60% when compared with the conventional treatment group (Nathan and Group, 1993, Steffes et al., 2003b).

1.2.3. Mortality and Cardiovascular Disease

T1D is associated with a 2- to 8-fold increase in mortality rates compared with the general population (Rawshani et al., 2017, Lind et al., 2014, Soedamah-Muthu et al., 2006). While, in developed nations, advancement in care has resulted in temporal improvements in the relative risk of death (Secrest et al., 2010, Rawshani et al., 2017), with one study estimating that T1D results in estimated reduction in life expectancy of 11.1 years (Livingstone et al., 2015). For individuals under 30 years of age, acute complications such as ketoacidosis and hypoglycaemia largely explain increased mortality, while at older ages, cardiovascular disease (CVD) is the main cause of morbidity and death (Figure 1.2) (Secrest et al., 2010, De Ferranti et al., 2014). Clinical trial data from the DCCT (Gubitosi-Klug, 2016, Bebu et al., 2020) and cohort studies such as Lind et al. (2014) demonstrate that improved glycaemic control in patients reduces the incidence of and mortality caused by CVD, encompassing coronary heart disease (CHD), cerebrovascular disease, peripheral artery disease (PAD), heart failure and cardiomyopathy. CVD risk factors such as chronic inflammation, hypertension, dyslipidaemia, dysglycaemia (including hyperglycaemia, hypoglycaemia and glycaemic variability) are all more common in patients with T1D and considerable research focus has been put on management of these risk factors (Schofield et al., 2019). The risk of CVD does not appear to be as attenuated by intensive glycaemic control as microvascular complications (DiMeglio et al., 2018). Even in carefully managed individuals with risk factors within target levels, the incidences of CVD and mortality are still elevated (Rawshani et al., 2017), with a longer duration of diabetes (Al Khalifah et al., 2016, Conway et al., 2009) and early age of diagnosis (Rawshani et al., 2018) associated with increased risk. Additionally, the protection against CVD offered by the female sex is lost (Harjutsalo et al., 2014, Huxley et al., 2015).

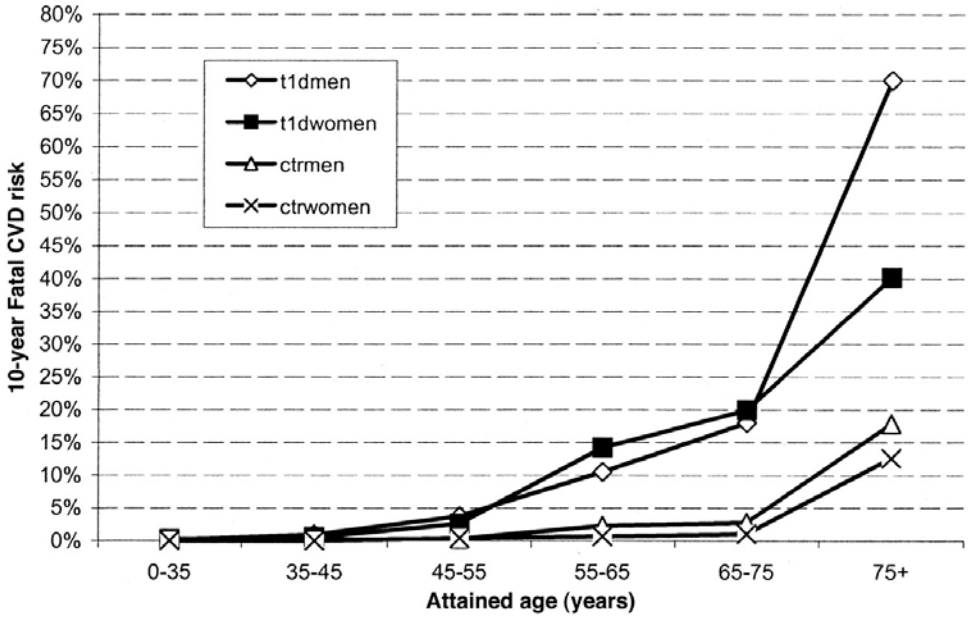

Figure 1.2 Estimated 10-year fatal CVD risk by current age in T1D men and women compared with nondiabetic comparison group. Images reproduced with permission (appendix I) from Soedamah-Muthu et al. (2006)

1.3. Residual β-cell function measurement in T1D

Measuring endogenous insulin production by the residual β-cells has increased in clinical practice within recent years (Jones and Hattersley, 2013), with a specific focus on correctly diagnosing an individual's diabetes to ensure most appropriate treatment and management is provided. An alternative measure of residual β-cell function is needed besides serum insulin for individuals diagnosed with T1D, as exogenous insulin is detected by insulin assays (Clark, 1999). Additionally, insulin has a half-life of only a few minutes (3 to 5 minutes), and is extensively metabolized on the first pass of the liver (≈50%) with varying rates of clearance in the liver and peripheral tissue (Brundin, 1999, Field, 1973, Licinio-Paixao et al., 1986, Polonsky et al., 1986).

C-peptide is an appropriate alternative to measure insulin secretion. As detailed below (Figure 1.3), insulin is produced in pancreatic β-cells within the islets of Langerhans. Here, C-peptide role is promote the biosynthesis of insulin by promoting folding and the bonds between the A and B chains to create proinsulin (Steiner et al., 1967). Proinsulin is mostly cleaved into insulin and C-peptide, which are secreted at the same time and in equimolar amounts by the pancreas into the bloodstream, although some proinsulin is secreted as well (Kitabchi, 1977). Peripheral C-peptide levels are a more

accurate reflection of portal insulin secretion than measurement of peripheral insulin, even in non-insulin treated individuals. This is due to a much longer half-life of 30 minutes than insulin, and that it passes through the liver before the majority is metabolized by the kidneys, with 5 to 10% excreted in urine (McDonald et al., 2009, Horwitz et al., 1977).

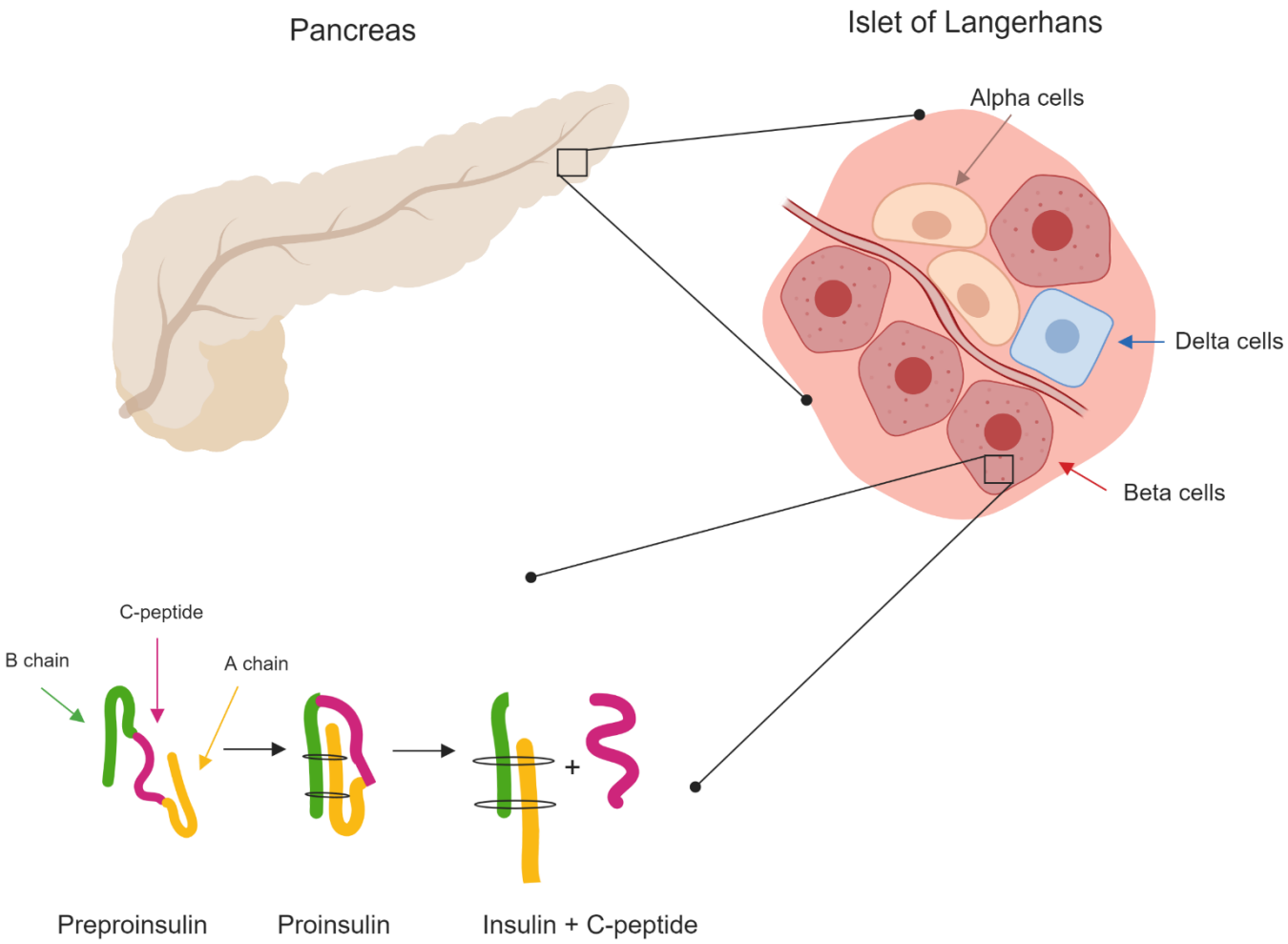

Figure 1.3 Schematic of insulin and C-peptide creation and secretion in the pancreatic β-cells

Serum C-peptide level following a stimulus such as a mixed meal tolerance test (MMTT), intravenous glucose tolerance test (IVGTT), or stimulation by arginine or glucagon provides a gold-standard measure of endogenous insulin secretion (Palmer et al., 2004, Mirel et al., 1980, Ludvigsson, 1983, Ludvigsson, 2016). However, they are impractical for clinical use because of extended time frame, numerous blood draws

and side effects such as nausea. Alternatives include a fasting and non-fasting blood samples, which are insufficient to detect subtle rises in C-peptide and may require further testing if inconclusive results are returned. Urine C-peptide (UCP) can be used as simple non-invasive measure of β-cell function. It allows samples to be provided without outside assistance in an outpatient setting and is stable in urine for up to 72 h in boric acid preservative (Bowman et al., 2012). Two methods of measuring UCP have been developed, the twenty-four hour UCP and urinary C-peptide to creatinine ratio (UCPCR). The twenty-four hour UCP is time-consuming and inconvenient for patients (Aoki, 1991). The UCPCR is a spot test method that uses creatinine to correct for dilution and correlates well with 24hr UCP (McDonald et al., 2009) and 90 minute post MMTT serum values in a range of different populations with diabetes (Hope et al., 2016, Besser et al., 2011a).

1.3.1. C-peptide and classification of diabetes

Residual β-cell function, as measured by C-peptide, has been shown to be a sensitive and specific biomarker for discriminating between type 1 and type 2 diabetes and Maturity Onset Diabetes of the Young (MODY) (Besser et al., 2013, Besser et al., 2011b, Shields et al., 2017). A stimulated values <200 pmol/L of C-peptide indicates a severe insulin deficiency and 96% sensitivity and 98% specificity in differentiating T1D from T2D or MODY at 5 years post diagnosis (Besser et al., 2011b). Individuals with 200 to 600 pmol/L of C-peptide also likely need insulin management, although there is the possibility they have a MODY. Finally a value >600 pmol/L of C-peptide at >5 years post diagnosis is unlikely to be T1D (Besser et al., 2011b).

When screening their whole clinic, Western General Hospital in Edinburgh discovered that of the 953 individuals diagnosed with T1D for ≥3 years duration, 13.7% had a C-peptide >200 pmol/L who were later screened for autoantibodies and genetically tested. Of the 70 who had a C-peptide 200-600 pmol/L, eight were reclassified as T2D and four with monogenic diabetes. Five of the 19 individuals with a C-peptide 600-900 pmol/L were reclassified as T2D, with 2 monogenic diabetes, while 13 of the 14 patients with a C-peptide >900 were reclassified with T2D (Wise, 2019, Foteinopoulou et al., 2019). With the high proportion of individuals who are misdiagnosed with T2D diagnosed especially in individuals who develop T1D after the age of 30, it is likely that similar results would be seen in if the clinically diagnosed T2D population was screened for C-peptide (Thomas et al., 2019).

1.3.2. β-Cell destruction and the clinical presentation in T1D

It is often stated that approximately 90-95% of an individual's β-cells have been destroyed by the occurrence of symptoms and a clinical diagnosis of T1D (Gepts, 1965, Daaboul and Schatz, 2003). However, autopsies of individuals with recent onset T1D show that diagnosis can also occur when around two-thirds of the islets are devoid of the insulin producing β-cells (Foulis and Stewart, 1984, Willcox et al., 2009). The destruction of β-cell in the pre-diabetes phase was originally thought to be linear (Figure 1.4) (Eisenbarth, 1986b), however the consensus is now a relapsing and remitting progression of disease pathogenesis. β-cell destruction can take weeks to several decades before the clinical manifestation of the symptoms occurs, with the major reduction of beta cell mass occurring late during in the pre-diabetic phase (Chen et al., 2017, Von Herrath et al., 2007, Ziegler et al., 2013). Individuals who are older or have a higher BMI are likely to have greater amount of functioning β-cell at diagnosis (Leete et al., 2016, Thunander et al., 2012).

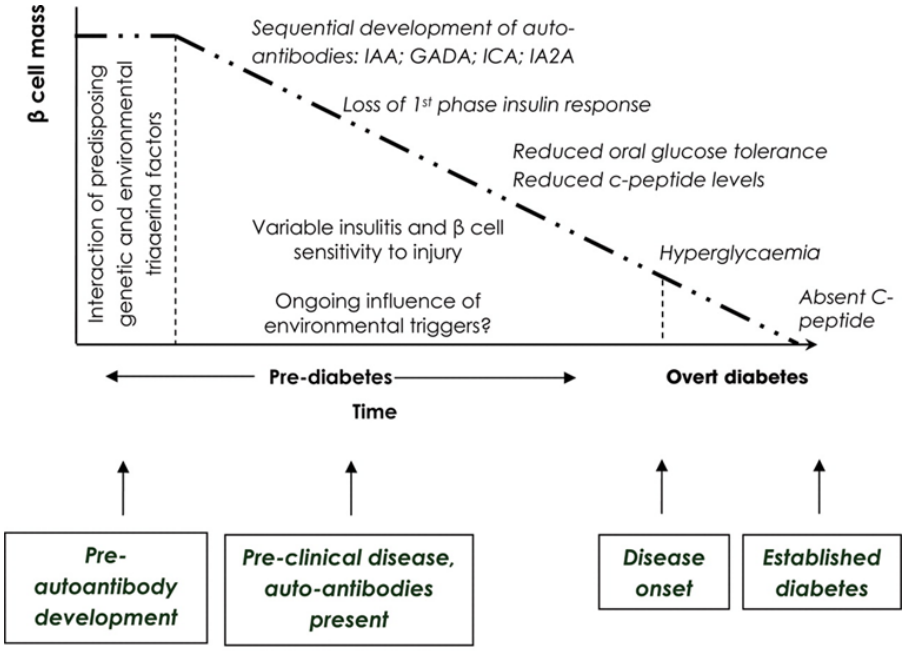

Figure 1.4 The natural history of type 1 diabetes. Images reproduced with permission (appendix I) from Thrower and Bingley (2011), adapted from the model proposed by Eisenbarth (1986a).

Progression of β-cell destruction carries on after diagnosis; however a remission or honeymoon period can often take place with the initiation of insulin therapy (Sochett

et al., 1987). It is hypothesised that the reduction in blood glucose following the starting of exogenous insulin treatment allows the restoration of endogenous insulin secretion in exhausted but not yet destroyed β-cells. The honeymoon partial remission phase is common, occurring in roughly 70% of cases and lasting an average of 7 months before the continued reduction in β-cell mass and increased amount of exogenous insulin is needed (Abdul-Rasoul et al., 2006, Snorgaard et al., 1992).

1.3.3. Residual β-Cell in long duration T1D

While the traditional model of the natural history of T1D (Figure 1.4) proposed by Dr Eisenbarth (1986b) describes a complete and inevitable annihilation of functioning β-cell mass with no present C-peptide, evidence of residual β-cell function is present even decades after diagnosis.

Histological analysis of donor pancreases exhibit the persistence of insulin-positive islets. Evidence from as far back as 1959 has demonstrated that individuals who had duration of T1D of 8.3 years still had some residual islet tissue (Maclean and Ogilvie, 1959). Similar results are seen elsewhere with a significant proportion of autopsies of long duration T1D (>3 years) having identifiable, yet markedly reduced β-cells which positively stain for insulin (Gepts and De Mey, 1978, Löhr and Klöppel, 1987, Meier et al., 2005, Rodriguez-Calvo et al., 2018, Lam et al., 2017, Butler et al., 2007). For example, a recent analysis of 47 donors with a duration of diabetes ranging from 0 to 41 years found that 67% of donors had insulin containing islets, with a total β-cell mass reduced by 88 to 95% (Lam et al., 2017). See Oram et al. (2019) for a detailed review.

These small number of identifiable β-cell in long duration T1D can also secrete endogenous insulin, with evidence that even at very low levels that they are functional and increase in response to caloric intake (Oram et al., 2014, McGee et al., 2014). Similar to the histological data, C-peptide secretion has been known about for decades, with Madsbad et al. (1978) finding 15% of individuals with a duration of T1D of 15 to 35 years having detectable levels. However, with the improvements in the sensitivity, specificity and usability of C-peptide testing, there has been renewed interest within the area in recent years. The development of highly sensitive C-peptide assays that dropped the lower limit of detection from 30 to 3 pmol/L (Oram et

al., 2014, Wang et al., 2012) has led to large cohorts of people with extended duration T1D being tested. Substantial numbers of this population have been found to have detectable residual β-cell function although the prevalence remains unclear. Estimates range from 17% (McGee et al., 2014), 23% or 40% (Davis et al., 2015), 35% (Williams et al., 2016), 36% (McKeigue et al., 2019), 63% (Wang et al., 2012), 73% (Oram et al., 2014) to 80% (Oram et al., 2015) of participants having detectable C-peptide at >5 years post-diagnosis (Williams et al., 2016, Oram et al., 2015). The wide variation in these studies is likely explained by the selection criteria for the cohort selection criteria, especially age, age of diagnosis and duration of diabetes, and sample storage and differing assay sensitivities (Oram et al., 2019).

An analysis of individuals with >50 years of T1D, the Joslin Medalists population, highlighted persistent C-peptide (>30 pmol/l) in 67.4% of the cohort and insulin positive β-cell in nine medallist who had post-mortem examination of their pancreases (Keenan et al., 2010). While this study has potential survivor bias (see 1.3.4 for persistent residual β-cells influence upon clinical and glycaemic outcomes), it highlights that even in ultra-long duration T1D C-peptide persistence is possible. When this cohort was followed up by Yu et al. (2019), only 32.4% retained detectable C-peptide levels, with levels increasing in 12.2% and decreasing in 37% of the participants. This waxing and waning of C-peptide suggests β-cell loss may be episodic and recovery of some mass possible. Interestingly, when screened for monogenic diabetes 27.5% of the cohort possessed rare variants, with it likely that for 7.9% of the cohort their diabetes was caused by pathogenic variants. This further highlights the difficulty in diagnosing diabetes (see 1.3.1).

An individual's remaining residual β-cell function appears to be mainly influenced by the duration of their T1D and age of diagnosis. In longitudinal studies, C-peptide has an initial exponential decline (Greenbaum et al., 2012, Hao et al., 2016, Dabelea et al., 2012), with some early evidence suggesting that after 7 years this phase is followed by a stabilising of residual β-cell function (Shields et al., 2018). It is unknown if this is because of a change in the immune response or if this population of remaining β-cells is somehow protected. Persistent C-peptide secretion is much more common in individuals diagnosed at an older age, with only 19% of those diagnosed before the age of 15 having detectable levels at 15 years post diagnosis compared to 42% of individuals diagnosed after 35 years (McKeigue et al., 2019).

Some evidence suggests that this may because of a less severe rate of decline in β-cell mass in older individuals (Greenbaum et al., 2012, Barker et al., 2014), although other studies find conflicting results (Shields et al., 2018, Hao et al., 2016). The fact that younger children have less β-cell mass than adults, meaning there is less to be destroyed, may also play a role in the reduced C-peptide seen in those diagnosed at younger ages (Davis et al., 2015). C-peptide persistence appears to be influenced by HLA haplotypes that differ from those that increase risk of an early-onset T1D, with known risk loci for T1D only accounting for a small proportion of the genetic effects on persistence (McKeigue et al., 2019). A recent paper by Leete et al. (2020) along with their previous research (Arif et al., 2014, Leete et al., 2016), suggests there may be two distinct endotypes that segregate according to age at diagnosis (<7 years and >12 years), with differing immune responses that are distinguishable by histology. The patients who were diagnosed at <7 years had much lower stimulated C-peptide levels than patients diagnosed at ≥13 years at > 5 years post diagnosis.

1.3.4. Residual β-Cell and diabetes complications

The Diabetes Control and Complications Trial (DCCT) established the initial evidence that persistent C-peptide secretion has clinical benefits in individuals with T1D (Steffes et al., 2003a). A retrospective analysis showed that in participants who had a stimulated C-peptide >200 pmol/L at study entry and who were randomised into the intensive control arm (3+ insulin injections a day (Nathan and Group, 1993)) had reduced development of retinopathy and nephropathy, lower HbA1c and lower incidences of serve hypoglycaemia in the subsequent 7 year follow up period compared to those with <200 pmol/L (Lachin et al., 2014, Steffes et al., 2003a). Specifically, individuals with sustained C-peptide of >200 pmol/L had a reduced risk of retinopathy by 58%, sustained retinopathy by 79% and neuropathy by 43% in the follow up period. HbA1c was an average of 6.6 mmol/mol lower for the responders and they had a 45% lower risk of an episode of severe hypoglycaemia. In the general T1D population, it is estimated that 8-16% of individuals diagnosed as an adult have this relatively high C-peptide level, compared to 5-6% of individuals with childhood onset of diabetes (Oram et al., 2015, Davis et al., 2015, Williams et al., 2016).

Further analysis on the DCCT cohort and other research has demonstrated that even much lower levels of C-peptide secretion have a have positive impact on rates of diabetic complications, with an apparent sustained positive relationship between the

variables (Panero et al., 2009, Lachin et al., 2014, Sjöberg et al., 1991). A cross-sectional study by Kuhtreiber et al. (2015) showed that a C-peptide level >10 pmol/l was associated with protection from the onset of diabetes-specific complications, with individuals below 10 pmol/L 3.1 times more likely to develop retinopathy, foot ulcer amputations, neuropathy or kidney disease. These results are similar to those found by Almeida et al. (2013) in another cross sectional study, where a detectable C-peptide >2 pmol/L was linked to a lower frequency of chronic complications (3.7% vs. 21.7%, $p=0.029$).

Currently there has been no studies investigating if residual β-cell function influences CVD risk in individuals with T1D. However, as detailed in chapters 1.3.5 and 1.2.3, endogenous insulin secretion can improve glycaemic control and subsequent improved glycaemic control can reduce the risk of CVD, showing a clear pathway that residual β-cell function may influence CVD. Additionally, as detailed in chapter 1.3.6, C-peptide itself may be a biologically active peptide that has vasoprotective properties, helping to reduce the damaging signalling pathways that dysglycaemia can cause to endothelial cells and potentially reducing vascular damage. In a large nationwide, register-based cohort study carried out in Sweden by Rawshani et al. (2018), they reported a significantly increased risk of mortality and cardiovascular disease in 27195 individuals with T1D compared with 135178 matched non-diabetic controls. There main findings from the study was that age of onset of T1D is an important determinant of survival and cardiovascular outcomes, independent of the duration of the diabetes and age. Individuals diagnosed at 0–10 years had a greater than five times increased risk of CVD than those diagnosed at 25-30 years, which contributed to a shorter life expectancy (a loss of 17.7 years for women and 14.2 years for men) than being diagnosed at 26–30 years (loss of 10.1 years for women and 9.4 years for men). While not measured, having detectable C-peptide is also heavily influenced by age of diagnosis, and could account for increased cardiovascular risk.

1.3.5. Residual β-Cell and glycaemic control

Evidence of residual β-cell impact upon glycaemic control within individuals with T1D is an emerging area. Preservation of 10-50 pmol/L C-peptide has also been associated with lower self-reported rates of symptomatic, asymptomatic and severe hypoglycaemia compared to C-peptide negative individuals (Kuhtreiber et al., 2015, Marren et al., 2019). Those with persistent β-cell function appear to have a residual

counter-regulatory responses to hypoglycaemia, including increased glucagon secretion (Zenz et al., 2018, Rickels et al., 2020), and endogenous glucose production (Zenz et al., 2018). However, these results are not replicated everywhere, with Sherr et al. (2013) finding no relationship between C-peptide and glucagon response during a hypoglycaemic clamp, while Rickels et al. (2020) and Madsbad et al. (1978) found no increase in endogenous glucose production in those with higher C-peptide. There is, however, a reduction in biochemical hypoglycaemia and an increase in glucagon response to hypoglycaemic clamp in C-peptide positive islet transplant recipients (Brooks et al., 2015). The α-cell's ability to secrete glucagon in response to hypoglycaemia is impaired around diagnosis of T1D (Arbelaez et al., 2014), with further functional losses observed as duration of diabetes increases (Siafarikas et al., 2012). It is hypothesized that functioning β-cells within the islet of Langerhans protect some residual α-cell function which may offer some hypoglycaemia protection, although underlying mechanisms remain unclear (McCrimmon and Sherwin, 2010).

While the DCCT (Lachin et al., 2014, Steffes et al., 2003) and Kuhtreiber et al. (2015) trials show that higher C-peptide is associated with lower HbA1c, Marren et al. (2019) and Gibb et al. (2020) recently found no difference in between individuals with low (<10 pmol/L) and preserved (>20 pmol/L and >10 pmol/L, respectively) C-peptide within the UK T1D population. The authors postulate that the lack of relationship between C-peptide and HbA1c may be a failure to have intense glycaemic management in individuals who would be at lower risk of hypoglycaemia. Both the Lachin et al. (2014) and Kuhtreiber et al. (2015) studies had significantly lower mean HbA1c than those found in Marren et al. (2019) and Gibb et al. (2020), and currently in the UK T1D population (Primary Care Domain, 2016) .

There is scarce research upon preserved C-peptide secretion influence upon CGM parameters. In the recently diagnosed, tracked from diagnosis to 2 years post diagnosis, and post islet transplantation patients, studies demonstrate that as residual β-cell function declines, CGM parameters such as time in euglycaemia (time in range 3.9-10 mmol/L) and coefficient of variation (CV) worsen (Buckingham et al., 2015, Brooks et al., 2015, Vantyghem et al., 2012). The nature of these T1D populations are that they have much higher C-peptide levels than most individuals with long duration T1D, thus it was unknown if residual β-cell and C-peptide

secretion impacts beyond the first few years after diagnosis and if very low levels influence CGM outcomes.

Two very recent papers by Rickels et al. (2020) and Gibb et al. (2020) have attempted to explore these unknowns by completing mechanistic and observational studies assessing how low levels of C-peptide secretion impacts CGM outcomes in longer duration T1D diabetes. Rickels et al. (2020) demonstrating that in individuals with duration of T1D of > 2 years and a very high stimulated C-peptide (>400 pmol/L) had greater time in euglycaemia (72%) and reduced time in hyperglycaemia (22%) compared to negative (euglycaemia 58%, hyperglycaemia 35%), low (17-200 pmol/L) (euglycaemia 52%, hyperglycaemia 43%) and intermediate (200-400 pmol/L) (euglycaemia 59%, hyperglycaemia 34%) C-peptide groupings. However, there was no differences in time spent in hypoglycaemia (negative 7±10%, low 5±4%, intermediate 6±7% and high 7±5%). When viewed as a continuous variable, C-peptide had significant relationships with mean glucose, time in euglycaemia ($r = 0.456$; $P < 0.001$), time with glucose >10 mmol/L ($r = -0.376$; $P = 0.003$), and glucose CV glycaemic outcomes.

This study also comprehensively assessed islet cell responsivity, showing that while glucagon secretion during caloric intake is equally dysfunctional across C-peptide groups, individuals with higher residual β-cell had greater responsivity to glucose and α-cell responsivity to hypoglycaemia, resulting in greater increases in glucagon. It is unclear why this increased glucagon response did not translate into increased endogenous glucose production or reduced time spent in hypoglycaemia in the free-living period. Hypoglycaemia is difficult to measure in research, with difficulties including the lag time in CGM readings compared to blood glucose (Zaharieva et al., 2019b), and that three-quarters of episodes of hypoglycaemia are asymptomatic (Henriksen et al., 2019). This may have played a role in the lack of findings in this study compared to studies that have reported C-peptides' influence on reducing self-reported rates of symptomatic, asymptomatic and severe hypoglycaemia (Kuhtreiber et al., 2015, Marren et al., 2019). Additionally, the collection period of 1 week instead of the recommended two (Battelino et al., 2019) and the group sizes of 15/17 per group may not have been statistically strong enough to identify this small but clinically significant percentage of time. Whilst the participants were clinically diagnosed as having T1D in specialised diabetes centres, with no autoantibody

screening it's possible that participants in these groups may have had MODY or T2D, especially in the intermediate and high C-peptide groups. Finally, the Rickels et al. (2020) only reported the hypoglycaemia CGM parameter percentage time <3.9 mmol/L, rather than time spent <3.0 mmol/L a potentially more clinically relevant marker (Danne et al., 2017, Battelino et al., 2019).

A large observational study (n=290) conducted by Gibb et al. (2020) reported that individuals > 3 years post diagnosis with preserved C-peptide (either 10 to 200 pmol/L or 10 to 50 pmol/L) had fewer low-glucose events and lower glucose variability by flash monitoring compared to low (<10 pmol/L) secretors. Median time spent <3.9 mmol/L in the low secretors was 5% (interquartile range (IQR) 3 to 9%) compared to 3% (IQR – 2 to 6%) for the 10 to 200 pmol/L group (p< 0.001) or 4% (IQR – 2-6%) for the 10 to 50 pmol/L group (p= 0.034). Compared to Rickels et al. (2020), the much greater sample size and 2 week data collection period observed in this study was potentially needed to identify these very small but meaningful results. No differences were found in time spent in euglycaemia or hyperglycaemia between groups, which may be explained by individuals with greater >200 pmol/L of C-peptide being excluded from the study. Combined with Rickels et al. (2020) results, it suggests there may be a C-peptide threshold, around or greater than 200 pmol/L, needed to reduce time spent in hyperglycaemia. Excluding these participants reduced the possibility of having individuals with insulin deficiency not caused by T1D, without having to undertake autoantibody or genetic screening. However, it also excludes a large subgroup of the T1D population, with up to 16% having this level of endogenous β-cell function (Oram et al., 2014). The glucose data collection method may also have influenced the results in Gibb et al. (2020). The Freestyle Libre flash glucose monitors requires a participant to actively engage in it wears, and using it actively reduces time spent in hypoglycaemia (Bolinder et al., 2016, Oskarsson et al., 2018), factors that could influence the results. Again the more clinically significant marker time spent <3.0 mmol/L, which equates to a clinically significant hypoglycaemia requiring immediate attention, was not displayed in this study even though it is recommended that more importance be placed on this reading than time spent <3.9 mmol/L (Danne et al., 2017).

1.3.6. C-peptide as a biologically active peptide

As well its role in the formation of insulin, and its measurement as a marker of endogenous insulin secretion, C-peptide appears to be a biologically active peptide (Wahren et al., 2007). C-peptide has been shown to bind to the cell membranes via G-protein and Ca^{2+} dependent pathways, eliciting intracellular signalling (Rigler et al., 1999, Shafqat et al., 2002). Recently reviewed in detail elsewhere (Yaribeygi et al., 2019, Souto et al., 2020, Alves et al., 2019), there is significant evidence of the beneficial action of C-peptide, mainly in those who lack it. Briefly (Figure 1.5), in vitro studies demonstrate that C-peptide reduces the production of reactive oxygen species (ROS) formation and induced apoptosis in endothelial cell during hyperglycaemia (Cifarelli et al., 2011). Additionally, C-peptide downplays VEGF overexpression which play a prominent role in vascular permeability (Lim et al., 2014), and may also reduce cytokine release leading to a decreased expression of adhesion molecules on the endothelium (Haidet et al., 2012). Finally, C-peptide increases the release of ATP and $NA^{+}K^{+}$-ATPase by erythrocytes, which stimulates NO production by platelets and endothelial cells (Giebink et al., 2013). This C-peptide stimulation of NO inhibits erythrocyte adherence to endothelium (Johnstone et al., 1993), reduces platelet activation and aggregation (Emerson et al., 1999), and increases vasodilation, endothelial function and blood flow (Johansson et al., 2003). This has resulted in C-peptide being described as having anti-oxidative, vasoprotective, anti-apoptotic and anti-inflammatory effects (Yaribeygi et al., 2019).

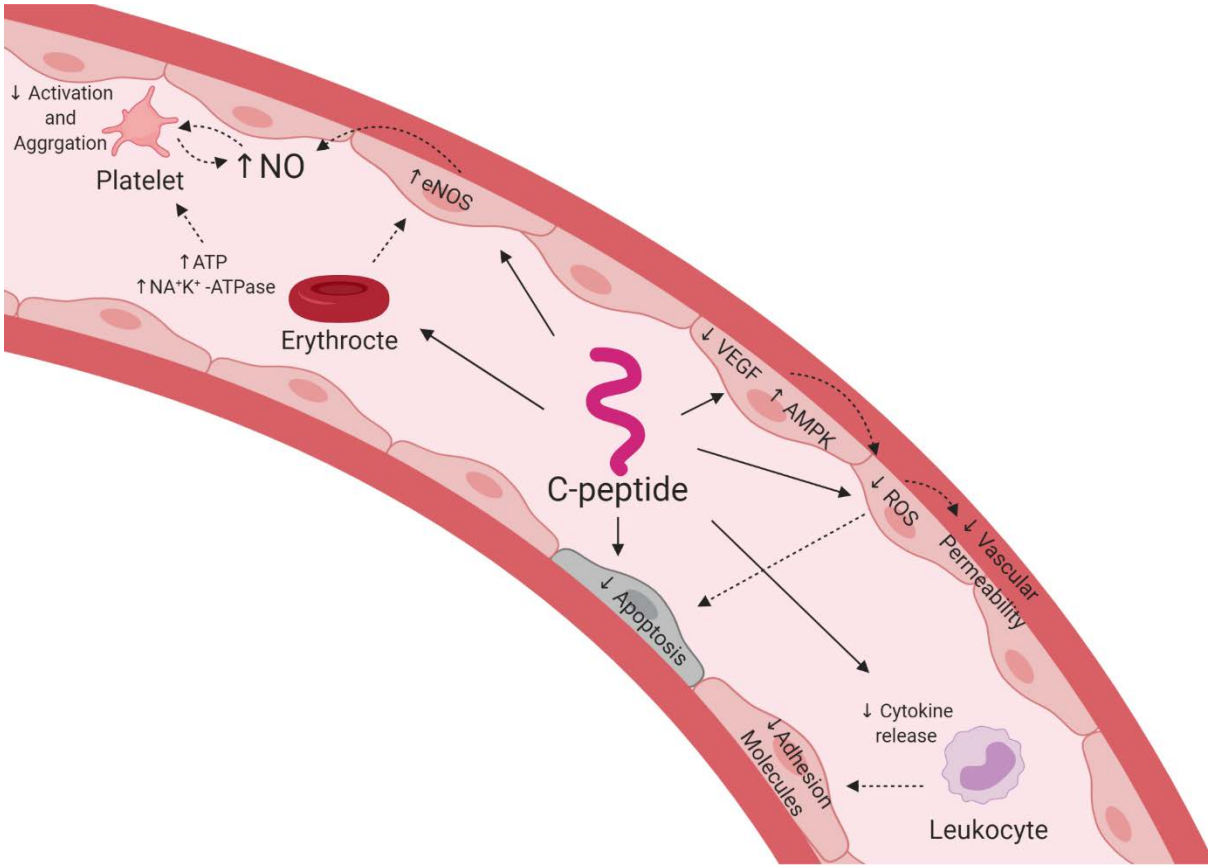

Figure 1.5 A schematic of C-peptide signalling pathways in vasculopathy protection. Solid lines represents C-peptides direct impact on the vasculature, dotted lines represent downstream impacts of increased C-peptide.

Abbreviations - AMPK, AMP-activated protein kinase alfa; ATP, adenosine triphosphate; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

Within type 1 diabetic animal models, C-peptide infusion has demonstrated functional and structural benefits on complications affecting the vasculature, kidney, and peripheral and central nervous systems (Souto et al., 2020). The replacement of C-peptide for 8 months has been shown to counter the diabetes-induced deterioration of the nerve conduction by 70% and increase nerve fibre regeneration (Sima et al., 2001), as well as reversing the damage to renal tissue (Elbassuoni et al., 2018). It is reported that C-peptide supplementation reduces microvascular leakage (Lim et al., 2014), increases microvascular blood flow by increasing recruitment of capillaries (Lindström et al., 1996) and enhancing wound healing (Lim et al., 2015).

Results in early clinical trials with T1D participants are mixed, with some small studies suggesting that C-peptide supplementation could be a potential therapeutic treatment target for reducing diabetes complications. However, a weekly long acting dose of C-peptide for 12 months in 250 patients with T1D and peripheral neuropathy resulted in

no difference in bilateral sural nerve conduction velocity (SNCV) compared to the placebo, but improvements in vibration perception threshold, both markers of peripheral neuropathy (Wahren et al., 2016). It is unclear why no improvement was seen in this study when two previous shorter studies with native C-peptide infusion found improvements in SNCV and vibration perception (Ekberg et al., 2003, Wahren et al., 2007). In patients with T1D and nephropathy, 1 and 3 month C-peptide infusion demonstrated reduced glomerular filtration rate (FR) and urinary albumin excretion (>40%), as well as improved heart rate variability and other indices of cardiac autonomic innervation (Johansson et al., 1993, Johansson et al., 2000). However a systematic review and meta-analysis of GFR revealed no difference between control and C-peptide, potentially explained by the lack of studies, small study sizes leading to large within group variation and varying degrees of renal disease (Shaw et al., 2015). C-peptide also appears to affect microvascular blood flow, improving flow mediated vasodilatation (Fernqvist-Forbes et al., 2001) and, interestingly for this thesis, increasing blood flow and capillary diffusion capacity of exercising skeletal muscles (Johansson et al., 1992).

While it is clear that C-peptide is biologically active and appears to have beneficial effects at attenuating diabetic complications, care should be taken when expanding these results to individuals with residual β-cell function whom create their own endogenous C-peptide. Specifically, while small amounts of endogenous insulin may influence glycaemic control (see 1.3.5), it is unknown if beneficial effects are seen with microscreating C-peptide. With previous research having explored the effects of supplementing C-peptide to a non-diabetic level, studies are needed to explore if the low physiological amounts of endogenous C-peptide seen in individuals with T1D has a positive effect on the vasculature.

1.4. Endothelial Progenitor Cells

As well as having a negative impact upon the vascular endothelium, diabetes also impacts upon endothelial repair. Blood vessels can be repaired and new vessels grown through two mechanisms; angiogenesis and postnatal vasculogenesis. Angiogenesis is the formation of new vessels from the existing vasculature network, and is often achieved by circulating cells having a paracrine effect causing endothelial cells to proliferate (Adair and Montani, 2010, Pankajakshan and Agrawal, 2014). Postnatal vasculogenesis describes the in situ differentiation and growth of blood

vessels derived from non-resident cells (Andrique et al., 2020). Endothelial progenitor cells (EPCs) were first described in 1997 by Asahara et al. (1997) as circulating mononuclear cells which, when isolated from human peripheral blood, could differentiate into endothelial cells in vitro, and could incorporate into site of active angiogenesis in animal models. These results have been confirmed elsewhere, with $CD34^+$ cells incorporating into the vascular wall and maturing into endothelial cells (Takahashi et al., 1999, Kocher et al., 2001, Grant et al., 2002). These cells can also form tubes on fibronectin-coated plates (Asahara et al., 1997, Tasev et al., 2016a), while a number of studies have shown that EPCs have the ability to stimulate neovascularization in both rodent and human models (Asahara et al., 1999a, Xia et al., 2012b, Reyes et al., 2002, Zengin et al., 2006).

Since the discovery of EPCs, a plethora of studies into their origin, mobilisation, role in the vascular system and cell-surface antigen marker have been published and debated.

1.4.1. Endothelial Progenitor Cells in culture

The term EPCs encompasses heterogeneous populations, with two distinct populations having been agreed to appear when grown in in vitro culture; Myeloid angiogenic cells (MACs) (also called circulating angiogenic cells (CACs), early EPCs, colony-forming unit-ECs (CFU-ECs or CFU-Hill) and endothelial colony-forming cells (ECFCs) (late EPCs, endothelial outgrowth cells) (Hur et al., 2004). MACs appear quickly in culture, secreting pro-angiogenic cytokines and growth factors, such as VEGF and IL-8 but have poor proliferative capacity and do not form tube-like structures (Hill et al., 2003). ECFCs appear later in culture (up to 12 weeks), produce more NO, and form capillary tube-like structures to a greater extent than early EPCs (Ingram et al., 2004). It is believed that MACs play an important role in the process of vessel injury and repair through paracrine function (Medina et al., 2017, Asahara et al., 2011), however, they are not an actual endothelial progenitor that is able to fully integrate as bona fide endothelial cells (Medina et al., 2011). In contrast, ECFCs have potent intrinsic clonal proliferative potential and are able to contribute to *de novo* blood vessel formation in vivo (Tasev et al., 2016b, Lin et al., 2000). Table 1.1 displays the diverse names, actions and surface markers that differentiate the two pro-angiogenic groups found in cell culture. While these cells are intrinsically different, they likely collaborate in the re-vascularization process, with MACs mobilised to the damaged

endothelial where their paracrine function helps recruit ECFCs, which migrate to, proliferate and incorporate into the vascular wall whilst MACs guide the process (Banno and Yoder, 2018, Ingram et al., 2005, Hubert et al., 2014)

Table 1.1 Phenotyping and characteristics of pro-angiogenic cells grown in culture

	MACS (Myeloid Angiogenic Cells)	**ECFCS (Endothelial Colony Forming Cells)**
Alternative Names Used	Early EPCs	Late EPCs
	Hematopoietic EPCs	Non Hematopoietic EPCs
	Circulating Angiogenic Cells (CACs),	Outgrowth endothelial cells (OECs)
	Myeloid Angiogenic Cells (MACs),	Endothelial Outgrowth Cells
	Colony-Forming Unit-EPCs (CFU-EPCs Or CFU-Hill)	
Cell Shape In Culture	Spindle shaped	Cobblestone shaped
Appearance In Culture	< 1 week	2-4 weeks
Lifespan	3-4 weeks	≈12 weeks
Neovascularization	Paracrine	Differentiate into endothelial cells
Cell-Surface Markers		
CD34	+	+
CD45	+	- or ^{dim}
CD133	- or +	-
VEGFR2	+	++
CD31^{+}	+	++
CD14	- , + or ^{dim}	-
VE CADHERIN	+	++
VWF	+	++

1.4.2. Endothelial Progenitor Cells – flow cytometry surface markers

An alternative to using cell culture to identify EPCs is enumerating by fluorescence activated cell sorting (FACS) and flow cytometry. EPCs can be prospectively identified by selecting sub-populations of mononuclear cells (MNCs) based on specific cell surface antigen expression. However, there is widespread debate on the exact cell-surface antigens of EPCs, with no combination of markers having been reliably adopted and no unique marker to differentiate the cells from other circulating cells. While surface markers of mature MACs and ECFCs in culture have been identified

(Table 1.1), the issue remains in identifying the markers of the more immature circulating cells that differentiate into these populations.

Normally, cell surface antigens chosen to define EPCs include a hematopoietic stem cell (e.g. $CD133^+$, $CD34^+$) and endothelial cell markers (e.g. $VEGFR2^+$, $CD31^+$). $CD34^+$ is a popular marker of the circulating EPCs as it has been shown to give rise to cells with endothelial properties in vitro and in vivo (Asahara et al., 1997). $CD34^+$ is a marker used to isolate human hematopoietic stem and progenitor cells (Sidney et al., 2014), while also expressed on mature endothelial cells (Fina et al., 1990), likely as an adhesion molecule for the interactions between endothelial cells and haematopoietic precursors (Cheng et al., 1996). However, as $CD34^+$ is widely expressed on mesenchymal, epithelial and cancer stem cells (Hirschi et al., 2008), further surface markers are required to discriminate EPCs from other circulating cells.

Other surface antigens measured include $VEGFR2^+$ (KDR+), an endothelial marker that regulates migration and proliferation, and when used in conjunction with $CD34^+$ a useful biomarker for cardiovascular risk (Werner et al., 2005). A similar endothelial marker to $VEGFR2^+$, that is also expressed on both MACs and ECFCs and has angiogenic properties is $CD31^+$ (Kim et al., 2010). Unfortunately, $CD34^+VEGFR2^+$ and $CD34^+CD31^+$ may also identify circulating mature endothelial cells sloughed from the endothelium (Lachmann et al., 2012), thus research groups have needed to explore further markers.

$CD133^+$ is another marker of hematopoietic widely used to identify EPCs (Prater et al., 2007). While $CD133^+$ has been shown to retain the ability to differentiate into endothelial cells (Gehling et al., 2000, Wu et al., 2007), there is evidence to the contrary, demonstrating $CD133^+$ is never expressed on mature endothelial cells (Peichev et al., 2000), devoid of vessel forming activity and remain hematopoietic in vitro (Case et al., 2007, Timmermans et al., 2007). $CD133^+$ expression decreases in circulation as the cell transforms into a more mature endothelial like cells (Quirici et al., 2001). Additionally, more than 99% of $CD34^+VEGFR2^+CD133+$ positive cells also strongly express $CD45^+$ (Case et al., 2007), a panleukocyte marker (Nakano et al., 1990), which is negative on the endothelial lineage. As $CD34^+CD45^+$ cells form hematopoietic progenitor cells (HPCs) but do not yield EPCs (Case et al., 2007), it is

likely that $CD34^{+}CD133^{+}VEGFR2^{+}$ are MACs, mobilising to ischemia and having a paracrine effect but being unable to mature into endothelial cells.

With the advancement of flow cytometry techniques and sensitivity, a distinction between CD45 negative, dim and bright positive events has been possible (Schmidt-Lucke et al., 2010). This has resulted with identifying most (≈90%) CD34+ progenitor cells express CD45 at low intensity ($CD45^{dim}$), resulting in less than 10% that are CD45-negative (Sambataro et al., 2014). The majority of circulating $CD34^{+}VEGFR2^{+}$ cells also express $CD45^{dim}$, a marker able to differentiate EPCs from circulating endothelial cells (CECs) which are negative for $CD45^{+}$ (Tropea et al., 2014, Huizer et al., 2017). While ECFC that appear within in vitro culture never express $CD45^{+}$, ECFC appear to initially express and develop from $CD45^{dim}$ $CD31^{+}$ cells (Timmermans et al., 2007, Bieback et al., 2013). 'True' circulating EPCs, which can differentiate into the endothelium, may therefore be part of the $CD34^{+}CD45^{dim}VEGFR2^{+}/CD31^{+}$ cell population detected by flow cytometry (Van Craenenbroeck et al., 2013), although there is a lack of in vivo evidence showing circulating EPCs differentiating into endothelial cells, and it may be that these circulating cells are paracrine in nature.

The identity of an EPC is elusive and definitions are a work-in-progress, despite the calls to end the nomenclature (Medina et al., 2017, Sandhu et al., 2018). In this thesis, we used the cell surface markers $CD34^{+}$ and $CD34^{+}CD45^{dim}$ to identify hematopoietic progenitor cells (HPCs, also called hematopoietic stem cells), a self-renewing population with the developmental potential to give rise to all types of mature blood cells (Pei, 1999). For EPCs, we used the markers $CD34^{+}VEGFR2^{+}$ and $CD34^{+}CD31^{+}$, which likely include other cells alongside EPCs, and $CD34^{+}CD45^{dim}VEGFR2^{+}$ and $CD34^{+}CD45^{dim}CD31^{+}$ and $CD34^{+}CD45^{dim}VEGFR2^{+}CD31^{+}$, which are a more specific markers of "true" EPCs that can differentiate into endothelial cells (Van Craenenbroeck et al., 2013, Bieback et al., 2013).

1.4.3. Mobilisation, recruitment and action of EPCs

Circulating EPCs are rare within the peripheral blood, making up between 0.0001 and 0.01% of all circulating mononuclear cells in human blood (Patel et al., 2015, Khan et al., 2005). Cells are mobilised into the peripheral blood in response to tissue ischemia or injury (Aicher et al., 2005), with damaged tissue able to increase the circulating numbers by up to 500% (Ritz et al., 2014).

There is controversy within the field of where these cells originate. While the majority of research suggests that EPCs are bone marrow derived (Asahara et al., 1999a), recent research from bone marrow transplant patients suggest this is not the case (Fujisawa et al., 2019b). It may well be the different phenotypes of EPCs have different origins, with MACs originating from the bone marrow (Stamm et al., 2003) and ECFCs from the vessel wall (Tura et al., 2013), or that there are pockets of tissues resistant cells around the body (Chopra et al., 2018).

When endothelial cells are under ischemic conditions, the hypoxia stimulates the expression of cytokines and growth factors such as VEGF, platelet-derived growth factor, insulin-like growth factor and fibroblast growth factor (Patel-Hett and D'Amore, 2011) (Figure 1.6). Another hypoxia-induced factors that is released, and a key driver of angiogenesis, is SDF-1. Many stem and progenitor cells express CXCR4, the receptor for SDF-1, with the SDF-1:CXCR4 signalling axis involved with the homing of cells from their niche within the bone marrow into circulation (Floege et al., 2009, Shen et al., 2011). Similar mechanisms for mobilisation from the bone marrow have been found for G-CSF, HIF-1α, VEGFR and NO (Silva et al., 2012, Natori et al., 2002, Li et al., 2006, Aicher et al., 2003).

Once mobilised into the peripheral blood, EPCs hone to the area of vascular damage traveling along the SDF-1 and VEGFR chemotactic gradients (Tang et al., 2009, Ackah et al., 2005, Shen et al., 2011). Indeed, when in vitro studies block CXCR4 or $VEGFR2^{+}$ it impairs or blocks migration of EPCs across a semi-permeable membrane (Tang et al., 2009, Sun et al., 2013). As detailed in 1.4.1 the exact role of EPCs at vasculature damage is varied and not completely understood. MACs secrete pro-angiogenic cytokines and growth factor, such as vascular endothelial growth factor, placental growth factor, transforming growth factor-β, thrombopoietin, hepatocyte growth factor, fibroblast growth factors and interleukin-8, which activate self-regeneration and stimulates endothelial cell proliferation (Medina et al., 2011, Peplow, 2014). ECFCs have been shown to contribute to vessel formation in vivo, with ECFCs implanted into mice and seeded in matrigel able to form de novo blood vessels (Melero-Martin et al., 2007) and human ECFCs demonstrated to form human endothelial cell also within mice (Shepherd et al., 2006).

It has been suggested that circulating MACs hone to the damaged tissue and their paracrine factors recruit ECFCs from either the circulation or the local vascular wall. These ECFCs migration and proliferation to the injured site to restore the endothelial integrity of the vascular wall (Banno and Yoder, 2018).

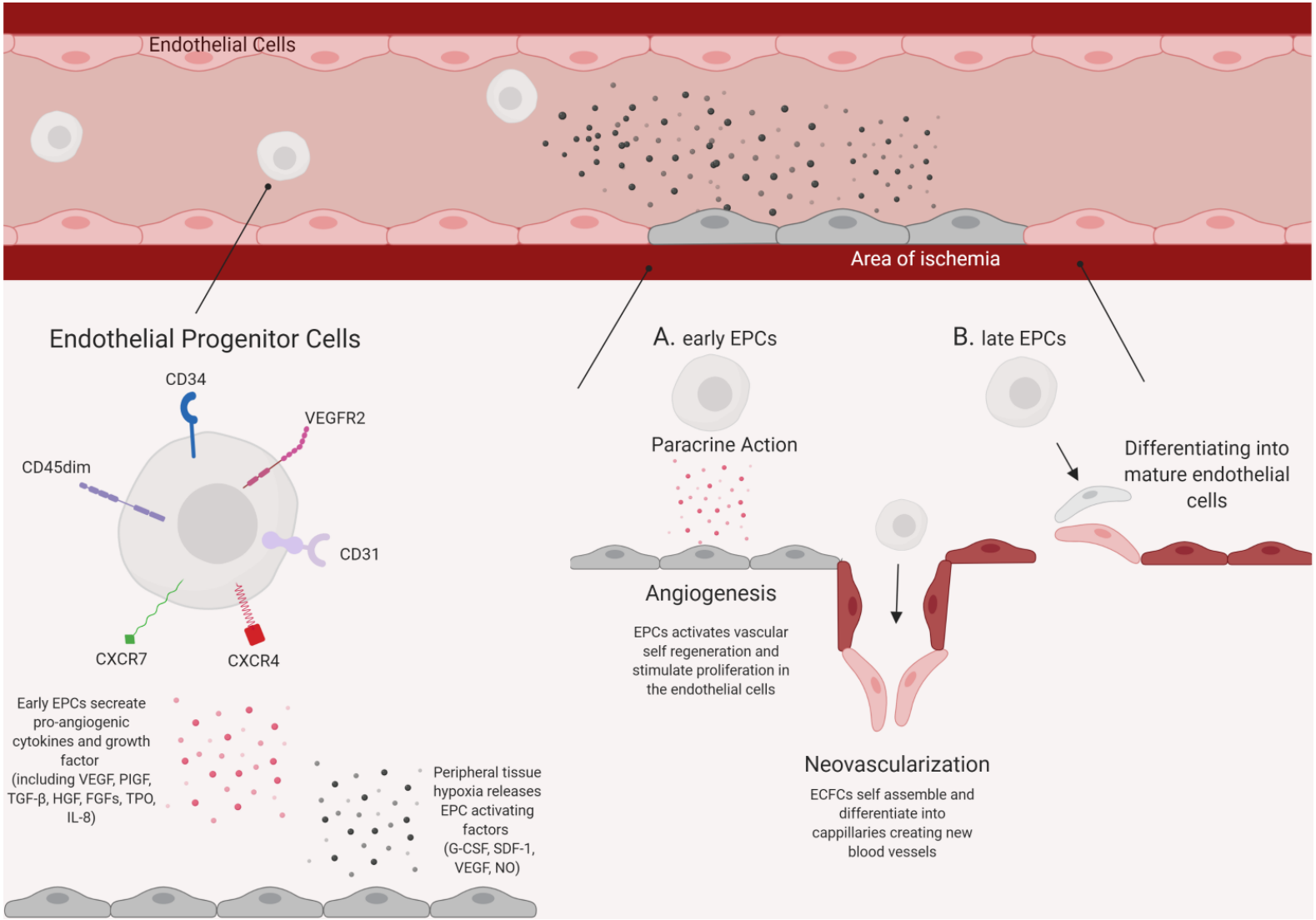

Figure 1.6 EPCs are circulating angiogenic cells in the blood with cell surface antigen markers of hematopoietic ($CD34^+$) and endothelial cells ($CD31^+$, $VEGFR2^+$) or pan-leukocyte that are expressed on both ($CD45^{dim}$). EPCs can promote endothelial cell repair, regeneration and proliferation, as well as differentiating into mature endothelial cells. Neovascularization by EPCs can occur through (A) early EPCs paracrine secretion of pro-angiogenic cytokines and growth factor, (B) late EPCs self-assemble and differentiate into endothelial cells creating new blood vessels. Areas of endothelial hypoxia release EPCs activating factors, which chemokine receptors (CXCR4 and CXCR7) upon the EPCs mobilise and home towards.

Abbreviations: vascular endothelial growth factor (VEGF), placental growth factor (PlGF), transforming growth factor-β (TGF-β), thrombopoietin (TPO), hepatocyte growth factor (HGF), fibroblast growth factors (FGFs), interleukin-8 (IL-8), Stromal cell-derived factor 1 (SDF-1), nitric oxide (NO), granulocyte-colony stimulating factor (G-CSF).

1.4.4. Exercise and EPCs

Exercise has a potent cardiovascular effect, reducing the development and reversing plaque formation (Szostak and Laurant, 2011, Madssen et al., 2015), improving endothelial function (Pagan et al., 2018) and angiogenesis (Laufs et al., 2004, Chinsomboon et al., 2009), and lowering the risk of mortality and CVD events (Lear et al., 2017).

Acute exercise can mobilise EPCs into circulation, with number elevated for up to 72 hours after a single bout (Van Craenenbroeck et al., 2008, Ross et al., 2014, Van Craenenbroeck et al., 2010a, Bonsignore et al., 2010). While most studies have found increased numbers post exercise in healthy individuals, some studies have failed to find the expected mobilisation in the post exercise period (Thijssen et al., 2006, Adams et al., 2008). The number of EPCs mobilised with exercised appears to be linked to the duration and intensity, with increases ranging from 20 to 160% for individuals in one study (Laufs et al., 2005). Changes in circulating EPCs is linked to increased chemoattractants within the blood, with correlations found with levels of NO, MM-9, VEGF, G-CSF and SDF-1α (Chang et al., 2015, Möbius-Winkler et al., 2009, Ross et al., 2014, Yang et al., 2007, Krüger et al., 2015)

As well as being a potent mobiliser into circulation, exercise improves the function and reduces apoptosis of circulating EPCs (Hoetzer et al., 2007, Laufs et al., 2004). The cells have enhanced migratory capacity to VEGF and SDF-1 in vitro after acute exercise (Van Craenenbroeck et al., 2010a), which may be due to sheer stress enhancing the cell surface expression of CXCR4 (Xia et al., 2012b).

Regular physical activity also has a training effect upon EPCs, increasing circulating numbers at rest (Xia et al., 2012a, Yang et al., 2013, Choi et al., 2014, Laufs et al., 2004). Additionally exercise training improves the function of EPCs, enhancing the migration to VEGF and SDF-1 and increasing secretion of NO (Schlager et al., 2011, Van Craenenbroeck et al., 2010b, Sonnenschein et al., 2011). These enhancements of EPCs are correlated with improvements in endothelial function with exercise, and may be a contributing factor (Steiner et al., 2005).

1.4.5. EPCs in chronic diseases and aging

In a pivotal study, Werner et al. (2005) demonstrated that lower count of EPCs lead to increased CVD events in the future. During a 12-month observational period, patients

with low baseline levels of EPCs had a significantly higher occurrence of a first major cardiovascular event and incidence of cardiovascular caused death. Hill et al. (2003) had previously demonstrated a strong relationship between EPCs and endothelial function in healthy but increased CVD risk individuals, with EPC numbers potentially a better predictor than traditional risk factors.

There has since been a plethora of research demonstrating that the number and function of EPCs are reduced in chronic vascular related diseases such as hypertension (Luo et al., 2016), diabetes (Ambasta et al., 2017, Yu et al., 2016, De Vriese et al., 2000) and cardiovascular disease (George et al., 2004). It appears that circulating EPCs numbers increase rapidly in the acute phase of ischaemic stroke and myocardial infarction (Shintani et al., 2001, Chu et al., 2008), with increased numbers of EPCs in the days after an event related to improved functional outcomes, reduced infarct growth and neurological improvements (Yip et al., 2008, Cesari et al., 2009). The increased requirement for vascular repair in chronic conditions may deplete EPCs within the bone marrow. Haematopoietic and mesenchymal progenitor cells have been found to be reduced in the bone marrow after 5 days of stimulation by VEGFR in mice (Tashiro et al., 2014), while the count of circulating and bone marrow resistant EPCs are reduced in critical limb ischemia patients compared to healthy controls (Teraa et al., 2013). Additionally the high glucose, inflammatory and oxidative stress conditions may impair mobilization and increased apoptosis resulting in reduced circulating numbers of EPCs in both type 1 and type 2 diabetes (Fadini et al., 2005, Sibal et al., 2009, Jiraritthamrong et al., 2012, Lombardo et al., 2012).

Older adults also display a lower number and reduced function of EPCs compared to younger individuals. Thijssen et al. (2006) and Thum et al. (2007b) demonstrated reduced counts of ECFC and MACs EPCs, respectively, in older adults compared to younger adults. The function of these EPCs is also reduced with Williamson et al. (2013), Hoetzer et al. (2007) and Kushner et al. (2010) reporting reduced migration, proliferation and paracrine function, which were independent predictors of endothelial function in both young and old individuals (Thijssen et al., 2006, Heiss et al., 2005).

It appears that exercise induced mobilisation of EPCs is also attenuated in chronic diseases and in older adults. Both absolute and delta change in number of EPCs after exercise is attenuated in diseases such as type 2 diabetes (Lutz et al., 2016), heart

failure (Van Craenenbroeck et al., 2011) and CVD (Adams et al., 2004, Sandri et al., 2005) compared to healthy controls. Similarly, reductions are found in older individuals with 60 to 75 year olds having reduced count compared to 18 to 25 year olds (Ross et al., 2018).

1.5. T1D and EPCs

Most research on T1D patients suggest that individuals have lower circulating number of EPCs compared to healthy controls, with this found in children (Zahran et al., 2019, Hörtenhuber et al., 2013), adolescents (Palombo et al., 2011, DiMeglio et al., 2010), and adults (Sibal et al., 2009, Loomans et al., 2004). Females appear to have a lower number of cells than males with T1D (Maiorino et al., 2018), the opposite of that found in non-diabetes individuals (Fadini et al., 2008), and may explain the greater excess risk of fatal and nonfatal vascular events showed by women with T1D compared with men. However Głowińska-Olszewska et al. (2013) found increased frequency of EPCs in children with T1D, which was negatively correlated with endothelial function. Arcangeli et al. (2017) also found increased numbers in children with T1D compared to non-diabetic controls, however lower circulating numbers of EPCs were found in adult patients.

A study by Hernandez et al. (2014) demonstrates that in the Joslin Medalist cohort of individuals who have had T1D for 50 years or longer, count of circulating progenitor cells (CPCs) and EPCs were similar to age-matched controls without diabetes. Despite the extreme duration of diabetes, the medallist had higher levels than both younger T1D participants and patients with type 2 diabetes who were of a similar age. When divided into Medalists with and without CVD, both CPCs and EPCs were decreased in the presence of CVD and nephropathy (Hernandez et al., 2014). Previous investigation exploring these individuals with long-duration disease demonstrated that the majority (67%) retained a random fasting level of serum C-peptide greater than 30 pmol/L, the lower limit of detection at the time (Keenan et al., 2010). Potentially, residual β-cell function may play a role in protecting the Medalists from the loss of CPCs and EPCs, attenuating the development of cardiovascular complications and enhancing survival.

Reduced EPC count in diabetes is likely due to a range of reasons affecting the production, survival and utilisation of these cells. Bone marrow from individuals with

type 2 diabetes has been found to be affected by diabetic microangiopath (Spinetti et al., 2013). Mouse models of T1D have also found microvascular rarefaction, increased permeability and depletion of stem cells with the bone mass (Oikawa et al., 2010, Mangialardi et al., 2013), which may explain the reduced mobilisation into the peripheral blood in response to ischemia (Fadini et al., 2006).

Once in circulation, hyperglycaemia causes dysfunction of these cells in individuals with T1D. EPCs isolated from healthy subjects and exposed to high glucose conditions or EPCs isolated from patients with diabetes have been shown to exhibit; reduced number of colony forming units and impaired abilities of differentiation into endothelial cells, reduced proliferation, adhesion and migration, impaired paracrine secretion, mobilization and homing, but increased senescence (Kang et al., 2017). When circulating progenitor cells (CPCs) from non-diabetic individuals were cultivated under hyperglycaemic conditions, there was both an increased rate of apoptosis and a decrease in proliferation (Kränkel et al., 2005), as well as a decreased ability to target damaged tissue and incorporate into tubular structures (Marchetti et al., 2006). Hyperglycaemia accelerates reactive oxygen species and advanced glycation products formation, which stimulates transcription factors such as nuclear factor kappa B and p53, subsequently resulting in inflammatory protein production and accelerated EPCs senescence (Yu et al., 2016). EPCs cultivated from individuals with T1D also had impaired angiogenic paracrine secretion of nitric oxide (NO) and other cytokines (Zhang et al., 2013a).

1.5.1. Residual β-cell function and EPCs

No previous research has examined if increased residual β-cell function results in a higher count and function of EPCs in individuals with T1D. With the reported improvements in microvascular complications seen in individuals with T1D who secrete endogenous insulin (Kuhtreiber et al., 2015, Lachin et al., 2014, Steffes et al., 2003a), even at very low C-peptide levels (Kuhtreiber et al., 2015), there may be a role that EPCs play in this.

Evidence from after islet transplantation may suggest a relationship between residual β-cell function and EPCs (Petrelli et al., 2010). Although count of circulating angiogenic cells (CAC) was similar between non-diabetic controls, T1D controls and insulin-independent islet-transplanted patients, restoration of β-cell function with islet

transplantation had a positive impact on the circulating angiogenic cells function and vitality (Petrelli et al., 2010). While further research is clearly needed, there are multiple pathways that residual β-cell function could influence the count and function of EPCs, including improved glycaemic control, the anti-inflammatory properties of insulin and the direct biological action of C-peptide.

1.5.2. Glycaemic control and EPCs

Within individuals with T1D, increased residual β-cell function may improve glycaemic control; reducing time in hyperglycaemia and hypoglycaemia, and reduced glycaemic fluctuations, as detailed in 1.3.5. When circulating progenitor cells (CPCs) from individuals without diabetes were cultivated under hyperglycaemic conditions, there was both an increased rate of apoptosis and a decrease in proliferation, resulting in a reduced count (Kränkel et al., 2005). Hyperglycaemia also causes decreased ability to target damaged tissue and incorporate into tubular structures (Marchetti et al., 2006). These results may explain the inverse correlation between EPCs number and HbA1C levels in individuals with T1D (Loomans et al., 2004), and the increase in EPC count in children who improved their HbA1c and a decrease in those who worsened (Hörtenhuber et al., 2013).

In addition, initial evidence suggests that both incidents of hypoglycaemia and increased glycaemic variability (GV) could be associated with reduced numbers of EPCs (Fadini et al., 2018, Maiorino et al., 2015). Fadini et al. (2018) suggested a single episode of mild hypoglycaemia in individuals could impair circadian rhythm of EPC, but not significantly reduce the count. Evidence from individuals with T2D suggest that in those who were hospitalised with severe hypoglycaemia had reduced count of EPCs compare to outpatient and non-hypoglycaemic diabetic controls (Fadini et al., 2018). Additionally in people with T2D initiating basal insulin, hypoglycaemia prevented the increase in EPCs (Fadini et al., 2015).

Glycaemic variability can contribute to the development of chronic vascular complications of diabetes and potentially, oscillating glucose levels may have a greater deleterious effect on endothelial cells and CVD than persistently high glucose exposure (Nusca et al., 2018). Maiorino et al. (2015) demonstrated that mean amplitude of glycaemic excursion (MAGE) but not standard deviation (SD) glucose variability over 72 hours is associated with reduced circulating EPCs. While Inaba et

al. (2018) found that J index, which reflects both hyperglycaemia and glycaemic variability, was associated with a reduction in the $CD34^+$ count, no other measure of GV (including MAGE) correlated. However, while 5 days of CGM data were used, EPC measurements were taken on either the first or the seventh day, questioning the validity of the data. People with T1D who switched to CSII from MDI for 24 weeks had an increase in circulating levels of EPCs ($CD34^+KDR^+$ and $CD34^+KDR^+CD133^+$) associated with the reduction of glucose variability. With the absence of significant changes in HbA1c level in the CSII or control MDI groups, the decrease in MAGE was the only predictive factor explaining the raised EPCs number (Maiorino et al., 2016). A recent paper by Boscari et al. (2020) demonstrated that in individuals with T1D circulating hematopoietic stem/progenitor cells (HSPCs) ($CD34^+$, $CD133^+$, $CD34^+CD133^+$ and $CD34^+CD45^{dim}$) but not EPCs ($CD34^+VEGFR2^+$, $CD133^+$ $VEGFR2^+$, $CD34^+CD133^+$ $VEGFR2^+$) were reduced compared to controls and that duration of diabetes inversely correlated with HSPC levels. Surprisingly, 3 months of flash glucose monitoring found a positive associations between GV (specifically CV%) or time in hypoglycaemia and higher HSPC levels. When the T1D population was split by duration, these associations were only found in those with the shorter disease duration (<16 years). The authors speculate that the reduction of HSPCs in early T1D is counteracted by the mobilizing effect of GV and hypoglycaemia. In longer duration T1D this compensatory mechanism is lost leading to a further decline in HSPC.

1.5.3. Anti-inflammatory properties of insulin and EPCs

While hyperglycaemia causes oxidative and inflammatory stress, insulin can attenuate this high glucose and free fatty acid mediated inflammation, as well as having its own anti-inflammatory properties (Sun et al., 2014). Insulin supresses inflammatory mediators and cytokines in vitro (Aljada et al., 2000), and in vivo in both rat models (Jeschke et al., 2004) and humans (Chaudhuri et al., 2004). Additionally, insulin has been shown to reduce the activation of the pro-inflammatory transcription factor NFκB in peripheral blood mononuclear cells in obese but non-diabetic participants (Dandona et al., 2001), which facilitates the attachment of monocytes to endothelial cells. Additionally, Egr-1, another pro-inflammatory transcription factor, is reduced in mononuclear cells with insulin treatment (Aljada et al., 2002), while insulin can also reduce macrophage apoptosis (Leffler et al., 2007), and shift T helper cells to secrete inflammatory mediators and proliferate (Viardot et al., 2007, Zhang et al., 2013b).

In individuals with T2D, it has been found that 6 months after the initiation of insulin treatment the count of EPCs is increased (Fadini et al., 2011). While a shorter study (4 months) found no increase in count, the colony forming ability in vitro of EPCs were improved with both insulin glargine and insulin NPH compared to increased oral medication, despite similar improvements in HbA1c (Oikonomou et al., 2014). In a streptozotocin-induced diabetic mouse model, insulin treatment improved EPC mobilisation from the bone marrow and enhanced post ischemic vasculogenesis and angiogenesis through eNOS-related pathways (Dong et al., 2011). However, these results are conflicting to those found by Gallagher et al. (2007), where insulin did not mobilise EPCs, all be it in a more hyperglycaemic model. In vitro, adding insulin to EPCs stimulates the outgrowth and tube formation of adult endothelial cells in both cells taken from participants with T2D and non-diabetic controls. While insulin treatment is uniform for individuals with T1D, potentially, residual β-cell function and endogenous insulin secretion into the portal vein and circulation may enhance the anti-inflammatory benefits of insulin compared to the peripheral administration of exogenous insulin. In a rat model, delivering the same dose of insulin into the intraperitoneal space, resulting in higher concentrations of insulin in the portal vein, resulted in lower hepatic and overall oxidative stress and inflammation compared to subcutaneous insulin delivery (Dal et al., 2015). However, in patients with T1D, there was no difference in systemic oxidative stress between intraperitoneal and subcutaneous insulin delivery (van Dijk et al., 2019)

1.5.4. C-peptide and EPCs

It is unknown if C-peptide directly influences the count or function of EPCs in individuals with T1D. Within a diabetic mice model, Lim et al. (2015) demonstrated that C-peptide infusion stimulates angiogenesis, promoting endothelial cell proliferation and migration, and increasing tube formation, resulting in an increased rate of skin wound closure. It is known that high glucose results in less production of NO in EPCs (Kränkel et al., 2005, Chen et al., 2007), which is required for survival, migration and angiogenesis and promotes the release of EPC from the bone marrow (Dimmeler et al., 2000, Aicher et al., 2003). Theoretically, C-peptides acting on intracellular signalling pathways that increases NO production (as highlighted in 1.3.6), may result in an increased number, function and mobilization of EPCs, attenuating endothelium damage independent of the vasodilatory effects of NO. Indeed, eNOS-

deficient mice have impaired function and mobilization of EPCs, while clinical drugs studies report that statins and metaformin increase NO availability and reduce oxidative stress, resulting in increased number and function of circulating EPCs in individuals with T1D (Aicher et al., 2003, Nakagawa et al., 2006, Ahmed et al., 2016, Ambasta et al., 2017). While C-peptide downplays VEGF, a powerful promoter of angiogenesis that promotes EPCs migration to areas of damage (Asahara et al., 1999b), the hyperglycaemic-hypoxic environment within diabetes results in an increased VEGF production, inducing abnormal endothelial cell proliferation and migration resulting in endothelial dysfunction and hyperpermeability in eyes and kidneys (Cooper et al., 1999, Ishida et al., 2000). The beneficial effect of VEGF are mediated in part by its ability to stimulate NO, which in high glucose conditions is lost as VEGF uncouples from NO (Nakagawa et al., 2006). Indeed, within rat models uncoupling of NO has been identified in diabetic bone marrow, as well as in glucose-treated EPCs and EPCs from patients with diabetes, resulting in a reduction of EPC levels and impairment of EPC function (Thum et al., 2007a). Taken together, the multifaceted action of C-peptide on the vasculature could also impact upon number and function of EPCs, although research is needed to explore this.

1.5.5. EPCs and T1D complications

Decreased numbers and function of EPCs may play a role in increased risk of diabetes complications, however there is currently a lack of compelling evidence (Fadini, 2014, Yu et al., 2016). Research comparing individuals with T1D against individuals with T1D and CVD, nephropathy, peripheral vascular disease (Hernandez et al., 2014), microalbuminuria (Dessapt et al., 2010), early markers of atherosclerosis (Palombo et al., 2011, Sibal et al., 2009) or nonproliferative retinopathy (Brunner et al., 2009) have all found reduced circulating numbers of EPCs in those with diabetes complications. Other studies have demonstrated impaired function and premature senescence in individuals with microvascular damage (Loomans et al., 2004, Ingram et al., 2008).

However, in individuals with proliferative retinopathy, a dramatic increase of mature EPCs has been observed (Tan et al., 2010, Brunner et al., 2009). There is also some evidence that the diabetic conditions may functionally impair cells, resulting in EPCs that fail to repair injuries and potentially play a role in the development of microvascular damage (Nobuta et al., 2019, Li et al., 2012). There is mounting evidence that some

EPC phenotypes may be involved in the pathogenesis of diabetic retinopathy, even when others are considered as potential cell therapies for diabetic macular edema and proliferative diabetic retinopathy (Lois et al., 2014).

1.5.6. EPCs in T1D and exercise

Two previous studies have investigated EPCs' mobilisation with exercise in individuals with T1D suggests that individuals have a blunted EPC response to acute exercise (West et al., 2015, Waclawovsky et al., 2015). However, both studies failed to observe the expected post aerobic exercise mobilisation in non-diabetic controls, making it hard to draw conclusions. West et al. (2015) demonstrated an increased count the following morning after exercise in the control group but not T1D participants, however as the testing commenced at 5 pm, and the blood samples were taken 60 minutes pre, 60 minutes post, as well as the following morning, circadian rhyme may have also influenced the count. While Waclawovsky et al. (2015) documented a mobilisation post resistance exercise in the controls, post aerobic exercise count of EPCs was decreased for the non-diabetic group with no change in the diabetic group. Because of this unusual non-mobilisation with exercise, it is difficult to state that EPCs response to acute exercise appears blunted in T1D. Further limitations include both studies measuring circulating EPCs as a percentage of leucocytes and not correcting for changes in blood volume around exercise. Exercise mobilisation could have been masked within changes in leucocytes around exercise (Saito et al., 2003). Both studies were small with unequal numbers between the T1D and non-diabetic groups, especially the Waclawovsky et al. (2015) study that compared 14 participants against 5 controls. These small numbers and the fact that both studies only used one phenotype definition of EPCs ($CD34^{+}CD45^{dim}VEGFR2^{+}$) may have reduced the likelihood of observing any changes.

Interestingly, resting counts were comparable between the T1D and non-diabetic groups, in contradiction to most other research into EPCs in individuals with T1D (see 1.5). Both studies were performed in young, male participants (mean age of T1D participants in West et al. (2015) – 27 years old and Waclawovsky et al. (2015b) – 30 years old), who had high levels of fitness (VO_{2peak} - 51 and 37.1 ml/kg/min, respectively) and good glycaemic control (HbA1c of 53.3 and 61.1 mmol/L). While a reduced HbA1c is associated with increased circulating numbers of EPCs (Loomans et al., 2004, Hörtenhuber et al., 2013), it is unknown if increased fitness or regular

exercise training results in levels comparable to non-diabetic controls. It has previously been reported that in the medallist population a higher count of EPCs is correlated with increased exercise, however as the method of assessing the quantity of exercise and the strength of the correlation are not mentioned and the p value was not significant (p= 0.07), further research is needed to explore this topic (Hernandez et al., 2014).

1.6. Type 1 Diabetes and Exercise

For individuals with T1D regular physical activity is encouraged by the American Diabetes Association (ADA), Diabetes UK and the NHS (Colberg et al., 2016). Specifically, guidelines match those given to non-diabetic populations that 150 minutes of accumulated moderate intensity physical activity should be performed a week, with no more than two consecutive days of no physical activity (Colberg et al., 2016). Resistance exercise is also recommended to be undertaken two or three times a week. Physical activity is defined as any bodily movement produced by the skeletal muscles that results in energy expenditure (Caspersen et al., 1985), with any moderate or harder intensity activity such as fast walking and cycling counting towards the recommended guidelines.

1.6.1. Health benefits of exercising with type 1 diabetes

Regular physical activity and exercise is recommended for individuals with T1D because of the range of health and wellbeing benefits it can deliver (Riddell et al., 2017, Colberg et al., 2016, Chimen et al., 2012a). The benefits of exercise for people with T1D have been demonstrated from studies dating back to the 1950's, where 5 months of cross country ski runs 3 times a week improved maximal oxygen uptake and lactate threshold a similar amount to non-diabetic controls (Larsson et al., 1964). Randomised control trials from the 1980's demonstrated that 12 weeks of vigorous aerobic exercise in children and adolescents improved lean body mass, VO_{2peak} and LDL-cholesterol compared to non-exercising individuals (Campaigne et al., 1984, Campaigne et al., 1985). Since, a range of studies have shown improved cardiorespiratory fitness, vascular health, decreased insulin requirements, reduced cardiovascular disease risk factors, and better self-rated quality of life (Wu et al., 2019). A large 6-month study by Salem et al. (2010) that randomised 196 participants into usual physical activity, an exercise session once a week, or an exercise session three times a week demonstrated improvements in HbA1c,

improved dyslipidemia and reduced insulin requirements, as well as a reduction in BMI and waist circumference, whilst not increasing the incidents of hypoglycemia. Increased frequency of exercise appeared to show additional, although non-significant, benefits. Two systematic reviews with a meta-analysis have found that in children, adolescents and adults with T1D, regular exercise training improves body composition, cardiorespiratory fitness, endothelial function, daily insulin dosage, blood lipid profiles and HbA1c (Quirk et al., 2014, Wu et al., 2019). Most studies included in the systematic reviews found at least one significant improvement in a health outcome after a physical activity intervention. As cardiovascular disease is the leading cause of mobility and mortality in young and old people with T1D (Miller et al., 2016, Katz et al., 2015), these improvements are all important as they can reduce this risk.

While Pierre-Louis et al. (2014) completed their testing on individuals with T2D and healthy individuals, a higher individuals' fitness level corresponds to a lower risk of coronary artery disease, myocardial ischaemia, and stroke for both groups. This effect is likely to be seen in individuals with T1D, where activity level is inversely related to mortality risk. Sedentary males (expending < 1,000 kcal/week) were three times more likely to die than active males (expending >2,000 kcal/week) (Moy et al., 1993). Studies of fitness levels in individuals with T1D suggest that they have lower fitness compared to non-diabetic controls who complete similar levels of physical activity levels (Moy et al., 1993, Nadeau et al., 2010, Mosher et al., 1998, Niranjan et al., 1997). While not all studies have found lower fitness levels (Veves et al., 1997) and no evidence exists for older adults, it is likely that abnormalities in the autonomic nerve function and cardiac muscle such as left ventricular torsion and reduced cardiac energetics found in individuals with T1D, reduces fitness levels (Piya et al., 2011). Another contributing factor is likely the increased up regulation of fatty acid utilization and oxidation within the myocardium due to an increase in plasma fatty acid levels and a decrease in myocardial extraction of glucose (Herrero et al., 2006). Oxidizing fatty acid has an increased oxygen cost compared to glucose (Grynberg and Demaison, 1996), and an overreliance on cardiac fatty acid metabolism could lead to an energy deprivation state due a decline in oxidative capacity (Fillmore et al., 2014). Physical activity interventions do however increase VO_{2max} in people with T1D (Laaksonen et al., 2000, Wallberg-Henriksson et al., 1982, Yki-Järvinen et al.,

1984), with improvements of 27% found after 4 months of cycling two or three times a week in both male and female middle aged participants (Fuchsjäger-Mayrl et al., 2002). Similar improvements in VO_{2max} were seen between people with T1D and aged matched healthy control over a 12 week cycle training program (Zinman et al., 1984).

Cross-sectional and prospective studies with long follow-ups have found that endothelial dysfunction and dyslipidaemia are markers of vascular disease and cardiovascular mortality in individuals with T1D (Ladeia et al., 2014, Buse et al., 2007). Studies investigating the effects of up to 4 months exercise training in this population have found reduction in LDL cholesterol and triglycerides of up to 15%, and an increase of between 8-30% in HDL cholesterol (Mosher et al., 1998, Laaksonen et al., 2000, Wallberg-Henriksson et al., 1982, Yki-Järvinen et al., 1984, Fuchsjäger-Mayrl et al., 2002, Rigla et al., 2000). Regular exercise also greatly improves insulin sensitivity and glucose metabolism, resulting in a reduction from 6 to 18% in daily insulin requirements (Yki-Järvinen et al., 1984, Fuchsjäger-Mayrl et al., 2002). Vascular function also improves following long term training protocols, however not to the same extent as it does in individuals without diabetes (Fuchsjäger-Mayrl et al., 2002, Mason et al., 2006). As insulin resistance is independently associated with the risk of developing both macro- and microvascular complications in individuals with T1D, the improvement seen with insulin sensitivity with exercise could play a large role in reducing the risk (Chaturvedi et al., 2001). A large cross-sectional study of 18028 adults with T1D found that the 17.8% of individuals who reported they exercised more than twice a week had reduced HbA1c, a more optimal body mass index, reduced occurrence of hypertension and dyslipidaemia, and fewer diabetes related complications (Bohn et al., 2015). Increased physical activity within the study was also found to be associated with less diabetic ketoacidosis and severe hypoglycaemia incidences. This was true for every age group apart from women aged 45 to 80 years, where active individuals were more likely to have severe hypoglycaemic events.

In healthy and a range of patient populations, single bouts of exercise have been shown to acutely raise EPCs, contribute to “endothelial repair” by homing to areas of dysfunctional endothelium and either differentiating into new endothelial cells or secreting growth factors that promote endothelial growth and angiogenesis (Rehman

et al., 2004). However, limited evidence suggests this response is blunted in individuals with T1D (Schaan et al., 2015, West et al., 2015). This lack of mobilisation of EPCs is likely due to impaired bone marrow function (Westerweel et al., 2013), and could indicate a blunted endothelium regenerating capacity.

Overall, it appears that physically active adults with T1D are significantly less likely to develop microvascular complications such as retinopathy, nephropathy and microalbuminuria, and neuropathy than their inactive peers (Kriska et al., 1991; Bohn et al., 2015). This also seems to be the case for macrovascular complications. The Pittsburgh IDDM Morbidity and Mortality study demonstrating that 25 years after T1D diagnosis, men who participated in team sports at school were three times less likely to have reported macrovascular diseases as well as three times lower mortality rates than those who did not take part (LaPorte et al., 1986). Moy et al. (1993) also found that physical activity levels in adulthood predicted likelihood of mortality within the next 6 years.

1.6.2. Barriers to exercise for individuals with T1D

Although the benefits of regular physical activity to health appear to be clear, a large cross-sectional study has shown that only 17% of patients reported doing aerobic exercise more than two times per week, and 63% of participants did no structured exercise at all (Bohn et al., 2015). In the Finnish Diabetic Neuropathy Study, 44% of patients with T1D were doing less than 1 session of exercise per week (Wadén et al., 2008). Tielemans et al. (2013) found across Europe 36% of individuals with T1D were doing none or only very mild physical activity. Whilst there is some evidence that individuals with T1D that are young and complication-free undertake similar amounts of physical activity as people without diabetes, these levels remain suboptimal (Moy et al., 1993).

Three studies have explored the barriers to regular physical activity in individuals with T1D (Lascar et al., 2014, Brazeau et al., 2008, Kennedy et al., 2018). Many of the barriers and motivators to complete exercise in patients with T1D are similar to patients with other chronic diseases and the general public, with work and time commitments ranked highly by all populations. On top of these barriers, people with T1D also have the fear that exercise will cause hypoglycaemia and/or worsen their glycaemic control. A lack of knowledge of effective strategies to avoid hypoglycaemia

is also often stated as a reason to not partake in physical activity. Around half of participants reported a decline in exercise levels around diagnosis, with a fear of hypoglycaemia a more potent barrier in the newly diagnosed (Kennedy et al., 2018). Hypoglycaemia is often seen as *the* limiting factor for managing T1D (Davis et al., 1997, Cryer, 2008, McCrimmon and Sherwin, 2010) and exercise is frequently identified as a major cause of both severe (low blood glucose levels that requires assistance from another person to treat) (Bhatia and Wolfsdorf, 1991) and mild hypoglycaemic events (Malik and Taplin, 2014).

1.6.3. Exercise influence on HbA1c in individuals with type 1 diabetes

Currently there is limited evidence for the impact of physical activity on long term glycaemic control in T1D with conflicting outcomes from meta-analyses (Chimen et al., 2012b). A meta-analysis by Quirk et al. (2014) found an average reduction in HbA1C of around 8.5 mmol/mol in T1D under the age of 18 with exercise training studies. In adults, Yardley et al. (2014a) stated that there is currently insufficient well-designed studies to ascertain the true effect of exercise training on HbA1c, while meta-analyses by Kennedy et al. (2013) found no impact and Tonoli et al. (2012) found a positive reduction. A number of individual studies have demonstrated that exercise training can worsen glycaemic control (Huttunen et al., 1989, Roberts et al., 2002, Ebeling et al., 1995).

A number of factors may contribute to the lack of a clear benefit of physical activity on glycaemic control in individuals with T1D, unlike the clear evidence that exercise improves HbA1c levels in individuals with type 2 diabetes (Thomas et al., 2006, Umpierre et al., 2011). The majority of studies found that calorie intake is increased around the time of physical activity in individuals with T1D (Kennedy et al., 2013). Increased energy intake could either be as a source of fuel, a reward or to avoid hypoglycaemia, and this may counteract any glucose-lowering effect of physical activity. Another potential explanation is that the majority of studies are not long enough to obtain a reduction in HbA1c (Chimen et al., 2012b), with Kennedy et al. (2013) calculating that 25 weeks of a physical activity intervention is needed to obtain an HbA1c reduction in the region of 0.5% (5.5 mmol/mol). Finally, the variation in exercise intervention (intensity, duration, type of exercise, diet and insulin dose adjustments) could also contribute to the range of results found, with distinctive

metabolic responses to different forms of exercise (Riddell et al., 2017) as well as a large inter-individual variation in response to exercise (Temple et al., 1995).

HbA1c may also not be the most appropriate measure of glycaemic control in active individuals (Kennedy et al., 2013). While the measurement reflects average plasma glucose over the previous eight to 12 weeks (Nathan et al., 2007), it does not take into account the acute variation in glucose over that time frame. Physical activity can increase the time spent in both hypo and hyperglycaemia and increase glycaemic fluctuation (Kapitza et al., 2010). Continuous glucose monitors (CGM) has enabled researchers to explore these acute changes in glycaemia around exercise, and in particular discover post-exercise glucose responses to different modalities of exercise, becoming a staple of most T1D exercise studies (Houlder and Yardley, 2018).

1.6.4. Blood glucose changes during exercise in T1D

Within a healthy individual, blood glucose fluctuates naturally throughout the day and tight physiological controls keep the concentration maintained between 3.9-7.9 mmol/L. The blood glucose concentration is regulated within these relatively narrow boundaries by balancing glucose entering into the circulation (glucose appearance) by the rate of glucose removal (glucose disappearance). Circulating blood glucose is derived from intestinal absorption of exogenous carbohydrates, glycogenolysis (the breakdown of glycogen), and gluconeogenesis (the formation of glucose primarily from lactate and amino acids during the fasting state), while insulin release from the pancreatic β-cells clears blood glucose out of circulation into storage within skeletal, adipose and hepatic tissue (Fernandes et al., 2011). Blood glucose concentration is constantly sensed at the hypothalamus and pancreatic islets. Corrective actions is undertaken if glucose concentrations are too high (with the secretion of insulin from β-cells) or too low (with release of the counter regulatory hormones cortisol, glucagon, epinephrine, and norepinephrine) (De Feo et al., 1986, De Feo et al., 1991, De Feo et al., 1989, Rizza et al., 1979).

During any type of physical activity theses homeostatic mechanisms are put under a variable amount of stress (Galassetti and Riddell, 2013). During aerobic exercise, repeated and continuous contractions of large muscle groups that rely primarily on aerobic energy-producing systems (e.g. jogging, swimming, cycling), insulin secretion

decreases and glucagon secretion increases into the portal vein to facilitate glycogenolysis and gluconeogenesis from the liver to match the rate of glucose uptake into the working muscles (Camacho et al., 2005). Muscular contractions require a substantial increase in energy turnover, with the metabolic requirements being increased up to 100 fold compared to at rest (Sahlin et al., 1998). At the initiation of aerobic exercise muscle glycogen predominantly fuels the working muscle, but as exercise progresses, non-esterified fatty acids (NEFA) and blood glucose become the major fuel sources (Coyle, 1995). To maintain the contribution of carbohydrate to the fuel metabolism, glucose uptake from the circulation into the working muscle dramatically increases, up to 50 times than at rest (Ploug et al., 1984), by translocation of glucose transporter proteins (GLUT 4) to the surface of the muscle cell independently of insulin signalling (Wojtaszewski et al., 2000). During higher intensity exercise (high intensity interval training and sprinting) or bouts of resistance training (strength based exercises including free weights, bodyweight exercise etc.), the body is mainly powered by anaerobic energy-producing systems. Circulating insulin concentrations do not decrease as much as during aerobic activities (Henriksen, 2002). This is likely due to the duration of activity being shorter, but also to offset the dramatic elevations in catecholamines, free fatty acids, and ketone bodies, all of which impair muscle glucose utilization (Bjorkman et al., 1988, Howlett et al., 1999).

The glycaemic response to exercise bouts in individuals with T1D is dependent upon a multitude of factors. These include the amount of active insulin in circulation, where the insulin was injected, the current blood glucose concentration and whether it is rising or falling, composition and timing of the last caloric intake, and the modality, intensity and duration of the exercise activity (Riddell et al., 2017). Major disruption of the complex equilibrium in T1D can result in (Figure 1.7)(Yardley and Sigal, 2015);

- The inability to maintain euglycaemia and blood glucose dropping, resulting in a hypoglycaemic episodes during or after exercise, usually associated with prolonged submaximal physical activity (Clarke et al., 1997, Winter, 2007).
- Transient acute hyperglycaemia usually resulting from shorter, very intense bout of physical activity (Chassin et al., 2007).
- Stable blood glucose, often during a mixed intensity activities, including team sports such as football and basketball, or resistance training (Bally et al., 2016, Guelfi et al., 2005).

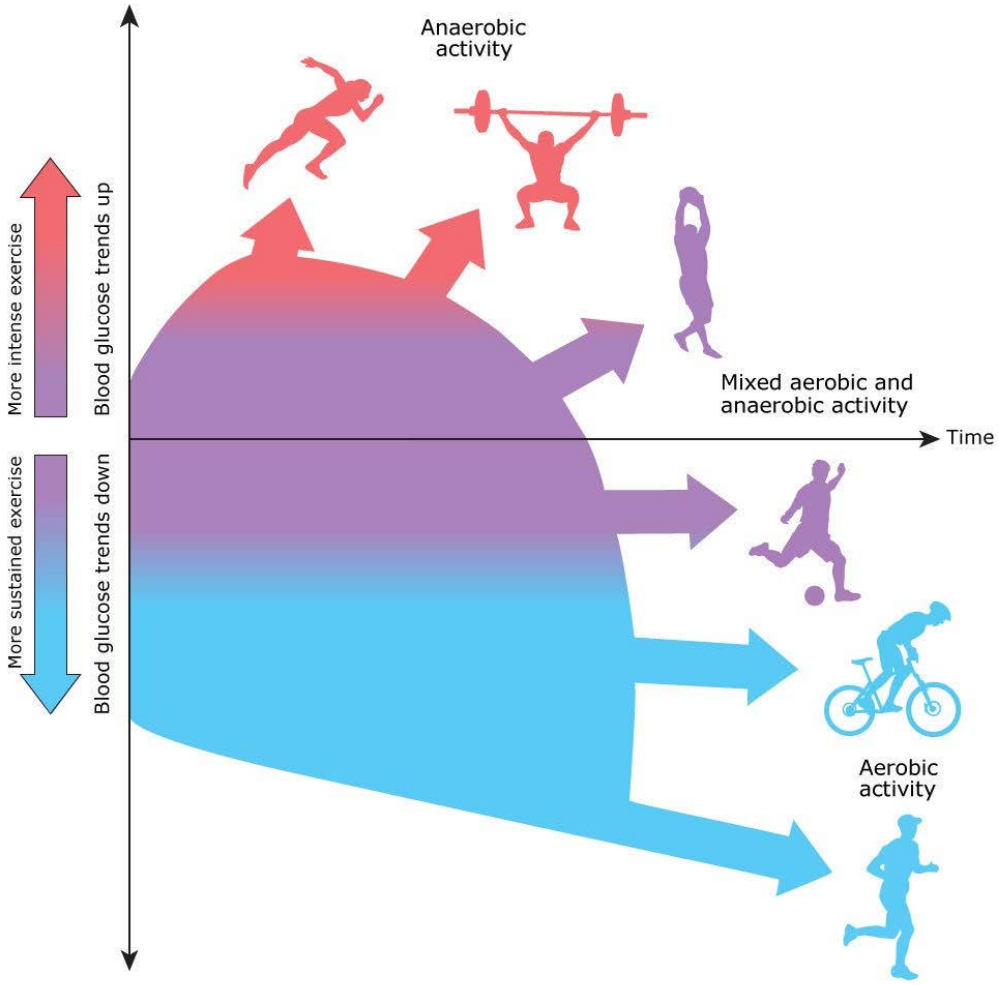

Figure 1.7 Schematic of varying blood glucose responses to different modalities of exercise from .Riddell and Hoppin (2019). While arrows and colours denote general trends, there is a high amount of inter-individual variation in glucose response during exercise. Reproduced with permission from: Riddell MC. Management of exercise for children and adolescents with type 1 diabetes mellitus. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on [10th June 2020].) Copyright ©2020 UpToDate (Appendix I).

During aerobic physical activity in individuals with T1D, the circulating insulin concentrations from the previously administered insulin dose is not reduced and concentrations tend to increase in the circulation (Mallad et al., 2015). Insulin concentrations likely rise because of the increased blood flow to subcutaneous adipose tissue during exercise (Frayn and Karpe, 2014). This promotes increased glycogenesis relative to hepatic glycogenolysis, as well as delaying the shift away from glucose toward NEFA as the predominant fuel source (Riddell et al., 2017). This increased reliance on glucose as a fuel often results in hypoglycaemia (Diabetes Research in Children Network Study, 2006, García-García et al., 2015). Without

insulin or carbohydrate adjustments, most individuals will develop hypoglycaemia within 45 minutes of exercising (Riddell et al., 1999, DirecNet StudyDirecNet, 2006, Garcia-Garcia et al., 2015)

During anaerobic exercise the massive adrenergic activation, which is driven by the magnitude of cardiovascular response to intense exercise, increases endogenous glucose production to a greater extent that the peripheral tissue needs (Sigal et al., 1996). Increased circulating levels of catecholamines (adrenaline and noradrenaline) result in the release of glucose from the liver (Kjaer et al., 1986), while increasing levels of growth hormone spare circulating blood glucose in favour of other fuel supplies (Yardley and Sigal, 2015, Møller et al., 1991). In individuals with T1D, insulin cannot be secreted in response to this hyperglycaemia, resulting in blood glucose increases during exercise that may persist after exercise until an insulin correction is administrated (Marliss and Vranic, 2002).

As well as dysglycaemia during exercise, individuals with T1D are at greater risk of hypoglycaemia and hyperglycaemia for at least 24 hours (Gomez et al., 2015, Zander et al., 1983). Following exercise, glycogen replenishment of depleted stores is a high priority for the body (Jentjens and Jeukendrup, 2003). Increased glucose uptake into the muscle is maintained by the contraction induced insulin independent GLUT 4 translocation to the cell surface which is maintain for several hours after exercise (Richter and Hargreaves, 2013), as well as an enhanced sensitivity to insulin which can last up to 48 hours (Cartee et al., 1989, Mikines et al., 1988). Afternoon and evening exercise in particular can cause nocturnal hypos (Campbell et al., 2015b), which in severe cases can lead to death (Tanenberg et al., 2009). After intense exercise, elevated glycaemia can last for several hours, with an insulin correction dose to return to euglycaemia often advisable (Turner et al., 2015). However, anaerobic exercise can also cause delayed-onset hypoglycaemia in recovery (Yardley et al., 2013a).

As demonstrated above, maintaining euglycaemia around exercise is a significant challenge for individuals with T1D. A current push is currently ongoing within research to develop safe and effective glycaemic management strategies before, during and after exercise. Strategies (as outline in the international consensus (Riddell et al., 2017) Table 1.2) involve; reduction in basal and bolus insulin (Kourtoglou, 2011),

nutritional modifications (such as taking 60g of carbohydrate per hour of exercise) (Campbell et al., 2014, Zaharieva and Riddell, 2015) and modifying exercise bout (e.g. resistance training before aerobic and including sprints in aerobic workouts) (Yardley et al., 2012, Yardley and Sigal, 2015).

Carbohydrate consumption both before, during and after activity bouts is used to fuel exercise and prevent hypoglycaemia (Scott et al., 2019). For moderate intensity physical exercise for up to 60 minutes, Riddell et al. (2017) suggest that 10–15 grams of carbohydrates are required throughout the exercise to protect against hypoglycemia and maintain euglycemia under low insulin levels. In contrast, Moser et al. (2019b) demonstrated that under both full (100%) or a reduced (75%) basal dose, participants needed a median 36g of carbohydrates per hour during exercise to maintain euglycaemia, with pre-exercise blood glucose concentration a key determinant of carbohydrates needed.

Insulin modification and carbohydrate consumption are often intertwined when it comes to managing glycemia during and after exercise. For example, Campbell et al. (2014) showed a 25% rapid-acting insulin dose with a carbohydrate bolus 60 minutes before 45 minutes of treadmill running, in combination with a low GI carbohydrate meal and 50% rapid-acting insulin dose at 60 minutes post exercise was able to provide hypoglycaemia protection during and ~8 h post exercise. However, participants were still at risk of late nocturnal hypoglycemia. When followed up with a study that included a 20% reduction in basal insulin on the day of the exercise, the ten young male adult participants were protected from nocturnal and following day hypoglycemia (Campbell et al., 2015a). However, If the bolus insulin reduction and carbohydrate intake is taken more than an hour before the exercise the protection against hypoglycaemia during the exercise bout is lost (West et al., 2011). It is also likely that increased carbohydrate intake and bolus insulin reduction is needed for both longer and harder aerobic exercise (Shetty et al., 2016, Rabasa-Lhoret et al., 2001). For individuals on MDI, reduction in basal insulin can be impractical for unplanned exercise bouts and could potentially cause a deterioration of glycaemic control. For individuals on CSII however, reduction of basal insulin rates for up to 2 hours before exercise (60 minutes prior for total suspension), reduces the risk of hypoglycaemia during the exercise, although increases hyperglycaemia after exercise (Tsalikian et al., 2006). A reduction of 80%

in basal infusion rate prior to and during exercise may be most beneficial for maintaining euglycaemia (Franc et al., 2015).

Modifying bouts of exercise can also protect against hypoglycaemia during and after exercise, with a 10 second maximal sprint before or after a moderate intensity exercise bout prevents glycaemia from falling during early recovery (Bussau et al., 2006, Bussau et al., 2007). When combined with carbohydrate intake, repeated sprints did not cause any further significant increase in blood glucose levels during and after fasted morning exercise compared to moderate aerobic exercise with prior carbohydrate intake (Soon et al., 2019). This is in comparison to Guelfi et al. (2005) findings where moderate-intensity exercise, with and without repeated sprints and performed in a postprandial state, was shown to result in a significant fall in blood glucose level, albeit to a lesser extent with the latter. Performing resistance exercise before aerobic exercise, in comparison to aerobic then resistance exercise, also improves glycemic stability throughout the whole exercise bout and reduces the duration and severity of post exercise hypoglycemia (Yardley et al., 2012). This may be due to increased circulating growth hormone when performing anaerobic exercise first increasing hepatic glucose production and lipid mobilization (Yardley et al., 2014b). It is likely that performing the same exercise but at a different time of the day leads to altered metabolism and varying glycaemic responses. For example, Toghi-Eshghi and Yardley (2019b) demonstrated an increase in plasma glucose during morning fasted resistance exercise, while the same bout of activity in afternoon largely resulted in a drop in glucose. Similar differences are seen with aerobic exercise with Ruegemer et al. (1990) finding that morning exercise increased blood glucose concentration from 6.7±0.4 to 9.1±0.4 mmol/L, whereas during afternoon exercise a small non-significant decline was found.

Table 1.2 Current recommended strategies (adjustment of insulin and/or food intake) to minimise dysglycemia events during and after prolonged aerobic exercise and brief high intensity aerobic and anaerobic exercise for both MDI and CSII T1D. Table reproduced with permission from Riddell et al. (2017) (Appendix I).

	Prolonged endurance exercise (predominantly aerobic)	Brief intense exercise (aerobic and anaerobic)
Bolus insulin dose reduction at the meal before exercise	Advised when exercise occurs within ~120 min of bolus dose; the magnitude of reduction varies according to timing, type, duration, and intensity of exercise	Bolus reduction not advised; might require additional conservative bolus dose correction if hyperglycaemia develops
Before exercise, basal insulin dose reduction (of ~20%) in patients on multiple daily injections	Useful especially if exercise is done less than every 3 days or if the frequency of exercise is high throughout the day; might also be useful if patients are on twice daily intermediate insulin	Basal insulin dose reduction not advised
Basal nocturnal insulin dose reduction (of ~20%) after exercise in patients on multiple daily injections and continuous subcutaneous insulin infusion to reduce the risk of nocturnal hypoglycaemia	Particularly important if the exercise was done in the afternoon or early evening	Useful for helping to prevent hypoglycaemia after a high intensity interval training exercise session
Temporary basal rate change (continuous subcutaneous insulin infusion)	Basal rate can be reduced by up to 100% (ie, pump suspension) during exercise, however, keeping some basal insulin delivery is preferred; to take into account rapid acting insulin pharmacokinetics, a basal rate reduction should ideally occur well before exercise is started (up to 90 min); normal basal rates can be resumed either at the end of exercise, or later in recovery depending on glucose trends	Increased basal rate might be needed to help prevent or treat hyperglycaemia either during or immediately after exercise
Carbohydrate intake before exercise	See table 1 for details	Not usually needed
Carbohydrate intake during exercise	Typically up to 60 g/h if no insulin dose adjustments have been made (see table 1 for additional information)	Not usually needed
Carbohydrate intake after exercise	Useful to reduce the risk of hypoglycaemia and improve recovery; might need a specified bolus insulin dose depending on the length and intensity of exercise (eg, a reduced insulin to carbohydrate ratio)	Useful to reduce the risk of hypoglycaemia and enhance recovery but should be delayed if hyperglycaemia is initially observed; might need a specified bolus insulin strategy (eg, a reduced insulin to carbohydrate ratio)
Sprint before or after exercise (alternative or complementary approach not related to insulin or food intake)	Might help reduce the risk of hypoglycaemia	Might increase the risk of hyperglycaemia; consider a prolonged aerobic cool down

1.6.5. Inter-individual variation in glycaemic response during and after exercise in individuals with T1D

While the consensus statement by Riddell et al. (2017) highlighted the progression research has made in protecting against exercise-related dysglycaemia, there are still considerable challenges surrounding maintaining glycaemic control during acute PA (Minnock et al., 2019). One major obstacle to providing exercise management support to people with T1D is a high inter-individual variability in the blood glucose responses to exercises (Riddell et al., 2017). Indeed, while the current recommendations can be a good starting point for strategies, most individuals have to use past experiences and a trial and error approach to managing their blood glucose around exercise (Dizon et al., 2019). Additionally, most research around exercise in T1D has been conducted in relatively young, fit, male participants, with small sample sizes (Yardley et al., 2018). Even within tightly controlled research studies that have adopted a strict inclusion criteria, recruited a homogenous cohort of T1D participants, have standardized insulin and dietary intake and used a CGM to stabilise pre-trial glucose, a large unexplained

inter-individual variability in the glycaemic responses to exercise remains during (Temple et al., 1995, Abraham et al., 2017, DirecNet, 2006, Kilbride et al., 2011, Riddell et al., 2019a) and after exercise (Kapitza et al., 2010). Outside of formal research, both clinical observations and feedback from patient support groups report potential for both an improvement and detrimental impact of regular exercise on long-term glycaemic control. Wide-ranging challenges in successfully avoiding hypoglycaemia persist, despite advancement and availability of supportive strategies including CGM and patient education.

This is currently an area of considerate interest within research, with groups using secondary analysis of previous studies to investigate this inter-individual glycaemic variation (Table 1.3). Riddell et al. (2019b) found the best predictor of the magnitude of drop in glucose for adolescents undertaking prolonged aerobic exercise was the pre-exercise glucose level, with age, BMI, disease duration, total daily insulin dose, HbA1c or sex having no impact. A higher pre exercise glucose tended to result in a greater drop in glucose, although they still had a reduced risk of a hypoglycaemia event. Meanwhile, Al Khalifah et al. (2016) demonstrated that hypoglycaemia during aerobic exercise happened more often in a higher fitness versus a poor fitness level group. This could have been the result of increased insulin sensitivity during the exercise, or the fact that they performed at a greater work threshold during the submaximal bout. An additional factor that could influence glycaemia around aerobic exercise is body composition, with leg and trunk fat mass positively and whole body lean mass negatively associated with glucose post exercise (Tagougui et al., 2019). The authors again speculate these results are due to insulin sensitivity, although unfortunately, this was not measured and body composition did not predict the drop in glucose during exercise.

Sex-related differences exist during exercise in individuals with T1D, with males having a greater drop in glycaemia during an acute bout of resistance exercise and had an increased risk of hypoglycaemia after the exercise than females (Brockman et al., 2020). This may be due to oestrogen promoting lipid oxidation and sparing blood glucose and glycogen stores during exercise (Devries et al., 2006, Isacco et al., 2012), consequently reducing the hypoglycaemia risk in recovery when the liver and muscles replenish their stores (Devries et al., 2006). While the exercise bout occurred in the follicular phase of the menstrual cycle where oestrogen levels are lowest and can be

comparable to males (Fragala et al., 2011), both resistance and endurance exercise acutely increase oestrogen (Kraemer et al., 1995, Copeland et al., 2002). This may be dependent on the where in the phase the exercise occurs (Nakamura et al., 2011). A study by Galassetti et al. (2002) demonstrated that in female individuals with T1D there was an attenuated catecholamine response and increased lipolysis rate. However this did not result in a reduced endogenous glucose production rate or need for an increased exogenous glucose infusion rate to maintain glucose during exercise, replicating the results found in non-diabetic individuals (Pullinen et al., 2002).

While these studies show novel findings, the nature of the secondary data analysis, including analysing multiple different studies with different protocols as one, unmatched groups and small sample sizes, have clear limitations. These findings do however highlight the need for future studies into the inter-individual variability in glycaemic responses to exercise in T1D, allowing for more personalised insulin dose adjustments and carbohydrate intake, as well as for the development of a successful artificial pancreas system that can handle the considerable challenge of exercise (Yardley, 2020). It has been argued that exercise may be one of the more difficult challenges for an artificial pancreas system (Riddell et al., 2015). A sensor able to detect the onset of exercise and intensity of the exercise is potentially needed (Colberg et al., 2016), as well as a system that understands and predicts how different exercise and physiological variables (age, sex, fitness, body composition, ect.) will influence the glycaemic response (Yardley et al., 2018).

No previous research has explored how residual β-cell function impacts glycaemia around exercise. With the potentially beneficial impact maintained endogenous C-peptide secretion has on glycaemic control during free-living periods, even at microsecreting levels (see 1.3.4) (Rickels et al., 2020, Gibb et al., 2020), it's possible that residual β-cell function may play a role in this inter-individual variability. In a study examining submaximal exercise in insulin independent post-transplant patients, Yardley et al. (2019) found a largely comparable counter regularity response to exercise compared to non-diabetic controls. While the circulating concentration was higher and reduction in insulin was less in the post-transplant patients, suggesting that there may be impaired sympathetic stimulation of the islet resulting in a greater declines in plasma glucose, glucagon response to exercise was similar between both groups. Despite the greater drop in plasma glucose, only one individual experienced

glucose level in the hypoglycaemic range and recovered without the need for exogenous carbohydrate. Additionally no hypoglycaemia was recorded in the free-living hours after the exercise, suggesting that the islet transplant and improved residual β-cell function impacts glycaemic control both during and after exercise. The lack of a non-transplant T1D control group within this study means it’s hard to exactly judge the influence of residual β-cell function upon the counter-regulatory responses to exercise.

Table 1.3 Summary of the literature investigating inter-individual variation of the glucose response to exercise in individuals with T1D

Reference	Variable	Design	Main Outcomes
Al Khalifah et al. (2016)	Fitness	Secondary analysis of 2 studies (Haidar et al., 2013, Haidar et al., 2015) 44 participants – 34 adults / 10 adolescents. 26 male / 22 female Categorised into poor or good VO_{2max} (< and > 25th age and sex percentile) Exercise 2.5 hours after last meal. Patients were allowed to suspend and/or reduce their basal rate as they saw fit Exercise – 60 minutes on a treadmill (n=29) or 30 minutes on a bike (n=15) at 60% $VO_{2max.}$ Low fitness (11 bike / 9 treadmill) and high fitness (4 bike / 19 treadmill) 32g CHO snack before the treadmill if blood glucose <15 mmol/L, 15g CHO snack before the bike if blood glucose <6 mmol/L	Participants who have a good fitness level were more at risk of developing exercise-associated hypoglycaemia, occurring in 17 of the 23 participants in the high fitness group (74%) compared to 8 of the 21 participants in the low fitness group (38%). Correlation between the nadir glucose and maximum oxygen uptake was r = −0.38 (p = 0.01)
Riddell et al. (2019b)	Pre-exercise blood glucose	Secondary analysis of 4 studies (Group, 2006, Riddell et al., 1999, Admon et al., 2005, Association, 2006) 120 participants – Mean age 15±2 years. 49 male / 71 female	Change in glucose during the exercise was negatively correlated with the pre-exercise glucose concentration (r^2 = 0.44, p< 0.001). Hypoglycaemia occurred during or immediately after exercise on 53 of 120 sessions (44%). Adolescents with a higher pre exercise glucose tended to have a greater drop in glycaemia during the activity, although they had a reduced risk of a hypoglycaemia event. The drop in

		45 to 60 minutes of aerobic exercise (2 bike and 2 treadmill) post meal (100 minutes to 4 hours) at 55 to 65% of $VO_{2max.}$ 2 morning and 2 afternoon trials with no meal bolus adjustment of insulin. Admon et al. (2005) reduced basal by 50% during the exercise and gave a pre exercise 20g CHO snack.	glucose tended to be greater in those on MDI as compared to CSII. No other measured variables (age, height, weight, BMI, BMI percentile, diabetes duration, total daily insulin, and sex) were associated with the change in glucose during exercise.
Brockman et al. (2020)	Sex	Secondary analysis of 2 studies (Yardley et al., 2013b, Toghi-Eshghi and Yardley, 2019a) 23 participants – 13 male / 10 female Resistance Exercise – 3 sets of 8 repetitions of 7 exercises. Rest period between sets was 90 seconds. CGM inserted day before exercise bout and for 24 hours after. Exercise bout at 5pm. MDI - 10% reduction in long-acting insulin the night before or morning of the exercise. CSII - 50% reduction in basal rate an hour before and during the exercise. Further 25% reduction if blood glucose <5 mmol/L. Participants consumed a 25g CHO an hour before exercise. Additional CHO was given if blood glucose <5.5 mmol/L before or during the exercise (n=3)	Plasma glucose decreased significantly in males from 8.6±2.5 to 6.3±2.1 mmol/L ($p< 0.001$), whereas females experienced no significant change (7.2±1.3 to 7.3±1.3 mmol/L, $p= 0.999$) during the exercise. In the 6 hours post-exercise, males developed significantly more time in hypoglycaemia monitoring. 6 of the 12 males had hypoglycaemic events, compared to 3 of 9 females. There was no CGM differences in the preceding 24 hours between groups.
Tagougui et al. (2019)	Body composition	Secondary analysis of 1 study (Haidar et al., 2015)	Leg and trunk fat mass were positively correlated with blood glucose level at the end of the exercise and after 30 minutes of recovery. Whole

	30 participants – 20 male / 10 females, 20 adults / 10 adolescents, aged 12 to 74. All using CSII Body composition was measured by dual-energy X-ray absorptiometry (DXA) Participants consumed a meal at 5.30pm with normal insulin bolus. Exercise was performed 90 minutes post finishing the meal. Exercise – 60 minutes on the treadmill at 60% VO_{2peak} Participants (n=11) were given 16g CHO if blood glucose <3.3 mmol/L with symptoms or <3 mmol/L. Participants maintained their usual basal and boluses rates to regulate glucose, without adjusting insulin for the exercise	body lean mass was negatively correlated with blood glucose after 30, 60 and 90 minutes of recovery. There was relationship between all the parameters of body composition and blood glucose level at rest, or the drop in plasma glucose levels from start to end of exercise.

1.6.6. Intra-individual variation in glycaemic response during and after exercise in individuals with T1D

Despite the large inter-individual response to exercise, there appears to be a high inter-individual reproducibility in the plasma glucose response to aerobic (Temple et al., 1995, Kilbride et al., 2011, Abraham et al., 2017, Biankin et al., 2003) and HIIT (Riddell et al., 2019a) under standardised protocols. Riddell et al. (2019a) demonstrated that despite a mean coefficient of variation for the plasma glucose change across the four HIIT sessions and 16 subjects of 33.9 ± 39.3%, within subject visit to visit variation was well correlated (r= 0.58). Other studies have demonstrated even higher reproducibility during aerobic exercise (Temple et al., 1995, Abraham et al., 2017). Reproducibility may be higher in fasting conditions than post prandial (Biankin et al., 2003), with poor reproducibility when carbohydrates are ingested before or during exercise (Kilbride et al., 2011). However, this is likely due to glucose levels not being stable or adequately matched prior to exercise. Even in the studies that found high reproducibility, it is unclear if this is the case in free-living circumstances. While background insulin is likely to be similar day-to-day, having similar plasma glucose concentration, occurring at the exact same time of day and the type, duration and intensity of exercise all be similar could be a difficult to achieve.

1.7. Summary of the literature

Residual β-cell function and endogenous insulin secretion occurs in a substantial numbers of individuals with T1D. Currently it is not fully understood the role this remaining β-cell function plays in glycaemic control in individuals with T1D. This is especially true around exercise where maintaining glycaemic control during exercise is challenging, and a large inter-individual variation exists. Micro amounts of endogenous insulin secretion appears to offer some level of protection against vascular damage and diabetes complications. Endothelial progenitor cells (EPCs) are important for the repair and growth of blood vessels, with circulating number increased by exercise. However, the count and function of EPCs appears to be reduced in individuals with T1D. Further research is required into whether residual β-cell function in individuals with T1D influences these circulating cells.

1.8. Summary of experimental hypotheses and aims

The overarching aim of this thesis is to investigate the effects of residual β-cell function in individuals with T1D and its influence on glycaemic control under free-living condition and after an acute bout of aerobic exercise, as well on the count of circulating EPCs. The aims and hypotheses for each of the studies are detailed below:

Chapter 3:

The aim of this study was to examine the impact of residual β-cell function on glycaemic control during a free-living observational week in people with T1D. The primary outcome was percentage of the free-living observational week spent with an interstitial glucose in euglycaemia (3.9-10 mmol/L). Secondary outcomes were glycaemic variability (standard deviation (SD) and coefficient of variance (CV)), percentage time spent in hypoglycaemia and percentage time spent in hyperglycaemia. We hypothesized that individuals with greater C-peptide will have increased amount of time with an interstitial glucose in euglycaemia.

Chapter 4:

The aim of this study was to examine the impact of residual β-cell function on glycaemic control after a bout of aerobic exercise in people with T1D. The primary outcome was amount of time with an interstitial glucose in euglycaemia (3.9-10 mmol/L). Secondary outcomes were glycaemic variability (standard deviation (SD) and coefficient of variance (CV)), time spent in hypoglycaemia and time spent in hyperglycaemia. We hypothesized that individuals with greater C-peptide will have increased amount of time with interstitial glucose in euglycaemia.

Chapter 5:

The aim of this study was to examine the count of EPCs and hematopoietic progenitor cells between T1D participants and age, sex, BMI and fitness matched controls at rest, immediately and 1hr post exercise. The T1D group were further explored by examining EPCs mobilisation between residual β-cell groups. We hypothesized that the T1D group will have diminished count of resting and exercise mobilised EPCs compared to the control, while the T1D with high residual β-cell function will have augmented numbers compared to the lower C-peptide groups

Chapter 2. General Methodology

2. Chapter 2. General Methodology

Data collection commenced 1/11/16 and was completed on the 26/07/19, with a total number of 241 study visit days and 526 hours spent in the laboratory with participants. Figure 2.1 displays numbers of participants contacted and recruited, and the protocols participants undertook for this thesis.

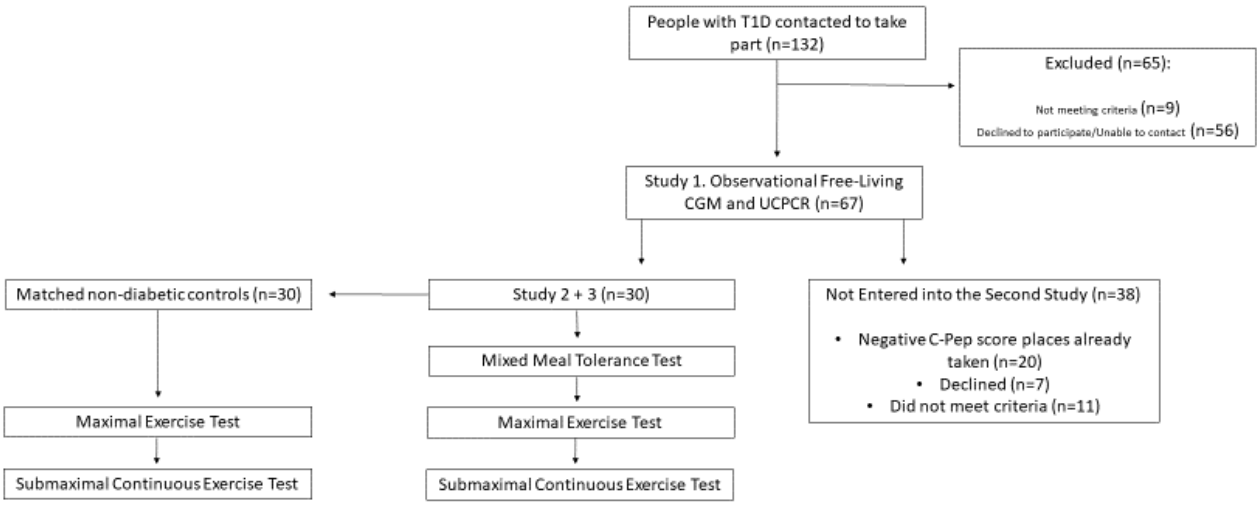

Figure 2.1 Schematic of the thesis recruitment and study protocols

2.1. Recruitment of T1D participants

Participants with T1D were recruited from the Newcastle Diabetes Centre, with potential participants being approached in clinic by their Diabetes Clinician and details passed to the research team if they were interested. Advertisement was also placed in local newspapers and posters were positioned around Newcastle University, Newcastle Diabetes Centre and the Newcastle Clinical Research Facility (CRF) in the Royal Victoria Infirmary hospital. Prospective participants were contacted and sent a participant information sheet (Appendix A). Those participants who were willing to partake in the study were subsequently screened against the inclusion/exclusion criteria and invited to attend the CRF for the first visit. Participants willing to take part signed the informed consent (Appendix B)

2.2. C-Peptide

C-peptide can be measured in whole blood serum, plasma and urine with the SI units of nmol/L and pmol/L (Leighton et al., 2017). C-peptide concentration has also been reported in nmol/mol and ng/mL. Table 2.1 has the conversion factors between the units.

Table 2.1 C-peptide concentration conversion factors

CONVERSION FACTORS:
ng/mL (µg/L) x 0.33333 = nmol/L
ng/mL x 333.33 = pmol/L
nmol/L x 3.0 = ng/mL
nmol/L x 100 = nmol/mmol
pmol/L x 0.003 = ng/mL
nmol/L x 1000 = pmol/L

2.3. Urine C-peptide to Creatinine Ratio test (UCPCR)

The measurement of C-peptide in urine is a convenient, non-invasive way of measuring β-cell function and insulin secretion. Within a 24h period, ~5-10% of C-peptide secreted from the pancreas is excreted into urine compared to around 0.1% of secreted insulin (Horwitz et al., 1977).

Within chapter 3, T1D participants completed a home Urine C-peptide to Creatinine Ratio test (UCPCR) to measure β-cell function and endogenous insulin secretion. Participants were provided with 2 urine pots containing boric acid preservation and instructed to collect a urine sample immediately before their largest meal of the day (highest carbohydrate content meal) and one 2 hours post the meal. The content of the meal was not specified and the participants were instructed to maintain their normal basal and bolus insulin and any other medication on the day of the sample collection, in line with previous validation studies (Besser et al., 2011a, Jones et al., 2011). Participants were asked to not consume any further food in the postprandial period until the 2 hour urine sample had been collected, unless experiencing a hypoglycaemic event, in which case an alternative urine kit was provided for completion upon another day (McDonald and Perry, 2016). The UCPCR kits were stored in a cool, dark place before being posted to Exeter Clinical Laboratory, to arrive within 36 hours of collection.

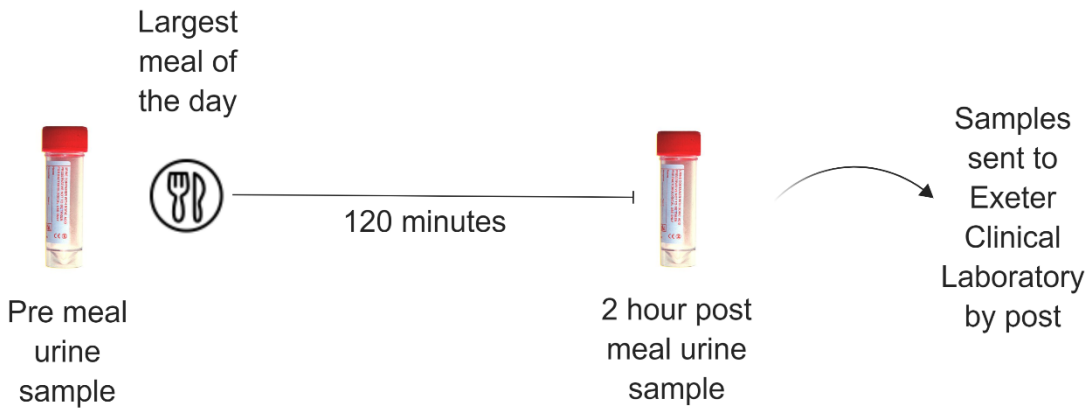

Figure 2.2 Schematic of the UCPCR collection procedure

UCPCR samples collected in boric acid have been shown to be stable for up to 72 hours at room temperature, with a mean of 103% of the sample tested at baseline and none dropping to <90% (McDonald et al., 2009). Upon arrival at Exeter Clinical Laboratory, samples were either analysed within 36 hours or frozen and analysed within the week. Urine C–peptide was analysed using a direct electrochemiluminescence immunoassay on the 602 module of the Cobas 8000 Roche E170 automated platform (Roche Diagnostics, Mannheim, Germany) at the Exeter Clinical Laboratory. See Serum C-peptide (chapter 2.4) for further details. Urinary creatinine was analysed on the Roche P800 platform using creatinine Jaffé reagent (standardized against isotope dilution mass spectrometry) and used to adjust for urine dilution to obtain UCPCR (nmol/mmol).

UCPCR measured during a mixed meal tolerance test (MMTT) or after a home meal is sensitive and specific method for measuring endogenous insulin secretion, with a 120 minute postprandial evening meal UCPCR highly correlated to the gold standard, 90 minute MMTT serum C-peptide ($r= 0.91$, $P < 0.001$) (Besser et al., 2011a). The test has been validated in people without diabetes, individuals with T1D and T2D, adults and children (Hope et al., 2013, Besser et al., 2013, Bowman et al., 2012, Jones et al., 2011, Oram et al., 2013, Besser et al., 2011b).

2.4. Serum C-peptide

Samples from the Mixed Meal Tolerance Test were shipped to Exeter Clinical Laboratory to determine serum C-peptide, with results used in chapter 4 and 5.

Serum C-peptide was analysed using a direct electrochemiluminescence immunoassay on the 602 module of the Cobas 8000 Roche E170 automated platform (Roche Diagnostics, Mannheim, Germany) at the Exeter Clinical Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK. The assay is a 2-site immunoassay with 20µL of serum reacted with a biotinylated monoclonal anti-C-peptide antibody (mouse), and a monoclonal anti-C-peptide antibody (mouse) labelled with ruthenium complex to form a sandwich complex. Samples, calibrators and controls were at 20-25 °C prior to measurement. The Cobas 8000 is standardized against the WHO International Reference Reagent for C-peptide of human insulin for immunoassay (IRR code 84/510) from the National Institute for Biological Standards and Control (Bristow and Das, 1988).

The lower limit of detection of the assay is 0.003 nmol/L (3.3 pmol/l). Values below the lower detection limit were classified as undetectable. Hope et al. (2016) reported an intra-assay coefficient of variation of 3.3% and inter-assay coefficient of variation of 4.5% for C-peptide measurements using the Roche E170 at the Exeter Clinical Laboratory. The serum C-peptide assay uses antibodies that show cross-reactivity with proinsulin and proinsulin conversion products. However, because the concentration of proinsulin is 100 times lower during fasting condition the cross-reactivity is ~10% and seen as negligible and having no clinical significance (Palmer et al., 2004).

2.5. Continuous Glucose Monitor (CGM)

Participants with T1D had a new generation Enlite® sensor (Enlite, Medtronic Diabetes, Medtronic Minimed, USA) inserted and iPro^{TM}2 Professional CGM (Medtronic Diabetes, Medtronic MiniMed, USA) attached for chapter 3 and chapter 4. In chapter 3, participants had the CGM fitted during the visit at the start of the observational week. While for chapter 4, participants attended the CRF 42 to 24 hours before the submaximal visit for insertion of the CGM.

The Enlite® sensor is a subcutaneous tissue sensor that provides an interstitial fluid glucose measurement. The sensor encases three platinum electrodes with glucose oxidase enzyme disseminated across the surface and where a constant voltage is maintained (Bailey et al., 2014). When glucose and oxygen pass through the semipermeable membrane, the enzyme-mediated oxidation of glucose generates

hydrogen peroxide that releases an electron per glucose and oxygen molecule (Keenan et al., 2012). This produces a fluctuating electrical current which is transmitted to the iPro™2 receiver (Cappon et al., 2017). Sensor readings are acquired every 10 seconds, and averaged values reported every 5 minutes. The sensor has an upper and lower limit of detection of 2.2 mmol/L and 22.2 mmol/L, respectively.

The Enlite® sensor was inserted into the subcutaneous tissue of the anterior superior lateral abdomen around the right or left lumbar region above the iliac crest. This is the location recommended by Medtronic and Keenan et al. (2012) for comfort and to minimise the physiological time-lag between blood and interstitial glucose concentration (figure 2.3).

When placed in the abdomen and calibrated three or more times per day, the Enlite® sensor provides accurate readings over a 6-day period in adults with T1D, with a mean absolute relative deviation (ARD) of 13.6% (Bailey et al., 2014).

After 5 to 10 minutes of allowing the sensor to become hydrated with interstitial fluid, the iPro™2 receiver was attached. The iPro™2 is a blinded CGM that stores the sensor readings in its device memory. Upon completion of the testing events, the device was removed and the data downloaded into the CGM software (CareLink, Medtronic Diabetes, Medtronic Minimed, USA) and analysed. While the iPro™2 records interstitial fluid glucose values every 5 minutes, the real time readings are not available to the participants to minimise influencing their behaviour around glucose control. Participants were required to record ≥ 4 blood glucose readings per day in the Food, Glucose and Insulin diary (appendix E).

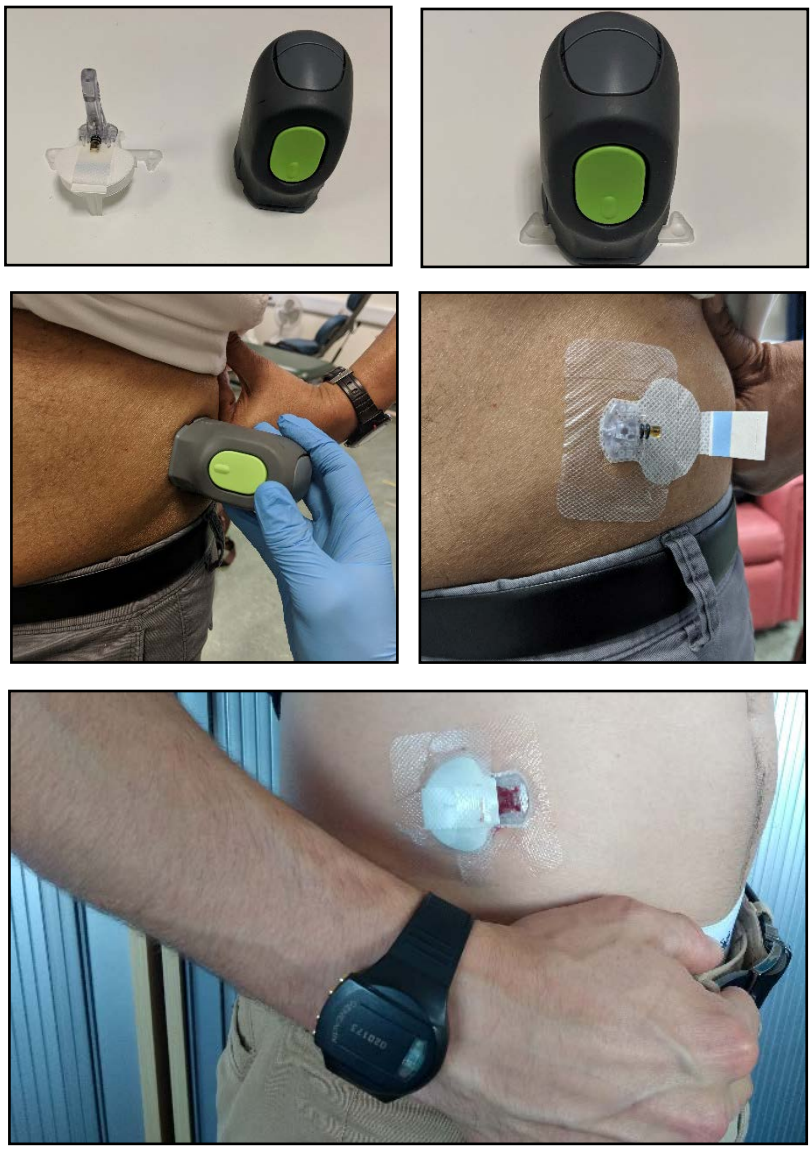

*Figure 2.3 Insertion of the Enlite® sensor and iPro™2. **A.** An Enlite® sensor in its pedestal casing (left) and the Serter automated insertion device (right). **B**. The Enlite® sensor loaded into the Serter device. **C.** Enlite® sensor being inserted into anterior superior lateral abdomen. The green button releases the sensor into the subcutaneous tissue. **D** The imbedded sensor, after the insertion needle had been removed and adhesive tape attached. **E.** The iPro™2 fully attached to the fitted Enlite® sensor and secured with overtape.*

2.6. CGM data processing

Upon downloading the data, the blood glucose readings were retrospectively entered into the CareLink software for automatic calibration by linear regression with 5 minute average interstitial glucose values attained for the duration of wear. Raw data were downloaded for analyses in Microsoft® Excel (Microsoft, USA).

To determine optimal accuracy of the cgm data, the CareLink software automatically performed checks of the acceptance thresholds (Table 2.2) on each individual day of data. If a day's data, from midnight to midnight, failed any of the thresholds or had

missing data greater than 15 minutes segments, subsequent collected data were deemed suboptimal and not used. In chapter 3, the CGM had to collect 4 valid days of data to be accepted; while in chapter 4, the exercise and following day had to be valid to be accepted. If the iPro^{TM}2 failed to collect this data the testing process was repeated.

Table 2.2 displaying the acceptable thresholds for the checks automatically performed in the CareLink software to determine the suitability and use of the data.

Available at: https://professional.medtronicdiabetes.com/para/carelink-pro-software

THRESHOLDS	EXPLANATION
VALID CALIBRATIONS	≥ 3 valid blood glucose readings are required per calendar day, with day not meeting this threshold unable to be properly calibrated. Only BG meter readings between 2.2 and 22.2 mmol/L are able to be used for calibration.
MAD% Mean absolute difference % - average difference between sensor and BG expressed	The threshold for MAD% varies depending on the range of BG meter values: - A range ≥ 5.6 mmol/L then a MAD% of 28.0 or less is considered optimal. - A range < 5.6 mmol/L then an MAD% of 18.0 or less is considered optimal.
CORRELATION	A correlation ≥ 0.79 between BG and CGM was considered optimal.

2.7. Calculation of time in range and glycaemic variability

The recent international consensus reports upon Continuous Glucose Monitoring Data Interpretation, endorsed by both the American Diabetes Association and European Association for the Study of Diabetes, outlined which advanced metrics of assessing CGM data should be used (Danne et al., 2017, Battelino et al., 2019). While Battelino et al. (2019) streamlined the CGM metrics for use in clinical practice (table 2.3) and focussed upon developing clinical targets for time in range (figure 2.4), this thesis will also use the extended metrics agreed in Danne et al. (2017) (table 2.4) to explore the CGM data in further detail.

Table 2.3 displaying the Continuous Glucose Monitoring Data interpretation guidelines recommended by Battelino et al. (2019) and used within this thesis.

STANDARDIZED CGM METRICS FOR CLINICAL CARE: 2019
1. **Number of days CGM worn** (recommend 14 days)
2. **Percentage of time CGM is active** (recommend 70% of data from 14 days)
3. **Mean Glucose**
4. **Glucose management indicator** (GMI) (formally estimated HbA1c)
5. **Glycaemic variability** Coefficient of variation (%CV) target < 36%
6. **Level 2 hyperglycaemia** - Time above range (TAR): % of readings and time > 13.9 mmol/L
8. **Time in range** (TIR): % of readings and time 3.9–10.0 mmol/L
10. **Level 2 hypoglycaemia** - Time below range (TBR): % of readings and time < 3.0 mmol/L

Table 2.4 displaying the additional Continuous Glucose Monitoring Data interpretation guidelines used within this thesis recommended by Danne et al. (2017).

STANDARDIZED CGM METRICS: 2017 INTERNATIONAL CONSENSUS
1. **Level 1 hyperglycaemia** - Time above range (TAR): % of readings and time > 10 mmol/L
2. **Level 1 hypoglycaemia** - Time below range (TBR): % of readings and time < 3.9 mmol/L
3. **Glycaemic variability** Standard deviation (SD)
4. **Hypoglycaemia and hyperglycaemia episodes** - >15 min at level 1 and 2 hypoglycaemia and hyperglycaemia. End of a CGM event: readings for 15 min > 3.9 mmol/L or < 10 mmol/L

Raw data were downloaded into excel, with time in each glycaemic range calculated as percentage of total time in chapter 3 and percentage of stipulated time periods in chapter 4 (12 hours pre exercise, 12 hours post exercise, 24 hours post exercise, 48 hours post exercise and 24 to 48 hours post exercise). Participant's time in each glycaemic range was compared against the recommendations made by the international consensus (figure 2.4).

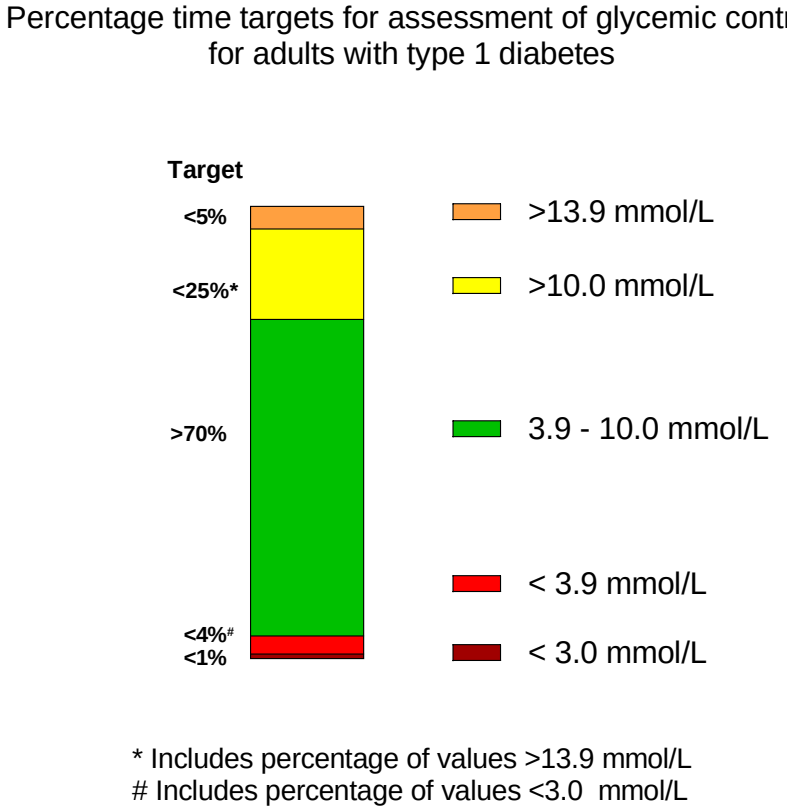

Figure 2.4 Schematic of recommendations for percentage of time spent in glycaemic ranges adapted from Battelino et al. (2019)

Glycaemic variability measured were calculated using the EasyGV© Microsoft Excel workbook (Oxford University, UK) available for use at www.easygv.co.uk. Coefficient of variation was calculated by dividing standard deviation by the mean.

2.8. Cannulation and blood sampling

A 22-gauge cannula (Vasofix®, B.Braun Melsungen AG, Germany) was inserted into the antecubital or dorsal hand vein using aseptic non touch technique (ANTT) by a trained phlebotomist. Participants were instructed to attend the visits in a well hydrated condition to aid in the insertion of the cannula. A 3M™ Tegaderm™ I.V. securement dressing (3M Health Care, Germany) was used to fixate the cannula, while a leur encased in a barrel was connected to draw samples. A Stylet (Madrin/Stylet Introcan®, B Braun, Germany) was inserted into the cannula in between sample draws to prevents thrombus formation and catheter obstruction at rest and during exercise.

2.9. YSI

The YSI 2300 STAT PLUS Glucose and Lactate Analyzer (YSI Inc, Xylem Analytics, USA) is a laboratory instrument intended to quantify blood glucose and L-lactate in whole blood and plasma. The YSI 2300 analyser aspirates 25 µl of whole blood collected in BD Fluoride or EDTA vacutainers®, which is diluted with 475 µL buffer to create a 1/20 solution. Total glucose is measured after the intracellular glucose diffuses out of the erythrocytes by using glucose oxidase to convert glucose and oxygen to gluconic acid and hydrogen peroxide (H_20_2) thus creating an evaluable electrical signal that corresponds with glucose concentration (Astles et al., 1996). Glucose concentrations are determined by a comparison to an internal 10 mmol/L glucose calibration standard, with daily calibration carried out with external 10 and 25 mmol/L glucose standards. Acceptable range for the external samples are the following:

10 mmol/L – 9.8 mmol/L and 10.2 mmol/L

25 mmol/L – 24.2 mmol/L and 25.8 mmol/L

2.10. HbA1c

An additional 4ml EDTA vacutainer® was drawn at 0 min from the T1D participants during the mixed meal tolerance test (see 4.2.3.2) and 0 min of the submaximal exercise bout for healthy controls (see 5.2.2.3). Samples were sent to and analysed using a TOSOH G11 analyser at the Newcastle Laboratory at Royal Victoria Infirmary, Newcastle-upon-Tyne for glycosylated haemoglobin (HbA1c).

2.11. Health Screening

Pre-exercise screening was delivered in accordance with the standing operating procedures for exercise testing in the Medical Research Council Muscle Performance & Exercise Training Laboratory based upon the American College of Sports Medicine's (ACSM) guidelines for exercise testing (Wilkins, 2017). Screening was undertaken by an ACSM Certified Clinical Exercise Physiologist.

Participants completed a medical history and physical examination (appendix C and D.) before being stratified into low, moderate or high risk of an adverse response to exercise determined by using the ACSM pre-participation screening algorithm before a resting ECG and stress test was performed.

2.11.1. Electrocardiogram (ECG)

A modified 12 lead electrocardiogram (ECG) (Mason and Likar, 1966) was used to assess the electrical activity of the heart at rest and during exercise to check for any cardiac abnormalities. Any abnormalities or contraindications to exercise prior to exercise were checked with an on call physician. The exercise protocol was terminated if any adverse events occurred during the testing bout and patient care managed by the clinical cover. Contraindications to exercise, relative and absolute indicators for terminating the exercise test were derived from ACSM guidelines (Wilkins, 2017).

2.11.2. Protocol for an unsuccessful screening

If an individual was identified as having a resting or exercise induced cardiac abnormalities or contraindications to exercise, they were referred to their general physician (GP). A report of the findings was sent to the GP and the patient advised to avoid exercise until they had been seen and cleared. Two T1D individuals who participated in the study demonstrated abnormal resting ECGs and the protocol above followed. Both participants were later cleared of any problems and entered back into the study. All other T1D and healthy participants in these studies showed normal cardiac function.

2.12. VO_{2peak} testing

A maximal graded exercise test was performed on a Lode Valiant 2 CPET treadmill (Lode, Groningen, The Netherlands) to determine peak oxygen uptake (VO_{2peak}) and peak heart rate (HR_{peak}). The Bruce protocol, a continuous incremental walking to running test series of 3 minute stages of steady state exercise (Table 2.5), was used (Bruce et al., 1973). Participants started exercising at a treadmill speed of 2.7 $km \cdot h^{-1}$ and an incline of 10% gradient with workload subsequently increased in a simultaneous way until volitional exhaustion was reached (Table 2.5). The protocol duration was aimed for between 8 to 12 minutes long (Yoon et al., 2007), with strong verbal encouragement given throughout (Moffatt et al., 1994) to maximise the peak VO_2 attainted. Throughout the exercise test a Meta Control 3000 (Cortex, Germany) measured expired air and ECG continuously, while blood pressure was measured at rest, the last minute of each stage and for 10 minutes after cessation of the test. Participants wore a face mask (Hans Rudolph 7450 Series V2 mask) to collect expired air which was immediately measured by an online gas analyser (Metalyzer® 3B-R3 CPET, Cortex, Germany) and a 12-channel PC ECG device (Custo cardio 110, Cortex,

Germany). The test was considered a maximal effort if participants met two of the following criteria: a distinct plateau in oxygen consumption, a peak hr ≥ 90% age predicted maximum HR (220-age), a rating of perceived exertion (RPE) of ≥ 18 and / or respiratory exchange ratio (RER) of 1.15 or greater (Edvardsen et al., 2014).

Table 2.5 Speed and gradient for each stage of the Bruce maximal treadmill test

STAGE	SPEED (KM·H^{-1})	GRADIENT (%)
1	2.74	10
2	4.02	12
3	5.47	14
4	6.76	16
5	8.05	18
6	8.85	20
7	9.65	22
8	10.46	24
9	11.26	26
10	12.07	28

2.13. Gas Analysis

Expired gas was captured by Hans Rudolph 7450 Series V2 facemask and immediately measured by a Metalyzer® 3B-R3 CPET online gas analyser. Before calibration, the Metalyzer® 3B-R3 system was switched on for a minimum of 30 minutes to allow the sensors to warm up and ensure that the oxygen and carbon dioxide sensors were stabilised. To calibrate the O_2 and CO_2 sensors, ambient air and calibration gas cylinder with a known concentration (17.1% O_2, 5.0% CO_2: BOC, Industrial Gases, Linde AG, Munich, Germany) were used, while a 3 litre syringe (Series 5530, Hans-Rudolph Inc, USA) was used to check sample volume. As part of the calibration, the system automatically checked ambient temperature, humidity and pressure, and expired gas samples were corrected to standard temperature and pressure. Breath by breath volume of oxygen and carbon dioxide (VO_2 and VCO_2) were calculated within the Metasoft software (Cortex Biophysik GmbH, Germany) using standard metabolic algorithms employing the Haldane transformation. VO_2 data were collected in 10 second averages.

2.14. Determining VO_{2peak} and maximal heart rate

The peak rate of oxygen uptake and utilization during the maximal intensive exercise (VO_{2peak}) was determined by averaging the highest consecutive 30 seconds VO_2 value before voluntary exhaustion.

The highest number of beats per minute recorded during maximal exercise test was determined as maximal heart rate (HR_{peak}).

2.15. Calculating energy expenditure and substrate oxidation

Measurement of oxygen consumption and excretion of carbon dioxide during the submaximal exercise bout were used to estimate substrate oxidation rates using stoichiometry calculations as described by Jeukendrup and Wallis (2005) (Table 2.6).

Table 2.6 Jeukendrup and Wallis equations for calculating carbohydrate and fat oxidation from gas measurements at moderate to high intensity exercise (50 to 75% $\dot{V}O_{2peak}$)

Carbohydrate Oxidation (g/min)	4.210 V̇CO2 – 2.962 V̇O2 – 2.37 n
Fat Oxidation (g/min)	1.695 V̇O2 – 1.701 V̇CO2 - 1.77 n
Energy from 1g of carbohydrate (20% glucose and 80% glycogen)	4.07 kcal
Energy from 1g of fat	9.75 kcal
Energy Expenditure*	0.550 V̇CO2 + 4.471 V̇O2

n = urinary nitrogen excretion

* Calculations of energy expenditure assume negligible contribution to energy expenditure from protein oxidation

Previous calculations of carbohydrate oxidation have use different substrate breakdowns. Calculations are predominantly for use at rest, thus glucose has been used as the representative carbohydrate (Frayn, 1983). To account for muscle glycogen contribution during moderate exercise alternative calculations have been developed, with the traditional equations potentially overestimating the carbohydrate oxidation by 8% (Jeukendrup and Wallis, 2005). While the contribution to carbohydrate oxidation is repeatedly reported to be around 15 to 25% from plasma glucose and 75 to 85% from muscle glycogen at moderate to high intensity endurance activities (Jeukendrup, 2003), these calculations are based upon individuals without diabetes and it is unknown whether these assumptions can be applied to individuals with T1D. While Robitaille et al. (2007) demonstrated that substrate and exogenous glucose oxidation rates are similar between T1D and healthy controls, plasma glucose oxidation was lower with muscle glycogen compensating for this short fall. Conversely, research by Raguso et al. (1995) who suggest that the shortfall in plasma glucose is compensated for by an increased intramuscular triglyceride oxidation. Hyperglycaemia has also been shown to shift fuel metabolism further towards carbohydrate oxidation in T1D compared to euglycaemia (Jenni et al., 2008). Within this thesis, participants were fed a carbohydrate snack prior to exercise with T1D

participants withholding bolus insulin, typically resulting in hyperglycaemia during the submaximal exercise bout. Despite these problems with calculating substrate oxidation and energy expenditure, recent studies have followed these or similar assumptions (Zaharieva et al., 2019a, Campbell et al., 2016).

2.16. Calculating treadmill speed and gradient for the sub maximum exercise bout

Sixty percent of the participant's individual VO_{2peak} was used to determine the gradient needed upon the treadmill during the submaximal test. As humans prefer to walk at or near the speed that minimizes their gross energy expenditure (Ralston, 1958), and individuals preferred walking speed is influenced by factors such as height, sex and age (Finnis and Walton, 2008), a range of treadmill speeds (4.5kph, 5.1kph and 6kph) were chosen and the gradient determined for each. Participants were allowed to choose whichever speed they found most comfortable.

The ACSM walking metabolic equation (Glass et al., 2007) was used to determine the percentage gradient (Table 2.7).

Table 2.7 displaying ACSM walking metabolic equation (Glass et al., 2007)

TARGET VO_2 (ml.kg.min) = 3.5 + (0.1 X SPEED) + (1.8 X SPEED X % GRADE)

While limited data exists looking at the accuracy of this metabolic equation in a range of populations, the ACSM walking equation can accurately predict VO_2 and energy expenditure and may be the most suitable prediction equation to use (Hall et al., 2004).

2.17. Glycaemic strategies during exercise testing

The participant's with T1D blood glucose control was managed according to the current international consensus (Riddell et al., 2017). Participant's capillary blood glucose concentrations were checked before, during and after exercise using a handheld CONTOUR® blood glucose meter (Contour Next, Ascensia Diabetes Care, USA).

2.17.1. Glycaemic strategies during the maximal exercise test

Participants blood glucose was checked upon arrival, with a blood glucose concentration 5 - 6.9 mmol/L treated with 10g of carbohydrates from glucose tablets (Glucotabs, BBI Healthcare, UK) while participants with < 5 mmol/l consumed 15g of carbohydrate from a glucose shot (Glucojuice, BBI Healthcare, UK). Participants with

a blood glucose of >15 mmol/L had their blood ketones checked upon a KEYA® smart meter (Inside Biometrics International Ltd, UK), with visits rearranged if the individual had modestly elevated ketones >0.6 mmol/L. Patients commenced exercise when blood glucose was ≥7 mmol/L for a minimum of 15 minutes.

After completion of the incremental exercise test (see 2.17.1), participants were discharged from the CRF following completion of the exercise test if blood pressure had returned to resting values and blood glucose was ≥7 mmol/l for a minimum of 15 minutes.

2.17.2. Glycaemic strategies during the submaximal exercise test

If participants had a hypoglycaemic event overnight prior to the study visit, the visit was rearranged, while if participants awoke with blood glucose >10mmol/L they were instructed to have a small bolus of insulin upon waking (<2 units). Participants were instructed to maintain their basal insulin as normal.

After arriving and insertion of a cannula (See 2.8), participants were fed a carbohydrate snack (Belvita Soft Bakes Chocolate Chip, Mondelēz International, USA), providing 204kcal of which 31g CHO. After resting for 20 minutes, the bout of exercise was started if the participants blood glucose was >7 mmol/L.

Interval capillary blood samples were collected throughout the bout of exercise, with participants given a 100ml drink with 10g of glucose if their blood glucose fell <7 mmol/L.

2.18. Statistical Analysis

Throughout this thesis GraphPad Prism 8.0.1 (San Diego, USA) and IBM SPSS Statistics (version 24, IBM, Armonk NY) software package were used to analyse the data. Statistical analysis that were repeated throughout this thesis are detailed below.

Differences between C-peptide groups were assessed by the use of a one-way ANOVA with Tukey-Kramer post-hoc analysis. Data were assessed for normality and outliers by Shapiro-Wilk test and boxplots, with excessively skewed data SQRT or LOG10 transformed or assessed by Kruskal-Wallis H test. When the assumption homogeneity of variances was met, a one-way ANOVA was used to determine statistical significance, with significant results interpreted to determine possible group comparisons by Tukey-Kramer post hoc test. Assumption of homogeneity of variances

were assessed by Levene's test for equality of variances. When the assumptions were violated, a Welch ANOVA was used to determine statistical significance with significant results interpreted by Games-Howell post hoc test. The distributional assumption was used to determine if the Kruskal-Wallis H test was used to compare medians or distribution.

Pearson product-moment or Spearman's rank-order correlation were used to determine the strength and direction of a linear relationship between C-peptide and CGM data.

A two-way repeated measures mixed ANOVA was used to examine for interactions between time and C-peptide groups during the MMTT in chapter 4, as well as for comparing EPCs mobilisation with between the T1D and control groups and between the C-peptide groups (chapter 5). Significant time and group main effects were analysed using post-hoc Bonferroni adjusted pairwise comparisons. Outliers were assessed by examination of studentized residuals for values greater than ±3, while normality was assessed by Shapiro-Wilk's test of normality on the studentized residuals. Assumption of sphericity was evaluated by Mauchly's test with violated data asssed by Greenhouse-Geisser or Huynh-Feldt. When values where missing, a fitted mixed effects model with Geisser-Greenhouse as implemented in GraphPad Prism 8.0 was used. The mixed model uses a compound symmetry covariance matrix, and is fit using Restricted Maximum Likelihood (REML). In the absence of missing values, this method gives the same P values and multiple comparisons tests as repeated measures ANOVA. In the presence of missing values (missing completely at random), the results can be interpreted like repeated measures ANOVA. Excessively skewed data were transformed using square root and logarithmic transformation.

Chapter 3. The influence of residual β-cell functions upon free-living glycaemic control in individuals with type 1 diabetes

Research team members and responsibilities:

Guy Taylor – Study design, led participant recruitment, collected informed consent, data collection and processing (continuous glucose monitoring, accelerometer, questionnaires, diaries, anthropometric measurements, medical history and notes), and statistical analysis.

Dr Daniel West – Study design, obtained funding, resources and ethical approval, reviewed data and chapter.

Professor Emma Stevenson – Reviewed chapter

Professor Jim Shaw – Study design, participant recruitment and provided medical consultation on exclusion of suspected non-T1D participant.

Dr Ayat Bashir - Participant recruitment.

Dr Anneliese Flatt - Participant recruitment.

Dr Timothy McDonald - Analysed Urine C-peptide to Creatinine Ratio tests.

Dr Richard Oram - Analysed Urine C-peptide to Creatinine Ratio tests.

3. Chapter 3. The influence of residual β-cell functions upon free-living glycaemic control in individuals with type 1 diabetes

3.1. Abstract

Objective

To investigate the impact of residual β-cell function CGM outcomes during a free-living observational week in people with T1D.

Research Design and Methods

Sixty-seven participants with T1D for ≥1 years were recruited. Participants wore a blinded CGM and GENEActiv accelerometer for 7 days of free-living data capture. Participants completed a home UCPCR test which was sent to Exeter Clinical Laboratories Medical for analysis. Medical history questionnaire data were collected, and participants completed questionnaires on their hypoglycaemia awareness, and a blood glucose, food, insulin and exercise diary. UCPCR was used to allocate participants into undetectable ($Cpep_{und}$ <0.001 nmol/mmol), low ($Cpep_{low}$ 0.001–0.019 nmol/mmol) or high C-peptide groups ($Cpep_{high}$ ≥0.20 nmol/mmol).

Results

UCPCR was positively associated with percentage time spent in euglycaemia (r= 0.341, p= 0.005), and negatively associated with time in hyperglycaemia (percentage of time >13.9 mmol/L; r= -0.412, p= 0.001), but not time in hypoglycaemia (percentage of time <3.9 mmol/L; r= -0.200, p= 0.874). UCPCR was a significant predictor of the above outcomes in a stepwise multiple regression including clinical variables. No differences exist between $Cpep_{und}$ and $Cpep_{low}$ for any outcome, $Cpep_{high}$ had statically significantly higher time in euglycaemia (71.8±17.0%) compared to Cpepund (60.5±14.6%, p= 0.031) but not Cpeplow (60.1± 14.9%, p= 0.091)

Conclusions

People with T1D who have higher residual β-cell function show improved glycaemic control during free-living periods, driven by reduction in hyperglycaemia and glycaemic variability. Quantifying C-peptide could potentially be used to personalise glycaemic targets.

3.2. Introduction

T1D is an autoimmune disease, originating from the selective destruction of insulin producing β-cells in the pancreatic islets of Langerhans by immune cells (Anderson and Bluestone, 2005, Bluestone et al., 2010, Willcox et al., 2009). Throughout the autoimmune destruction of the β-cells, insulin secretion declines eventually resulting in dysregulation of glycaemia and clinical diagnosis (Gepts, 1965, Daaboul and Schatz, 2003). With the initiation of exogenous insulin therapy a partial remission phase is common, where exhausted but not yet destroyed β-cells are able restoration some of their endogenous insulin secretion (Abdul-Rasoul et al., 2006, Snorgaard et al., 1992). However, the destruction of the β-cells continues, with the traditional Eisenbarth model of T1D (Eisenbarth, 1986b) stating that complete and inevitable destruction occurs.

Recent research has challenged this dogma that all β-cell are destroyed, with insulin-positive islets discoverable in autopsy studies (Keenan et al., 2010, Gepts, 1965, Lam et al., 2017, Rodriguez-Calvo et al., 2018) and C-peptide measureable (Keenan et al., 2010, Oram et al., 2014, Oram et al., 2015, Wang et al., 2012, Davis et al., 2015, McGee et al., 2014), even decades after diagnosis. The development of highly sensitive C-peptide assays (Oram et al., 2014, Wang et al., 2012) has led to substantial numbers of the T1D population having detectable residual β-cell function, although the prevalence remains unclear, with estimates of between 35 and 80% of participants having detectable C-peptide at >5 years post-diagnosis (Williams et al., 2016, Oram et al., 2015). Detectable C-peptide appear to be associated with disease duration and age at diagnosis, with those diagnosed at an older age being more likely to have persistent C-peptide (Davis et al., 2015).

The Diabetes Control and Complications Trial (DCCT) established the initial evidence that persistent C-peptide secretion has a clinical benefit (Steffes et al., 2003a). Participants who had a stimulated C-peptide > 200 pmol/l at study entry and were randomised into the intensive control arm had reduced development of retinopathy and nephropathy, lower HbA1c and lower incidences of serve hypoglycaemia (65%) in the subsequent 7 year follow up period compared to those with < 200 pmol/L (Lachin et al., 2014, Steffes et al., 2003a). It is estimated that 8-16% of individuals diagnosed with T1D as an adult have this relatively high C-peptide level, compared to 5-6% of individuals with childhood onset of diabetes (Oram et al., 2015, Davis et al., 2015,

Williams et al., 2016). Additionally, further research has demonstrated that even much lower levels (>10 pmol/L) of C-peptide secretion have also been shown to a have positive impact on rates of diabetic complications (Panero et al., 2009, Lachin et al., 2014).

Evidence of residual β-cell impact upon glycaemic control within individuals with T1D is an emerging area. Preservation of 10-50 pmol/L C-peptide has also been associated with lower self-reported rates of symptomatic, asymptomatic and severe hypoglycaemia compared to C-peptide negative individuals (Kuhtreiber et al., 2015, Marren et al., 2019). While the DCCT (Lachin et al., 2014, Steffes et al., 2003a) and Kuhtreiber et al. (2015) trials show higher C-peptide is associated with lower HbA1c, Marren et al. (2019) and Gibb et al. (2020) recently found no difference in HbA1c between low (<10 pmol/L) and preserved (>20 pmol/L and >10 pmol/L, respectively) C-peptide groups within UK populations.

In recently diagnosed and post islet transplantation patients, studies demonstrate that as residual β-cell function declines, CGM parameters such as time in euglycaemia (time in range 3.9-10 mmol/L) and CV% worsen (Buckingham et al., 2015, Brooks et al., 2015). These populations have higher C-peptide levels than most individuals with long duration T1D, yet two very recent papers have assessed how low levels of C-peptide secretion impacts CGM outcomes in longer duration T1D diabetes. Rickels et al. (2020) demonstrating that in individuals with duration of T1D of >2 years and a very high stimulated C-peptide (>400 pmol/L) had greater time in euglycaemia and reduced time in hyperglycaemia compared to negative, low (17-200 pmol/L) and intermediate (200-400 pmol/L) C-peptide groupings, with no differences in time spent in hypoglycaemia. A large observational study (n=290) conducted by Gibb et al. (2020) reported that individuals >3 years post diagnosis with preserved C-peptide (either 10 to 200 pmol/L or 10 to 50 pmol/L) had fewer low-glucose events and lower glucose variability by flash monitoring compared to low (<10 pmol/L) secretors. However, no differences in time in euglycameia or hyperglycaemia existed. With these varying results, further research exploring endogenous insulin productions impact on glycaemic control in long duration T1D is clearly warranted.

This study examined the impact of residual β-cell function on glycaemic control during a free-living observational week in people with T1D. We hypothesized that individuals

with greater C-peptide will have increased amount of time with interstitial glucose in euglycaemia (3.9 to 10 mmol/L) – the primary outcome.

3.3. Methods

The study was an observational trial, examining how residual beta cell function impacts free-living glycaemia in individuals with T1D.

3.3.1. Participants

Participants were recruited as described (see 2.1). Briefly, interested potential participants from the Newcastle Diabetes Centre and local area were sent a participant information sheet. Those willing to partake were subsequently screened against the inclusion/exclusion criteria. Those who meant the criteria and were willing to partake attended the Newcastle Clinical Research Facility (CRF) and provided written informed consent. This study was approved by the local National Health Service Research Ethics Committee (code: 16/NE/0192).

Eligibility criteria comprised a clinical diagnosis of T1D (primary osmotic symptoms, weight loss, hyperglycaemia, ketosis, insulin initiation at diagnosis); age 18-70 years with diabetes duration >1 years at enrolment; HbA1c <86 mmol/mol (10.0%); and stable Multiple Daily Injection (MDI) or Continuous Subcutaneous Insulin Infusion (CSII) regimen without changes over the preceding 6 months.

3.3.2. Protocol

Participants attended the Newcastle CRF for fitting of a continuous glucose monitor (CGM) (as described in 2.5) and wrist worn GENEActiv accelerometer, completing a medical history questionnaire, and given questionnaires on their hypoglycaemia awareness (Gold score and HypoA-Q), a blood glucose, food, insulin and exercise diary (Appendix E) and a home UCPCR test (see 2.3) to complete at home. The CGM, accelerometer and blood glucose, food, insulin and exercise diary were completed over the subsequent 7-8 days after the visit. During the free-living observational week participants maintained their standard care, including use of their own CGM/Flash glucose monitor (FGM).

Participant's height, weight (Seca 220 height measure / Seca 889 scales, Seca, Germany) and medical history were taken (Appendix C). A blinded CGM unit (Enlite®

sensor with iPro^{TM}2 Professional CGM Medtronic Diabetes, Medtronic MiniMed, USA) was inserted into lateral abdomen (as described in 2.5). The participants were instructed to self-record blood glucose concentrations, using their normal capillary glucose monitor, at least four times per day into the blood glucose, food, insulin and exercise diary (Appendix E) for calibration purposes.

Participants were given a wrist worn accelerometer (GENEActiv, Activinsights Ltd. Cambridge, UK) to be worn upon their non-dominant arm at all times. The GENEActiv has a tri-axial, ± 6 g seismic acceleration sensor, with a near body temperature sensor to help improve the confirmation of wear and non-wear time, and is calibrated for physical activity against indirect calorimetry (Esliger et al., 2011).

A home UCPCR kit was given to the participant to complete within the free-living observational week with instructions to collect urine samples immediately before their largest meal of the day (highest carbohydrate content meal) and 2 hours post the meal (as described in 2.3). Participants sent the sample from home to Exeter Clinical Laboratories in a free post envelope where they were analysed (as described in 2.3 and 2.4).

Post meal UCPCR result was used to assess C-peptide status. For further analysis, individual's post meal UCPCR was used to confirm which C-peptide groups participants were sorted into; undetectable ($Cpep_{und}$, <0.001 nmol/mmol), low ($Cpep_{low}$, 0.001 to 0.19 nmol/mmol) and high ($Cpep_{high}$ ≥0.2 nmol/mmol). The $Cpep_{high}$ threshold was based upon the threshold found in the Diabetes Control and Complications Trial to have some clinical benefits (Lachin et al., 2014).

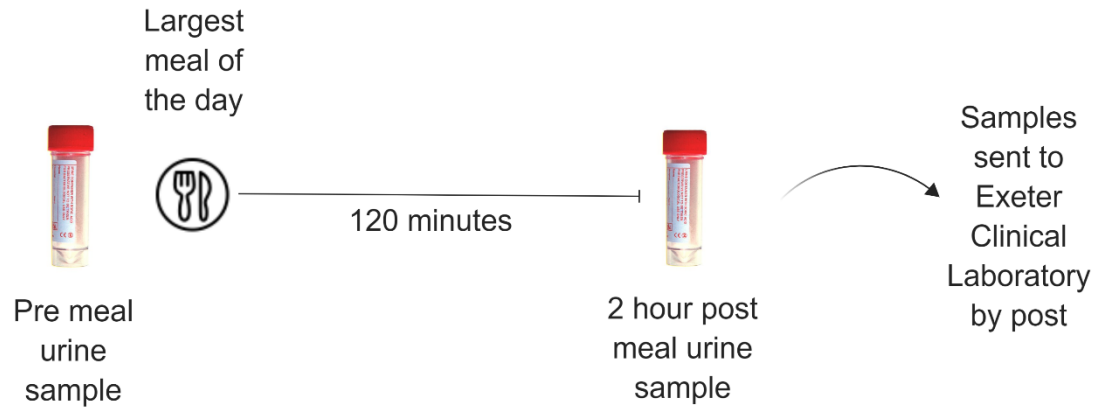

Figure 3.1 Schematic of the UCPCR collection procedure

After a week of wearing the CGM and accelerometer, participants either self-removed the CGM and the monitors were collected or participants returned to the CRF for removal. Upon removal, participants completed questionnaires on their hypoglycaemia awareness; Gold score (Gold et al., 1994) and Hypoglycaemia Awareness Questionnaire (Hypo A-Q) (Speight et al., 2016). Participants most recent HbA1c was accessed from clinical notes

3.3.3. Data processing

CGM sensor data were retrospectively processed using CareLink iPro software (Medtronic Diabetes). Data from the blood glucose, food, insulin and exercise diary was used to calibrate the iPro^{TM}2 data. If a day's CGM recording, from midnight to midnight, failed any of the Carelink optimal data thresholds (Valid calibrations, MAD%, Correlations) (Medtronic, 2017) or had missing data greater than 15 minutes segments, data from throughout that day were deemed sub-optimal and not used. The CGM had to collect 4 valid days of data to be accepted. If the iPro^{TM}2 failed to collect sufficient data the testing process was repeated.

Accelerometer data were downloaded to .bin files and converted to 15 second epoch .csv files using PC software version 3.2, before processing in R using R-package GGIR version 1.9 (Migueles et al., 2019). Threshold for moderate activity or greater was set as ≥100 mg. 18 hours of daily wear time was considered an acceptable threshold for data inclusion and only moderate and vigorous activity bouts (MVPA) of ≥10 minutes duration were analysed (van Hees et al., 2013, Sabia et al., 2014). Data were displayed as average minutes of MVPA per day. The accelerometer had to collect 4 valid days of data to be accepted. If the GENEActiv failed to collect sufficient data the testing was repeated the following week, or if the iPro^{TM}2 had failed to collect data, the accelerometer data collection were also redone during the repeated testing week.

3.3.4. Statistical and data analysis

Data are presented as mean±standard deviation throughout unless otherwise stated with statistical significance set at $p<0.05$. The primary outcome was percentage of the free-living observational week spent with an interstitial glucose in euglycaemia (3.9-10 mmol/L). Secondary outcomes were glycaemic variability (standard deviation (SD)

and coefficient of variance (CV)), percentage time spent in hypoglycaemia and percentage time spent in hyperglycaemia. CGM ranges were defined as 3.9-10 mmol/L (euglycaemia), <3.9 mmol/L (hypoglycaemia 1), <3.0 mmol/L (hypoglycaemia 2), >10 mmol/L (hyperglycaemia 1), >13.9 mmol/L (hyperglycaemia 2) as recommended by international consensus (Danne et al., 2017, Battelino et al., 2019). CV was calculated as SD divided by mean glucose.

Pearson product-moment or Spearman's rank-order correlation were used to determine the strength and direction of a linear relationship between post meal UCPCR and CGM data.

Stepwise multiple regressions were performed with UCPCR, sex, age, age at diagnosis, BMI, gold score and MVPA inputted to assess their prediction on glycaemic outcomes. These variables were included on the basis of previous research and discussions with clinicians on appropriate predictors. Sex (Manicardi et al., 2016), age (Beck et al., 2012), age at diagnosis (Clements et al., 2014), BMI (Lee et al., 2017a), insulin dose (Strich et al., 2017), gold score of hypoglycaemia unawareness (Gold et al., 1994) and physical activity (Kennedy et al., 2013) all been associated with changes in glycaemic control. It was determined that duration of diabetes would be excluded from the multiple regression to avoid 1st-order autocorrelation with age and age of diagnosis. The one in ten rule for number of events per variable in a logistic regression analysis was followed to avoid biasing the regression coefficients (Peduzzi et al., 1996).

Statistically significant differences between the means of $Cpep_{und}$, $Cpep_{low}$ and $Cpep_{high}$ were determined by one-way ANOVA with Tukey post-hoc analysis. Data were assessed for normality and outliers by Shapiro-Wilk test and boxplots, with excessively skewed data transformed or assessed by Kruskal-Wallis H test.

GraphPad Prism 8.0.1 (San Diego, USA) and IBM SPSS Statistics (version 24, IBM, Armonk NY) software package were used to analyse the data.

3.4. Results

Data were collected for 68 participants. One participant was excluded because of a non-diabetic C-peptide value and expected MODY. Participant demographic data is displayed in Table 3.1.

Table 3.1 Participants Characteristics (n=67)

	Mean ± SD	Range
N	67	
Male / Female	35 / 32	
UCPCR (nmol/mol)	0.22 ± 0.42	0.0 to 1.88
Median	0.00	
Undetectable / Positive	34 / 33	
Age (years)	41.1 ± 12.2	18 To 68
Age At Diagnosis	19.9 ± 11.7	1 To 56
Duration Of Diabetes (years)	21.2 ± 12.7	1 To 47
BMI (kg.m^2)	25.6 ± 3.5	20.1 To 35.5
HbA1c (mmol/mol)	58.1 ± 10.3	34 to 83
(%)	7.5 ± 3.1	5.3 to 9.7
Method Of Insulin Control (MDI / CSII)	37 / 30	
Daily Insulin (Units)	40.55±19.10	12.83 to 121.84
Insulin units/kg/day	0.53±0.20	0.15 to 1.17
CGM (None / Flash / CGM)	50 / 10 / 7	
Moderate To Vigorous Physical Activity (Minutes A Day)	24.2 ± 18.3	0 to 84.5

During the observational week, when assessed by Spearman's rank correlations, UCPCR was positively associated with percentage time spent in euglycaemia (3.9 to 10 mmol/L) (Figure 3.2).

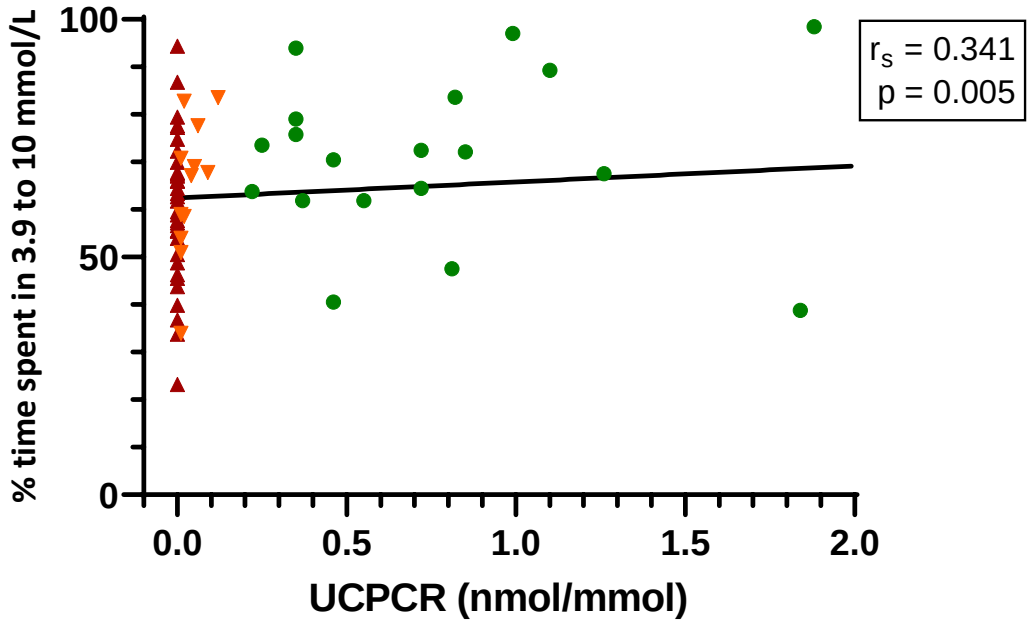

Figure 3.2 Scatter plots displaying linear relationship between 120 minutes post meal urine C-peptide creatinine ratio vs percentage of time spent in euglycaemic control 3.9 to 10 mmol/L during the free-living observational week.

Red triangles = undetectable UCPCR (<0.001 nmol/mmol) (n=34), Orange triangles = low UCPCR (0.001-0.2 nmol/mmol) (n=13), Green circles = high UCPCR (>0.2 nmol/mol) (n=20).

Post meal UCPCR was also negatively associated with time in hyperglycaemia, mean glucose, HbA1c and glycaemia variability, although notably it did not predict time in hypoglycaemia (Table 3.2)

Table 3.2 Displaying the Spearman's correlations (r_S) and significance between 120 minutes post meal urine C-peptide creatinine ratio and glucose parameters during the free-living observational week.

Glycaemic Outcome	**r_S**	**P**
Time Spent <3.0 mmol/L	-0.122	0.323
Time Spent <3.9 mmol/L	-0.200	0.874
Time Spent 3.9-10 mmol/L	0.341	**0.005**
Time Spent >10 mmol/L	-0.309	**0.011**
Time Spent >13.9 mmol/L	-0.412	**0.001**
Mean Glucose (mmol/L)	-0.288	**0.018**
SD	-0.439	**<0.001**
CV (%)	-0.286	**0.019**
HBA1C (mmol/mol)	-0.303	**0.013**

3.4.1. Multiple Linear Regressions

To determine the most important factors on the free-living observational week glycaemic outcomes, stepwise multiple regressions were performed with UCPCR, sex, age, age at diagnosis, BMI, daily insulin, gold score and MVPA. Table 3.3 shows that UCPCR, BMI, gold score, insulin, age and age of diagnosis are associated with different measures of glycaemic outcomes. The stepwise regression model demonstrated BMI and UCPCR statistically significantly predicted % time spent in 3.9 to 10 mmol/L, $F(2, 56) = 8.382$, $p= 0.001$, $R^2 = 0.230$. Figure 3.3 displays the predicted % time spent in euglycaemia from the UCPCR and BMI model compared to the measured results. Interestingly, none of the parameters predicted HbA1c.

Table 3.3 Stepwise multiple linear regression model for glycaemic outcomes (n=67)

Glycaemic Outcome	Predictors in Model	*R*	R^2	*B*	*SEB*	*β*	*F*	*p*	*t*	*p*
% Time Spent <3.0 mmol/L	Age	0.354	0.125	0.078	0.027	0.344	8.143	0.006	2.854	0.006
% Time Spent <3.9 mmol/L	Gold Score	0.453	0.205	1.752	0.608	0.344	7.218	0.002	2.882	0.006
	Age at diagnosis			0.161	0.063	0.306			2.565	0.013
% Time Spent 3.9-10 mmol/L	BMI	0.480	0.23	-1.926	0.563	-0.416	8.382	0.001	-3.421	0.001
	UCPCR			14.091	4.591	0.373			0.373	0.003
% Time Spent >10 mmol/L	BMI	0.534	0.285	1.830	0.622	0.361	7.321	< 0.001	2.943	0.005
	UCPCR			-15.513	5.028	-0.375			-3.085	0.003
	Gold Score			-4.206	1.806	-0.288			-2.329	0.024
% Time Spent >13.9 mmol/L	Gold Score	0.387	0.149	-2.637	0.918	-0.371	4.921	0.011	-2.872	0.006
	UCPCR			-5.366	2.605	-0.266			-2.060	0.044
Mean Glucose (mmol/L)	Age at diagnosis	0.481	0.232	-0.048	0.016	-0.352	8.445	0.001	-3.005	0.004
	Gold Score			-0.450	0.155	-0.339			-2.896	0.005
SD	Insulin	0.510	0.260	0.016	0.005	0.384	10.197	< 0.001	3.192	0.002
	UCPCR			-0.634	0.213	-0.336			-2.974	0.004
CV (%)	UCPCR	0.436	0.190	-5.879	2.249	-0.327	6.821	0.002	-2.615	0.010
	Insulin			0.116	0.047	0.289			2.444	0.018
HbA1c (mmol/mol)	N/A									

***B** = unstandardized regression coefficient, **SEB** = Standard error of the coefficient, **β** = Standardized coefficient, **F** = F-statistic, **t =** T-statistic.*

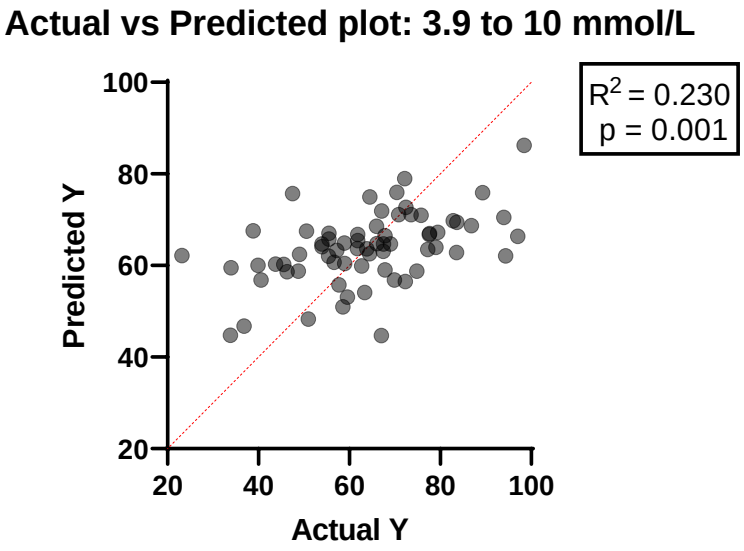

Figure 3.3 Scatter Plot Displaying the UCPCR and BMI Multiple Linear Regression Actual vs Predicted results for % time spent in 3.9 to 10 mmol/L

To further analyse the influence of the UCPCR on glycaemic outcomes, participants were sorted into one of three urinary C-peptide groupings based on previously reported distribution of residual C-peptide (Davis et al., 2015) and improvements in clinical outcomes (Steffes et al., 2003a): undetectable ($Cpep_{und}$, <0.001 nmol/mmol), low ($Cpep_{low}$, 0.001 to 0.19 nmol/mmol) and high ($Cpep_{high}$ ≥0.2 nmol/mmol). Demographic group data are shown in Table 3.4. Age, BMI and moderate to vigorous physical activities (MVPA) were comparable between groups. However, the $Cpep_{high}$ group had significantly higher age of diagnosis and shorter duration of diabetes than the $Cpep_{und}$ and $Cpep_{low}$.

Table 3.4 Displaying mean ± SD group demographic information for serum C-peptide groups

C-PEPTIDE GROUPING	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$
N	34	13	20
Male / Female	18 / 16	6 / 7	11 / 9
Age (Years)	42.6 ± 12.0	40.8 ± 12.1	38.7 ± 12.8
Age At Diagnosis	17.7 ± 10.5	13.8 ± 8.0	27.6 ± 12.2 *#
Range (Years)	1 to 42	8 to 32	12 to 56
Duration Of Diabetes K-W T	26.82 ± 13.24	21.89 ± 13.34	10.70 ± 6.15 *#
Range (Years)	4 to 47	12 to 46	1 to 30
BMI (kg/m^2)	25.4 ± 2.9	25.4 ± 4.5	26.1 ± 3.7
Method Of Control (MDI / CSII)	18 / 16	5 / 8	14 / 6
Daily Insulin (units) K-W T	42.62±15.01	41.59±20.77	36.45±23.76
Insulin units/kg/day K-W T	0.56±0.22	0.55±0.18	0.46±0.18
CGM (None / Flash / CGM)	22 / 7 / 4	10 / 2 / 1	17 / 1 / 2
MVPA (Minutes A Day)	22.9 ± 12.7	17.1 ± 7.6	20.8 ± 10.1

K-W T – Kruskal-Wallis Test * Significantly different to $Cpep_{und}$ # Significantly different to $Cpep_{low}$

Group glycaemic outcomes are displayed in Figure 3.4. While no differences exist between $Cpep_{und}$ and $Cpep_{low}$ for any outcome, $Cpep_{high}$ had statistically significantly higher time in euglycaemia (71.8 ± 17.0%) compared to $Cpep_{und}$ (60.5±14.6, p= 0.031) but not $Cpep_{low}$ (60.1± 14.9, p= 0.091)(Figure 3.4C). $Cpep_{high}$ also had significantly less time in hyperglycaemia (Figure 3.4E + F) and lower SD glycaemic variability (Figure 3.4G) than $Cpep_{und}$. No group differences existed in hypoglycaemia (Figure 3.4A + B).

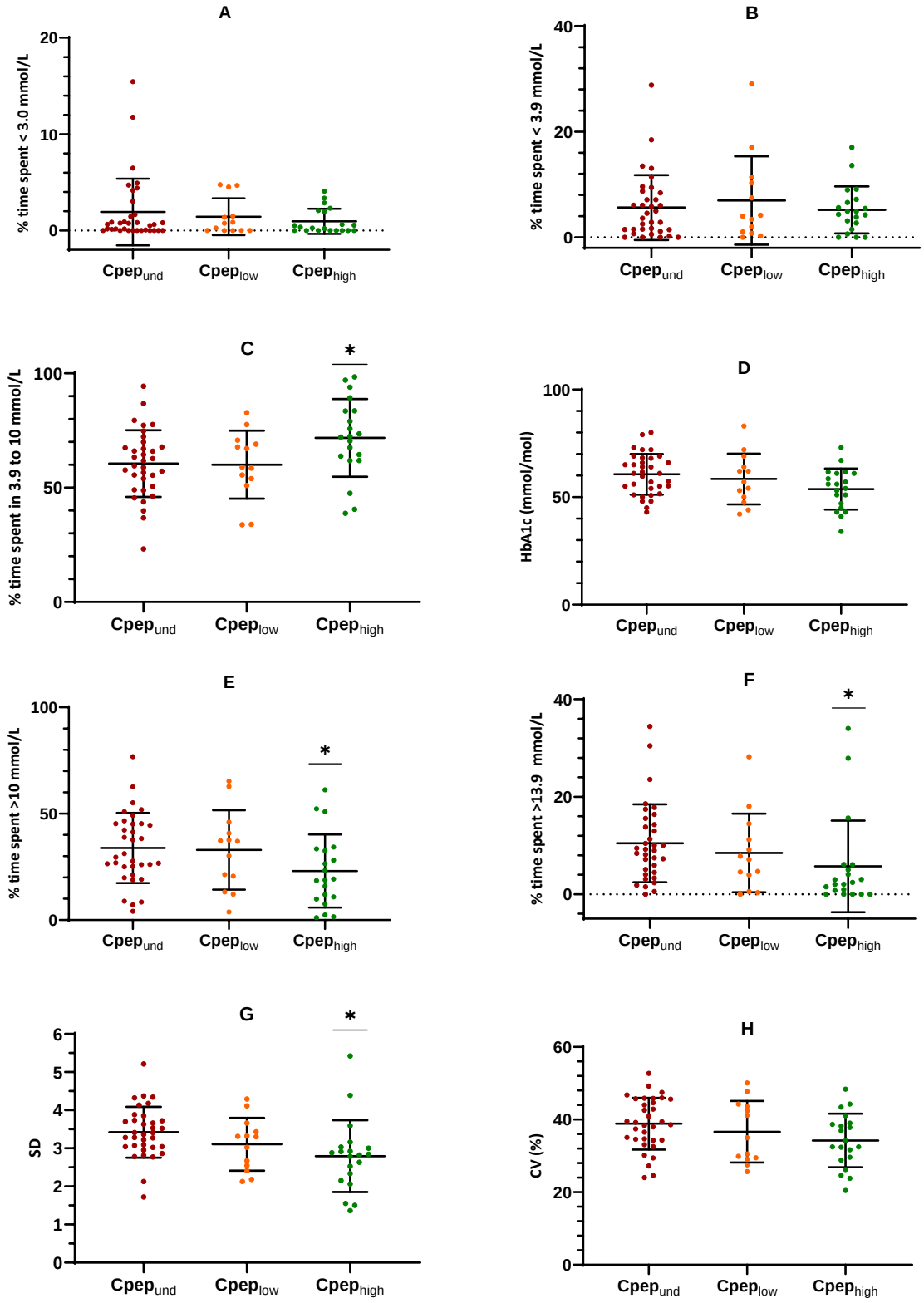

*Figure 3.4 Group mean±SD and individual data points for time spent; less than 3 mmol/L (A), less than 3.9 mmol/L (B), euglycaemic range 3.9 to 10 mmol/L (C), more than 10 mmol/L (E), more than 13.9 mmol/L (F). Last available HbA1C (D) and Glycaemic variability SD (G) and coefficient if variance (H) are also displayed. Red circles = Cpep$_{und}$ (n=34), Orange circles = Cpep$_{low}$ (n=13), Green circles = Cpep$_{high}$ (n=20). * indicates significantly different to Cpep$_{und}$*

3.5. Conclusion

We investigated how residual β-cell function influences glycaemic control during a free-living observational week. We show that in this cohort, post meal UCPCR correlates with greater amount of time spent in euglycaemia, less time in hyperglycaemia (level 1 and 2) and less glycaemic variability (SD and GV). However, UCPCR did not associate with time spent in hypoglycaemia (level 1 or 2). Furthermore, when other variables where included in a stepwise multiple regression, UCPCR was still a significant predictor of the above outcomes. Finally, to explore if specific thresholds of C-peptide are needed for glycaemic control, we demonstrate that there was no difference between groups with undetectable or low (0.001 to 0.19 nmol/mmol) C-peptide, while individuals with T1D with higher residual β-cell function (UCPCR ≥ 0.2 nmol/mmol) displayed a substantially greater amount of time spent in euglycaemia compared to the undetectable group.

Before 2020, only a paediatric cohort of participants with recently diagnosed T1D who were investigated for 2 years post diagnosis (Buckingham et al., 2015) and post islet transplantation patients followed for a mean 18 months (Brooks et al., 2015), had been explored for how residual β-cell functions influences CGM outcomes in T1D. Both studies demonstrated that as high levels of C-peptide declined, concomitant CGM outcomes measuring glycaemic control also deteriorated, including time spent in euglycaemia and glycaemic variability. As newly diagnosed and post-transplant patients have high amounts of residual β-cell function it was unknown if these benefits translated into longer duration T1D where most C-peptide positive individuals create micro amounts of endogenous insulin.

Two recent studies have explored this, with our findings similar to those published by Rickels et al. (2020). In this study, they demonstrated that individuals with >400 pmol/L of C-peptide spent greater time in euglycaemia and less time in hyperglycaemia, compared to negative, low (17-200 pmol/L) and what they have defined as intermediate (200-400 pmol/L) groups, where there was no differences between. In our study, we found that the group with UCPCR ≥0.2 nmol/mmol (equivalent of >200 pmol/L serum C-peptide) also had greater time in euglycaemia and less time in hyperglycaemia, as well as less glucose SD, while we also found no difference between our undetectable and low groups. While technically we found differences in the CGM outcomes at a lower C-peptide threshold than Rickels et al. (2020), in our

study 13 of the 20 high C-peptide group had a UCPCR >0.4 nmol/mmol (equivalent of >400 pmol/L). When evaluated as a continuous relationship, similar significant relationships between C-peptide and glycaemic outcomes were shown between ours and Rickels et al. (2020) studies, with mean glucose, time in euglycaemia ($r = 0.341$; $P = 0.005$ vs $r = 0.456$; $P < 0.001$), time with glucose >10 mmol/L ($r = -0.309$; $P = 0.011$ vs $r = -0.376$; $P = 0.003$), and glucose CV comparable.

Interestingly, neither the present study, Buckingham et al. (2015) or Rickels et al. (2020) found an association between higher C-peptide and less time spent in hypoglycaemia (<3.9 mmol/L). These results are contrary to the recently study published by Gibb et al. (2020) which also explored C-peptides influence on glycaemic outcomes in longer duration T1D. This large observational study (n=290) found individuals > 3 years post diagnosis with preserved C-peptide (either 10 to 200 pmol/L or 10 to 50 pmol/L) had fewer low-glucose events and lower glucose variability by flash monitoring, as well as less self-reported asymptomatic hypoglycaemia, compared to low (<10 pmol/L) secretors. However, no differences in time in euglycameia or hyperglycaemia existed between the groups.

Divergent results between studies may be due to a multitude of reasons. As the median time spent <3.9 mmol/L in the low secretors was 5% (interquartile range (IQR) 3 to 9%) compared to 3% (IQR – 2 to 6%) for the 10 to 200 pmol/L group ($p< 0.001$) or 4% (IQR – 2-6%) for the 10 to 50 pmol/L group ($p= 0.034$), the much greater sample size seen in this study are potentially needed to spot these very small but meaningful results (Gibb et al., 2020). Additionally, differences in glucose data collection methods may also have influenced the results, with use of a CGM blinded to the participants and collected for one week in ours and Rickels et al. (2020) studies, compared to use of a Freestyle Libre flash glucose monitors collected for 2 week in Gibb et al. (2020). This increased duration of measurement, as recommended by the international consensus on CGM data interpretation (Battelino et al., 2019, Danne et al., 2017), could significantly differentiate these small differences between groups. Additionally, the use of a monitor that participants actively engage in and reduces time spent in hypoglycaemia (Bolinder et al., 2016, Oskarsson et al., 2018), could also explain the differences.

The significant differences in time spent <3.9 mmol/L and self-reported asymptomatic hypoglycaemic incidents in Gibb et al. (2020) is consistent with previous research showing higher C-peptide reduces the incidences of self-reported hypoglycaemia (Marren et al., 2019, Kuhtreiber et al., 2015, Hope et al., 2018). This reduction in hypoglycaemia seen in C-peptide positive patients may be due to a more pronounced glucagon concentration production in response to a hypoglycaemia found in some (Zenz et al., 2018, Rickels et al., 2020) but not all studies (Sherr et al., 2013). Indeed, while Rickels et al. (2020) found a weak relationship between C-peptide and glucagon response to hyperglycaemia, this did not translate into an increase in endogenous glucose production and may explain why both this study and our own results found no correlation between percentage of time spent <3.9 mmol/L and peak C-peptide. Further research is needed to explore if residual β-cell function does contribute to hypoglycaemic counterregulation by protecting or enhancing the α-cells glucagon secretion, as the exact mechanisms behind this is unknown (McCrimmon and Sherwin, 2010).

Our primary finding that higher C-peptide resulted in more time in euglycaemia was largely a result of reduced time spent in level 1 and 2 hyperglycaemia. These results are contrary to Gibb et al. (2020) recent findings of no difference in time in euglycaemia or reduced time in hyperglycaemia between C-peptide groups. This discrepancy in results is likely due to Gibb et al. (2020) excluding participants with a C-peptide >200 pmol/L, with the current and Rickels et al. (2020) studies only finding group differences above this C-peptide threshold. When peak MMTT C-peptide was plotted against change in C-peptide from baseline, the relationship was strongly apparent ($r = 0.99$; $P < 0.0001$), with incrementally greater responses in the low, intermediate, and high C-peptide groups (Rickels et al., 2020). The present study, in combination with previous (Rickels et al., 2020, Gibb et al., 2020), suggests a minimum threshold of peak C-peptide (~200 to 400 pmol/L) required to smooth out the peaks in glucose and reduce time spent in hyperglycaemia in “real world” settings.

This study demonstrates for the first time that even when other variables are considered, C-peptide is a predictor of glycaemic control. Only BMI and UCPCR predicted the primary outcome in the present study, together predicting 23% of the variability, with BMI having a negative association and UCPCR having a positive relationship with the amount of time spent in euglycameia. Previous studies that

included C-peptide amongst multiple other variables only used a baseline C-peptide value to track changes in HbA1c from diagnosis (Hesse et al., 2018). Considering β-cell function is known to have an initial exponential fall for around 7 years post diagnosis (Shields et al., 2018), and that change in C-peptide strongly predicts change in HbA1c in the first three years (Buckingham et al., 2015, Grönberg et al., 2020), unsurprisingly did not find C-peptide as a predictor. BMI has previously been demonstrated to have a U-shaped correlation with HbA1c, with both those with low and high BMI having increased average glucose level and a BMI of between 21-23 kg/m^2 (Lee et al., 2017b, Williams et al., 1999, Eeg-Olofsson et al., 2007). In contrast, an increased BMI in the recently diagnosed and in young adults is associated with improved control (Ferriss et al., 2006, Purnell et al., 1998), likely because of weight gain from intensive insulin therapy. In this study we found a negative relationship between BMI and time in euglycaemia, likely due to the small number of individuals with a low BMI and the increased insulin resistance associated with a higher BMI (Priya and Kalra, 2018). Other results from the multiple linear regression largely replicate previous research. We found a higher Gold score (a greater hypoglycaemia unawareness) was a predictor of increased time in hypoglycaemia <3.9 mmol/L, replicating Geddes et al. (2007) and true even when individuals are using a CGM (Lin et al., 2019). Interestingly, we did not find it predicted percentage time <3.0 mmol/L, with increasing age the only predicting variable. It is unclear why Gold score predicted only hypoglycaemia level 1 and not 2, but increased age is associated with greater hypoglycaemia unawareness (Brierley et al., 1995) and impairment of counterregulatory responses to hypoglycaemia (Matyka et al., 1997) in older non-diabetic individuals which may explain the findings in this study.

A higher Hba1c has previously been shown to associate with an increased insulin dosage (Priya and Kalra, 2018) and increased glycaemic variability (Pickup et al., 2006), which may explain the findings that increased insulin dose was associated with greater glycaemic variability (both SD and CV). However, insulin did not predict any other measure of glycaemic control. The higher C-peptide group had a reduced daily insulin dosage (36.45±23.76 units) and insulin dose per kg (0.46±0.18 units/kg/day) compared to the low (41.59±20.77 units and 0.55±0.18 units/kg/day) and undetectable groups (42.62±15.01 units and 0.55±0.22 units/kg/day), although not significantly ($p=0.115$ and $p=0.279$, respectively). This may explain the comparable HbA1c levels

between groups. With the DCCT (Lachin et al., 2014, Steffes et al., 2003a) and Kuhtreiber et al. (2015) trials show higher C-peptide is associated with lower HbA1c, Marren et al. (2019) and Gibb et al. (2020) recently found no difference in HbA1c between low (<10 pmol/L) and preserved (>20 pmol/L and >10 pmol/L, respectively) C-peptide groups within UK populations. While this study had different C-peptide grouping, the median C-peptide in the preserved group in Marren et al. (2019) was a relatively high 114 pmol/L. The authors propose that the non-intensive treatment found within the UK cohorts (HbA1c ≈68 mmol/mol) results in comparable insulin levels despite lower incidences of hypoglycaemia, and that more intensive treatment is needed within high C-peptide individuals to get the full benefit of their endogenous insulin secretion.

While the 'real-world' design is a strength in terms of generalisability, the unbalanced groups and higher proportion of participants with undetectable C-peptide who used a CGM/FGM during the study period may have influenced the results, specifically the percentage of time spent in hypoglycaemia. Because time in hypoglycaemia is such a small proportion of total time, it is likely that two weeks of CGM data is needed to see statistical differences. Future studies should carefully consider how to most meaningfully measure hypoglycaemia in free-living conditions, with a combination of CGM, diaries and questionnaires likely to be needed (Henriksen et al., 2019). We did not measure T1D autoantibodies, therefore it is possible that our cohort contained individuals with different forms of diabetes, especially in individuals with the high C-peptide. However, as the central tenet of this project was to explore the relationship between C-peptide and euglycameia, we did not see this as a major problem. The main aim of the study was to explore the association between C-peptide and free-living glycaemic outcomes, therefore the results from the multiple linear regression exploring different variables must be interpreted with caution. The study protocol of measuring UCPCR and CGM during the same week is a strength. Previous studies have not disclosed the time between C-peptide and glucose measurements (Gibb et al., 2020), with C-peptide decline possible even years after diagnosis (Shields et al., 2018).

With the conflicting findings of C-peptides influence on hypoglycaemia, further research is needed in free-living settings, especially exploring lower glucose levels (<3.0 mmol/L). Understanding how functioning β-cells may influence the counter

regulation responses to hypoglycaemia, including the α-cell ability to secrete glucagon, and how C-peptide influences glycaemic variability, are also areas of interest. Finally, with the rise of automated – closed loop – insulin pumps, understanding how different variables influence blood glucose in isolation and in vivo is also important to fully optimise these systems.

In conclusion, people with T1D who have higher residual beta-cell function show improved glycaemic control during free-living periods, driven by reduction in hyperglycaemia and glycaemic variability. Based on the influence residual β-cell function has on glycaemia, C-peptide could potentially be used to personalise glycaemic targets. These findings should further highlight the positives of treatments that aim to protect β-cell function in T1D, with preservation of endogenous insulin production resulting in substantial clinical benefits.

Chapter 4. The influence of residual β-cell functions upon post-exercise glycaemic control in individuals with type 1 diabetes

Research team members and responsibilities:

Guy Taylor – Study design, led participant recruitment, collected informed consent, data collection and processing (anthropometric measurements, medical history and notes, continuous glucose monitoring, mixed meal tolerance test – including phlebotomy and sample processing, health screening, maximal exercise test – including resting and exercising electrocardiography, CPET and blood glucose management, main trial exercise bout – phlebotomy and sample processing, exercise testing), and statistical analysis.

Dr Daniel West – Study design, obtained funding, resources and ethical approval, reviewed data and chapter.

Professor Emma Stevenson – Reviewed chapter.

Professor Jim Shaw – Study design, participant recruitment and provided medical consultation on adverse findings.

Kieran Smith – Assisted with exercise tests

Dr Tess Capper – Assisted with exercise tests

Jadine Scragg - Assisted with exercise tests

Dr Ayat Bashir - Participant recruitment, helped on difficult phlebotomy and provided clinical cover during exercise testing.

Dr Anneliese Flatt - Participant recruitment, helped on difficult phlebotomy and provided clinical cover during exercise testing.

Dr Timothy McDonald - Analysed C-peptide, Glucagon and Autoantibodies.

Dr Richard Oram - Analysed C-peptide, Glucagon and Autoantibodies.

4. Chapter 4. The influence of residual β-cell functions upon post-exercise glycaemic control in individuals with type 1 diabetes

4.1. Abstract

Objective

To investigate the impact of residual β-cell function on CGM outcomes following acute exercise in people with T1D.

Research Design and Methods

Thirty participants with T1D for ≥3 years were recruited. Firstly, participants wore a blinded CGM for 7 days of free-living data capture. Secondly, a 3 hour mixed meal test, assessed stimulated C-peptide and glucagon. Peak C-peptide was used to allocate participants into undetectable ($Cpep_{und}$ <3 pmol/L), low ($Cpep_{low}$ 3–200 pmol/L) or high C-peptide groups ($Cpep_{high}$ >200 pmol/L). Finally, participants completed 45 minutes of incline treadmill walking at 60%VO_{2peak} followed by a further 48 hours' CGM capture.

Results

CGM parameters were comparable across groups during the free-living observation week. In the 12 (12hr) and 24 hours (24hr) post-exercise periods the $Cpep_{high}$ group had significantly greater amount of time spent with glucose 3.9-10 mmol/L (12hr: 73.5±27.6%, 24hr: 76.3±19.2%) compared to $Cpep_{low}$ (12hr: 43.6±26.1%, p=0.027, 24hr: 52.3±25.0%, p=0.067) or $Cpep_{und}$ (40.6±17.0%, p=0.010, 24hr: 51.3±22.3%, p=0.041). Time spent in hyperglycemia (12hr and 24hr glucose >10 and >13.9 mmol/L, p<0.05) and glycemic variability (12hr and 24hr SD, p<0.01) were significantly lower in the $Cpep_{high}$ group compared to $Cpep_{und}$ and $Cpep_{low}$. Change in CGM outcomes from pre to 24hr post-exercise was divergent: $Cpep_{und}$ and $Cpep_{low}$ experienced worsening (glucose 3.9-10 mmol/L: -9.1% and -16.2% respectively), with $Cpep_{high}$ experiencing improvement (+12.1%)(p=0.017).

Conclusions

Residual β-cell function may partially explain the inter-individual variation in the acute glycemic benefits of exercise in individuals with T1D. Quantifying C-peptide could aid in providing personalized and targeted support for exercising patients.

4.2. Introduction

Regular physical activity (PA) and exercise can have considerable health benefits for individuals with T1D. These include reduced risk of microvascular damage, improved cardiovascular risk factors, enhanced mental wellbeing, as well as extended life expectancy (Bohn et al., 2015, Moy et al., 1993, Kriska et al., 1991, LaPorte et al., 1986, Zoppini et al., 2003, Imayama et al., 2011, Chimen et al., 2012a). Despite this, PA levels appear to be lower in T1D compared to the general population, with less than 20% of patients managing to do aerobic exercise more than two times per week (Bohn et al., 2015). The lower PA levels may be due to disruptions to glucose control caused by exercise, in particular by causing hypoglycaemia, which can be complex to manage (Riddell et al., 2017, Adolfsson et al., 2018). Other barriers include a general lack of knowledge around exercise management and a fear of hypoglycaemia, as well as the increased forethought and planning needed for insulin and nutritional strategies around PA (Lascar et al., 2014, Jabbour et al., 2016, Brazeau et al., 2008).

One major obstacle to providing exercise management support to people with T1D is a high inter-individual variability in the blood glucose responses to exercises (Riddell et al., 2017). Even in tightly controlled research studies that have adopted a strict inclusion criteria, recruited homogenous groups, have standardized insulin and dietary intake and used CGMs to stabilise glucose in the run up to the study, a large unexplained inter-individual variability in the glycaemic responses to exercise remains during (Temple et al., 1995, Abraham et al., 2017, DirecNet, 2006, Kilbride et al., 2011) and after exercise (Kapitza et al., 2010). This is currently an area of considerate interest. Using secondary analysis, research groups have demonstrated that pre-exercise blood glucose levels is a predictor of the magnitude of drop in glucose during aerobic activity (Riddell et al., 2019b), that patients with good fitness level appear to be more prone to hypoglycaemia during exercise (Al Khalifah et al., 2016), that leg and trunk fat mass positively and whole body lean mass negatively associated with glucose post exercise (Tagougui et al., 2019), and that males may have a greater risk of hypoglycaemia during and after resistance exercise than females (Brockman et al., 2020). Additionally, outside of formal research, both clinical observations and feedback from patient support groups report potential for both an improvement and detrimental impact of regular exercise on long-term glycaemic control.

With the development of an ultra-sensitive C-peptide assay, recent research has shown that even in long duration T1D, β-cell function can persist. There is some disparity within the evidence regarding the prevalence of residual β-cell function within the T1D population, with numbers ranging from 30 to 80% thought to be insulin microsecreators at >5 years post diagnosis (Oram et al., 2015, Oram et al., 2014, Williams et al., 2016, Wang et al., 2012, Davis et al., 2015). Moreover, it is estimated that 8-16% of individuals diagnosed with T1D as an adult have a relatively high C-peptide level, above the threshold found in the Diabetes Control and Complications Trial to have some clinical benefits (Lachin et al., 2014). This compares to 5-6% of individuals with childhood onset of diabetes with this high level of C-peptide (Oram et al., 2015, Davis et al., 2015, Williams et al., 2016).

Evidence from recently diagnosed individuals and patients after an islet transplantation, when consequently C-peptide levels are relatively high, demonstrates that as residual β-cell function declines, CGM parameters such as time in euglycaemia (time in range 3.9-10 mmol/L) and CV% worsen under normal free-living conditions (Buckingham et al., 2015, Brooks et al., 2015). A recent paper by Rickels et al. (2020) demonstrated that individuals with a very high stimulated C-peptide (>400 pmol/L) had greater time in euglycaemia at rest compared to negative, low (17-200 pmol/L) and intermediate (200-400 pmol/L) C-peptide groupings, where no differences in glycaemia existed between groups. Another recent paper by Gibb et al. (2020), found no difference in euglycameia but reduced hypoglycaemia in individuals with preserved (>10 pmol/L) C-peptide. How this translates to around exercise, is unclear. Potentially, diminished but functioning β-cells may convey some level of intrinsic glucose regulation that offers benefits under an intense metabolic stressor (including increased metabolic rate, carbohydrate oxidation and insulin sensitivity) such as exercise. Moreover, it can be hypothesized that β-cell function is associated with CGM outcomes explaining (at least in part) inter-individual variability in the exercise response. This information could be valuable for provision of targeted exercise support, based on C-peptide status.

This study examined the impact of residual β-cell function on glycaemic control after a bout of aerobic exercise in people with T1D. We hypothesized that individuals with greater C-peptide will have increased amount of time with an interstitial glucose in euglycaemia (3.9 to 10 mmol/L) – the primary outcome. As well as reduced glycaemic

variability, time spent in hypoglycaemia and time spent in hyperglycaemia – the secondary outcomes.

4.3. Method

The study was an acute observational trial, examining how residual beta cell function impacts glycaemia at rest and after exercise in individuals with long duration T1D. The trial design is displayed in the below schematic (figure 4.2).

4.3.1. Recruitment of T1D participants

Thirty participants with a range of UCPCR who successfully completed study 1 and met the criteria for study 2 were invited into the study. UCPCR results were used to preliminary place participants in three C-peptide groupings, undetectable ($Cpep_{und,}$ <0.001 nmol/mmol), low ($Cpep_{low,}$ 0.001 to 0.2 nmol/mmol) and high ($Cpep_{high}$ >0.2 nmol/mmol).

Eligibility criteria comprised of being clinically diagnosed with T1D (primary osmotic symptoms, weight loss, hyperglycaemia, ketosis, insulin initiation at diagnosis), aged between 18 and 65 years with a duration of T1D >3 years upon enrolment, an HbA1c <86 mmol/mol / 10.0%, absence of diabetes-related complications apart from retinopathy; and stable Multiple Daily Injection (MDI) or Continuous Subcutaneous Insulin Infusion (CSII) regimen without changes over the preceding 6 months (Table 4.1).

All patients who participated provided written informed consent and this study was approved by the local National Health Service Research Ethics Committee (code: 16/NE/0192, ISRCTN registry: ISRCTN50072340).

Table 4.1 displaying inclusion/exclusion criteria for chapter 4.

INCLUSION	EXCLUSION
Clinically diagnosed with T1D	T1D participants duration of disease less than 3 year
Aged 18-65 years	HbA1c >86 mmol/mol (10%)
Treated with exogenous insulin (MDI or CSII)	Unable to complete a maximal exercise test
Free from diabetes complications apart from retinopathy	

4.3.2. Sample Size

Sample size was calculated from available C-peptide and CGM data (Brooks et al., 2015). Based on this data, using percentage time in range 3.9–10 mmol/L during a 5-day CGM capture from islet transplant recipients with stimulated C-peptide >200 pmol/L (71±21%) and <150 pmol/L (45±16%). With an estimated difference of at least 10% in the primary outcome, a sample of 10 participants per group would be needed to test the null hypothesis that mean time within range (3.9-10.0 mmol/L) of all groups is equal with a probability of 0.8. Type 1 error associated with this calculation is 0.05.

4.3.3. Protocol

Participants had previously attended the Newcastle CRF for study 1, with a further 3 visits for study 2.

4.3.3.1. Pre study; UCPCR and observational free-living CGM week

Participants had previously completed study 1, investigating how residual β-cell function influences glycaemic control during a free-living observational week in people with T1D. Participants had worn a CGM (see 2.5) and completion of a home UCPCR test (see 2.3) as described in chapter 3.

4.3.3.2. Visit 1. Mixed Meal Tolerance Test (MMTT)

Participants who entered into study 2, attended the Newcastle CRF at ~8.30am after an overnight fast and having withheld bolus insulin from midnight. Individuals were instructed to maintain their normal basal insulin regime, including amount and timings. If participants had a hypoglycaemic event or needed to bolus insulin overnight or in the morning, the visit was rearranged. Participants were instructed to avoid exercise and alcohol consumption the day before the visit and transport was provided the morning of the visit. Upon arrival, participant's capillary blood sample was tested with the visit going ahead if it was between 4 and 12 mmol/l, before a cannula was inserted (see 2.8).

A standard but extended multiple time point mixed meal tolerance test (MMTT) protocol was used with participants given 240ml of Fortisip (Nutricia, Trowbridge, UK) consisting of: 360 kcal, 14.4g protein, 13.92g fat and 44.16g carbohydrate, to drink within 2 minutes (Greenbaum et al., 2008, Leighton et al., 2017). Blood samples were drawn at baseline and every 30 minutes up to and including 180 minutes (figure 4.1). A total of 12ml of whole blood samples was collected into 2 BD vacutainers® at each time point, a 2ml EDTA and 10ml SST™ II Advance. An additional 4ml EDTA vacutainer® was drawn at 0min and analysed for HbA1c.

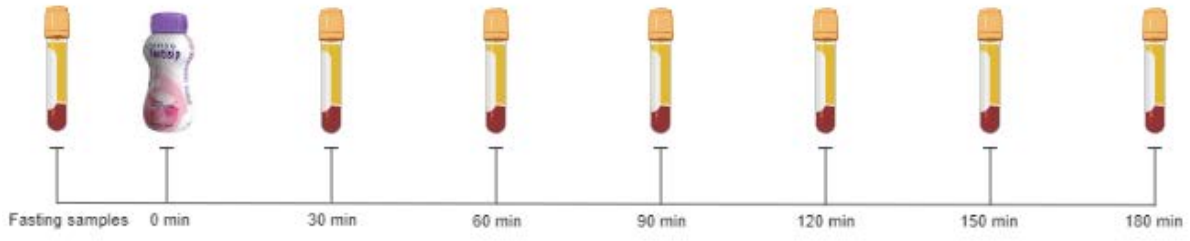

Figure 4.1 Schematic of the protocol for the MMTT

4.3.3.2.1. Sample processing

1ml of whole blood was drawn from the 2ml EDTA vacutainers® into a 5ml aliquot and presented to the YSI 2300 STAT PLUS for immediate analysis of whole blood glucose (see 2.9).

The 10ml SSTTM II Advance vacutainers® was allowed to clot for 30 minutes before being centrifuged at room temperature for 10 minutes at 1300G. Immediately after being centrifuged, samples were processed and stored in 10ml aliquots tubes at -80 °C in long-term storage at the Newcastle Biobank facility. Serum samples were transported to Exeter Clinical Laboratory for analysis of serum C-peptide, glucose, glucagon, insulin, proinsulin and autoantibodies. No freeze/thaw happened prior to the samples being tested.

An additional 4ml EDTA vacutainer® was drawn at baseline for measurement of HbA1C (see 2.10)

4.3.3.2.2. Blood Sample analysis

Serum was transported to Exeter Clinical Laboratory and analysed for C-peptide as described (see 2.4). Lower limit of detection of the assay was 3.3 pmol/l with a reported

intra- and inter-assay coefficient of variation of 3.3% and 4.5% Hope et al. (2016). Individual's peak serum C-peptide recorded during the MMTT was used to confirm which C-peptide groups. Participants were sorted into; undetectable ($Cpep_{und}$) peak <3 pmol/L, low ($Cpep_{low}$) 3 – 200 pmol/L and high ($Cpep_{high}$) >200 pmol/L. Serum glucagon was measured using a Mercodia Glucagon ELISA (Mercodia AB, Uppsala, Sweden) on the Dynex DS2 automated platform (Dynex Technologics, Worthing, U.K) with a lower limit of detection of 1.5 pmol/L.

4.3.3.2.3. Autoantibody

Autoantibody analysis was performed using ELISA assays (RSR Ltd., Cardiff, UK) on the Dynex DS2 automated platform (Dynex Technologics, Worthing, U.K) as previously reported (McDonald et al., 2011). Glutamic acid decarboxylase (GAD65), tyrosine phosphorylase-related protein 2 (IA2) and zinc transporter 8 (ZnT8) were analysed using ELISA kits from RSR limited, per the manufacturers instruction, with a sensitivity and specificity of 98% and 92%, 98% and 76%, and 99% and 72% respectively. The cut-offs for positivity were: ≥7.5 U/mL (IA-2); ≥11 U/mL (GAD65); ≥65 U/mL (ZnT8) if aged < 30 years or ≥9.1 U/mL if aged >30 years. Positive result defined as above the 97.5th centile of 1,559 control participants without diabetes (McDonald et al., 2011), with the laboratory participating in the International Autoantibody Standardization Program.

Participants with one or more antibody above the reference ranges were classified as autoantibody-positive. Individuals within the $Cpep_{high}$ group who were autoantibody-negative were included in the initial analysis before being excluded from the further analysis to reduce the possibility of misdiagnosed cases of type 2 diabetes influencing the results.

4.3.3.3. Visit 2. Health screening and Maximal Exercise Test

Participants stature and mass (Seca 889 and Seca 220, Seca, Germany), National Early Warning Score (NEWS), and pre-exercise health screening, including medical history and examination (appendix C and D) were taken. Participants underwent a modified 12-lead resting and exercising electrocardiogram (ECG) to screen for any cardiac abnormalities (See 2.11), with any concerns or a score upon the NEWS chart checked by the clinical cover.

A maximal graded walking treadmill (Valiant 2 CPET, Lode, Groningen, Netherlands) test (Bruce protocol; Bruce et al. (1973)) was performed to determine peak oxygen uptake (VO_{2peak}) (see 2.12 and 2.14) and peak heart rate (HR_{peak}) (see 2.14). Glycaemic strategy was managed as per the guidance of Riddell et al. (Riddell et al., 2017) (see 2.17.1).

4.3.3.4. Visit 3. Main trial Exercise Bout

Prior to the submaximal exercise phase, participants attended the Newcastle CRF 24 to 48 hours before the final testing visit, to have a CGM sensor inserted (See 2.5). Individuals arrived at the exercise lab at ~8.30am after an overnight fast with transport provided. Blood glucose and NEWS were checked upon arrival at the CRF, and a cannula was inserted (see 2.8) with fasting blood samples taken. A carbohydrate snack (Belvita Soft Bakes Chocolate Chip, Mondelēz International, USA), providing 204kcal of which 31g CHO, was then consumed and participants remained rested for 20 minutes. Blood Glucose management is detailed in 2.17.2.

To account for varying stride length, the ACSM walking calculations was used to determine treadmill gradient needed at 5.1kph, 6kph and 4.5kph to elicit 60% of the participants $\dot{V}O_{2peak}$ (see 2.16). Participants walked at this intensity for 45 minutes whilst heart rate (Polar H7, Polar Electro Oy, Finland) and expired air was captured and analysed throughout by the meta control 3000 (see 2.13).

Upon completion of the exercise bout, blood samples were drawn from the cannula. Participants remained rested a further 60 minutes, with further blood samples drawn at 30 and 60 minutes post exercise. Participants were discharge from the laboratory, with the CGM capturing free living interstitial glucose responses and participants tracking blood glucose, food and carbohydrate intake and insulin dosage (appendix E) for the 48 hours following the exercise bout.

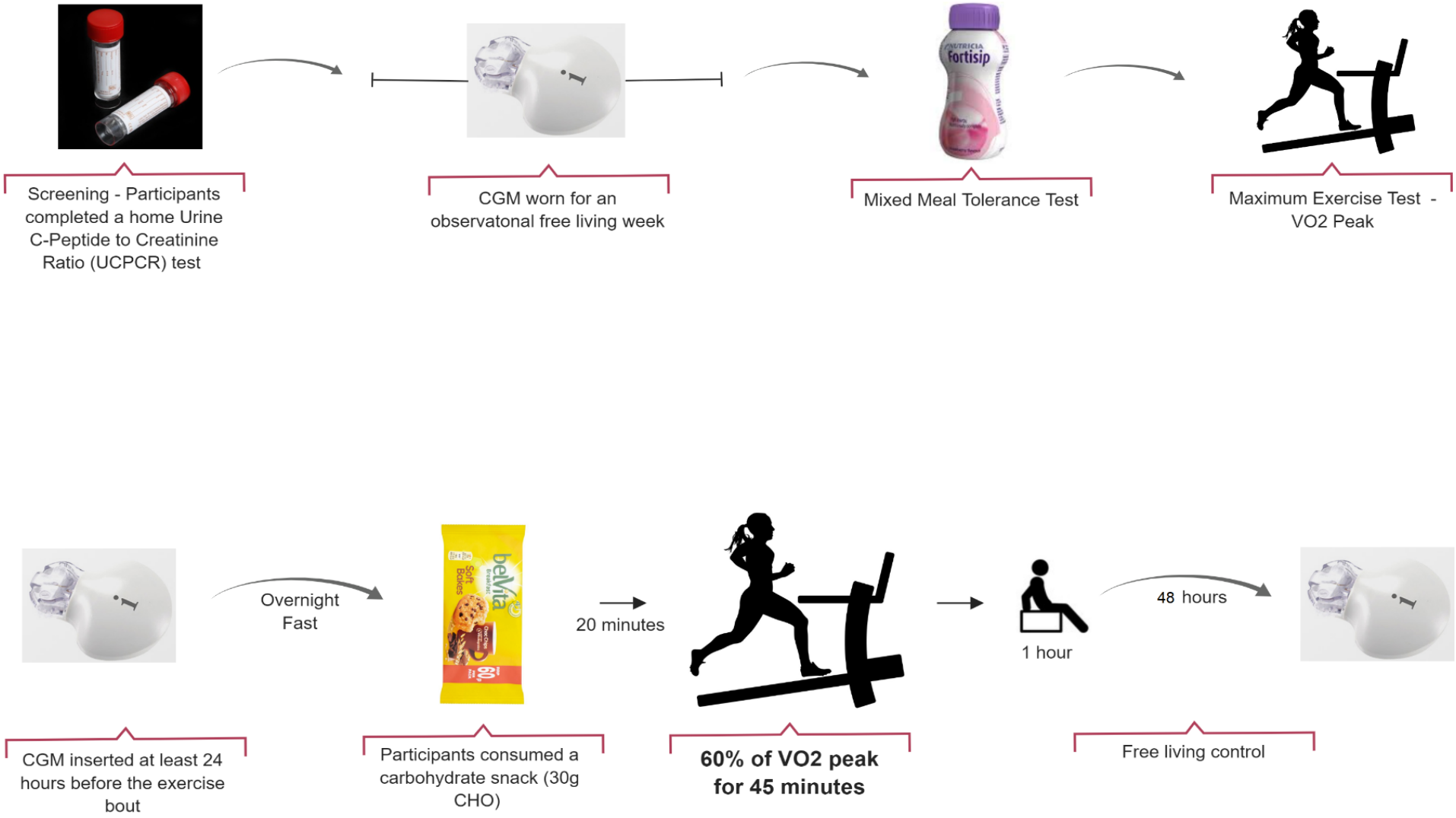

Figure 4.2 Schematic of the study 2 protocol

4.3.4. Statistical analysis

Data is presented as mean ± standard deviation throughout unless otherwise stated. The primary outcome was amount of time with an interstitial glucose in euglycaemia (3.9-10 mmol/L). Secondary outcomes were glycaemic variability (standard deviation (SD) and coefficient of variance (CV)), time spent in hypoglycaemia and time spent in hyperglycaemia. CGM ranges were defined as 3.9-10 mmol/L (euglycaemia), <3.9 mmol/L (hypoglycaemia 1), <3.0 mmol/L (hypoglycaemia 2), >10 mmol/L (hyperglycaemia 1), >13.9 mmol/L (hyperglycaemia 2) as recommended by international consensus (Danne et al., 2017, Battelino et al., 2019). CV was calculated as SD divided by mean glucose.

Data described as delta change from free-living to 24 hours post exercise times were calculated by subtracting the continuous glucose monitor free-living data away from the 24 hours post-exercise data.

Statistical significance differences between the means of $Cpep_{und}$, $Cpep_{low}$ and $Cpep_{high}$ were determined by the use of a one-way ANOVA with Tukey post-hoc analysis. Data were assessed for normality and outliers by Shapiro-Wilk test and boxplots, with excessively skewed data assessed by Kruskal-Wallis H test. Pearson product-moment or Spearman's rank-order correlation were used to determine the strength and direction of a linear relationship between peak MMTT serum C-peptide and glucagon vs CGM data.

Two-way repeated measures mixed ANOVA was used to examine for interactions between time and group during the MMTT. Significant time and C-peptide group effects were analysed using post-hoc Bonferroni adjusted pairwise comparisons. When values where missing, a fitted mixed effects model with Geisser-Greenhouse was implemented in GraphPad Prism 8.0. The mixed model uses a compound symmetry covariance matrix, and is fit using Restricted Maximum Likelihood (REML).

To explore the lowest important C-peptide measurement upon glycaemic control following a bout of submaximal exercise, T-tests were used to compare participants above and below a C-peptide threshold with skewed data assessed by Mann-Whitney U test instead. A biphasic linear regression model of best fit with a slope of the first portion of the biphasic linear regression constrained to zero was also attempted to be fitted to the data.

GraphPad Prism 8.0.1 (San Diego, USA) and IBM SPSS Statistics (version 24, IBM, Armonk NY) software package was used to analyse the data.

4.4. Results

Three participants who were initially recruited with a 'Low' UCPCR, subsequently demonstrated an undetectable peak serum C-peptide. Additionally, two participants initially recruited with 'Undetectable' UCPCR subsequently showed 'Low' C-peptide positivity during the MMTT.

4.4.1. Group demographic

Participants were allocated into three groups according to MMTT peak serum C-peptide. Demographic and MMTT group data is presented in table 4.2. Age, HbA1c, BMI, VO_{2peak} and peak Glucagon were comparable between groups. However, the $Cpep_{high}$ group had significantly different age of diagnosis and duration of diabetes. Although C-peptide metrics differed between groups (in keeping with the study design), MMTT glucagon values were comparable.

Table 4.2 Displaying mean ± SD group demographic information for serum C-peptide groups

C-PEPTIDE GROUPING	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$
N	11	9	10
Male/Female	5/6	6/3	5/5
Age (Years) $_{K\text{-}W\ D}$	40.09 ± 11.18	38.67 ± 14.73	35.80 ± 10.98
Age At Diagnosis $_{K\text{-}W\ D}$	13.27 ± 4.50	16.56 ± 8.57	25.10 ± 8.20 *
Range (Years)	8.00 to 24.00	8.00 to 32.00	13.00 to 35.00
Duration Of Diabetes $_{K\text{-}W\ M}$	26.82 ± 13.24	21.89 ± 13.34	10.70 ± 6.15 *
Range (Years)	13.00 to 47.00	9.00 to 44.00	3.00 to 20.00
HbA1c (mmol/mol)	61.64 ± 10.64	58.11 ± 7.11	55.40 ± 8.47
(%)	7.8 ± 3.1	7.5 ± 2.8	7.2 ± 2.9
BMI (kg/m^2) $_{K\text{-}W\ M}$	25.65 ± 3.27	24.20 ± 4.13	25.67 ± 4.04
Method Of Control (MDI/CSII)	5/6	4/5	6/4
VO_{2peak} (ml/kg/min) $_{K\text{-}W\ D}$	35.61 ± 7.69	43.93 ± 9.03	35.67 ± 10.77
Daily Insulin (units) $_{K\text{-}W\ D}$	39.93 ± 15.15	47.88 ± 23.21	38.30 ± 31.23
Insulin (units/kg/day) $_{K\text{-}W\ D}$	0.54 ± 0.19	0.63 ± 0.25	0.49 ± 0.29
MIXED MEAL TOLERANCE TEST			
Peak C-Peptide (pmol/L)	0.00 ± 0.00	42.00 ± 32.58 *	671.70 ± 435.15 * #

Range (pmol/L)	0 to 0	4 to 83	221 to 1640
Median	0.00	53.00	568.50
$AUC_{0\text{-}180min}$ C-Peptide ($pmol/L_{per180min}$)	0.00 ± 0.00	6026 ± 4452 *	89459 ± 48095 * #
Peak Glucagon (pmol/l)	14.04 ± 6.74	18.60 ± 13.49	12.45 ± 4.34
$AUC_{0TO180min}$ Glucagon ($pmol/L_{per180min}$)	1557 ± 905.8	2072 ± 1370	1259 ± 674.5
Pre Glucose (mmol/L)	10.12 ± 3.38	9.55 ± 1.62	8.47 ± 3.15
Peak Glucose (mmol/L) $_{K\text{-}W\ D}$	21.91 ± 2.75	20.03 ± 2.34	17.74 ± 3.59*
Delta change Pre to Peak Glucose (mmol/L) $_{K\text{-}W\ D}$	11.76 ± 2.77	10.48 ± 2.12	9.27 ± 3.02*
Autoantibody Positivity	6/11	7/9	8/10

$_{K\text{-}W\ M}$ – Kruskal - Wallis Comparison of Medians, $_{K\text{-}W\ D}$ – Kruskal - Wallis Comparison of Distribution, * Significantly different to $Cpep_{und}$, # Significantly different to $Cpep_{low}$

4.4.2. Autoantibody Status

C-peptide group autoantibody status is presented below in table 4.3. 23 of the 30 participants were autoantibody positive for at least one of GAD, IA2 or ZnT8. Individual autoantibody positivity status is displayed in Appendix F. Two participants within the $Cpep_{high}$ group were auto-antibody negative (duration of diabetes: 17 and 20 years, peak C-peptide 532 and 1170 pmol/L, respectively). To reduce the possibility of misdiagnoses of type 2 or monogenic diabetes influencing the results, we reassessed the data excluding these participants.

Table 4.3 displaying total and C-peptide group autoantibody status

C-PEPTIDE GROUPING	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$	TOTAL
AUTOANTIBODIES NEGATIVE	5/11	2/9	2/10	7/30
MULTIPLE AUTOANTIBODIES POSITIVE	1/11	4/9	5/10	10/30
POSITIVE GAD	5/11	6/9	5/10	16/30
POSITIVE IA2	2/11	4/9	5/10	11/30
POSITIVE ZnT8	4/11	3/9	5/10	12/30

4.4.3. Observational week

Data were collected for an average 5.1±0.96 days, with no differences between groups (p=0.730). For the participants in study 2, there were no differences between the C-peptide groups in time spent in euglycaemia, hypoglycaemia or hyperglycaemia, mean glucose, SD or CV during the free-living observational week (Table 4.4). When assessed as a continuous variable by Pearson's product-moment and Spearman's rank-order correlation, MMTT C-peptide and glucagon values did not predict any CGM outcomes during the observational week (P>0.05) (Figure 4.3 and table 4.4).

Table 4.4 Displaying C-peptide group means±SD of the glycaemic outcomes for the observational week

	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$	P
< 3 mmol/L $_{K\text{-}W\ M}$	0.72 ± 1.40	1.33 ± 1.94	0.88 ± 1.18	0.710
< 3.9 mmol/L $_{K\text{-}W\ D}$	3.47 ± 3.24	8.66 ± 9.69	5.67 ± 5.39	0.540
3.9 to 10 mmol/L	60.44 ± 14.15	68.56 ± 7.40	64.14 ± 13.94	0.130
> 10 mmol/L	36.09 ± 14.66	22.79 ± 9.99	30.19 ± 16.33	0.129
> 13.9 mmol/L $_{K\text{-}W\ D}$	8.81 ± 5.91	4.33 ± 3.38	6.83 ± 8.56	0.206
Mean Glucose	9.05 ± 1.15	7.81 ± 1.28	8.53 ± 1.64	0.149
SD $_{K\text{-}W\ D}$	3.23 ± 0.64	3.03 ± 0.58	3.06 ± 0.59	0.604
CV	36.71 ± 7.59	38.22 ± 7.30	36.52 ± 6.02	0.848

$_{K\text{-}W\ M}$ – Kruskal - Wallis Comparison of Medians, $_{K\text{-}W\ D}$ – Kruskal - Wallis Comparison of Distribution

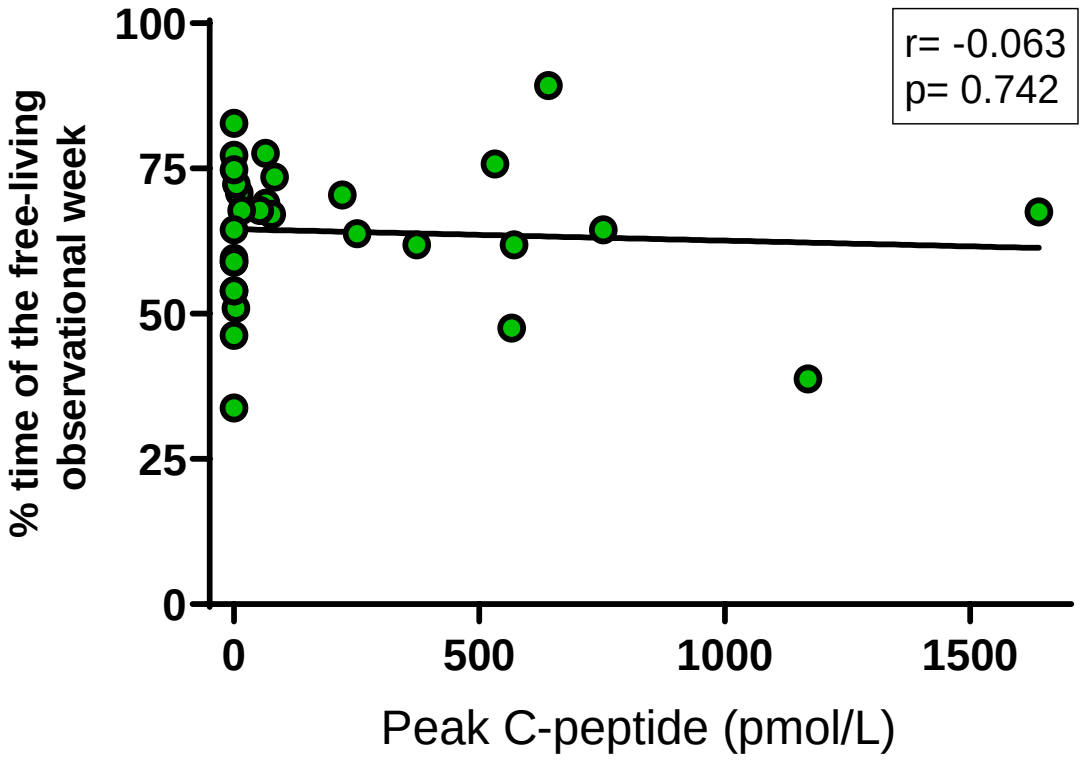

Figure 4.3 Scatter plots displaying linear relationships as assessed by Pearson's correlation between peak serum C-peptide and percentage of time spent in a glycaemic range 3.9 to 10 mmol/L

Table 4.5 Displaying the Pearson's (r) and Spearman's correlations (r_s) and significance between the Peak serum C-peptide and interstitial glucose parameters during the observational week.

CGM MEASURES	r	p
% of Time <3 mmol/L $_s$	0.047	0.806
% of Time <3.9 mmol/L $_s$	0.144	0.447
% of Time 3.9 – 10 mmol/L	-0.063	0.742
% of Time >10 mmol/L	0.078	0.684
% of Time >13.9 mmol/L $_s$	-0.296	0.112
Mean	0.076	0.689
SD	-0.133	0.484
CV	-0.111	0.558

$_s$ Assessed by Spearman's rank-order correlation.

4.4.4. Mixed Meal Tolerance Test

Group C-peptide, glucose and glucagon results from the mixed meal tolerance test (MMTT) are displayed below.

4.4.4.1. C-peptide

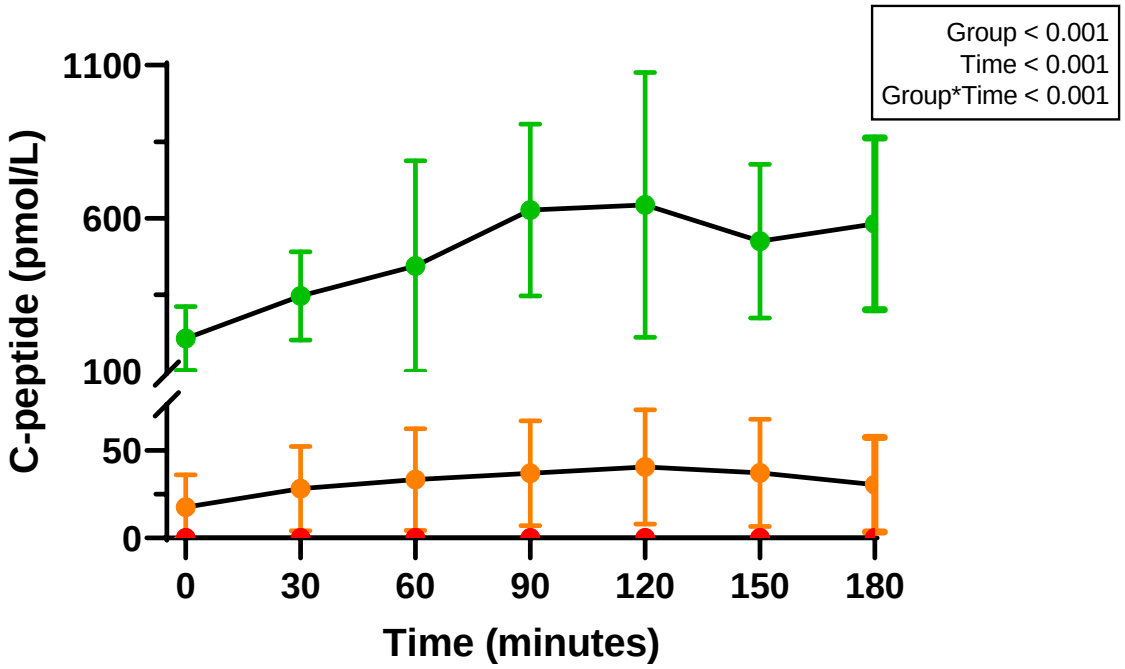

Figure 4.4 Time-course changes in C-Peptide (pmol/L) over the MMTT. Data presented as mean ± SD. Red circles = $Cpep_{und}$ (n=11), Orange circles = $Cpep_{low}$ (n=9), Green circles = $Cpep_{high}$ (n=10).

Figure 4.4. displays the 2-way mixed effects model results of C-peptide at baseline and every 30 minutes up to and including 180 minutes after the intake of the Fortisip for the different groups. In keeping with the study design, there was a statistically significant difference in mean C-peptide concentration between each of the groups. There was a significant group*time interaction, F (12, 144) = 9.980, p < 0.001, with C-peptide concentration significantly increased from 0 to 30 minutes in the $Cpep_{low}$ group (10.56 pmol/L, 95% CI 0.72 to 20.39 pmol/L), while C-peptide significantly increased from 0 to 30, 60, 90, 150 and 180 minutes in the $Cpep_{high}$. Peak and $AUC_{0To180min}$ C-peptide was significantly different between groups (Table 4.6.)

Table 4.6 Displaying One Way ANOVA results for C-peptide during the MMTT

	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$	P
PEAK C-PEPTIDE (pmol/L)	0.00 ± 0.00	42.00 ± 32.58*	671.70 ± 435.15* #	**<0.001**
$AUC_{0TO180min}$ C-PEPTIDE ($pmoL/L_{per180min}$)	0.00 ± 0.00	6026 ± 4452*	89459 ± 48095* #	**<0.001**

* Significantly different to $Cpep_{und}$ # Significantly different to $Cpep_{low}$

4.4.4.2. Glucose

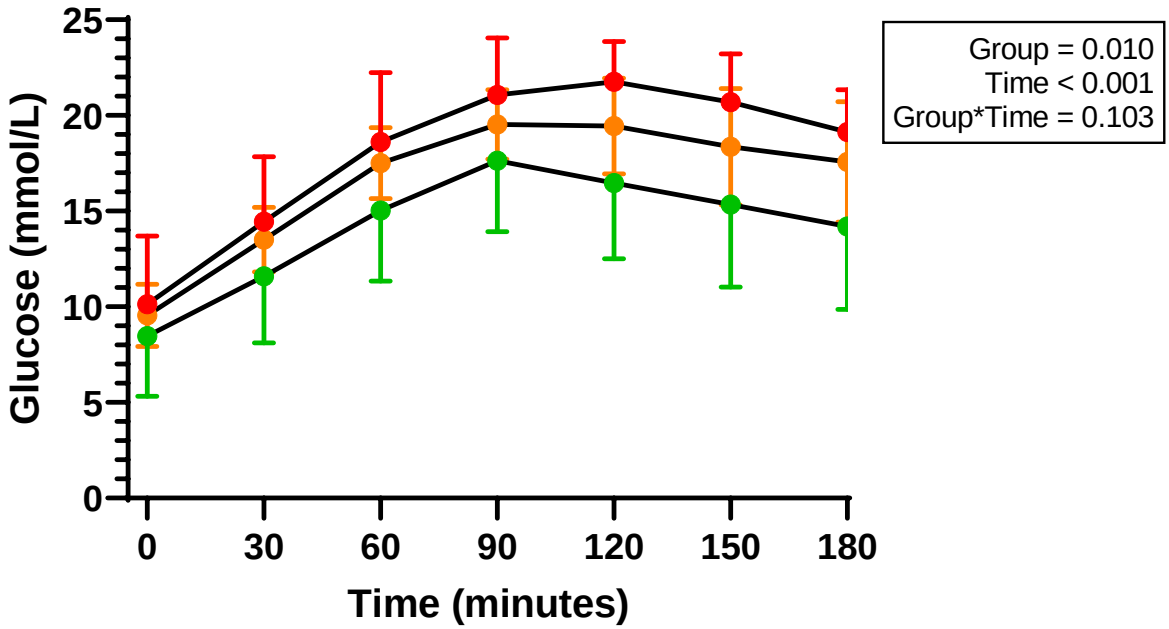

Figure 4.5 Time-course changes in Glucose (mmol/L) over the MMTT. Data presented as mean ± SD. Red circles = $Cpep_{und}$ (n=11), Orange circles = $Cpep_{low}$ (n=8), Green circles = $Cpep_{high}$ (n=10).

Figure 4.5. displays the 2-way mixed effects model results of glucose at baseline and every 30 minutes up to and including 180 minutes after the intake of the Fortisip for the different groups. One participant from the $Cpep_{low}$ group was excluded because of suspected bolus insulin intake preceding the MMTT trial. There was a statistically significant difference in mean glucose between groups, with $Cpep_{high}$ (14.04 ± 3.12 mmol/L) significantly lower than $Cpep_{und}$ (18.02 ± 4.23 mmol/L, p< 0.001) and $Cpep_{low}$ (16.36 ± 3.67 mmol/L, p= 0.013), with no significant difference between $Cpep_{und}$ and $Cpep_{low}$ (p= 0.100). There was no statistically significant interaction between the C-peptide group and time (p= 0.103), with all groups having a similar increase from baseline across time (p < 0.001). $Cpep_{high}$ had significantly lower peak glucose and AUC compared to $Cpep_{und}$ but not $Cpep_{low}$ (Table 4.7).

Table 4.7 Displaying One Way ANOVA and Kruskal-Wallis Comparison results for glucose during the MMTT

	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$	P
PEAK GLUCOSE (mmol/L) $_{K\text{-}W\ M}$	21.91 ± 2.75	20.04 ± 2.34	17.74 ± 3.59*	0.016
$AUC_{0TO180min}$ GLUCOSE ($mmol/L_{per180min}$) □	3336 ± 513.4	3057 ± 336.9	2622 ± 629.3*	0.0145

□ Assessed by Walsh ANNOVA $_{K\text{-}W\ M}$ – Kruskal - Wallis Comparison of Medians * Significantly different to $Cpep_{und}$

4.4.4.3. Glucagon

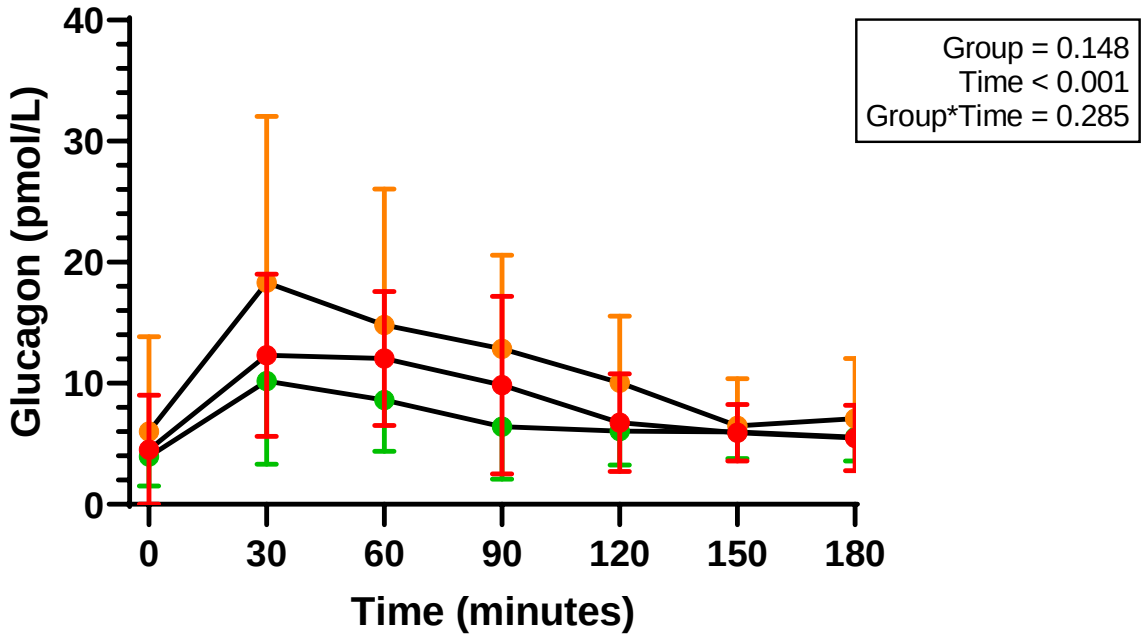

Figure 4.6 Time-course changes in Glucagon (pmol/L) over the MMTT. Data presented as mean ± SD. Red circles = Cpepund (n=11), Orange circles = Cpeplow (n=9), Green circles = Cpephigh (n=10).

Figure 4.6. displays the 2-way mixed effects model results of glucagon at baseline and every 30 minutes up to and including 180 minutes after the intake of the Fortisip for the different groups. There was no statistically significant group * time interaction (p= 0.285) or group mean differences (p= 0.148) for glucagon. The Fortisip drink did cause a significant increase over time (p < 0.001), peaking at 30 minutes post before declining back to baseline levels. There was no difference in individual peak or $AUC_{0TO180min}$ glucagon between C-peptide groups (table 4.8)

Table 4.8 Displaying One Way ANOVA and Kruskal-Wallis Comparison results for glucagon during the MMTT

	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$	P
PEAK GLUCAGON $_{K\text{-}W\ D}$	14.04 ± 6.74	18.60 ± 13.49	12.45 ± 4.34	0.802
$AUC_{0TO180min}$ GLUCAGON (pmol/$L_{per180min}$) □	1557 ± 905.8	2072 ± 1370	1259 ± 674.5	0.2518

□ Assessed by Walsh ANNOVA $_{K\text{-}W\ D}$ – Kruskal - Wallis Comparison of Distribution

4.4.5. Submaximal CGM data

4.4.5.1. 12 hours pre exercise phase

In the 12 hours prior to the submaximal exercise bout there was no difference in mean interstitial glucose ($Cpep_{und}$ 9.46 ± 3.29 vs $Cpep_{low}$ 8.48 ± 2.71 vs $Cpep_{high}$ 8.14 ± 1.57 mmol/L, p= 0.507), CV (p= 0.349), or any time spent in glycaemic ranges (p> 0.05). While percentage time spent in glycaemic range 3.9 to 10 mmol/L increased from $Cpep_{und}$ (52.10 ± 29.54%), to $Cpep_{low}$ (60.78 ± 30.74%), to $Cpep_{high}$ (78.62 ± 19.64%) groups in that order, the difference between the groups was not statistically significant (p = 0.093). Seven participants spent more than 4% of the preceding 12 hours in hypoglycaemic level 1.

4.4.5.2. Laboratory phase - Exercise bout

On average, participants exercised at 59.42 ± 4.06% of their VO_{2peak}, with no differences between the C-peptide groups ($Cpep_{und}$ 58.34 ± 4.15 %, $Cpep_{low}$ 60.24 ± 4.84 %, $Cpep_{high}$ 59.92 ± 3.17 % p = 0.542). There was no difference in carbohydrate oxidation ($Cpep_{und}$ 1.54 ± 0.58 g/min, $Cpep_{low}$ 2.04 ± 0.83 g/min, $Cpep_{high}$ 1.59 ± 0.78, p = 0.279), fat oxidation ($Cpep_{und}$ 0.15 ± 0.11 g/min, $Cpep_{low}$ 0.18 ± 0.12 g/min, $Cpep_{high}$ 0.17 ± 0.10 g/min, p = 0.884) or energy expenditure ($Cpep_{und}$ 348.88 ± 103.45 kcal, $Cpep_{low}$ 451.62 ± 153.97 kcal, $Cpep_{high}$ 364.87 ± 143.34 kcal, p= 0.215) between the groups.

The $Cpep_{und}$ group had higher capillary blood glucose on arrival ($Cpep_{und}$ 9.83±2.17, $Cpep_{low}$ 7.96±3.11, $Cpep_{high}$ 7.25±1.52 mmol/L, p=0.045), pre ($Cpep_{und}$ 11.42±2.76, $Cpep_{low}$ 9.37±1.61, $Cpep_{high}$ 8.30±1.14 mmol/L, p=0.007) and post-exercise ($Cpep_{und}$ 13.00±4.38, $Cpep_{low}$ 9.26±4.37, $Cpep_{high}$ 9.00±2.83 mmol/L, p=0.048), as well on leaving the laboratory at 1 hour post-exercise ($Cpep_{und}$ 13.34±3.21, $Cpep_{low}$ 11.23±3.86, $Cpep_{high}$ 9.32±2.58 mmol/L, p=0.029), compared to the $Cpep_{high}$ but not the $Cpep_{low}$ group. There were no incidents of hypoglycaemia within the laboratory phase of the study, either during the exercise bout or throughout the 60 minute post-exercise recovery period. Six participants (1 $Cpep_{und}$, 2 $Cpep_{low}$ and 3 $Cpep_{high}$) were given 10g of additional carbohydrates during the exercise bout as their blood glucose had dropped below 7 mmol/L.

The interstitial glucose over the time spent in the exercise laboratory for each C-peptide group are presented in figure 4.7. There was no statistically significant group*time interaction for interstitial glucose concentration (p= 0.706). The main effect of time showed a significant post exercise rise in interstitial glucose (p = 0.001). There was a statistically significant difference in mean (± SEM) interstitial glucose between groups, with $Cpep_{high}$ (8.73 ± 0.78 mmol/L) significantly lower than $Cpep_{und}$ (11.80 ± 0.75 mmol/L, p= 0.026) but not $Cpep_{low}$ (9.67 ± 0.83 mmol/L, p= 1.00).

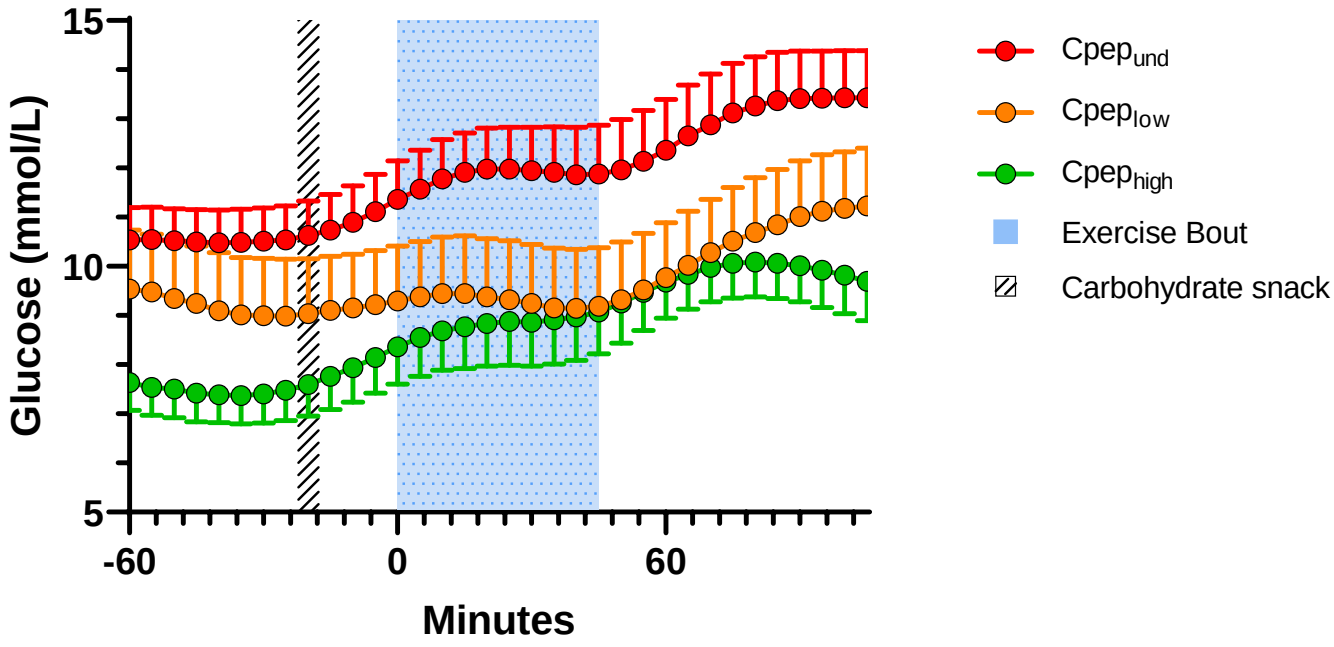

Figure 4.7 Time-course changes in interstitial glucose over the laboratory visit hours. Data presented as mean ± SEM. Red circles = Cpepund (n=11), Orange circles = Cpeplow (n=9), Green circles = Cpephigh (n=10). Blue dotted area indicates the submaximal exercise bout. Black thatched area indicates carbohydrate snack intake.

4.4.5.3. Post exercise

Twelve and 24 hour post-exercise interstitial glucose responses are presented in Figure 4.8B and C and Table 4.9. The $Cpep_{high}$ group spent 73.51 ± 27.64% of the 12 hours post-exercise in euglycaemia, compared to 43.58 ± 26.07% for $Cpep_{low}$ (p= 0.027) and 40.61±16.97% for $Cpep_{und}$ (p= 0.010) (Figure 4.9.B). The $Cpep_{high}$ group also had significantly less time spent in hyperglycaemia (Categories 1 and 2), lower mean glucose and SD compared to $Cpep_{low}$ and $Cpep_{und}$ (p< 0.05). No difference existed between groups for time spent with CGM glucose <3.9 mmol/L (p= 0.766) or

<3.0 mmol/L (p= 0.370), although notably mean time with CGM <3.0 mmol/L was zero in the $Cpep_{high}$ group (Table 4.9).

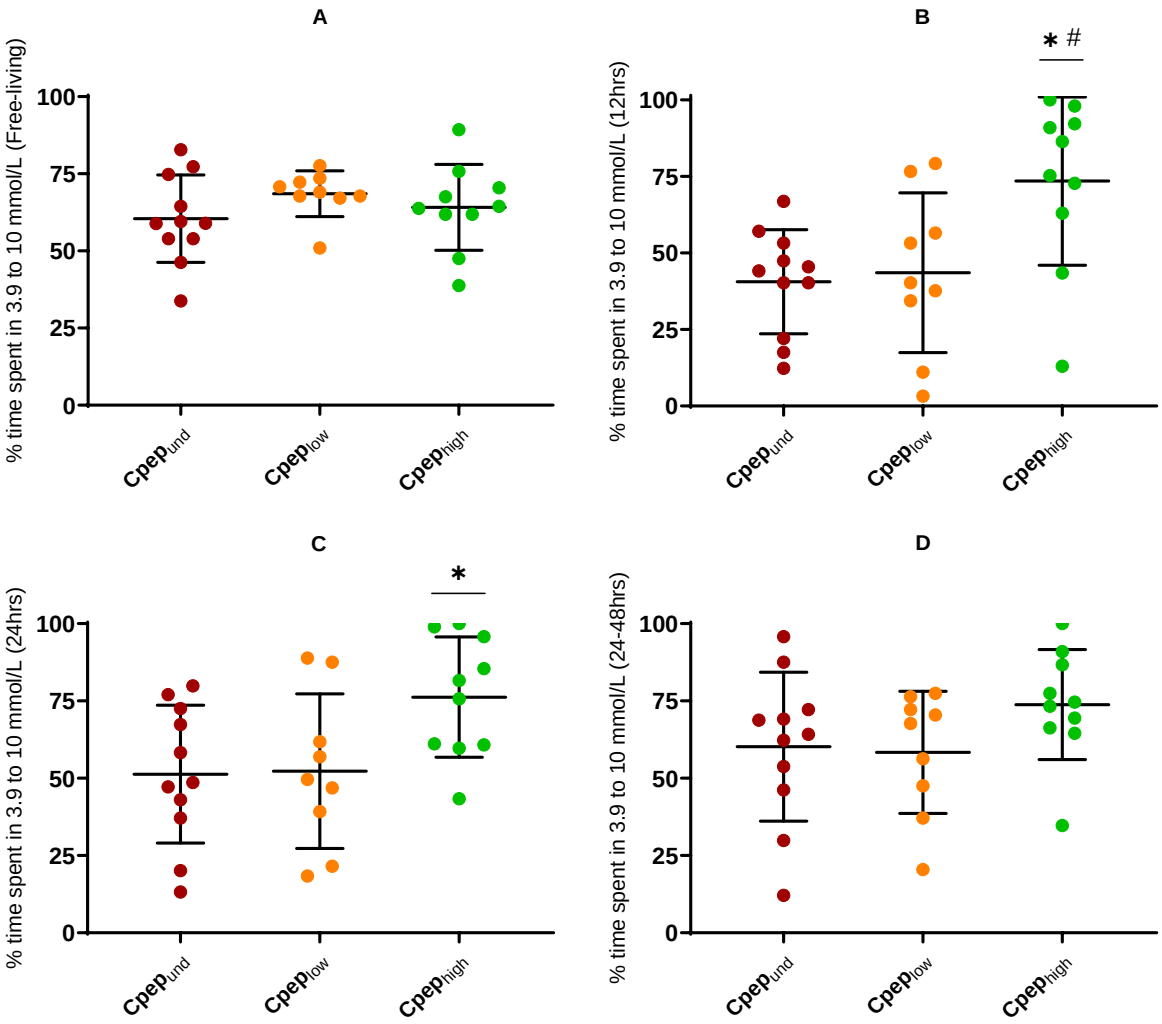

*Figure 4.8 Group mean±SD and individual data points for time spent in a euglycaemic range 3.9 to 10 mmol/L during (A) the observational free-living week, (B) 12 hours post submaximal exercise bout, (C) 24 hours post submaximal exercise bout, (D) between 24 and 48 hours post submaximal exercise bout. Red circles = Cpepund (n=11), Orange circles = Cpeplow (n=9), Green circles = Cephigh (n=10). * indicates significantly different to Cpepund, # indicates significantly different to Cpeplow.*

Similar patterns were observed in the interstitial glucose response in the 24 hours post-exercise period, with $Cpep_{high}$ having higher time in euglycaemia (76.25 ± 19.16%) than $Cpep_{und}$ (51.33 ±2 2.26%, p= 0.041), although not statistically higher than $Cpep_{low}$ (52.31 ± 24.98%, p= 0.067) (Figure 1.C). $Cpep_{high}$ had significantly lower amount of time spent in hyperglycaemia and reduced measures of GV compared to both $Cpep_{low}$ and $Cpep_{und}$ (Table 4.9.).

In the 24 to 48 hour following the exercise bout, the effects were largely lost with only time spent >13.9 mmol/L and SD significantly lower in the $Cpep_{high}$ group compared to $Cpep_{und}$ and $Cpep_{low}$ (Table 4.9. Figure 4.8D).

Table 4.9 One-way ANOVA and Kruskal-Wallis Comparison results for the CGM outcomes of each C-peptide grouping at different time points. Data is mean ± SD

	Observational Week				12 hours pre				12 hours post			
	$Cpep_{und}$	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**	**$Cpep_{und}$**	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**	**$Cpep_{und}$**	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**
< 3 mmol/L	0.72 ± 1.40	1.33 ± 1.94	0.88 ± 1.18	0.710	4.95 ± 11.12	5.52 ± 13.76	0.28 ± 0.87	0.503	0.71 ± 2.35	3.03 ± 8.39	0.00 ± 0.00	0.284
< 3.9 mmol/L	3.47 ± 3.24	8.66 ± 9.69	5.67 ± 5.39	0.540	6.83 ± 14.29	9.66 ± 16.55	2.21 ± 5.33	0.622	3.60 ± 5.13	5.91 ± 9.09	1.88 ± 3.24	0.586
3.9 to 10 mmol/L	60.44 ± 14.15	68.56 ± 7.40	64.14 ± 13.94	0.224	52.10 ± 29.54	60.78 ± 30.74	78.62 ± 19.64	0.093	40.61 ± 16.97	43.58 ± 26.07	73.51 ± 27.49* #	**0.007**
> 10 mmol/L	36.09 ± 14.66	22.79 ± 9.99	30.19 ± 16.33	0.129	41.06 ± 32.92	29.57 ± 31.13	19.17 ± 19.82	0.336	55.79 ± 17.52	50.51 ± 30.28	24.61 ± 27.63*	**0.015**
> 13.9 mmol/L	8.81 ± 5.91	4.33 ± 3.38	6.83 ± 8.56	0.206	11.79± 26.78	10.09 ± 17.13	2.83 ± 8.94	0.345	20.19 ± 15.71	23.59 ± 18.09	2.34 ± 5.97* #	**0.001**
Mean	9.05 ± 1.15	7.81 ± 1.28	8.53 ± 1.64	0.149	9.46 ± 3.29	8.48± 2.71	8.14 ± 1.57	0.507	10.69 ± 1.57	10.66 ± 2.85	8.17 ± 1.59*	**0.006**
SD	3.23 ± 0.64	3.03 ± 0.58	3.06 ± 0.59	0.604	2.28 ± 1.00	2.32 ± 1.01	1.72 ± 0.89	0.325	3.40 ± 1.15	3.69 ± 0.98	2.03 ± 0.96* #	**0.003**
CV (%)	36.71 ± 7.59	38.22 ± 7.30	36.52 ± 6.02	0.848	26.09 ± 13.68	30.78 ± 18.17	21.10 ± 10.00	0.349	32.48 ± 11.46	36.78 ± 14.15	24.76 ± 9.93	0.098
	24 hours post				**48 hours post**				**24 to 48 hours post**			
< 3 mmol/L	1.29 ± 3.66	5.25 ± 15.35	0.49 ± 1.54	0.773	0.82 ± 1.86	4.88 ± 12.92	0.82 ± 2.45	0.763	0.35 ± 0.78	4.63 ± 10.74	0.77 ± 2.32	0.759
< 3.9 mmol/L	3.22 ± 5.07	9.30 ± 16.15	4.10 ± 9.82	0.471	2.80 ± 2.83	7.95 ± 14.59	4.05 ± 9.22	0.306	2.37 ± 3.97	6.98 ± 14.62	3.67 ± 7.11	0.953
3.9 to 10 mmol/L	51.33 ± 22.26	52.31 ± 24.98	76.25± 19.43 *	**0.029**	56.39 ± 20.79	55.46 ± 19.12	73.19 ± 15.76	0.094	60.18 ± 24.08	58.41 ± 19.75	70.90 ± 16.16	0.385
> 10 mmol/L	45.45± 23.52	38.39 ± 24.84	19.65 ± 19.55 *	**0.043**	40.82 ± 21.94	36.59 ± 19.31	22.84 ± 17.69	0.107	37.45 ± 24.90	34.61 ± 18.27	25.43 ± 19.45	0.368
> 13.9 mmol/L	11.99 ± 10.22	19.10 ± 20.89	1.25 ± 3.19 * #	**0.001**	12.09 ± 8.48	16.42 ± 17.49	1.14 ± 1.83 * #	**<0.001**	12.46 ± 12.95	13.66 ± 16.16	0.89 ± 1.51 #	**0.009**
Mean	9.82 ± 1.72	9.64 ± 2.97	7.73 ± 1.53	0.065	9.58 ± 1.51	9.49 ± 2.57	7.93 ± 1.51	0.132	9.42 ± 1.72	9.31 ± 2.39	8.07 ± 1.60	0.262
SD	3.02 ± 0.87	3.84 ± 1.01	2.00 ± 0.73 * #	**<0.001**	3.30 ± 0.72	3.81 ± 0.89	2.09 ± 0.47 * #	**<0.001**	3.21 ± 1.18	3.52 ± 1.08	2.05 ± 0.37 * #	**0.013**
CV (%)	31.89 ± 10.80	42.06 ± 15.35	26.17 ± 9.62 #	**0.025**	35.30 ± 8.97	42.21 ± 14.14	26.70 ± 6.36 #	**0.013**	34.40 ± 11.15	39.42 ± 15.17	25.97 ± 5.42	0.054

* Significantly different to $Cpep_{und}$, # Significantly different to $Cpep_{low}$

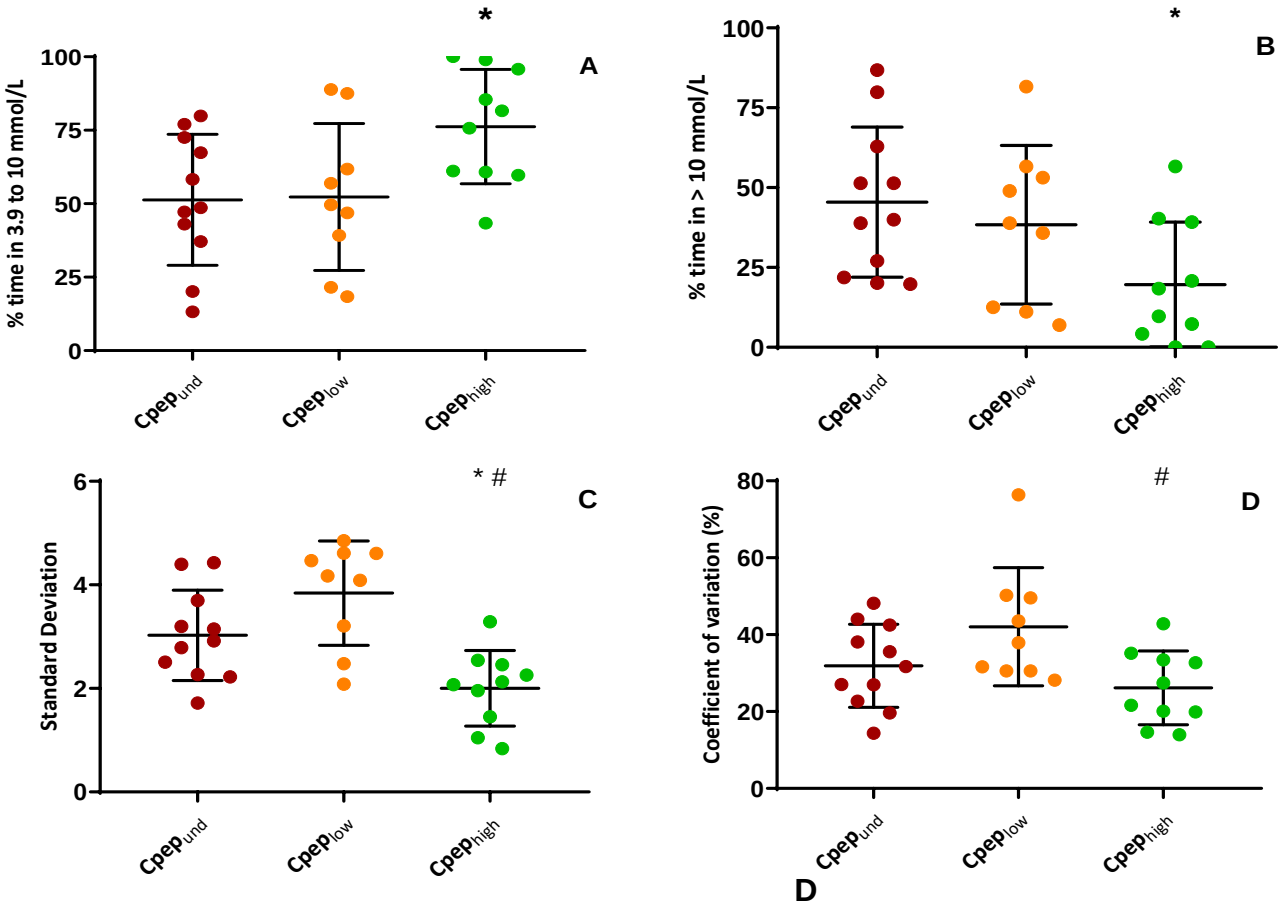

Figure 4.9. Group mean±SD with individual data points displaying C-peptide group's vs glycaemic control measures for the 24 hours post exercise. (A) Percentage of time spent in a glycaemic range 3.9 to 10 mmol/L, (B) Percentage of time spent in a glycaemic range > 10 mmol/L, (C) Standard Deviation, (D) Coefficient of Variation. Red circles = Cpepund (n=11), Orange circles = Cpeplow (n=9), Green circles = Cpephigh (n=10)

4.4.6. C-peptide vs glycaemic control correlations

Assessing the linear relationship between peak C-peptide and post exercise glycaemic outcomes demonstrated a positive relationship between C-peptide and time in range 3.9 to 10 mmol/L in the 12, 24 and 48 hours following the bout of submaximal exercise, facilitated by the relationship between C-peptide and time spent in hyperglycaemia (figure 4.10A and B, table 4.10). Similarly, measures of GV were strongly linked with residual β-cell function (table 4.10). No relationship was found between peak C-peptide and time spent in hypoglycaemic ranges within any time period. The positive relationship between C-peptide and time in range is diminished to a non-significant value when the time-period 24 to 48 hours were analysed ($r = 0.306$, $p = 0.107$) (figure 4.10C), indicative of a waning effect of the bout of exercise.

Peak stimulated glucagon did not predict time in hypoglycaemia or any CGM measure post exercise (p>0.05) (Table 4.10).

Table 4.10 Displaying the Spearman's correlations (r_s) and significance (p) between the peak serum glucagon and interstitial glucose parameters during the 24 hour post exercise period.

	rs	p
< 3 mmol/L $_S$	0.178	0.346
< 3.9 mmol/L $_S$	0.082	0.668
3.9 to 10 mmol/L	-0.152	0.421
>10 mmol/L	0.099	0.603
> 13.9 mmol/L $_S$	0.089	0.642
Δ Mean $_S$	0.103	0.586
Δ SD	-0.002	0.993
Δ CV (%)	-0.035	0.855

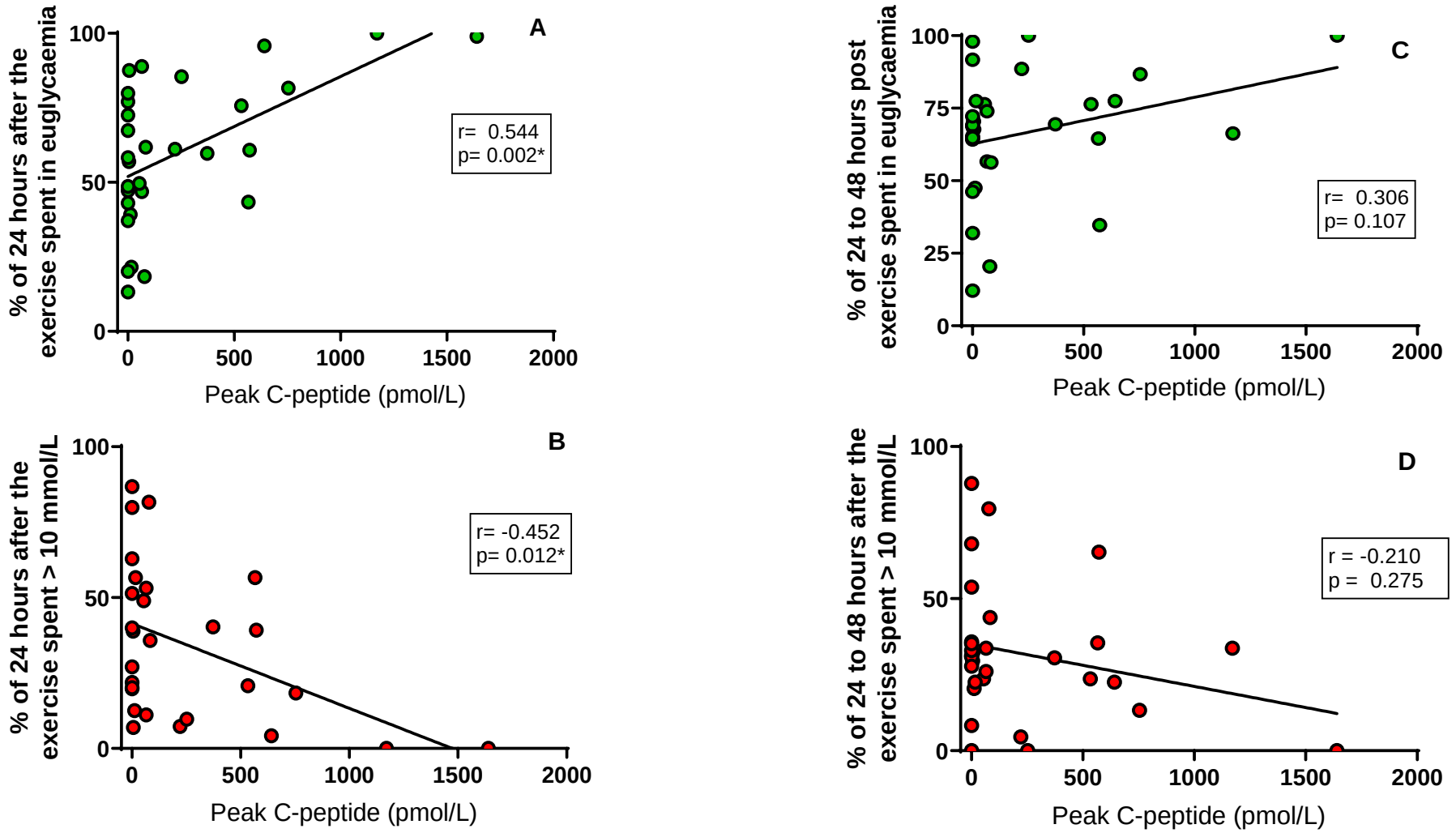

*Figure 4.10 Scatter plots displaying linear relationships between peak serum C-peptide vs glycaemic control measures for the 24 hours post exercise (A + B) or 24 to 48 hours post exercise (C +D). (A + C) Percentage of time spent in a glycaemic range 3.9 to 10 mmol/L and (B + D) Percentage of time spent in a glycaemic range > 10 mmol/L. * Significant correlation.*

Table 4.11 Displaying the Pearson's (r) and Spearman's correlations (r_s) and significance (p) between the Peak serum C-peptide and interstitial glucose parameters during the post exercise periods.

	12hr Post Exercise		24hr Post Exercise		48hrs Post Exercise		24 to 48hrs Post Exercise	
	r / rs	**p**	**r / rs**	**p**	**r / rs**	**p**	**r / rs**	**p**
< 3mmol/L	-0.189 $_s$	0.318	-0.192 $_s$	0.309	-0.171 $_s$	0.375	-0.090 $_s$	0.642
< 3.9mmol/L	-0.194 $_s$	0.304	-0.229 $_s$	0.224	-0.212	0.271	-0.069 $_s$	0.723
3.9 - 10mmol/L	**0.583**	**0.001***	**0.544**	**0.002***	**0.488**	**0.007***	0.306	0.107
> 10mmol/L	**-0.517**	**0.003***	**-0.452**	**0.012***	**-0.405**	**0.029***	-0.210 $_s$	0.275
> 13.9mmol/L	**-0.566 $_s$**	**0.001***	**-0.529 $_s$**	**0.003***	**-0.624 $_s$**	**<0.005***	**-0.468 $_s$**	**0.010***
Mean (mmol/L)	**-0.507**	**0.004***	**-0.388**	**0.034***	**-0.385**	**0.039***	-0.328	0.082
SD	**-0.613**	**<0.005***	**-0.632**	**<0.005***	**-0.676**	**<0.005***	-0.345	0.067
CV (%)	**-0.385 $_s$**	**0.036***	-0.258 $_s$	0.168	**-0.502**	**0.005***	**-0.383**	**0.040***

* Significant correlation, $_s$ Assessed by Spearman's rank-order correlation

4.4.7. Delta change from observational week to 24 hours CGM

Delta change (Δ) in interstitial glucose parameters from the observational week to 24 hours post-exercise showed significant correlations between peak C-peptide and time in euglycaemia (Figure 4.11A), time spent >10 mmol/L (Figure 4.11C), time spent >13.9 mmol/L and measures of glucose variability (Figure 4.11D). Participants with higher C-peptide had increased percentage time in euglycaemia in the 24 hours following the exercise bout compared to their free-living observational week (Δ12.11±21.54%), whereas individuals with low (Δ-16±24%, p= 0.018) or undetectable (Δ-9.1±18%, p= 0.073) C-peptide had reduced time in euglycaemia compared to the observational week (table 4.11, figure 4.11).

Table 4.12 Displaying the Pearson's (r) and Spearman's correlations (rs) and significance (p) between the Peak serum C-peptide and delta change (Δ) in interstitial glucose parameters from the observational week to 24 hours post exercise periods.

	r / rs	p
< 3 mmol/L $_s$	-0.142	0.454
< 3.9 mmol/L $_s$	-0.165	0.384
3.9 to 10 mmol/L	**0.595**	**0.001 ***
>10 mmol/L	**-0.502**	**0.005 ***
> 13.9 mmol/L $_s$	-0.257	0.170
Δ Mean $_s$	-0.317	0.088
Δ SD	**-0.562**	**0.001 ***
Δ CV (%)	**-0.450**	**0.013 ***

* Significant correlation, s - Assessed by Spearman's rank-order correlation

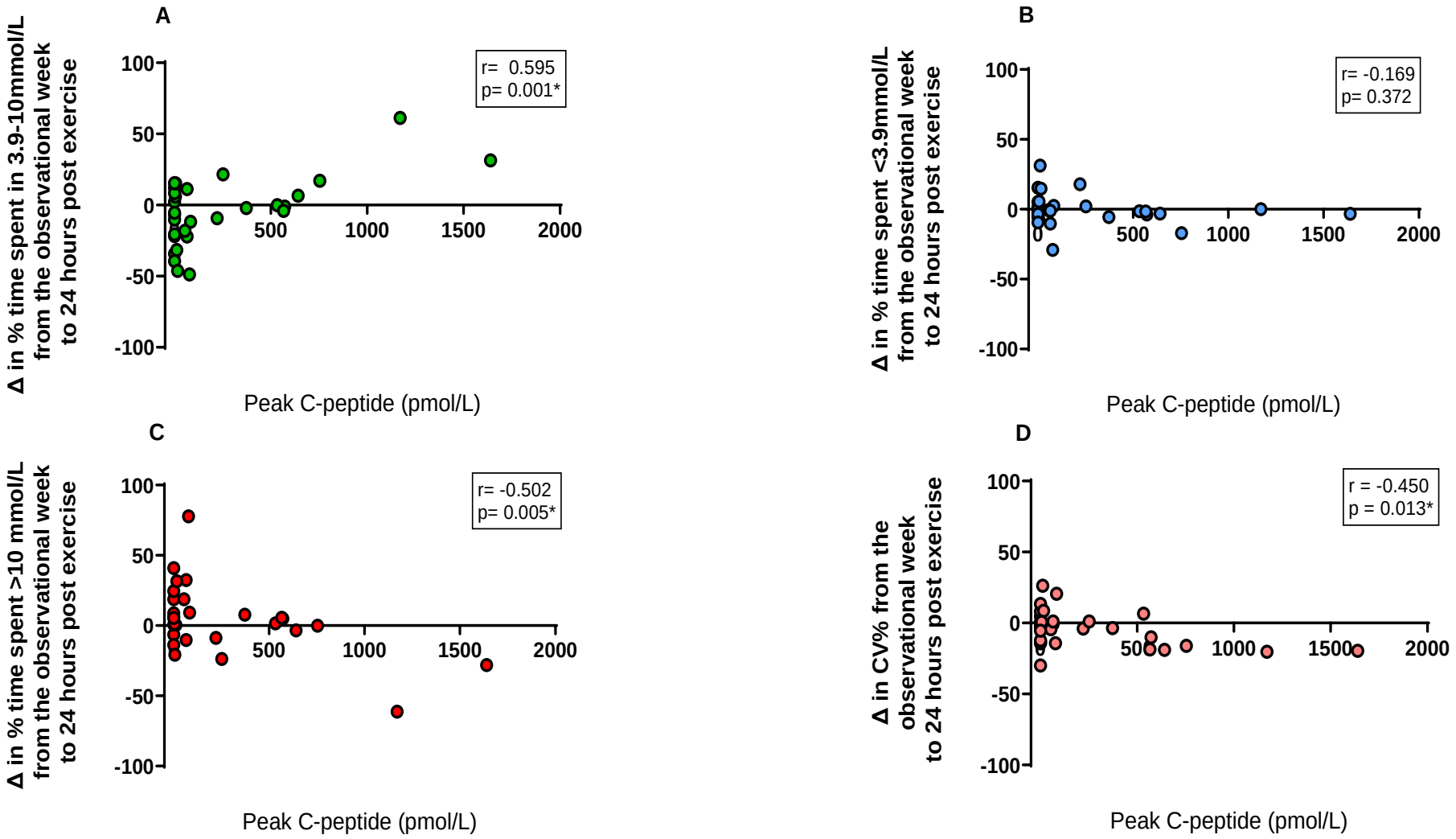

*Figure 4.11 Scatter plots displaying linear relationships between peak serum C-peptide vs the delta change in glycaemic control measures from the observational week to the 24 hours post exercise. (A) Delta change in the percentage of time spent in 3.9 to 10 mmol/L, (B) Delta change in the percentage of time spent <3.9 mmol/L, (C) Delta change in the percentage of time spent > 10 mmol/L and (D) Delta change in the CV%. * Significant correlation*

4.4.8. Exploration of lowest important C peptide level

To explore the lowest important C-peptide measurement upon glycaemic control following a bout of submaximal exercise, the participants were split into further groupings of <50 ($Cpep_{<50}$, n = 15) or >50 pmol/L ($Cpep_{>50}$, n = 15) and <70 ($Cpep_{<70}$, n = 18) or >70 pmol/L ($Cpep_{>70}$, n = 12).

Within the 12 hours post submaximal exercise both the $Cpep_{>50}$ and $Cpep_{>70}$ groupings had significantly greater time in glycaemic range 3.9 to 10 mmol/L, less time spent > 13.9 mmol/L and lower SD than $Cpep_{<50}$ and $Cpep_{<70}$ respectively. The $Cpep_{>70}$ also having significantly less time > 10 mmol/L, lower mean glucose and SD (Table 4.12). When the time was extended to 24 hours post, significance was largely lost for the glycaemic measures. $Cpep_{>50}$ and $Cpep_{>70}$ trended towards having more time in euglycaemic ranges.

A biphasic linear regression model of best fit within Graphpad Prism 8 with a slope of the first portion of the biphasic linear regression constrained to zero was unable to be fitted to the data.

Table 4.13 Displaying mean ± SD and T-test results for the glycaemic outcome of C-peptide groupings <50 and >50pmol/L, and <70 and >70pmol/L in the 12 and 24 hours proceeding the submaximal exercise bout.

	12 HOURS POST						24 HOURS POST					
	$Cpep_{<50}$	**$Cpep_{>50}$**	**p**	**$Cpep_{<70}$**	**$Cpep_{>70}$**	**p**	**$Cpep_{<50}$**	**$Cpep_{>50}$**	**p**	**$Cpep_{<70}$**	**$Cpep_{>70}$**	**p**
n	15	15		18	12		15	15		18	12	
< 3 mmol/L MWU	2.34 ± 6.68	0.00 ± 0.00	0.361	1.95 ± 6.12	0.00 ± 0.00	0.465	4.10 ± 12.05	0.32 ± 1.26	0.367	3.41 ± 11.05	0.41 ± 1.40	0.545
< 3.9 mmol/L MWU	5.71 ± 7.86	1.73 ± 2.84	0.217	4.91 ± 7.39	1.99 ± 3.10	0.391	7.69 ± 12.93	2.99 ± 8.09	0.081	6.48 ± 12.06	3.62 ± 8.97	0.249
3.9 to 10 mmol/L	41.69 ± 19.28	63.25 ± 30.60	**0.029**	41.56 ± 21.82	68.83 ± 27.56	**0.005**	51.32 ± 22.88	68.54 ± 23.59	0.052	53.07 ± 22.63	70.23 ± 24.34	0.058
> 10 mmol/L MWU	52.60 ± 22.71	35.02 ± 30.95	0.074	53.54 ± 24.48	29.22 ± 27.80	**0.015**	41.00 ± 23.86	28.47 ± 24.53	0.167	40.45 ± 23.10	26.16 ± 25.28	0.121
> 13.9 mmol/L MWU	33.29 ± 19.90	23.81± 21.89	**0.033**	20.49 ± 15.08	7.41 ± 16.15	**0.002**	11.60 ± 9.57	9.49 ± 18.63	0.700	12.02 ± 9.30	8.33 ± 20.47	0.508
Mean Glucose	10.38 ± 2.06	9.29 ± 2.47	0.202	10.58 ± 2.14	8.73 ± 2.17	**0.029**	9.40 ± 2.00	8.75 ± 2.53	0.443	9.44 ± 1.89	8.53 ± 2.72	0.289
SD	3.49 ± 1.15	2.58 ± 1.19	**0.041**	3.44 ± 1.07	2.42 ± 1.27	**0.026**	3.23 ± 0.99	2.63 ± 1.20	0.144	3.27 ± 0.97	2.43 ± 1.19	**0.043**
CV	34.97 ± 13.79	27.43 ± 10.10	0.099	31.89 ± 10.80	42.06 ± 15.35	0.148	36.34 ± 15.11	29.73 ± 10.62	0.176	36.16 ± 13.94	28.35 ± 11.12	0.115

4.4.9. Exclusion of individuals within $Cpep_{high}$ with negative autoantibodies

Individual autoantibody positivity status is displayed in Appendix F. Nine of the 30 participants were auto-antibody negative, including 2 participants within the $Cpep_{high}$ group (duration of diabetes: 17 and 20 years, peak C-peptide 532 and 1170 pmol/L, respectively). To reduce the possibility of misdiagnosed cases of type 2 or monogenic diabetes influencing the results, the data were reanalysed without these two participants.

Between group differences within the first 12 hours post exercise mirrored those seen within the whole group analysis, with time spent in euglycaemia significantly higher for $Cpep_{high}$ than $Cpep_{low}$ and $Cpep_{und}$ ($p= 0.023$). When extended out to 24 hours the trends persisted, with clinically relevant, but not statistically significant, mean differences ($Cpep_{und}$ 51.33±22.26, $Cpep_{low}$ 52.31±24.98, $Cpep_{high}$ 73.35±19.88%, $p=0.093$). Furthermore, the same relationships between C-peptide and Δ from the observational week to 24 hours post-exercise for euglycaemia ($r=0.473$, $p=0.041$), <3.9 mmol/L ($r=-0.192$, $p=0.328$), >10 mmol/L ($r=-0.355$, $p=0.064$) and CV ($r=-0.432$, $p=0.022$) exist.

4.5. Discussion

We investigated how residual β-cell function impacts glycaemic control following exercise in people with T1D. For the first time we showed that despite having a cohort who had comparable glycaemic control under free-living conditions, individuals with T1D with higher residual β-cell function (stimulated C-peptide of >200 pmol/L) displayed a substantially greater amount of time spent in euglycaemia in the hours following a bout of moderate intensity exercise. Higher C-peptide was also associated with participants spending less time in hyperglycaemia and having less glycaemic fluctuations post-exercise. Furthermore, we show divergence in the impact of exercise on glycaemic profiles, with high residual C-peptide associated with improved control compared with pre-exercise free-living conditions and low / absent C-peptide associated with worsened control following exercise.

Results from the baseline observational free-living CGM data are discussed in detail in chapter 3. While the higher C-peptide group had substantially greater time in euglycaemia in the larger cohort, no group differences existed between the participants who progressed into the current study. Results from the present study are not dissimilar to Rickels et al. (2020) study, where they demonstrated that individuals with C-peptide >400 pmol/L spent greater time in euglycaemia under free-living conditions, but no differences between the negative, low (0.017-200 pmol/L) and what they have defined as intermediate (200-400 pmol/L) groups. Differences between the free-living CGM data in chapter 3 and 4 may be due to a multitude of reasons. While the free-living study was a relatively straightforward single study visit and observational week for the participants, the current study was more intensive in terms of both time and protocols. This resulted in many more participants being interested and willing to partake in study 1 than study 2. Study 2 had a more homogenous group of participants in regards to time in euglycaemia, with the $Cpep_{high}$ group spending 64.1±13.9% compared to 71.8±17.0% in study 1, the $Cpep_{low}$ group spending 68.6±7.4% compared to 60.1±14.9%, and the $Cpep_{und}$ spending 60.4±14.2% compared to 60.5±14.6%. We speculate that participants recruited into study 2 were either already experienced and confident in glycaemic control around exercise or specifically interested in learning. The high C-peptide group had a much shorter duration of disease, which may result in less experience managing glucose around exercise. The differing inclusion criteria of ≥1 year from diagnosis in study 1 to ≥3 from

diagnosis in the current study also likely made a substantial difference in the high C-peptide group, excluding participants who were in the honeymoon period (Abdul-Rasoul et al., 2006, Schölin et al., 1999). As participants were motivated to take part in an intensive research study, all attended a single diabetes centre, were mainly in good to moderate long-term glycaemic control (HbA1c; 58.5±9.1 mmol/mol, 7.5±3%), had similar insulin treatment, and had access to the same clinical management and education, it is likely that C-peptide had minimal impact under stable free-living conditions. However, persistent C-peptide secretion may still have an influence in more extreme stimuluses such as hypoglycaemia, MMTT or during and after exercise, as demonstrated in the current study.

Glucose is the major fuel source during exercise and glucose uptake into the skeletal muscle during an exercise bout can increase by up to 50-fold (Sylow et al., 2017). During an exercise bout, increased skeletal muscle blood flow (Joyner and Casey, 2015) and substantial elevation in capillary recruitment within the muscle (Vincent et al., 2006) maintains glucose delivery to the working skeletal muscle fibres. With the onset of aerobic exercise, the availability of intra-muscular and extra-muscular substrates, the abundance of substrate transporter proteins and the activity of regularly enzymes are all regulators of the increase and interaction of carbohydrate and lipid metabolism (Hearris et al., 2018). In moderate to hard intensity aerobic exercise (60-85% VO_{2max}), muscle glycogenolysis, liver glycogenolysis and glucose uptake increase as carbohydrate metabolism becomes the dominant energy source. Contraction induced Increases in Ca^{2+} and substrate accumulation increases the flux through key regulatory enzymes such as phosphorylase , phosphofructokinase, lactate dehydrogenase and pyruvate dehydrogenase (PDH), increasing the breakdown of muscle glycogen (Hargreaves and Spriet, 2020). In addition, exercise independently increase glucose uptake into the skeletal muscles, with the contraction induced mechanisms including Ca^{2+} and Ca^{2+} dependent signalling proteins (Gehlert et al., 2015), mechanical stress (Sylow et al., 2015), AMPK activation (Koistinen et al., 2003) and nitric oxide (Bradley et al., 1999) all potentially able to increase the redistribution of GLUT4 glucose transporters to the cell membrane (Douen et al., 1990). A single bout of endurance exercise also increases insulin's action (Gulve et al., 1990), with sensitivity to insulin persisting for up to 48 hours post exercise (Mikines et al., 1988, Annuzzi et al., 1991, Devlin et al., 1987). This process appears linked to

the restoration of muscle glycogen reserves (Bogardus et al., 1983). Within individuals with T1D, increased glucose is required during and shortly after the completion of exercise and also approximately 7-11 hours post exercise to maintain euglycaemia as the muscle glycogen stores are replenished (McMahon et al., 2007). However, insulin stimulated GLUT4 translocation to the membrane of skeletal muscle cells does not fully explain the increase in glucose uptake after exercise. McConell et al. (2020) demonstrated that permeability of the skeletal muscle membrane to glucose increased around 17-fold with insulin stimulation in a rested muscle, and 36-fold in the exercised muscle, 4 hours after the exercise bout.

Our primary findings that individuals with higher C-peptide had substantially increased time in euglycaemia post-exercise compared to lower C-peptide individuals, in addition to the clear divergence in whether there is a positive or negative impact of exercise on parameters of glycaemic control depending on residual C-peptide status have not previously been reported. With the reduced glycaemic load from the MMTT and comparable glycaemic control in the free-living and pre-exercise phases, we hypothesize that the endogenous insulin secretion within the $Cpep_{high}$ group combined with increased insulin sensitivity following the exercise bout attenuated high blood glucose excursions. With the non-significant relationship between C-peptide and euglycaemia in the 24 to 48 hours post exercise and no differences between groups, our results further reflect this initial increase in insulin sensitivity, improving glycaemia for the high C-peptide group, which reduces over time. These mechanisms may contribute to the difficulties in maintaining glycaemic control after exercise in those with low C-peptide, while enhancing the beneficial impact of endogenous insulin secretion within higher C-peptide individuals.

Authors from previous secondary analysis of glycaemic control during and after exercise have postulated that insulin resistance may play a role in the inter-individual variability (Al Khalifah et al., 2016, Tagougui et al., 2019). As a longer duration of diabetes is associated with increased insulin resistance (Teixeira et al., 2014), and the high C-peptide group had a lower mean duration, this study cannot rule out the role insulin resistance plays in post-exercise glycaemic control. However, it is important to note that the BMI (25.22±3.73 kg/m^2), total daily insulin dose (41.77±23.40 units) and dose per kg (0.55±0.24 units/kg/day) were comparable across groups, and were not high enough to indicate insulin resistance. Additionally, Rickels et al. (2020) found no

difference in total-body, peripheral or hepatic insulin sensitivity across the negative, low, intermediate, and high C-peptide groups.

Results from the present study, on first viewing, look very similar to those in chapter 3 that higher residual beta-cell function show improved glycaemic control during free-living periods. However, as the current cohort had very similar glycaemic control during the free-living observational week and the clear divergence in post exercise glycaemic control, our results may in part explain the large inter-individual variation seen during and after exercise (Riddell et al., 2017). We believe this is the first study to explicitly study inter-individual variation in glycaemic responses to exercise, with previously published research having performed secondary analysis of participant data to explore this topic (Riddell et al., 2019b, Al Khalifah et al., 2016, Brockman et al., 2020, Tagougui et al., 2019). Riddell et al. (2019b) found the best predictor of the magnitude of drop in glucose for adolescents undertaking prolonged aerobic exercise was the pre-exercise glucose level, with age, BMI, disease duration, total daily insulin dose, HbA1c or sex having no impact. Meanwhile, Al Khalifah et al. (2016) demonstrated that hypoglycaemia during exercise happened more often in a higher fitness versus a poor fitness level group. This could have been the result of increased insulin sensitivity during the exercise, or the fact that they performed at a greater work threshold during the submaximal bout. An additional factor that could influence in glycaemia around exercise is body composition, with leg and trunk fat mass positively and whole body lean mass negatively associated with glucose post exercise (Tagougui et al., 2019). The authors speculate these results are due to insulin sensitivity, a similar conclusion to this present study. Finally, sex-related differences exist during exercise in individuals with T1D, with males having a greater risk of hypoglycaemia after an acute bout of resistance exercise than females (Brockman et al., 2020). This may be due to oestrogen promoting lipid oxidation and decreasing carbohydrate oxidation during exercise (Devries et al., 2006, Isacco et al., 2012), as the attenuated catecholamine response in females should result in an increased decline in blood glucose (Galassetti et al., 2002, Pullinen et al., 2002). It is tempting to speculate upon the role residual β-cell function could have played within their studies. Interestingly, Al Khalifah et al. (2016) higher fitness group appeared to have an noticeably earlier age of diagnosis, which potentially could result in a lower mean values of C-peptide at diagnosis and throughout the disease progression (Shields et al., 2018, Leete et al., 2016, Davis et

al., 2015). These findings highlight the need for future studies into the intra-individual variability in glycaemic responses to exercise in T1D, for more personalised insulin dose adjustments and carbohydrate intake, as well as for the development of a successful artificial pancreas system that can handle the considerable challenge of exercise (Yardley, 2020).

Avoidance of hypoglycaemia, in everyday life as well as during and after exercise, is of central importance for people with T1D. A wide range of methods, including nutritional and insulin adjustments have been reported and discussed, yet difficulties in maintaining euglycaemia around exercise are prevalent (Riddell et al., 2017). Previous studies have reported that preserved β-cell function was associated with reduced self-reported (Marren et al., 2019, Hope et al., 2018, Kuhtreiber et al., 2015) and CGM measured hypoglycaemia (Gibb et al., 2020), however neither the present or another recently published study have seen time spent in hypoglycaemia as measured by CGM influenced by C-peptide (Rickels et al., 2020). In the current study, time spent in hypoglycaemia (both below 3.9 and 3 mmol/L) in the post exercise period was 2-fold or more less in the $Cpep_{high}$ group, which may be clinically meaningful although not statistically different. While Gibb et al. (2020) demonstrated a significant difference in time spent in hypoglycaemia between individuals with low (<10 pmol/L) and preserved (>10 pmol/L) C-peptide, the 1% difference between groups likely needed the large study size (n=290) to see the significant difference. Future studies should carefully consider how to most meaningfully measure hypoglycaemia in free-living conditions, with a combination of CGM, diaries, recalls and large sample sizes likely to be needed (Henriksen et al., 2019).

This study provides further evidence that the paradoxical glucagon secretion in response to oral ingestion is not influenced by C-peptide status, and that peak glucagon measured by these methods does not associate with time spent in hypoglycaemia (Rickels et al., 2020, Thivolet et al., 2019). Hyperglucagonemia responses in the postprandial period are observed in youth with T1D within 1 to 2 years from diagnosis (Sherr et al., 2014, Brown et al., 2008). This glucagon response is likely due to individuals with T1D having an exaggerated α-cell response to amino acid stimulation (Dean, 2020), which appears not to be influenced by C-peptide status. However, recent research demonstrates that during a hyperinsulinemia hypoglycaemic clamp, those with persistent β-cell function have a residual counter-

regulatory responses to hypoglycaemia including increased glucagon (Zenz et al., 2018). Additionally, there is a reduction in biochemical hypoglycaemia and an increase in glucagon response to hypoglycaemic clamp in C-peptide positive islet transplant recipients (Brooks et al., 2015). The α-cell's ability to secrete glucagon in response to hypoglycaemia is impaired around diagnosis of T1D (Arbelaez et al., 2014), with further functional losses as duration of diabetes increases (Siafarikas et al., 2012). It is hypothesized that functioning β-cells within the islet of Langerhans enable some residual α-cell function and allowing some hypoglycaemia protection, although underlying mechanisms remain unclear (McCrimmon and Sherwin, 2010). Whether responses to a hyperinsulinemic clamp have significant impact in real world conditions requires studies such as the current one.

To further understand the participants' responses in our study, autoantibody status was assessed to minimise the possibility of misdiagnosed diabetes impacting the results, despite a large proportion of individuals with T1D being autoantibody negative at this longer duration of the disease (Tridgell et al., 2011). Tridgell et al. (2011) demonstrated that within 5 years from diagnosis, 19.4% of individuals had neither GADA nor IA-2A autoantibodies, rising to 31.7% who were antibody-negative by 6 to 13 years. Even in the high C-peptide group, the two autoantibody negative participants met our inclusion criteria of classical presentation of T1D at diagnosis. When these participants were excluded similar patterns were observed, with residual β-cells influencing post-exercise CGM outcomes. Moreover, the same positive relationship between C-peptide and the delta change in free-living to 24 hours post-exercise euglycaemia exists. While the central purpose of this project was to explore the relationship between C-peptide and euglycameia post exercise, the autoantibody testing demonstrates that residual β-cell function is associated with post-exercise glycaemic control in this T1D population. Other strengths of this study included the utilization of a highly sensitive C-peptide assay and extended MMTT to accurately identify and classify participants into groups. Where previous exercise studies in T1D have largely explored young, physically fit male populations potentially contributing to results reported, our studied cohort were diverse reflecting the grander T1D population. Our C-peptide groupings were well balanced in terms of the free-living glycaemic control, age, HbA1c, BMI and fitness, with only the expected differences of

age of diagnosis and duration of diabetes deferring between the groups (Shields et al., 2018).

Limitations of this study include participants being a single cohort from the same diabetes centre and predominantly being in moderate or good control. While the CGM capture was largely from free-living periods, the exercise bout was laboratory based with carefully managed blood glucose. Further limitations of this study include the length of time for some participants between the UCPCR and observational week, and completing the MMTT and exercise protocol. Changes in C-peptide status were possible, with 5 participants changing between $Cpep_{und}$ and $Cpep_{low}$ groups. While no participants were reclassified from the $Cpep_{high}$ group, changes were still possible with C-peptide declining for around 7 years post diagnosis (Shields et al., 2018). It thus remains unclear whether results can be generalized to the wider exercising T1D population.

Keeping in mind the potential for residual beta-cell function to help stabilize glycaemic control during and after exercise, future research should explore longer-term exercise and its associations with hypoglycaemia. Previous studies have demonstrated that exercise can blunt counter-regulatory responses to subsequent hypoglycaemia (Sandoval et al., 2006), and conversely, antecedent hypoglycaemia can blunt hormone responses to exercise (Galassetti et al., 2003). Potentially, residual β-cell function may limited the burden of hypoglycaemia by preserving some of these counter-regulatory responses to repeated bouts of physiological stress, helping facilitate effective and safe long-term exercise. Investigations into whether residual β-cell function influences the glycaemic responses to differing modalities of exercise (i.e. resistance, high intensity intermittent training), as well as under a range of different insulin and nutritional strategies around exercise (i.e. fasted morning exercise) are warranted. Finally, a large long term trial is needed to explore if C-peptide predicts HbA1c changes with exercise, as well as to explore further glycaemic and cardiovascular outcomes, teasing apart whether reported improvements in diabetes complications are due to glycaemic improvements and/or potentially a direct impact of C-peptide upon vasculature.

In conclusion, people with T1D who have higher residual β-cell function show improved glycaemic control following exercise. C-peptide may be useful in

identification of patients most at risk of exercise associated dysglycaemia. We show that future exercise research should consider level of C-peptide as a factor that may impact study outcomes.

Chapter 5. The mobilisation of endothelial and hematopoietic progenitor cells in response to an exercise bout in individuals with type 1 diabetes compared to non-diabetes controls and the influence of residual β-cell function

Research team members and responsibilities:

Guy Taylor – Study design, led participant recruitment, collected informed consent, data collection and processing (anthropometric measurements, medical history and notes, mixed meal tolerance test – including phlebotomy and sample processing, health screening, maximal exercise test – including resting and exercising electrocardiography, CPET and blood glucose management, main trial exercise bout – phlebotomy and sample processing, exercise testing, flow cytometry) and statistical analysis.

Dr Daniel West – Study design, obtained funding, resources and ethical approval, reviewed data and chapter.

Professor Emma Stevenson – Reviewed chapter.

Dr Mark Ross – Assisted with flow cytometry methodology, gating and analysis.

Professor Jim Shaw – Study design, participant recruitment and provided medical consultation on adverse findings.

Kieran Smith – Assisted with exercise tests

Dr Tess Capper – Assisted with exercise tests

Jadine Scragg - Assisted with exercise tests

Dr Ayat Bashir - Participant recruitment, helped on tricky phlebotomy and provided clinical cover during exercise testing.

Dr Anneliese Flatt - Participant recruitment, helped on tricky phlebotomy and provided clinical cover during exercise testing.

Dr Timothy McDonald - Analysed C-peptide.

Dr Richard Oram - Analysed C-peptide.

5. Chapter 5. The mobilisation of endothelial and hematopoietic progenitor cells in response to an exercise bout in individuals with type 1 diabetes compared to non-diabetes controls and the influence of residual β-cell function

5.1. Abstract

Objective

To examine the count of EPCs and HPCs at rest and after acute submaximal exercise in people with T1D compared to non-diabetes controls. Individuals with T1D were further grouped and compared by peak stimulated C-peptide.

Research Design and Methods

Thirty participants with T1D for ≥3 years, and 30 age, sex, BMI and fitness matched non-diabetes controls were recruited. All participants completed 45 minutes of incline treadmill walking at 60%VO_{2peak} with blood samples taken at rest, immediately and 1-hour post exercise. Fluorescent-labelled antibodies were added, and the HPCs and EPCs were quantified by flow cytometry. Additionally, the T1D participants completed a 3 hour mixed meal test to assess stimulated C-peptide. Peak C-peptide was used to allocate T1D participants into undetectable ($Cpep_{und}$ <3 pmol/L), low ($Cpep_{low}$ 3–200 pmol/L) or high C-peptide groups ($Cpep_{high}$ >200 pmol/L).

Results

All markers of HPCs and EPCs were lower at rest and all time points in the T1D group compared to the control ($p > 0.05$). While mobilisation from pre to post exercise was attenuated for $CD34^+CD31^+$ and $CD34^+VEGFR2^+$ EPCs in the T1D group ($p = 0.038$ and $p = 0.015$, respectively), all markers increased with exercise within both groups. Further analysis revealed that while there was no significant difference in count of HPCs or EPCs at rest ($p > 0.05$), only the individuals with a high amount of residual β-cell function (stimulated C-peptide > 200 pmol/L) had a post exercise mobilization of EPCs and HPCs.

Conclusions

People with T1D have significantly reduced counts of EPCs and HPCs, and reduced EPCs mobilisation pre to post exercise. People with T1D who have higher residual β-cell function show the capacity to mobilize these cells, a capacity which appears to be reduced in individuals with undetectable or low C-peptide.

5.2. Introduction

Individuals with T1D have a 2 - to 8-fold increase in mortality rates compared with the general population (Rawshani et al., 2017, Lind et al., 2014), with CVD the major cause of this increased morbidity and death (Secrest et al., 2010, De Ferranti et al., 2014b). Increased glucose concentration and fluctuations, characteristics of T1D, augment and combine with other traditional cardiovascular risk factors causing vascular damage (Maiorino et al., 2015). While improved glycaemic control is associated with reduced development of CVD (Gubitosi-Klug, 2016, Bebu et al., 2020), even in individuals who achieve desired risk factor thresholds, CVD is still elevated (Rawshani et al., 2017).

First described in 1997 by Asahara et al. (1997), endothelial progenitor cells (EPCs) are circulating mononuclear cells in human peripheral blood that, when isolated, can differentiate into endothelial cells in vitro, and able to incorporate into site of active angiogenesis in animal models. In the following two decades, studies have reported the vascular repair (Zampetaki et al., 2008), angiogenic (Sieveking et al., 2008) and de novo blood vessel formation ability of these cells (El Aziz et al., 2015). While there is a consensus within the literature that there are two distinct EPC populations in vitro culture; myeloid angiogenic cells (MACs) and endothelial colony-forming cells (ECFCs) (Hur et al., 2004), there is significant debate on which surface antigens represents these cells, where these cells are mobilised from and their exact role in vivo (Medina et al., 2017). MACs appear to promote angiogenesis through a paracrine mechanism but do not have the capacity to form endothelial cells (Hill et al., 2003), whereas ECFCs can form capillary tube-like structures and mature into endothelial cells (Ingram et al., 2004). These cells are mobilised from either the bone marrow (Asahara et al., 1999a) or endothelial lining niche (Lin et al., 2000, Fujisawa et al., 2019a) in response to vascular ischemia, homing to areas of injury or inflammatory (Aicher et al., 2005). Resting numbers are associated with endothelial function (Bruyndonckx et al., 2014), and may be a predictor of cardiovascular events and mortality (Werner et al., 2005).

Exercise is a potent mobiliser of EPCs into the circulation (Van Craenenbroeck et al., 2008, Ross et al., 2014), improving the function of these cells (Hoetzer et al., 2007, Laufs et al., 2004) and may be one of the beneficial pathways of physical exercise on endothelial function (De Biase et al., 2013). However, it appears that mobilisation is

attenuated in in chronic diseases such as type 2 diabetes (Lutz et al., 2016), heart failure (Van Craenenbroeck et al., 2011) and CVD (Adams et al., 2004, Sandri et al., 2005).

For individuals with T1D, the number and function of EPCs appear reduced at rest compared to healthy controls at all ages (Loomans et al., 2004, Sibal et al., 2009, DiMeglio et al., 2010, Palombo et al., 2011), with only Głowińska-Olszewska et al. (2013) reporting increased frequency of EPCs in children with T1D compared to non-diabetic controls. HbA1c, age and duration of the disease are all inversely related to count of EPCs (Arcangeli et al., 2017, Hörtenhuber et al., 2013). Limited research suggests that individuals with T1D have a blunted EPC response to acute exercise (West et al., 2015, Waclawovsky et al., 2015), although both studies failed to observe the expected post aerobic exercise mobilisation in non-diabetic controls, making it hard to draw conclusions.

β-cell function – as measured by C-peptide – can persist for many years after diagnosis, with it estimated that between 35 and 80% of individuals with T1D have detectable β-cell function at >5 years post-diagnosis (Williams et al., 2016, Oram et al., 2015). No previous research has explored if there is a relationship between residual β-cell function and count or function of EPCs. Hernandez et al. (2014) demonstrated that in a cohort of individuals who have had T1D for ≥50 years, who an increased proportion retained β-cell function compared to the wider T1D population (Keenan et al., 2010), count of circulating progenitor cells (CPCs) and EPCs were similar to age-matched controls without diabetes and higher than younger participants with T1D. Evidence from post-islet transplantation may also suggest a relationship between residual β-cell function and EPCs (Petrelli et al., 2010). Although count of circulating angiogenic cells (CAC) was similar between non-diabetic controls, T1D controls and insulin-independent islet-transplanted patients, restoration of beta cell function with islet transplantation had a positive impact on the circulating angiogenic cells function and vitality (Petrelli et al., 2010). Residual β-cell function, through improved glycaemic control, the anti-inflammatory properties of endogenous insulin secreted directly into circulation via the portal vein, or the direct impact of C-peptide on the vasculature, may play a role in protecting the loss of circulating EPCs, attenuating the development of cardiovascular complications and enhancing survival.

Therefore, we examined the count of EPCs and other circulating angiogenic cells (hematopoietic progenitor cells (HPCs)) between T1D participants and age, sex, BMI and fitness matched controls at rest, immediately and 1hr post exercise. The T1D group were further explored by examining EPCs mobilisation between residual β-cell groups. We hypothesized that the T1D group will have diminished counts of resting and exercise mobilised EPCs compared to the control, while the T1D with high residual β-cell function will have augmented numbers compared to the lower C-peptide groups.

5.3. Methods

The study was an acute observational trial, comparing EPCs and HPCs counts at rest and after acute submaximal exercise in clinically diagnosed people with T1D compared to non-diabetes controls. Individuals with T1D were further grouped and compared by undetectable, low and high C-peptide. The trial design is displayed in the below schematic (figure 5.1).

5.3.1. Recruitment of participants

Participants with T1D were recruited as described in chapter 4. Control participants were recruited by posters, email lists and word of mouth. Control participants were matched against T1D participants on a one to one basis by age, sex, BMI and fitness. All patients who participated provided written informed consent (appendix G and H) and this study was approved by the local National Health Service Research Ethics Committee (code: 16/NE/0192, ISRCTN registry: ISRCTN63739203).

Eligibility criteria for the T1D participants comprised of being clinically diagnosed with T1D, aged between 18 and 65 years with a duration of T1D >3 years upon enrolment, an HbA1c <86 mmol/mol / 10.0%, absence of diabetes-related complications, and stable insulin therapy without changes in the last 6 months. Eligibility criteria for the control participants comprised of being aged between 18 and 65 years, non-smoker, free from any muscular injuries and free from any history of chronic diseases (table 5.1 and 5.2).

Table 5.1 displaying inclusion/exclusion criteria T1D for chapter 5.

INCLUSION	EXCLUSION
Clinically diagnosed with T1D	T1D participants duration of disease less than 3 year
Aged 18-65 years	HbA1c >86 mmol/mol (10%)
Treated with exogenous insulin (MDI or CSII)	Unable to complete a maximal exercise test
Free from diabetes complications apart from retinopathy	

Table 5.2 displaying inclusion/exclusion criteria for controls for chapter 5

INCLUSION	EXCLUSION
Aged 18-65 years	Current or recent (< 6 months) smoker
Ability to be matched to a participant with T1D	Any previous chronic disease
	A current muscular injury
	Unable to complete a maximal exercise test

5.3.2. Protocol

T1D participants had previously completed a UCPCR test and free-living observational CGM week as described in chapter 3. This study was run in conjunction with study 2 with the T1D group undertaking the MMTT, maximal exercise test and submaximal exercise test visits, while the control group completed the maximal and submaximal exercise test visits, as described below.

5.3.2.1. Visit 1. Mixed Meal Tolerance Test

T1D participants who entered into study 2/3, completed an extended MMTT as described in chapter 4.2.3.2, with samples sent to Exeter Clinical Laboratory for analysis of serum C-peptide and autoantibodies (2.4). Individual serum peak C-peptide was used to confirm which C-peptide groups participants were sorted into; undetectable ($Cpep_{und}$) peak < 3 pmol/L, low ($Cpep_{low}$) 3 – 200 pmol/L and high ($Cpep_{high}$) > 200 pmol/L.

5.3.2.2. Visit 2. Health screening and Maximal Exercise Test

Both T1D and control participants attended the Newcastle clinical research facility for a pre-exercise health screening, including medical history and examination, as well as resting ECG (see 2.11), and to perform an incremental exercise test to determine peak oxygen uptake (VO2peak) (see 2.12). The T1D group's pre and post exercise glucose management was described in 2.17.1.

5.3.2.3. Visit 3. Sub-maximal exercise bout

The below schematic details the procedure for visit 3 (figure 5.1). Participants arrived at the exercise lab at ~8.30am after an overnight fast with transport provided. Glycaemic strategy for participants with T1D is described in 2.17.2.

Upon arrival at CRF all participants' blood glucose and NEWS were checked upon, and a 22-gauge cannula (Vasofix®, B.Braun Melsungen AG, Germany) was inserted into the antecubital vein (see 2.7). Two 4ml EDTA, 1 10ml EDTA, 2 6ml serum and 1 4ml Sodium Citrate vacutainers® (BD Biosciences, USA) were drawn from the cannula. To ensure that the blood was not contaminated with mature endothelial cells due to puncture of the vein, the first 4ml drawn was discarded. Capillary blood glucose was measured by a CONTOUR® blood glucose meter (Contour Next, Ascensia Diabetes Care, USA). Capillary blood was also collected in a microcuvette and analysed by a HemoControl to determine haematocrit and haemoglobin (EKF, Cardiff, UK).

A carbohydrate snack (Belvita Soft Bakes Chocolate Chip, Mondelēz International, USA), providing 204kcal of which 31g CHO, was then consumed and participants remained rested for 20 minutes. The exercise bout involved the participants walking at 60%VO_{2peak} for 45 minutes at a comfortable stride length as calculated by the ACSM walking metabolic equation (see 2.16). Heart rate and expired air was captured and analysed throughout by the Polar V800 and H7 strap and Meta control 3000 (see 2.13).

Upon completion of the exercise bout, a further two 4ml EDTA, 1 x 10ml EDTA, 2 x 6ml serum and 1 x 4ml Sodium Citrate vacutainers® (BD Biosciences, USA) were drawn immediately from the cannula. Participants remained rested a further 60 minutes, with further blood samples drawn at 1 hour post exercise. Capillary blood glucose, haematocrit and haemoglobin were also taken immediately and 1 hour post exercise as samples were drawn from the cannula.

Figure 5.1 Schematic of the sub-maximal testing visit

5.3.3. Flow Cytometry Enumeration of Hematopoietic and Endothelial Progenitor Cells

HPCs and EPCs were quantified on a flow cytometer (BD LSRFortessa™ X20; BD Biosciences, USA). Briefly, 200 µL of whole peripheral blood collected in EDTA vacutainer was incubated with 10 µL anti-CD34 FITC, 10 µL anti-VEGFR2 APC, 10 µL anti-CD45 BV421 (BioLegend, San Diego, CA, USA), 10 µL anti-CD31 BUV395 (BD Biosciences, USA), 10 µL anti-CXCR4 APC Cy7, and 10 µL anti-CXCR7 PE (BioLegend, San Diego, CA, USA) in BD Trucount™ tube with a known number of fluorescent beads, for 30 minutes in the dark at 4°C. A further 2mL of red blood cell lysis buffer (BD Pharm Lyse^{TM}, BD Biosciences, United Kingdom) was added and left to incubate for a further 30 minutes at 4°C in the dark before enumeration by flow cytometry. Reverse pipetting was used throughout. The samples were vortexed at low speed to resuspend beads and reduce cell aggregation before each sample was run for 45 minutes or until 500,000 $CD45^{+}$ events had been collected and analysed, whichever occurred first. Samples were analysed on an LSRFortessa equipped with a blue, yellow/green, red, violet and ultra violet lasers (488nm, 561nm, 635nm, 405nm and 355nm wavelengths, respectively).

Compensation, using BD™ CompBead (BD Biosciences, USA), was performed prior to collecting each participants data to correct for any spectral overlap. Samples containing no antibodies of CD31–BUV395, VEGFR2–APC, CXCR4–APC Cy7, and 10 µL CXCR7–PE were used as negative controls to help determine the gating of positive events in the positive samples for each participant. Between sample, FACS clean (BD Biosciences, USA) and deionized water were run for 5 minute each to decontaminate the flow cytometer.

Following data acquisition, flow cytometry files were analysed using FCS express 7 research edition (De Novo, California, USA). HPCs and EPCs are expressed as cells/mL (table 5.3), with the absolute count of the cell population (A) calculated by dividing the number of positive cell events (X) by the number of trucount bead events (Y), and then multiplying by the BD Trucount bead concentration (N/V, where N = number of beads per test and V = test volume (2260 µL)).

Table 5.3 Calculating absolute cell count by flow cytometry when using BD trucount beads

Table X. Calculating Absolute Cell Count (cells/mL)

$A = X/Y \times N/V$

Haematocrit and haemoglobin concentrations measures at pre, post and 1hr post exercise were used to adjust absolute cell counts changes in blood volume using the Dill and Costill (1974) method.

The density and colour-dot plots for enumeration of the HPCs ($CD34^+$, $CD34^+CD45^{dim}$) and EPCs ($CD34^+CD31^+$, $CD34^+VEGFR2^+$, $CD34^+CD45^{dim}CD31^+$, $CD34^+CD45^{dim}VEGFR2^+$, $CD34^+CD45^{dim}CD31^+VEGFR2^+$) are displayed in figure 5.2. We used the phenotypes $CD34^+CD45^{dim}VEGFR2^+$ and $CD34^+CD45^{dim}CD31^+$ as measure of “true” EPCs that can differentiate into endothelial cells (Van Craenenbroeck et al., 2013, Bieback et al., 2013), see 1.4.2.

Firstly, trucount beads were gated to determine absolute cell count (Figure 5.2A), before gating for doublet exclusion (Figure 5.2B). CD45+ PBMCs were gated (Figure 5.2C), followed by identification of SSC-low and CD34+ events (Figure 5.2D), subsequent low expression of CD45 ($CD45^{dim}$; Figure 5.2E), $CD31^+$ (Figure 5.2F) and VEGFR2+ events (Figure 5.2G) were identified.

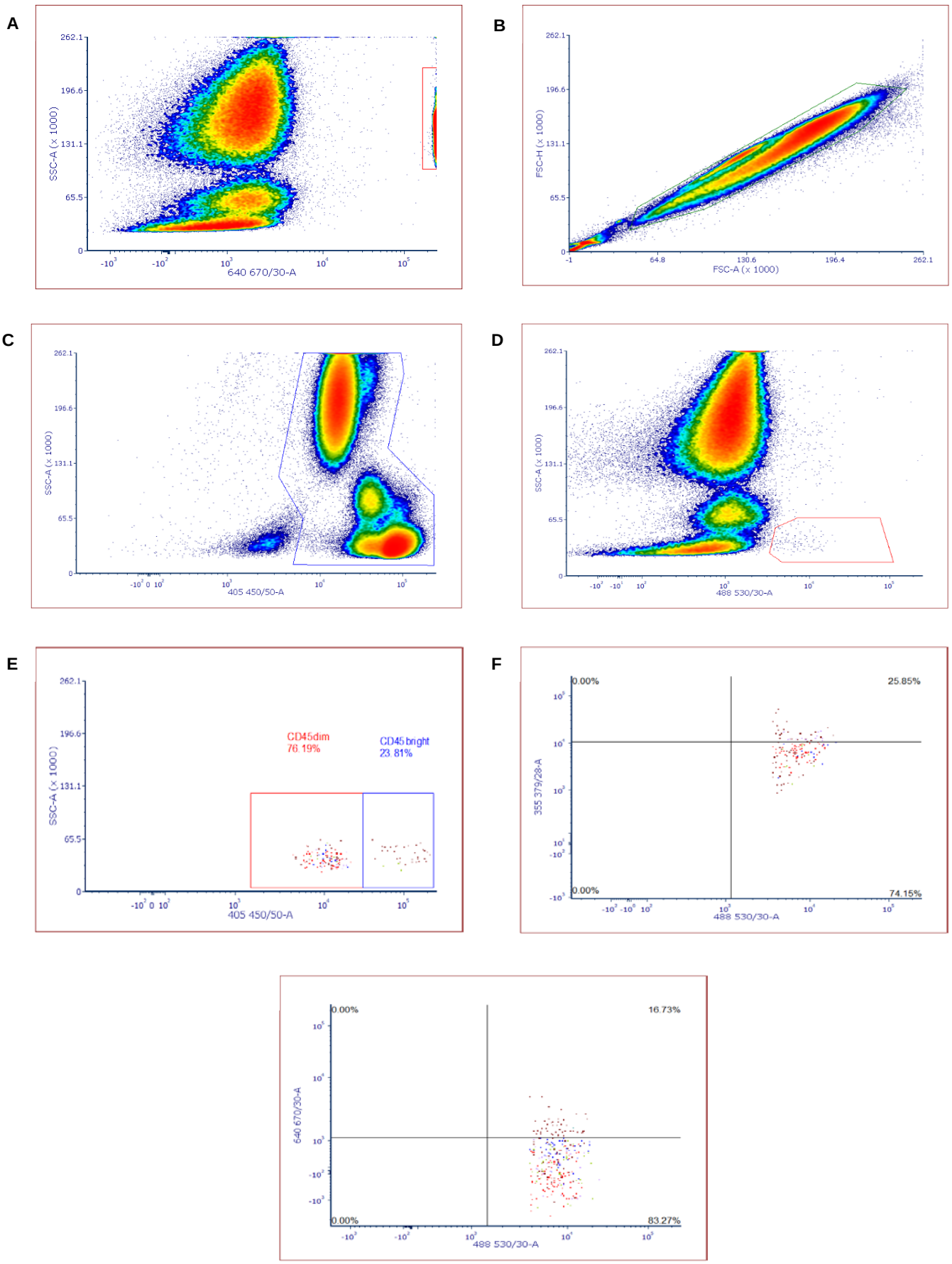

Figure 5.2 Enumeration of HCPs and EPCs by flow cytometry. A- Gating of the fluorescent beads from the Trucount™ Tubes. B- Forward scatter height versus forward scatter area density plot for doublet exclusion. C- Gating of $CD45^+$ mononuclear cells. D- Identification of $CD45^+CD34^+$ cells. E- Gating of $CD34^+CD45^{dim}$ cells. F- Identifying of $CD31^+$ subset of $CD34^+$ or $CD34^+CD45^{dim}$. G- Identifying of $VEGFR2^+$ subset of $CD34^+$ or $CD34^+CD45^{dim}$.

5.3.4. Statistical analysis

Data is presented as mean ± standard deviation throughout unless otherwise stated.

Statistically significant differences for group demographic, baseline count and delta change from pre to post exercise count between the T1D and control group were determined by independent sample T-test. Data were assessed for normality and outliers by Shapiro-Wilk test and boxplots, with excessively skewed data assessed by Mann-Whitney U Test. When the assumption of homogeneity of variances was violated a Welch T-test was used. The distributional assumption was used to determine if the Mann-Whitney U Test was used to compare medians or distribution.

Statistically significant differences for group demographic, baseline count and delta change from pre to post exercise count of $Cpep_{und}$, $Cpep_{low}$ and $Cpep_{high}$ group were determined by the use of a one-way ANOVA, with excessively skewed data assessed by Kruskal-Wallis H test.

For both the comparison between the T1D and control groups and between the C-peptide groups a two-way repeated measures mixed ANOVA was used to examine the interactions on cell count between sample time and group. Significant time and group main effects were analysed using post-hoc Bonferroni adjusted pairwise comparisons. When values where missing, a fitted mixed effects model with Geisser-Greenhouse as implemented in GraphPad Prism 8.0 was used. Excessively skewed data were transformed using square root and logarithmic transformation.

GraphPad Prism 8.0.1 (San Diego, USA) and IBM SPSS Statistics (version 24, IBM, Armonk NY) software package was used to analyse the data. Statistical significance set at $p \leq 0.05$.

5.4. Results

5.4.1. Type 1 Diabetes vs Control Group Demographic

Group demographic information is presented in table 5.4. As expected by the study design with matched controls; age, HbA1c, BMI and VO_{2peak} were comparable between groups.

Table 5.4 Displaying mean ± SD group demographic information

GROUPING	T1D	Control
N	30	30
Male/Female	16/14	16/14
Age (Years) $_{MWU}$	38.2 ± 12.0	37.6 ± 12.1
HbA1c (mmol/mol)	58.5 ± 9.1	33.5 ± 2.3 *
(%)	7.5 ± 3.0	5.2 ± 2.4
BMI (kg/m^2) $_{MWU}$	25.2 ± 3.7	24.7 ± 4.6
VO_{2peak} (ml/kg/min)	38.8 ± 9.5	42.4 ± 12.4
Age At Diagnosis	18.2 ± 8.6	-
Range (Years)	8 to 35	
Duration Of Diabetes	20.0 ± 13.0	-
Range (Years)	3 to 47	
Method Of Control (MDI/CSII)	15/15	-

* - Statistical significance between the groups, Mann-Whitney U Test – $_{MWU}$

5.4.2. Laboratory Phase

There was no differences in intensity of the exercise between groups, with the T1D group exercising at 58.7 ± 3.8% compared to 58.9 ± 5.1 % for the non-diabetic control (p= 0.907). There were no incidents of hypoglycaemia within the T1D group during the laboratory phase of the study, either during the exercise bout or throughout the 60 minute post-exercise recovery period.

5.4.3. Endothelial and Hematopoietic Progenitor Cells

5.4.3.1. Basal resting value

Basal mean ± SD resting counts of HPCs and EPCs are displayed in table 5.5. The T1D group had significantly lower median rest count of HPCs as assed by Mann-Whitney U test, with $CD34^+$ 27.2% and $CD34^+CD45^{dim}$ 24.3% lower than the control group.

All EPCs were also significantly lower for the T1D group in comparison to the controls ($p<0.05$), with median basal counts ranging from 15.9 to 42.3% lower across all samples.

Table 5.5 Displaying mean ± SD basal rest numbers of HPCs and EPCs (cells/ml) and unpaired t-test/ Mann-Whitney U test results between the T1D and control groups

	T1D	Control	p
$CD34^+$ $_{MWU}$	129.9 ± 54.0	181.3 ± 68.0	**0.001**
$CD34^+CD45^{dim}$ $_{MWU}$	105.2 ± 47.4	149.0 ± 67.7	**0.009**
$CD34^+CD31^+$	31.4 ± 9.1	52.5 ± 14.7	**<0.001**
$CD34^+VEGFR2^+$ $_{MWU}$	36.3 ± 14.1	58.7 ± 19.2	**<0.001**
*** $CD34^+CD45^{dim}CD31^+$ $_{MWU}$**	26.8 ± 8.9	45.5 ± 14.2	**<0.001**
*** $CD34^+CD45^{dim}VEGFR2^+$ $_{MWU}$**	25.8 ± 10.7	40.9 ± 15.7	**<0.001**
$CD34^+CD45^{dim}CD31^+VEGFR2^+$	13.9 ± 7.0	17.6 ± 7.2	**0.047**

Mann-Whitney U Test – $_{MWU}$, * - True EPCs

5.4.3.2. Time Course Change with Exercise

Figure 5.3. displays the Mixed Model ANOVA results of HPCs and EPCs at rest, post and 1hour post the sub-maximal exercise.

For $CD34^+$ HPCs (Figure 5.3.A) the main effects demonstrated the count was statistically significantly higher in the control than the T1D control ($p= 0.004$) and exercised mobilised the cells pre to post in both groups ($p< 0.001$). There was a statistically significant interaction between the groups and time ($p= 0.037$), with the control group having a higher count at rest and post exercise (240.0±126.1 vs 158.4±73.5, $p< 0.001$), but not 1hr post (185.4±72.4 vs 149.3±61.6, $p= 0.076$). The other marker of HPCs - $CD34^+CD45^{dim}$ (Figure 5.3.B), did not display a group*time interaction ($p= 0.176$), with the control group having a higher count (mean difference ± SE - 49.46±17.31, 95%CI 14.8 to 84.1, $p= 0.003$), and mobilising from pre to post exercise ($p< 0.001$) and remaining elevated at 1hr post ($p= 0.002$).

All EPC phenotypes were significantly higher in the control group compared to the T1D group ($p< 0.001$). The main effects of time demonstrated that exercise mobilized all EPC phenotypes, with significant increases from rest to post exercise, while $CD34^+CD31^+$ EPCs remained elevated from pre at 1hr post exercise ($p= 0.032$). Only the $CD34^+VEGFR2^+$ phenotypes has a group*time interaction ($p= 0.037$), with the 1hr post sample being significantly lower than the post exercise sample in the control ($p< 0.001$) but not the T1D group ($p= 0.121$).

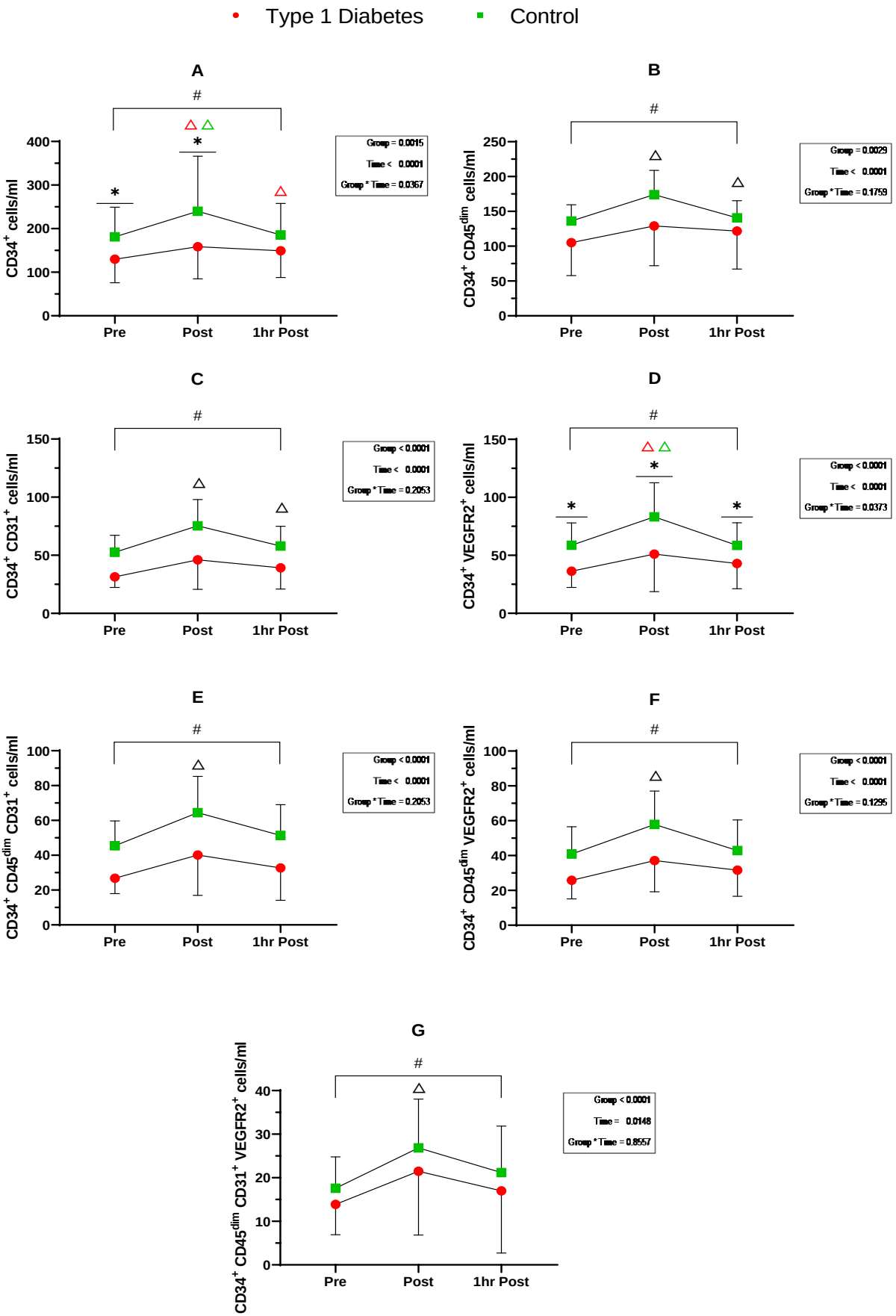

*Figure 5.3 Absolute mean ± SD mixed model ANOVA results for the T1D group (red circle) and the control group (green square) for A - CD34⁺, B - CD34⁺CD45^{dim}, C - CD34⁺CD31⁺, D - CD34⁺VEGFR2⁺, E - CD34⁺CD45^{dim}CD31⁺, F - CD34⁺CD45^{dim}VEGFR2⁺, G - CD34⁺CD45^{dim}CD31⁺VEGFR2⁺. #[- Significant main effect of group differences, △ – Significant main effect of time difference from baseline, *| - Significant group differences at timepoint, ⊿ - Significant time difference from baseline in T1D group, △ - Significant time difference from baseline in T1D group*

5.4.3.3. Delta Change Pre to Post exercise

To further explore the mobilisation of HPCs and EPCs with exercise the mean delta change (Δ) in rest to post exercise between groups is displayed in Table 5.6. Δ was substantially lower in all phenotypes in the T1D group compared to the control ranging from 18.3 to 51.4%, with $CD34^+CD31^+$ and $CD34^+$ $VEGFR2^+$ EPCs significantly so when assessed by Mann-Whitney U Test.

Table 5.6 Displaying mean ± SD delta change count of HPCs and EPCs (cells/ml) from pre to post exercise, with Mann-Whitney U test results between the T1D and control groups.

	T1D	Control	p
$CD34^+$ $_{MWU}$	28.5 ± 38.9	58.6 ± 79.6	0.209
$CD34^+CD45^{dim}$ $_{MWU}$	23.9 ± 33.0	47.7 ± 65.2	0.139
$CD34^+CD31^+$ $_{MWU}$	14.6 ± 25.0	22.8 ± 18.6	**0.038**
$CD34^+VEGFR2^+$ $_{MWU}$	14.7 ± 30.8	24.4 ± 24.1	**0.015**
*** $CD34^+CD45^{dim}CD31^+$ $_{MWU}$**	13.3 ± 22.5	19.0 ± 15.1	0.069
*** $CD34^+CD45^{dim}VEGFR2^+$ $_{MWU}$**	11.3 ± 18.6	17.1 ± 15.2	0.143
$CD34^+CD45^{dim}CD31^+VEGFR2^+$ $_{MWU}$	7.6 ± 15.8	9.3 ± 10.9	0.204

$_{MWU}$ = Mann-Whitney U Test, * - True EPCs

5.4.4. C-peptide Groups

5.4.4.1. Group demographic

Participants within the T1D group were separated into C-peptide grouping, undetectable ($Cpep_{und}$) peak < 3 pmol/L, low ($Cpep_{low}$) 3 – 200 pmol/L and high ($Cpep_{high}$) > 200 pmol/L, with demographic presented in Table 5.7. Age, HbA1c, BMI and VO_{2peak} were comparable between groups. However, the $Cpep_{high}$ group had significantly higher age of diagnosis and shorter duration of diabetes than the $Cpep_{und}$.

Table 5.7 Displaying mean ± SD group demographic information for serum C-peptide groups

C-Peptide Grouping	$Cpep_{und}$	$Cpep_{low}$	$Cpep_{high}$
N	11	9	10
Male/Female	5/6	6/3	5/5
Age (Years) $_{K\text{-}W\ D}$	40.09 ± 11.18	38.67 ± 14.73	35.80 ± 10.98
Age At Diagnosis $_{K\text{-}W\ D}$	13.27 ± 4.50	16.56 ± 8.57	25.10 ± 8.20 *
Range (Years)	8.00 to 24.00	8.00 to 32.00	13.00 to 35.00
Duration Of Diabetes $_{K\text{-}W\ M}$	26.82 ± 13.24	21.89 ± 13.34	10.70 ± 6.15 *
Range (Years)	13.00 to 47.00	9.00 to 44.00	3.00 to 20.00
HbA1c (mmol/mol)	61.64 ± 10.64	58.11 ± 7.11	55.40 ± 8.47
(%)	7.8 ± 3.1	7.5 ± 2.8	7.2 ± 2.9
BMI (kg/m^2) $_{K\text{-}W\ M}$	25.65 ± 3.27	24.20 ± 4.13	25.67 ± 4.04
Method Of Control (MDI/CSII)	5/6	4/5	6/4
VO_{2peak} (ml/kg/min) $_{K\text{-}W\ D}$	35.61 ± 7.69	43.93 ± 9.03	35.67 ± 10.77

$_{K\text{-}W\ M}$ – Kruskal - Wallis Comparison of Medians, $_{K\text{-}W\ D}$ – Kruskal - Wallis Comparison of Distribution

* Significantly different to $Cpep_{und}$, # Significantly different to $Cpep_{low}$

5.4.4.2. Basal Resting value

Mean ± SD resting counts of HPCs and EPCs between the C-peptide groups are displayed in table 5.8. No significant differences between groups were present when assessed by a 1-way ANOVA.

Table 5.8 Displaying mean ± SD basal rest numbers of HPCs and EPCs (cells/ml), analysed by a 1-way ANOVA between the C-peptide groups.

	$Cpep_{und}$	$Cpep_{low}$	$Cpep_{high}$	p
$CD34^+$	105.8 ± 25.3	132.3 ± 56.5	154.1 ± 67.0	0.120
$CD34^+CD45^{dim}$	88.7 ± 26.7	108.8 ± 50.1	120.0 ± 60.4	0.320
$CD34^+CD31^+$	28.6 ± 6.7	31.9 ± 8.0	34.1 ± 12.0	0.391
$CD34^+VEGFR2^+$	30.8 ± 6.2	35.7 ± 16.7	43.0 ± 16.1	0.136
*** $CD34^+CD45^{dim}CD31^+$**	25.0 ± 6.0	25.3 ± 6.9	30.1 ± 12.4	0.368
*** $CD34^+CD45^{dim}VEGFR2^+$**	22.2 ± 7.2	27.9 ± 11.3	28.0 ± 13.1	0.375
$CD34^+CD45^{dim}CD31^+VEGFR2^+$	11.8 ± 6.0	14.7 ± 5.9	15.5 ± 8.7	0.457

* - True EPCs

5.4.4.3. Time Course Change with Exercise

Figure 5.4 displays the 2-way mixed model ANOVA results of HPCs and EPCs at rest, post and 1hour post the sub-maximal exercise between the C-peptide groups.

For the HPCs phenotypes (Figure 5.4.A and B) there was no significant time*group interaction ($CD34^+$; p= 0.129, $CD34^+CD45^{dim}$; p= 0.099). $Cpep_{high}$ had significantly higher $CD34^+$ cell count than the $Cpep_{und}$ (mean difference ± SE; 66.4±24.2, 95%CI; 126.4 to 6.5 cells/ml, p= 0.026) but not $Cpep_{low}$ (mean difference ± SE; 34.40±25.43, 95%CI; -28.7 to 97.4 cells/ml, p= 0.367). The main effect of time demonstrated exercise mobilised the $CD34^+$ phenotype from pre to post exercise (p< 0.001), and remained elevated from baseline at 1hr post exercise (p= 0.005). No significant group differences exist for the $CD34^+CD45^{dim}$ cells ($Cpep_{und}$; 94.4±5.1, $Cpep_{low}$; 119.9±10.2, $Cpep_{high}$; 144.3±22.0 cells/ml, p= 0.073), with exercise again mobilising from pre to post (p< 0.001) and remaining elevated at 1hr post (p= 0.004).

$CD34^+VEGFR2^+$ and $CD34^+CD45^{dim}VEGFR2^+$ EPCs had a significant group*time interaction (p= 0.033 and p= 0.29, respectively). In these phenotypes there was no significant differences between groups at pre exercise. Only $Cpep_{high}$ significantly increased from pre to post exercise, with both the post and 1hr post samples significantly higher than the $Cpep_{und}$ and $Cpep_{low}$ groups. $CD34^+VEGFR2^+$ cells in the $Cpep_{high}$ group increasing from 43.0±16.1 cells/ml at rest to 78.2±40.1 cells/ml post exercise (p< 0.001), while no significant changes happened in the $Cpep_{und}$ (30.8±6.2 to 36.1±16.6 cells/ml, p= 0.676) or $Cpep_{low}$ (35.8±16.7 to 39.2±16.2 cells/ml, p= 0.810).

Whilst the other EPCs markers did not have statistically significant group*time interaction ($CD34^+CD31^+$; p= 0.077, $CD34^+CD45^{dim}CD31^+$; p= 0.075, $CD34^+CD45^{dim}CD31^+VEGFR2^+$; p= 0.090), the main effect of group showed $Cpep_{high}$ had significantly higher counts than the $Cpep_{und}$ ($CD34^+CD31^+$; p= 0.013, $CD34^+CD45^{dim}CD31^+$; p= 0.008) or both groups ($CD34^+CD45^{dim}CD31^+VEGFR2^+$ - $Cpep_{high}$ vs $Cpep_{und}$ mean difference ± SE; 12.4±3.4, 95%CI 4.1 to 20.7 cells/ml, p= 0.003, $Cpep_{high}$ vs $Cpep_{low}$ mean difference ± SE; 10.9±3.5, 95%CI 2.2 to 19.7 cells/ml, p= 0.035).

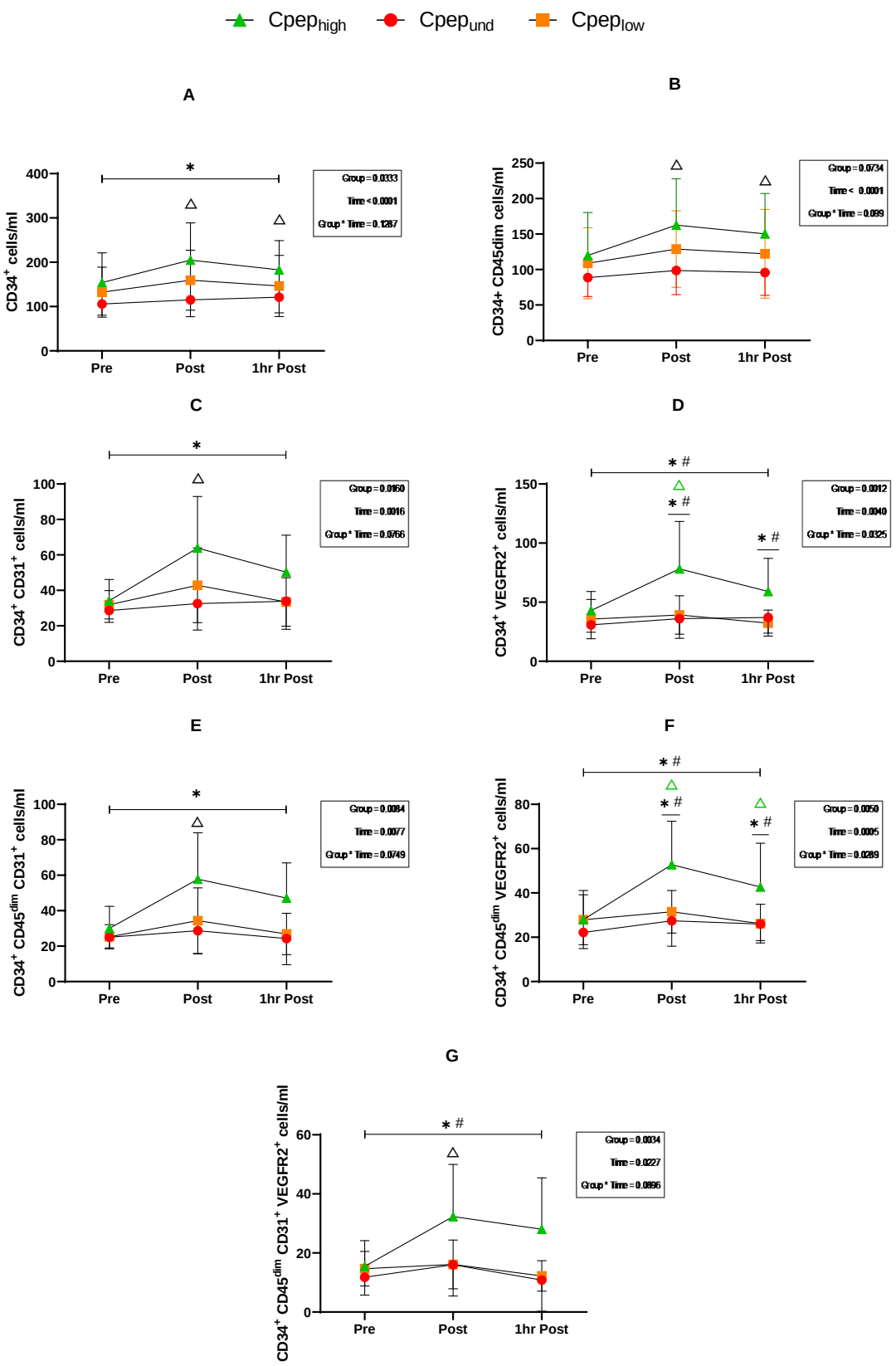

*Figure 5.4 Absolute mean ± SD mixed model ANOVA results for the Cpep$_{high}$ (green triangle), Cpep$_{low}$ (orange square) and the Cpep$_{und}$ (red circle) group (green square) for A - CD34$^+$, B - CD34$^+$CD45^{dim}, C - CD34$^+$CD31$^+$, D - CD34$^+$VEGFR2$^+$, E - CD34$^+$CD45^{dim}CD31$^+$, F - CD34$^+$CD45^{dim}VEGFR2$^+$, G - CD34$^+$CD45^{dim}CD31$^+$VEGFR2$^+$. *[- Significant main effect of group differences between Cpep$_{und}$ and Cpep$_{high}$, #[- Significant main effect of group differences between Cpep$_{low}$ and Cpep$_{high}$, △ – Significant main effect of time differences from baseline, *| - Significant group differences at timepoint between Cpep$_{und}$ and Cpep$_{high}$, #| - Significant group differences at timepoint between Cpep$_{low}$ and Cpep$_{high}$, △ - Significant time difference from baseline in Cpep$_{high}$ group*

5.4.4.4. Delta Change

To further explore the mobilisation of HPCs and EPCs with exercise, the mean delta change (Δ) in rest to post exercise between C-peptide groups is displayed in Table 5.9, and a 2-way mixed model ANOVA exploring absolute changes from baseline is displayed in Figure 5.5.

$Cpep_{high}$ had significantly higher Δ from rest to post exercise count for $CD34^+$ HPC and $CD34^+CD31^+$ EPCs than the $Cpep_{und}$, and $CD34^+CD45^{dim}VEGFR2^+$ EPCs than both the $Cpep_{und}$ and $Cpep_{low}$ (Table 5.9). While Kruskal–Wallis comparison determined that there was significant differences between groups for $CD34^+VEGFR2^+$ and $CD34^+CD45^{dim}VEGFR2^+$, and $Cpep_{high}$ had substantially higher count, bonferroni post-hoc tests were not significant (p> 0.05).

Table 5.9 Displaying mean ± SD delta change count of HPCs and EPCs (cells/ml) from pre to post exercise, with test results displaying 1-way ANOVA and Kruskal-Wallis comparison between the C-peptide groups.

	$Cpep_{und}$	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**
$CD34^+$	9.4 ± 27.1	27.3 ± 33.0	50.6 ± 45.0 *	**0.047**
$CD34^+CD45^{dim}$	10.0 ± 25.3	20.0 ± 27.1	42.7 ± 38.7	0.065
$CD34^+CD31^+$ $_{K\text{-}W\ D}$	3.9 ± 15.2	10.9 ± 23.0	29.8 ± 29.6 *	**0.045**
$CD34^+VEGFR2^+$ $_{K\text{-}W\ M}$	5.3 ± 15.6	3.4 ± 14.2	35.2 ± 43.5	**0.027**
*** $CD34^+CD45^{dim}CD31^+$ $_{K\text{-}W\ D}$**	3.7 ± 13.9	9.1 ± 18.9	27.7 ± 27.2	**0.050**
*** $CD34^+CD45^{dim}VEGFR2^+$**	5.3 ± 14.5	3.6 ± 11.0	24.8 ± 21.5 *#	**0.013**
$CD34^+CD45^{dim}CD31^+VEGFR2^+$	4.3 ± 11.7	1.4 ± 10.5	16.9 ± 20.2	0.142

$_{K\text{-}W\ M}$ – Kruskal - Wallis Comparison of Medians, $_{K\text{-}W\ D}$ – Kruskal - Wallis Comparison of Distribution

* Significantly different to $Cpep_{und}$, # Significantly different to $Cpep_{low}$ * - True EPCs

When equalised for baseline, the delta change ANOVA (Figure 5.5.) replicated the absolute ANOVA (Figure 5.4.). There was no interaction between group and time for the HPCs, while $CD34^+CD31^+$, $CD34^+VEGFR2^+$, $CD34^+CD45^{dim}CD31^+$ and $CD34^+CD45^{dim}VEGFR2^+$ EPCs had significant group*time interaction. Within the EPCs, $Cpep_{high}$ was the only group that was mobilised by exercise with $Cpep_{low}$ and $Cpep_{und}$ not increasing from rest at either post or 1-hour post exercise.

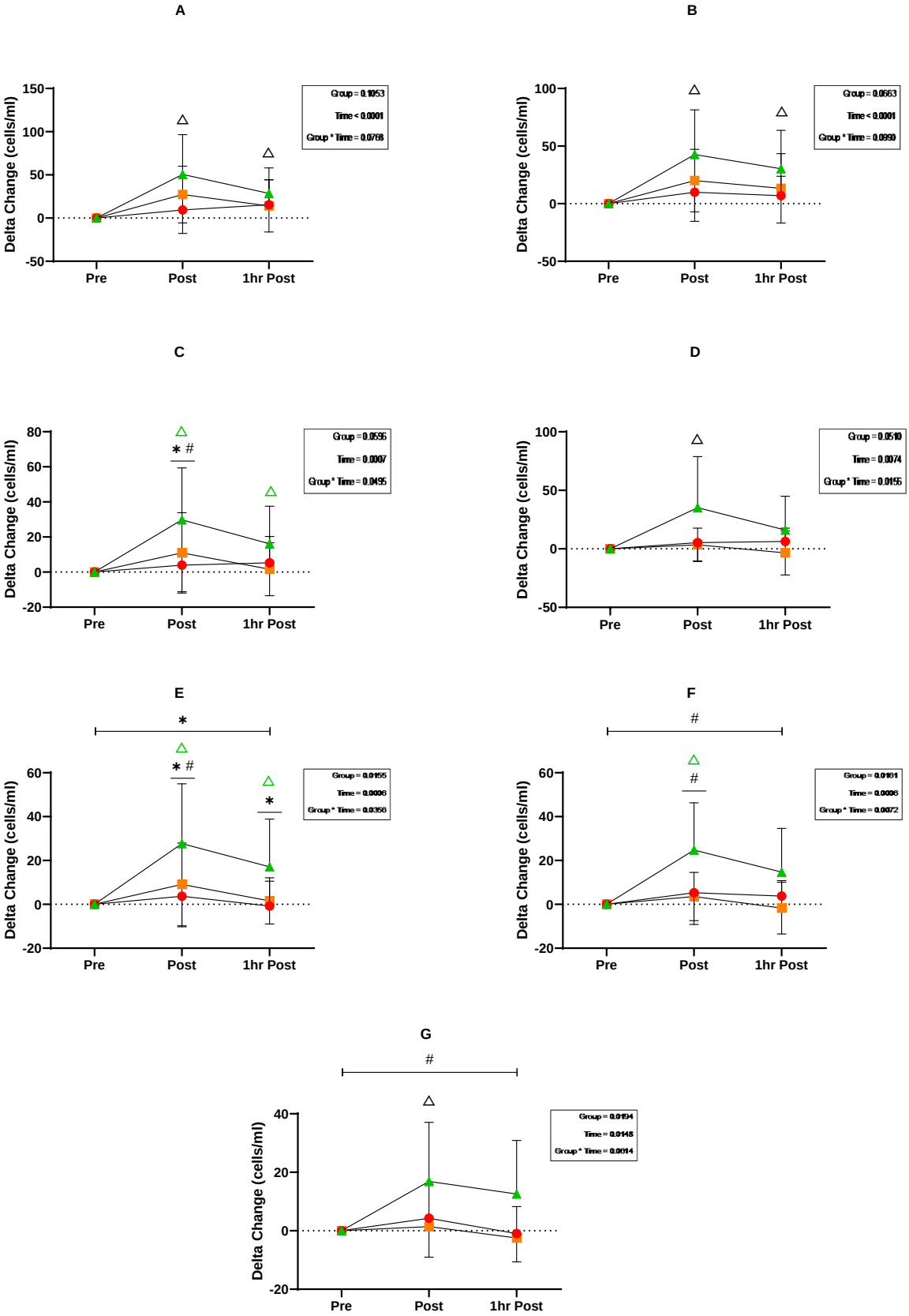

Figure 5.5 Delta change from baseline mean ± SD mixed model ANOVA results for the $Cpep_{high}$ (green triangle), $Cpep_{low}$ (orange square) and the $Cpep_{und}$ (red circle) group (green square) for A - $CD34^+$, B - $CD34^+CD45^{dim}$, C - $CD34^+CD31^+$, D - $CD34^+VEGFR2^+$, E - $CD34^+CD45^{dim}CD31^+$, F - $CD34^+CD45^{dim}VEGFR2^+$, G - $CD34^+CD45^{dim}CD31^+VEGFR2^+$. *[- Significant main effect of group differences between $Cpep_{und}$ and $Cpep_{high}$, #[- Significant main effect of group differences between $Cpep_{low}$ and $Cpep_{high}$, △ – Significant main effect of time differences from baseline, *| - Significant group differences at timepoint between $Cpep_{und}$ and $Cpep_{high}$, #| - Significant group differences at timepoint between $Cpep_{low}$ and $Cpep_{high}$, △ - Significant time difference from baseline in $Cpep_{high}$ group

5.5. Discussion

We investigated how the count of EPCs and HPCs changed at rest and after exercise in individuals with T1D compared to non-diabetic controls, and explored whether residual β-cell function in people with T1D influenced this count. We demonstrated that all markers of HPCs and EPCs were lower at rest and all time points in the diabetes group compared to the control. While mobilisation from pre to post exercise was attenuated for $CD34^+CD31^+$ and $CD34^+VEGFR2^+$ EPCs in the diabetes group, all markers increased with exercise within both groups. Further analysis revealed that while there was no significant difference in count of HPCs or EPCs at rest, only the individuals with a high amount of residual β-cell function (stimulated C-peptide > 200 pmol/L) had a post exercise mobilization of EPCs and HPCs.

Lower resting count of EPCs and HPCs compared to non-diabetic matched control is similar to the previous research in adolescents and adults with T1D (Loomans et al., 2004, Sibal et al., 2009, DiMeglio et al., 2010, Palombo et al., 2011). Reduced EPC count in diabetes is potentially due largely to hyperglycaemia causing dysfunction. EPCs isolated from healthy subjects and exposed to high glucose conditions or EPCs isolated from patients with diabetes have been shown to exhibit; reduced number of colony forming units and impaired abilities of differentiation into endothelial cells, reduced proliferation, adhesion and migration, impaired paracrine secretion, mobilization and homing, but increased senescence (Kang et al., 2017). When circulating progenitor cells (CPCs) from non-diabetic individuals were cultivated under hyperglycaemic conditions, there was both an increased rate of apoptosis and a decrease in proliferation (Kränkel et al., 2005), as well as a decreased ability to target damaged tissue and incorporate into tubular structures (Marchetti et al., 2006). VEGFR induced migration and mobilisation from the bone marrow to areas of ischaemia is also reduced in hyperglycaemic conditions (Fadini et al., 2006) and impairs paracrine secretion of nitric oxide (NO) and other angiogenic cytokines (Zhang et al., 2013a). As Werner et al. (2005) demonstrated in their pivotal study showing that lower count of EPCs lead to increased CVD events in the future, this reduced count of EPCs seen in individuals with T1D may contribute to the increased risk of CVD events seen.

For the first time we demonstrate that exercise can mobilise HPCs and EPCs in individuals with T1D, in contrast to previous research (West et al., 2015, Waclawovsky

et al., 2015). While neither of these studies observed the expected post aerobic exercise mobilisation in non-diabetic controls, West et al. (2015) demonstrated an increase count the following morning in the controls but not T1D participants. As the testing commenced at 5 pm and there was no morning rested sample on the day of the testing, and the blood samples were taken 60 minutes pre and post the exercise, as well as the following morning, circadian rhythm may have also influenced the count. While Waclawovsky et al. (2015) documented a mobilisation post resistance exercise in the controls, post aerobic exercise count of EPCs was decreased for the non-diabetic group with no change in the T1D group. Because of this unusual non-mobilisation with exercise, it is difficult to state that EPCs response to acute exercise appears blunted in T1D. As these studies measured circulating EPCs as a percentage of leucocytes and did not correct for changes in blood volume around exercise, it is possible that any mobilisation was masked within changes in leucocytes around exercise (Saito et al., 2003). This study used trucount tubes to calculate absolute cell count of the EPCs and adjusted these results for changes in the volume of (Dill and Costill, 1974). In this study, although all markers of HPCs and EPCs increased from pre to post exercise for the T1D group, delta change in $CD34^+CD31^+$ and $CD34^+VEGFR2^+$ EPCs was attenuated compared to the controls. A previous study by Fadini et al. (2013) revealed impaired hematopoietic ($CD34^+$, $CD133^+$ and $CD34^+CD133^+$) and endothelial progenitor cell ($CD34^+VEGFR2^+$, $CD133^+VEGFR2^+$ and $CD34^+$ $CD133^+VEGFR2^+$) mobilization 24 hours after direct bone marrow stimulation from human recombinant granulocyte colony–stimulating factor in both type 1 and 2 diabetes participants. It is unclear why we only saw significant impaired mobilisation in EPCs and not HPCs, however Fadini et al. (2005) found increased depletion of EPCs than HPCs in individuals with type 2 diabetes, suggesting that EPCs may be more susceptible to diabetic conditions. It is worth noting that baseline counts were comparable between the T1D and control groups in all the mobilisation studies, potentially influenced by having relatively young, well controlled and physically fit T1D participants. Indeed, future research is needed to explore if regular exercise in combination with improving fitness influences markers of vascular repair at rest and in response to exercise in this population.

Our primary findings that only individuals with higher C-peptide had significant mobilization of these cells after exercise, have not been reported before. While resting

count were not significantly different between residual β-cell groups, counts of all markers increased sequentially from the C-peptide negative to the C-peptide high group. It is likely that the sample size, 9 to 11 per group, lacked statistical power and if powered correctly the mean differences of the HPCs and $CD34^{+}VEGFR2^{+}$ EPCs between the groups would have been significant. This is despite comparable HbA1c and free-living CGM outcomes between the groups. While not displayed in this study, when compared by unpaired T-tests, the $Cpep_{high}$ group had significantly higher resting counts of HPCs and delta change in both HPCs and EPCs compared to $Cpep_{und.}$ Previously, it has been shown that in a cohort of individuals who have had T1D for ≥50 years (Joslin Medalist Cohort), counts of CPCs and EPCs were similar to age-matched controls without diabetes and higher than younger participants with T1D (Hernandez et al., 2014). It has previously been reported that this cohort have an increased proportion of whom retain β-cell function compared to the wider T1D population (Keenan et al., 2010), and that a high proportion of Medalists remain free from proliferative diabetic retinopathy, nephropathy, neuropathy, or cardiovascular disease (Sun et al., 2011). It has been hypothesised that within this population endogenous factors exist to attenuate the adverse effects of metabolic abnormalities associated with diabetes on vascular tissues, resulting in the unusual chronic survival of these patients. While it has not been explored directly, residual β-cell function may play a role in protecting from the decline in EPCs count within this population. Petrelli et al. (2010) report that while count was not significantly different, T1D participants who had islet transplantation and were insulin independent had improved survival and function of circulating angiogenic cells compared to T1D controls who were on the waiting list for an islet transplant. While individuals were not tracked pre to post transplant, the return of C-peptide and HbA1c to non-diabetic levels suggests that the normalization of glycaemic control through islet transplantation improves angiogenic ability of CACs. This may contribute to the improved cardiovascular outcome observed after islet transplantation (Fiorina et al., 2005).

We hypothesize that within individuals with high residual β-cell function, improved glycaemic control around exercise (as shown in chapter 4.), and the direct impact of endogenous insulin and C-peptide may play a role in protecting the production, survival and utilisation of HPCs and EPCs..

Increased residual β-cell function in individuals with T1D is associated with improved glycaemic control (Rickels et al., 2020, Lachin et al., 2014, Buckingham et al., 2015, Brooks et al., 2015), while improved glycaemic control in individuals with T1D is associated with increased count and function of EPCs (Loomans et al., 2004, Fadini et al., 2018, Hörtenhuber et al., 2013, Maiorino et al., 2015, Inaba et al., 2018). In Chapter 4, we demonstrated that the $Cpep_{high}$ had improved time in euglycaemia and reduced glycaemic variability around exercise. Could this blood glucose concentration that is closer to a normal physiological range allow for the enhanced survival and migration of EPCs in the circulation? Higher glucose is associated with increased apoptosis and a decrease in proliferation of EPCs, as well as a decreased ability to target damaged tissue and incorporate into tubular structures (Kränkel et al., 2005, Marchetti et al., 2006). Fluctuations in glucose levels may also have deleterious effect on EPCs, with chapter 4 demonstrating reduced glycaemic variability (SD and CV) after exercise for higher C-peptide individuals. Although different markers of GV, Maiorino et al. (2015) and Inaba et al. (2018) demonstrating that mean amplitude of glycaemic excursion (MAGE) or J index glucose were associated with reduced circulating EPCs or $CD34^+$ cells respectively. Finally, people with T1D who switched to CSII from MDI for 24 weeks had an increase in circulating levels of EPCs ($CD34^+KDR^+$ and $CD34^+KDR^+CD133^+$) associated with the reduction of glucose variability (Maiorino et al., 2016).

HbA1c is inversely correlated with EPCs count (Loomans et al., 2004) and improving HbA1c increases the count in children with T1D with the inverse also true (Hörtenhuber et al., 2013). While HbA1c was matched between groups in this study, chapter 3 demonstrates that individuals with higher C-peptide are likely to have improved time in euglycemic range and glycemic variation. As the present study is observational in nature, we cannot determine if an individual's long-term glycaemic control, such as years of increased hyperglycaemia exposure for lower C-peptide individuals, may have influenced the results. A major theory as to why counts of EPCs are decreased in chronic vascular diseases is the increased requirement for vascular repair may result in depleted EPCs reserves within the bone marrow (Tashiro et al., 2014, Teraa et al., 2013). Additionally, glycaemic control has been found to act as a predictor of skeletal deficits in several, but not all studies of bone marrow within individuals with T1D (Weber and Schwartz, 2016). Mouse models of T1D and bone

marrow from individuals with T2D have found to have been affected by diabetic microangiopath, with microvascular rarefaction and depletion of stem cells with the bone mass (Spinetti et al., 2013, Oikawa et al., 2010, Mangialardi et al., 2013). This bone marrow dysfunction may also be a pathway for explaining how long term glycaemic control can impact upon mobilisation into the peripheral blood in response to ischemia (Fadini et al., 2006).

The reported improvements in glycaemic control associated with C-peptide reported in this thesis and elsewhere (Riddell et al., 2017, Gibb et al., 2020, Buckingham et al., 2015, Brooks et al., 2015), may also result in improved markers of inflammation (Hoffman et al., 2016). Studies suggest that inflammation and oxidative stress modulate EPC bioactivity (Lin et al., 2013), with several clinical conditions, characterised by both increased inflammation and oxidative stress, associated with reduced numbers and impaired function of EPCs (Tousoulis et al., 2008). Persistent excessive inflammatory stimuli results in decreased numbers of EPCs in the circulation (Andreou et al., 2006), with increased levels of TNF-α, interleukin-1β, granulocyte macrophage-colony stimulating factor and stromal-derived factor-1 all shown modulate EPC mobilisation, recruitment and homing (Zhang et al., 2009).

As well its role in the formation of insulin, and its measurement as a marker of endogenous insulin secretion, C-peptide appears to be a biologically active peptide (Wahren et al., 2007). It is unknown if endogenous C-peptide directly influences the count or function of EPCs, however C-peptide has been shown to stimulate NO production in endothelial cells (Giebink et al., 2013), a key pathway for the mobilisation, survival, migration and angiogenesis of EPCs (Dimmeler et al., 2000, Aicher et al., 2003, Kränkel et al., 2005, Chen et al., 2007). With the reported improvements in microvascular complications seen in individuals with T1D who secrete endogenous insulin (Lachin et al., 2014, Steffes et al., 2003a), even at very low C-peptide levels (Kuhtreiber et al., 2015), EPCs may play a role in protecting the vasculature in these T1D populations.

Limitations of this study include not exploring whether clinical characteristics such as age, duration of diabetes and HbA1c predicted resting and mobilisation of HPCs and EPCs. While recommended as the best phenotype for sensitivity, specificity and reliability for quantifying EPCs (Van Craenenbroeck et al., 2013), it is unclear it these

cells are true EPCs that can differentiate into the endothelium or only have angiogenic paracrine function. In addition, the bout of exercise (60% VO_{2peak} for 45 minutes) may not have been at a high enough intensity or duration to mobilise EPCs for all participants (Silva et al., 2012).

Future research needs to understand if these differences are due to clinical characteristics, glycaemic control or the potential direct impact of C-peptide upon vasculature. Keeping in mind the action of C-peptide on the vasculature (Yaribeygi et al., 2019, Souto et al., 2020, Alves et al., 2019), could C-peptide treatment reduce the EPCs dysfunction in individuals with T1D? Finally, a large long term trial is needed to explore if increasing physical activity and fitness in T1D populations improves the count and function of EPCs.

In conclusion, people with T1D have significantly reduced count of EPCs and HPCs, and reduced EPCs mobilisation pre to post exercise. People with T1D who have higher residual β-cell function show the capacity to mobilize these cells, a capacity which appears to be reduced in individuals with undetectable or low C-peptide.

Chapter 6. General Discussion

6 Chapter 6. General Discussion

The research presented in this thesis examined the impact of residual β-cell function in individuals with T1D. Specifically, it explored its influence on free-living and post exercise glycaemic control, and levels of circulating cells shown to be involved with vascular growth and repair. This chapter will consider and collate the findings of chapters 3 to 5, how they relate to the wider T1D field and future research directions that this thesis highlights.

6.1 Residual β-cell function and glycaemic control in individuals with T1D

Before 2020 limited data existed on residual β-cells influence upon glycaemic control in individuals with long duration T1D, with only HbA1c and self-reported hypoglycaemia examined (Steffes et al., 2003, Lachin et al., 2014, Kuhtreiber et al., 2015, Marren et al., 2019, Brooks et al., 2015, Buckingham et al., 2015, Vantyghem et al., 2012). In combination with recent studies by Rickels et al. (2020) and Gibb et al. (2020), data from chapter 3 and 4 highlight that in individuals with longer duration T1D, residual β-cell function does influence CGM parameters.

The data presented in chapter 3 and 4 communicate similar findings to those presented by Rickels et al. (2020), showing high amounts of residual β-cells function increased euglycaemia by attenuating hyperglycaemia. At lower C-peptide concentrations, Gibb et al. (2020) found no difference in euglycaemia, but reduction in hypoglycaemia in individuals with greater residual β-cell function. These results highlight that a large study is needed to explore if all the differing beneficial outcomes of residual β-cell function, including glycaemic variability and time spent in hypoglycaemia and hyperglycaemia, is a continuum or if certain thresholds of C-peptide are needed. Modelled research by Brooks et al. (2015), who explored CGM parameters in post islet transplant patients, suggested that the improvements are a continuum down to negative C-peptide. However, the mechanistic research carried out by Rickels et al. (2020) found the low C-peptide group (17-200) did not behave any differently from the negative C-peptide group, in both response to hypoglycaemia or free-living CGM parameters. Knowing whether a certain threshold of C-peptide is needed for beneficial outcomes, and the exact impact increasing C-peptide has on reducing hyperglycaemia and hypoglycaemia, is important for a variety of reasons.

These include as a treatment targets for immunotherapy trials that try to preserve β-cell function in the recently diagnosed, as well as in studies trying to restore β-cell function through transplant or stem cells in individuals with longer duration T1D. It is to be determined if studies that are trying to preserve or restore β-cell function need to achieve a high amount of C-peptide, as demonstrated in this thesis and Rickels et al. (2020), or if low amounts of C-peptide would be beneficial for reducing time spent in hypoglycaemia for individuals who previously had undetectable β-cell function, as suggested by Gibb et al. (2020). Additionally, with the development of artificial pancreas and dual hormone insulin and glucagon pump systems, understanding how an individual's current C-peptide status influences the variation in glycaemic responses to everyday living will allow for improved systems (Nijhoff and de Koning, 2018). Indeed, while results from the MMTT and CGM outcomes from both chapter 3 and 4 found no significant differences between the undetectable and low C-peptide groups, the $Cpep_{low}$ had a lower mean peak MMTT glucose of 1.87 mmol/L than the $Cpep_{und}$ group, which may be clinically meaningful.

Sample size, inclusion criteria and study collection methods likely play a role in this thesis and Rickels et al. (2020) not finding residual β-cell function having any influence on time spent in hypoglycaemia as measured by CGM. This is in comparison to Gibb et al. (2020), which found differences as measured by FGM, and other studies that used self-reported hypoglycaemia measures (Marren et al., 2019, Kuhtreiber et al., 2015, Hope et al., 2018). These findings were despite hypoglycaemia (both below 3.9 and 3 mmol/L) in the post exercise period being ≥2-fold in the $Cpep_{high}$ group compared to the lower C-peptide groups in chapter 4. These results highlight the difficulties of measuring hypoglycaemia in research studies, with one study finding that self-reported symptomatic hypoglycaemia comprises only a minor fraction of the total burden of hypoglycaemia, with 77% of episodes <3.9 mmol/L and 52% of episodes <3.0 mmol/L asymptomatic in a cohort of largely hypoglycaemic aware T1D participants (Henriksen et al., 2019). Future research exploring hypoglycaemia should carefully design the studies, with a combination of CGM measuring for the recommended 2 weeks (Danne et al., 2017, Battelino et al., 2019), hypoglycaemia diaries and recall, as well as a large sample size likely to be needed.

6.2 Residual β-cell function and inter-individual glycaemic response to exercise in individuals with T1D

Chapter 4 demonstrates that there is a large variation in acute glycaemic control after a bout of exercise in individuals with T1D, with percentage time spent in euglycaemia in the 12 hours following the submaximal exercise bout ranging from 3.25 to 100%. While individuals' preferences and experience in managing post exercise glycaemia undoubtedly play a role in this large variation in free-living control, laboratory studies have also found large unexplained inter-individual variability in the glycaemic responses after exercise (Kapitza et al., 2010). Maintaining euglycaemia around exercise is a significant challenge for individuals with T1D with current recommended strategies include reducing basal and bolus insulin (Kourtoglou, 2011), nutritional modifications (Campbell et al., 2014, Zaharieva and Riddell, 2015) and modifying exercise modality and order (Yardley et al., 2012, Yardley and Sigal, 2015). However, chapter 4 clearly demonstrates that individuals who retain a large residual β-cell function have an easier time after exercise, with increased time in euglycaemia and reduced fluctuations in glucose.

Chapter 3 demonstrated that amount of time spent completing moderate to vigorous activity did not predict any marker of glycaemic control during the free-living week. Previous studies using accelerometers have found afternoon MVPA increases the likelihood of nocturnal and next day hypoglycaemia (Jaggers et al., 2019, Metcalf et al., 2014) and increased MVPA is associated with reduced HbA1c (Cuenca-García et al., 2012). In combination with other accelerometer studies, this data suggests that there is no clear relationship between total exercise and glycaemic control, with some participants able to achieve a large amount of MVPA with good glycaemic control, while the opposite is true for others. The data presented is not dissimilar to that found in meta-analyses that have explored physical activity on long term glycaemic control in T1D, where exercise training has no impact (Kennedy et al., 2013, Ostman et al., 2018) or limited improvements upon HbA1c (Yardley et al., 2014a, Tonoli et al., 2012). Most studies have pointed towards increased energy intake or variation in exercise intervention to explain a lack of improvement in HbA1c. However, the large variation in post exercise control demonstrated in chapter 4 and the fact most active individuals use a trial and error approach to managing blood glucose around exercise (Dizon et al., 2019) likely play a role to the lack of clear benefit in HbA1c seen with increased

exercise. While chapter 4 highlights the role residual β-cell function plays in this inter-individual variation in glycaemic responses to exercise, further research is needed to directly explore other factors that previous secondary analysis of data suggests may play a role. These include starting blood glucose concentration, sex, fitness and body composition (Riddell et al., 2019b, Al Khalifah et al., 2016, Brockman et al., 2020, Tagougui et al., 2019). Additionally, it is to be seen if C-peptide secretion can predict changes in HbA1c with exercise training.

Initial evidence from chapter 4 suggests that individuals with high C-peptide may have more stable glucose during exercise compared to individuals with undetectable C-peptide. However, specific research studies exploring C-peptide influences on glucose and substrate oxidation during exercise is needed. In chapter 4, it is speculated that the increased insulin sensitivity after exercise to replenish glycogen stores at the liver and skeletal muscle, combined with the relatively high endogenous insulin secretion may be the mechanism behind the improved time in euglycemic range after exercise in the high C-peptide group. Residual β-cell function may also influence glycaemic control during exercise through a range of other mechanism. These include endogenous insulin secreted from the pancreas into the portal vein directly inhibiting hepatic glucose output during intense exercise or exercise under high glycaemic conditions (Trefts et al., 2015, Gonzalez et al., 2016). Individuals with higher C-peptide may also have some preserved counter-regulatory responses to prolonged aerobic exercise. Studies have demonstrated that a previous hypoglycaemic event reduces the adrenaline, noradrenaline, cortisol, endogenous glucose production, and lipolytic responses by 40–80%, and abolishes the glucagon response all together, resulting in a threefold increase in exogenous glucose to maintain glycaemia in both individuals with and without T1D (Galassetti et al., 2003, Davis et al., 2000). As an improved counter-regulatory glucagon and endogenous glucose production to hypoglycaemia has been demonstrated in higher C-peptide individuals (Zenz et al., 2018, Rickels et al., 2020), and individuals with T1D have an impaired counter-regulatory response to exercise (Schneider et al., 1991, Lee et al., 2020), residual β-cell function may preserve some of these counter-regulatory responses to exercise. While a differing population, initial evidence suggests that post islet-transplant patients who no longer needed exogenous insulin may have at least partially restored counter-regularity function in response to exercise (Yardley et al., 2019).

Chapter 4 demonstrates that future exercise research in individuals with T1D should consider level of C-peptide as a factor that may impact upon study outcomes. Since the beginning of this PhD in 2016, many studies investigating glycaemic control around exercise in individuals with T1D have failed to account for C-peptide. This has included studies investigating combining exercise modalities (Farinha et al., 2018), reducing basal insulin dosage (Zaharieva et al., 2019a), and testing closed loop insulin pumps for improved glycaemic control (Hanaire et al., 2020). While other studies have used a C-peptide level as an inclusion/exclusion criteria, this threshold is not uniform. Indeed, some studies have used a thresholds as high as 300 pmol/L (Moser et al., 2017), 250 pmol/L (Moser et al., 2019a) and 230 pmol/L (Aronson et al., 2019). Chapter 4 demonstrated that individuals with a C-peptide >200 pmol/L had a substantially greater amount of time spent in euglycaemia in the hours following a bout of moderate intensity exercise. While the largely crossover designs of exercise research in individuals with T1D may offer some protection from this individual variation, the clear divergence in whether there is a positive or negative impact of exercise on parameters of glycaemic control depending on C-peptide status could highly influence these studies.

The findings presented in this thesis suggest that C-peptide should be screened and accounted for, either in the inclusion/exclusion criteria or in further analysis. The UCPCR has been shown to be a convenient, non-invasive test that has a high sensitivity and specificity for detecting endogenous insulin secretion (94% sensitivity/100% specificity for a C-peptide >200 pmol/L)(Besser et al., 2011a). While tempting to exclude individuals with higher C-peptide, 49.3% of participants who volunteered for chapter 3 had detectable C-peptide levels, while 29% had a C-peptide level above 200 pmol/L, which this thesis demonstrated to be clinically relevant. While chapter 3 was not designed to assess the C-peptide levels within the T1D population, previous studies have demonstrated up to 16% of individuals have a C-peptide level >200 pmol/L (Davis et al., 2015). Thus, only including individuals with a C-peptide level <200 pmol/L may result in a large proportion of the T1D population being excluded from research.

Thought should also be given to inclusion of individuals who are within a few years of diagnosis. C-peptide levels are likely to be higher, with individuals commonly still being

in the honeymoon period a year from diagnosis (Schölin et al., 1999). Furthermore, the ability of the α-cell's to secrete glucagon in response to hypoglycaemia are diminished as the duration of diabetes increases (Siafarikas et al., 2012), with individuals a year from diagnosis having impaired but still functioning counter-regularity glucagon responses (Arbelaez et al., 2014). While this research explored hypoglycaemia, it could also potentially be true for the counter-regulatory responses during exercise. The very high C-peptide seen around diagnosis limits the applicability of research in the newly diagnosed to the wider T1D population.

6.3 Residual β-cell function and EPCs to exercise in individuals with T1D

Evidence clearly demonstrates the beneficial impact of residual β-cell function on diabetes complications (Steffes et al., 2003a, Lachin et al., 2014, Sjöberg et al., 1991, Panero et al., 2009, Kuhtreiber et al., 2015, Almeida et al., 2013) in a range of differencing T1D populations. However, the exact pathways by which increased residual β-cell function results in reduced complications is unknown.

Circulating EPCs may play a role in protecting individuals with T1D from microvascular and macrovascular complications (Hernandez et al., 2014, Dessapt et al., 2010, Palombo et al., 2011, Sibal et al., 2009, Brunner et al., 2009, Loomans et al., 2004, Ingram et al., 2008). Glycaemic control in individuals with T1D appears to influence EPCs count, with higher HbA1c associated with lower count in vivo (Loomans et al., 2004, Hörtenhuber et al., 2013). Hyperglycaemia and glycaemic variation are also associated with reduced count, reduced function and increased apoptosis in vitro (Kränkel et al., 2005, Marchetti et al., 2006, Fadini et al., 2018, Maiorino et al., 2015). The data in chapter 5 demonstrated that only individuals with higher C-peptide had significant mobilization of these cells after exercise. However, post-exercise glucose was only significantly different between the high and undetectable C-peptide groups, with similar glucose levels between the low and high groups. Further research needs to explore if acute glucose level influences the resting count and the mobilisation of EPCs with exercise, as this may place an even greater importance on maintaining euglycaemia during exercise. Additionally, it would be advantages to understand how clinical and lifestyle factors, such as increased physical activity, influences EPCs in a T1D population. Restoration of β-cell function with islet transplantation has been shown to have a positive impact on the circulating angiogenic cells function and vitality although it did not increase the count in a study by Petrelli et al. (2010). There is a

need to disentangle any improvements in glycaemia resulting from endogenous insulin secretion from any potential effect of C-peptide and insulin directly impacting upon or mediating the effect of dysglycaemia upon EPCs. Figure 6.1 details some of the potential pathways that residual β-cell function could influences count and function of EPCs, as well as diabetic complications.

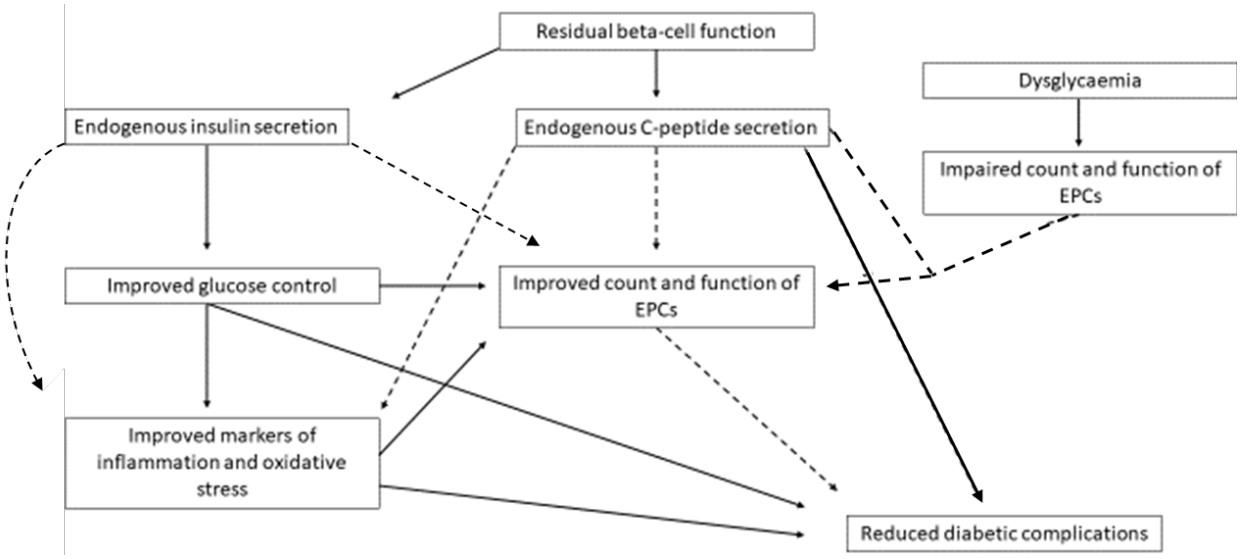

Figure 6.1 Schematic of potential pathways that residual β-cell function may influence the count and function of EPCs and diabetic complications. Solid lines indicate pathways that research has evidenced; hashed lines indicate hypothetical pathways which have yet to be researched.

Understanding C-peptides direct influence on the vasculature in individuals with residual β-cell function would also be beneficial. Currently limited data exists upon C-peptides influence on micro and macrovascular damage, with promising signs from animal models (Souto et al., 2020). However, C-peptide infusion as a strategy to treat neuropathy in individuals with T1D has had mixed results (Wahren et al., 2016, Ekberg et al., 2003, Wahren et al., 2007). There is a need to explore the impact C–peptide has as part of a dual hormone treatment in individuals with T1D on markers of vascular damage and diabetes complications.

6.4 Limitations of the studies presented in this thesis

Specific limitation for each study are addressed within the respective chapters. The findings in this thesis are based upon observational acute exercise studies, which requires more thorough evidence before it can be considered as part of the evidence base. Research in this area would benefit from long term observational studies, such

as exploring how residual β-cell function influences the long-term glycaemic response to exercise training.

Other limitations of the present studies include the length of time of data collection. Specifically, the gap between participants completing the free-living week and entering into the second study (chapter 4) could have influenced the data showing a clear divergence in glycaemia from the free-living period to post exercise control. Additionally, the research only studies one exercise model, with glycaemic, metabolic and vascular differences varying greatly across different types, durations and intensity of exercises in individuals with T1D. It remains to be seen if residual β-cell function has an influence during exercise and under different modalities. Research for this thesis was carried out and analysed by a single researcher. Especially for flow cytometry, which relies on a subjective gating analysis, these results could have been influenced by bias. Flow cytometry analysis of EPCs can be problematic, with the rare nature of the cells making detection difficult and results variable. A major issue with chapter 5 is the lack of an apoptosis marker, therefore it is likely that the resulting EPCs quantified may contain non-viable cells. Finally, while autoantibody data were collected in chapter 4 and used to screen for participants who may have had another type of diabetes, the main findings presented in all the chapters included all participants. While secondary analysis was included to make sure these participants did not influence the results, it was concluded that the focus of this thesis was upon residual β-cell function influence upon glycaemic and vascular outcomes and not T1D specifically.

6.5 Conclusions

The findings of this thesis demonstrate that residual β-cell function influences acute glycaemic and vascular outcomes in individuals with longer duration T1D. Increased C-peptide secretion is associated with improved glycaemic control under free-living condition and after an acute bout of exercise, as well as increasing the count of circulating EPCs that are mobilised with exercise.

Chapter 7. References

7. Chapter 7. References

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Chapter 8. Appendices

Participant Information Sheet

Part ONE:

1. Study title
   The role of residual beta-cell function on glycaemic variability before and after exercise in Type 1 diabetes

2. Invitation paragraph
   My name is Dr. Daniel West and I am a Senior Research Fellow in Clinical Exercise Physiology at Newcastle University. I invite you to take part in a study which will aim to assess the role that low levels of natural insulin production have on glucose control before and after exercise in people with Type 1 diabetes.

3. Why are we doing this research?
   Recent research has showed that people with long standing Type 1 diabetes can still produce small amounts of natural insulin. This small amount of insulin is suggested to influence people's diabetes control, and may play a role in preventing dangerously low blood glucose levels (hypoglycaemia). With this in mind, we wish to find out how important having some remaining pancreas beta-cell function (i.e. producing some natural insulin) is for glucose control when exercising in people with Type 1 diabetes. The findings from this study will potentially provide a useful tool to help refine strategies for more tightly controlled blood glucose concentrations for people with Type 1 diabetes who wish to regularly exercise, and contribute to improving overall health and well-being of people.

4. Why have I been asked to take part?

You have been identified as a person with type I diabetes who meets our inclusion criteria; a) 18 - 60 years old; b) treated with modern insulin, c) diagnosed over 2 years ago, d) HbA1c <10%. You may have struggled to manage your blood glucose levels when exercising in the past. By taking part in this study you can contribute to research attempting to improve blood glucose control for exercising people with Type 1 diabetes.

5. Do I have to take part?
   Taking part in the research is completely voluntary so you do not have to take part in this study. Also, you are free to withdraw from this study at any time during the course of the testing without giving reason. This will in no way affect the care that you will receive.

6. What will I have to do if I take part?
   Firstly you will be provided with a pack for the collection of a home urine sample, which you can put in the post and then we will use this to check your eligibility for the trial. If you are eligible to take part, you will attend the Clinical Research Facility (CRF) at the Royal Victoria Infirmary in Newcastle upon Tyne on 4 occasions.

   Visit 1: The first occasion will last ~15 minutes, and you will be fitted with a continuous glucose monitor which you will then wear for 7 days. This device is a very small unit which is inserted just beneath the skin and can continuously record glucose profiles. The device is small, discrete and waterproof and requires no input from you after it has been fitted. There may be some light skin discomfort where the device has been inserted.

   Visit 2: For the next visit you will be required to present to the CRF on a morning (~08:00 – 09:30), following an overnight fast, via a prearranged taxi, where you will be greeted by a researcher. During this visit blood samples (24 ml in total) will be collected from you and then you will undergo a mixed meal tolerance test. This test is used to assess how much insulin your body can still produce naturally. The test involves the consumption of a standard carbohydrate drink and then will collect blood samples frequently over a 2 hour period. During this time you will also complete questionnaires relating to your own experiences with hypoglycaemia and exercise. Once the questionnaires are complete you will have access to the facilities of the Clinical Research Facility which include WIFI and a TV. After the mixed meal test is complete you will be free to go and a taxi will be provided.

Visit 3: At least 48 hours after visit 2 you will be required to attend the CRF for a resting and exercising stress test. This is such that we can confirm that your heart responds normally to exercise and that it is safe for you to complete the exercise part of the study.

We will measure heart rate and oxygen consumption (the amount of air you breathe) using a chest strap and mouth-nose piece, respectively. You will be required to complete a walking test. This test starts off at a light intensity and gradually increases every 3 minutes until you feel you can no longer continue. This allows us to capture your heart rates responses gradually with changes in exercise intensity. We will also use this test to estimate how hard you will work for the main exercise test in the study.

Visit 4: At least 7 days after your last visit, you will attend the CRF for the main exercise trial. Upon arrival you will be fitted with a continuous glucose monitor. Prior to the test starting, we will provide you with a small carbohydrate snack and check your blood glucose concentrations are between 8 – 12 mmol/L, and it is ok for exercise to begin. You will then complete 45 minutes of treadmill walking. During the test we will capture your heart rate and oxygen consumption (the amount of air you breathe) using a chest strap and mouth-nose piece, respectively. During the test we will take capillary glucose from you to check your blood glucose concentrations are stable. After the test is complete, you will be free to go, and you will wear the CGM for another 3 days. During this time you will be required to record your food intake and insulin doses.

7. What are the possible disadvantages of taking part in this study?
   As with any research project there are always some potential risks you should be aware of. We will be taking blood from a vein in your arm by indwelling a cannula and it is possible you might experience some discomfort when it is being placed in the arm and/or bruising when it is taken out. The exercise intensity implemented in trials will be moderate but far from maximal and you can choose to terminate the test at any time. There will be medical cover present during your time at the laboratories. Moreover, you will be provided with contact information for you to call a member of the research team if you experience any difficulties before or after your laboratory visits. A member of the research team will be available for 24/7. As such, you will be provided with clinical cover during time it takes you to complete the study.

Although we will do our best to ensure the visits take place at a time that suits you, it may be that you incur some financial shortfall through taking part in this study (loss of earnings etc). Newcastle University will cover any travel expenses incurred from taking part in the trials, and will offer a one-off payment of £100 on completion of the study as compensation for time.

8. What are the possible benefits of taking part in this study?
   We cannot promise the study will definitely help you, but your results, and the results from other participants, may help provide a stronger understanding of how important natural insulin production is for glucose control when exercising and this may help develop more advanced ways of managing blood glucose for other people with Type 1 diabetes.

9. Will my taking part in the study be kept confidential?
   Yes, we value your input to this project and all data gathered from you will be treated in the strictest of confidence. Only members of the research team will have access to the data, which will be stored for 3 years, and the results will be anonymous so you cannot be directly identified. Also, although the results may be published, your data will be compiled with others and available as an average, so again you cannot be identified.

10. What are the possible side effects of the procedure?
    Although you have been chosen because you have Type 1 diabetes, there is a possibility that you might experience some dizziness or other discomfort to the exercise tests, as such there is a possibility of collapsing during the test. However, you will be constantly supervised and monitored in the Exercise Physiology Laboratory. If any problems develop when you leave the laboratory please contact me using the details below and I, or another member of the research team, will provide assistance straightaway.

Contact Details:

Dr. Daniel J West

Institute of Cellular Medicine

Newcastle University

NE2 4HH

Email address: Daniel.west@newcastle.ac.uk

Office (tel): (0191) 208 7076 Mobile (tel): 07557340319

Participant Information Sheet

Part TWO: Additional information you need to know if you still want to take part

1. What happens if new information about the experimental testing comes along?
   Sometimes during research, new things are found out which may result in unforeseen physiological changes. If we notice you developing any unwanted responses to the interventions that we have not controlled for we may have to stop you performing the trial and either reschedule the test or withdraw you from the study.

2. What happens if I don't want to carry on with the study?
   If you wish to withdraw from the study you can do so at any time by informing a member of the research team. All identifiable data and/or blood samples will be destroyed, but we may use the data collected up to your withdrawal. If you choose to withdraw, you will no longer be able to take part in the remaining trials. However, this does not mean that you cannot volunteer for future studies.

3. What happens when the research project stops?
   If requested, you will be given a small report which will give you a summary of your results from your visits. Later, you will be invited to attend an informal presentation that explains the results of all the research participants and reports the study's conclusions.

4. What if there is a problem or something goes wrong?
   There will be a research supervisor available at all times to oversee your health and safety.

5. What happens if I have an accident?
   In the unlikely event of an accident, you will be immediately attended to by the research supervisor, and medical staff that are present. If you are unhappy with any of the treatment provided during the study please

contact the Diabetes Research Group (DRG; 0191 282 0070) directly or a researcher on (0191) 208 7076. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence, you may have grounds for legal actions for compensation against the NHS Trust, but you may have to pay your legal costs. The trial is covered for claims; therefore if you wish to raise a complaint against a/the researcher/s or a member of staff you can do via the normal National Health Service complaints mechanisms using the details provided below.

Complaints Department In the Trust
Tel: (0191) 233 6161
Email: patient.relations@nuth.nhs.uk
Address: Patient Relations Department,
The Newcastle upon Tyne Hospitals NHS Foundation Trust
Headquarters: Freeman Hospital,
High Heaton,
Newcastle upon Tyne,
NE7 7DN

6. Will anyone else know I'm doing this?
   Only members of the research team will have access to the data. Some of your medical records may be checked to make sure that you are ok to take part in the study, your name, however, will not be disclosed outside the hospital. All information which is collected during this research study will be kept strictly confidential and your name and address will be removed so that you cannot be recognised from it. Any information you give or data collected will be retained for a maximum duration of 3 years.

7. What will happen to any samples I give?
   We will be taking blood samples for analysis of certain metabolites. However, because we cannot carry out all of the analysis straightaway we need to place some of the samples in storage. We have a controlled-access freezer in the Clinical Research Facility that will allow us to store the samples and when the samples are fully analysed they will be destroyed. Your name will not be used on any sample but you will be given a unique code at the start of the study which will only appear on the labels.

8. What will happen to the results of the research study?
   Results will be presented for the intention to publish in scientific journals. All results will be presented as grouped data and your individual results will not be identifiable. You will have access to your results after the completion of the study, or may be informed during, if we have discovered something that may benefit your current regimen.

9. Who is organising and funding the research?
   The study is funded by the Diabetes Research and Wellness Foundation through a grant that has been awarded to Newcastle University.

10. Who has reviewed the study?
    All research in the NHS is looked at by an independent group of people (the Research Ethics Committee), to protect your interests. This Study has received ethical approval by the NHS Research Ethics Committee

Contact Details:

Dr. Daniel J West

Institute of Cellular Medicine

4th Floor William Leech Building

Newcastle University

NE2 4HH

Email address: Daniel.west@newcastle.ac.uk

Phone: (0191) 208 7076

Mobile (tel): 07557340319

8.2. Appendix B. Informed Consent form for participants with T1D in study 1, 2 and 3 (Chapter 3,4 and 5)

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP
Tel: 0191 233 6161

Newcastle University

Study Number:____ Patient Identification Number:___

CONSENT FORM

Title of Project: The role of residual beta-cell function on glycaemic variability before and after exercise in Type 1 diabetes.

Name of Researcher: Dr Daniel J West

Please Tick Box and initial below the box:

1	I confirm that I have read and understand the information sheets dated..... for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐ ___
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without prejudice and without my medical care or legal rights being affected.	☐ ___
3	I understand that relevant sections of any of my medical notes and data collected during this study may be looked at by responsible individuals from Newcastle University, from regulatory authorities or from the NHS Trust where it is relevant to my taking part.	☐ ___

4	I give permission for the individuals described above to have access to my records, and I understand that accessible data will not be stored or copied.	☐
5	I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or NHS Trust, where it is relevant to my taking part in this research. I give my permission for these individuals to have access to my records.	☐
6	I agree to take part in this study.	☐

_____	_____	_____
Name of Patient	**Date**	**Signature**
_____	_____	_____
Name of Person Taking Consent	**Date**	**Signature**
_____	_____	_____
Researcher/s	**Date**	**Signature**

8.3. Appendix C. Medical History Questionnaire

Name: Date of Birth:

Do you have or suffer from a:			**Details:**
History of heart disease (eg. heart attack, surgery, angina etc)		YES NO	
Problems with the circulation	YES NO		
High blood pressure	YES NO		
Diabetes	YES NO		
Lung disease/breathing problems (eg. asthma, COPD etc)	YES NO		

Have you ever suffered from:			**Details:**
Discomfort in the chest, jaw, neck, back or arms (e.g. pressure, tingling, pain, heaviness, burning, tightness, squeezing or numbness)	YES NO		
Light headedness, dizziness or fainting?		YES NO	

Have you had any recent Illnesses?	YES	NO	**Details:**
(including hospitalisation, new medical diagnosis, surgery)			

Do you have any joint problems or anything which would make exercising difficult?

YES NO **Details:**

What medication are you taking?

(Please list)

Other habits

Caffeine	YES	NO	if yes, units per week	___
Alcohol	YES	NO	if yes, units per week	___
Tobacco	YES	NO	if yes, units per week	___

Family History			**Details:**
Heart disease	YES	NO	
Lung disease	YES	NO	
Diabetes		YES	NO
Stroke	YES	NO	
Sudden death	YES	NO	

Do you have any known allergies? YES NO **Details**

Diabetes related complications

			Details
Neuropathy (Nerve damage)			
Peripheral	YES	NO	
Autonomic	YES	NO	
Nephropathy (Kidney damage)	YES	NO	
Microalbuminuria	YES		NO
Retinopathy (Eye damage)	YES		NO
Diabetic ketoacidosis (DKA)	YES	NO	
Foot problems	YES	NO	

Any additional information:

Completed by _____ Date _____

8.4. Appendix D Physical examination

Name: _____ ***DOB:*** __/__/____

Body weight (kg): _____ **Waist Circumference** (cm): _____

%Fat Free Mass: _____ **% Fat Mass**: _____

Apical pulse rate(min): _____ **Rhythm**: OK / Not OK

Resting blood pressure, seated. ___ / ____

Auscultation of the lungs **OK / Not OK**

with specific attention to uniformity of breath *Comment:*_____

sounds in all areas (absence of rales and wheezes)

_____

Palpation of cardiac apical impulse **OK / Not OK**

point of maximal impulse

*Comment:*_____

_____

Auscultation of the heart **OK / Not OK**

with specific attention to murmurs,

*Comment:*_____

gallops, clicks and rubs. _____

Evaluation of the abdomen **OK / Not OK**

Bowel sounds, masses, visceromegaly, *Comment:*_____

and tenderness.

_____

Evaluation of lower extremities **OK / Not OK**

Oedema and presence of arterial pulse. *Comment:*_____

_____

Inspection of the skin **OK / Not OK**

focus on lower extremities in people with diabetes. *Comment:*

_____

Neurologic function **OK / Not OK**

Reflexes

*Comment:*_____

Any orthopedic or medical condition **YES / NO**

that would limit exercise. *Comment:*_____

Ventricular tachycardia **OK / Not OK**

*Comment:*_____

ST elevation (+1.0 mm) **OK / Not OK**

in leads without diagnostic Q-waves *Comment:*_____

(other than V_1 or aVR) _____

ST or QRS changes **OK / Not OK**

such as excessive ST suppression >2mm

*Comment:*_____

horizontal or down sloping ST-segment depression

_____

Arrhythmias other than: **OK / Not OK**

sustained ventricular tachycardia, including

*Comment:*_____

multiple PVCs, triplets of PVCs, supraventricular _____

tachycardia, heart block, or bradyarrhythmias. _____.

Cleared to start exercise test **YES / NO**

Competed by: _____ Date _____

Physical examination (page 2 on reverse of page 1)

Exercise Stress Testing

Exercise Protocol: _____

Absolute indicators for terminating the Exercise Stress test:

Drop in blood pressure of >10mm Hg **OK / Not OK**

from baseline blood pressure *Comment:*_____

despite an increase in workload,

_____

when accompanied by other _____

evidence of ischemia.

Any form of chest pain or **OK / Not OK**

shortness of breath *Comment:*_____

_____

Increasing nervous system symptoms **OK / Not OK**

(e.g. ataxia, dizziness or near syncope) *Comment:*_____

_____

Technical difficulties monitoring **OK / Not OK**

ECG or blood pressure

*Comment:*_____

_____

Ventricular tachycardia **OK / Not OK**

*Comment:*_____

ST elevation **OK / Not OK**

(+1.0 mm) in leads without *Comment:*_____

diagnostic Q-waves (other than V_1 or aVR)

_____

ST or QRS changes **OK / Not OK**

such as excessive ST suppression >2mm horizontal

*Comment:*_____

or down sloping ST-segment depression _____

Arrhythmias other than: **OK / Not OK**

sustained ventricular tachycardia,

*Comment:*_____

including multiple PVCs, triplets of PVCs, supraventricular

_____

tachycardia, heart block, or bradyarrhythmias. _____

Fatigue, shortness of breath, wheezing, **OK / Not OK**

leg cramps, or patient develops

*Comment:*_____

discomfort. _____

Development of bundle-branch block **OK / Not OK**

or intraventricular conduction delay *Comment:*_____

*that cannot be distinguished from ventricular tachycardia*_____

Hypertensive response **OK / Not OK**

Systolic blood pressure of > 250 mm Hg

*Comment:*_____

and / or diastolic pressure of >115 mm Hg

_____

Comments:

_____

_____

_____

Adverse reaction to exercise: **YES / NO**

Cleared to start exercise: **YES / NO**

Competed by: _____ Date _____

8.5. Appendix E Blood glucose, food, insulin and exercise diary

CONFIDENTIAL

Participant ID:_____

BLOOD GLUCOSE, FOOD, INSULIN AND EXERCISE DIARY

Please record everything you eat and drink, blood glucose values, insulin doses and amount of exercise you perform for the **7 days during the trial**

NSTRUCTIONS FOR USING THE BLOOD GLUCOSE, FOOD, INSULIN AND EXERCISE DIARY

Everything that you eat and drink over the course of the day, finger-stick blood glucose (BG) values, insulin doses and any exercise you perform over the course of the study should be recorded.

Blood Glucose Testing

On the first day:

- Take your first BG meter reading at 1 hour after the iPro2 continuous glucose monitor (CGM) is inserted.
- Take a second BG meter reading at 3 hours after the iPro2 CGM is inserted
- Collect at least one more meter reading before going to bed.

Collect at least <u>4 BG meter readings</u> each day throughout the study, such as <u>before breakfast, lunch, dinner, and going to bed.</u>

On the final day, please attempt to take at least 3 BG value before the sensor is removed

Food

- Do not forget to record second helpings and between meal snacks.
- Most people eat foods away from home each day, please do not forget to record these.

Names and descriptions of foods should be as detailed as possible, including the brand name and any other information available.

Time	**Blood Glucose** (mmol/l)	**Food/Drink** Description: (name/whether fresh, frozen, dried, canned/ cooked: boiled, grilled, fried, roasted/ type of fat food fried in)	**Quantity** (grams)	**Carbohydrate Estimate** (grams or carbohydrate portion)	**Insulin Dose** (units)	**Exercise** Description (time, type, intensity)
10:30 am	3.2mmol/l	McVities Digestive Biscuits (2)	32 g	20.8g	-	

Exercise

- Provide information about the type of exercise performed, how long for and the intensity of the exercise.

  e.g. Steady run, 35 minutes

General comments

- Write down any other events such as feeling hypoglycaemic, stress, or illness.

Any problems or questions please contact Guy Taylor on 0191 20 88 264 or G.Taylor3@newcastle.ac.uk

Day 1: Date:
___ /___ / ___

Time am/pm	**Blood Glucose** (mmol/l)	**Food/Drink** Description: (name/whether fresh, frozen, dried, canned/ cooked: boiled, grilled, fried, roasted/ type of fat food fried in)	**Quantity** (grams)	**Carbohydrate Estimate** (grams or carbohydrate portion)	**Insulin Dose** (units)	**Exercise** Description (time, type, intensity)

GENERAL COMMENTS:

8.6. Appendix F. Table of individual autoantibody positivity

Participant	C-peptide category	Autoantibody positivity	Autoantibodies		
			GAD (U/mL)	IA2 (U/mL)	ZNT8 (U/mL)
1	$Cpep_{und}$	No	<5.0	<7.5	<10.0
2	$Cpep_{und}$	Yes	68.6	<7.5	<10.0
3	$Cpep_{low}$	Yes	42.4	<7.5	<10.0
4	$Cpep_{und}$	Yes	<5.0	51.6	<10.0
5	$Cpep_{low}$	Yes	<5.0	71.4	<10.0
6	$Cpep_{low}$	No	<5.0	<7.5	<10.0
7	$Cpep_{high}$	Yes	55.7	<7.5	<10.0
8	$Cpep_{high}$	Yes	130.9	399.1	<10.0
9	$Cpep_{und}$	No	5.4	<7.5	18.7
10	$Cpep_{low}$	Yes	7.5	<7.5	10.9
11	$Cpep_{und}$	Yes	250.3	<7.5	87.6
12	$Cpep_{und}$	No	<5.0	<7.5	<10.0
13	$Cpep_{low}$	Yes	>2000.0	11.8	<10.0
14	$Cpep_{und}$	Yes	<5.0	<7.5	19.8
15	$Cpep_{und}$	No	<5.0	<7.5	<10.0
16	$Cpep_{und}$	No	<5.0	<7.5	<10.0
17	$Cpep_{low}$	Yes	16.3	85.4	379.1
18	$Cpep_{low}$	Yes	22.1	<7.5	<10.0
19	$Cpep_{high}$	Yes	352.1	<7.5	12.2
20	$Cpep_{high}$	No	<5.0	<7.5	<10.0
21	$Cpep_{high}$	No	<5.0	<7.5	<10.0
22	$Cpep_{low}$	Yes	5.4	45	<10.0
23	$Cpep_{und}$	Yes	30	<7.5	<10.0
24	$Cpep_{high}$	Yes	20.1	17.3	1667.9
25	$Cpep_{high}$	Yes	<5.0	13.3	85
26	$Cpep_{und}$	Yes	68.9	174.8	106.4
27	$Cpep_{high}$	Yes	19.2	63	14.9
28	$Cpep_{high}$	Yes	<5.0	<7.5	31.2
29	$Cpep_{high}$	Yes	<5.0	12.3	<10.0
30	$Cpep_{low}$	No	<5.0	<7.5	43.8

8.7. Appendix G. Participant information sheet for non-diabetic controls in study 3 (Chapter 5)

Participant Information Sheet

Factors affecting circulating numbers of Endothelial Progenitor Cells in response to an acute bout of exercise in healthy participants

Chief investigator: Dr. Daniel West

Physical Activity & Exercise Research

Institute of Cellular Medicine

Room M4.075

4th Floor William Leech Building

Medical School

Newcastle University

NE2 4HH

For further information, contact the study team at:

T: 0191 208 8264 E: G.Taylor3@newcastle.ac.uk

You are being invited to participate in a research study. Before you commit to whether you would like to take part or not it is important that you understand what the research will involve and why it is being undertaken. Please read this information sheet carefully and if you have any questions or require any more information, please do not hesitate to contact us. Details about the study are displayed below. Please take time to decide whether or not you wish to take part.

What is the purpose of the study?

Exercise mobilises endothelial progenitor cells (EPCs), a cell that circulates within the blood and plays a role in the repair and formation of new blood vessels in healthy individuals. Higher numbers of these cells is associated with improved vascular function and reduced cardiovascular disease. While we know that older individuals and individuals with lower fitness levels have reduced number of circulating EPCs at rest, it is not know to what extent clinical factors such as age, fitness level and inflammation influences the number of EPCs. The number of EPCs circulating within the blood is very low, thus the ability to mobilise these cells from the bone marrow and peripheral sources is important to repair the endothelial cells. However, it is not fully understood how EPCs are mobilised from the bone marrow and peripheral sources into the blood after exercise. The primary aim of this study is to understand how the clinical factors of age, inflammation and fitness impacts upon the number of circulating EPC at rest and the response to an acute submaximal exercise bout in healthy participants. We will also explore the relationship between number of EPCs and circulating amounts of potential mobilisation mechanisms.

Why have I been chosen?

You have been contacted because you have expressed an interest in our research and taking part. We are looking for 30 healthy individuals (males and females) between the ages of 18-60 with no muscular injuries or history of any chronic diseases. We will need a wide range of participants in terms of age and weight.

Do I have to take part?

It is completely up to you whether you wish to take part or not. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw at any time and without giving a reason.

What will happen if I want to take part?

We will contact you by phone or email to answer any questions you may have about the study. We will then ask you some questions about your medical history to check if you eligible to partake. You will not be able to participate in the study if you have any medical conditions or if you are taking medications that will affect the measurements in the study. If you are a suitable participant, we will invite you to attend Clinical Research Facility at the RVI hospital on two separate occasions.

What will I have to do?

Visit 1

You will be briefed on the full study at which point you will be invited to ask any questions or raise any concerns. Participation will depend on the satisfactory completion of a medical history questionnaire. If you are considered eligible and agree to take part you will sign an informed consent form.

You will then have your height and weight taken and complete a resting and exercising electrocardiogram (ECG) stress test. This is so we can confirm that your heart responds normally to exercise and that it is safe for you to complete the exercise part of the study. During the ECG test, electrodes will stuck around your chest to measure the electrical impulses within the heart. You will not feel any pain throughout this test.

We will measure oxygen consumption (the amount of air you breathe) throughout the exercise tests using a mask that covers the mouth and nose. The exercise test is a walking/running test that takes place on a treadmill. This test starts off at a light intensity and gradually increases every 3 minutes until you feel you can no longer continue. This allows us to capture your heart rate and breathing responses gradually with changes in exercise intensity. We will also use this test to estimate how hard you will work for the main exercise test in the study.

The first visit takes 1 hour 30 minutes to complete.

Visit 2

At least 3 days after your last visit, you will again attend the CRF for the main exercise trial. You will arrive fasted in the morning.

1. A capillary blood sample will be taken to measure your blood glucose, haemoglobin and haematocrit.
2. A cannula (a thin tube inserted into a vein) will be inserted by a trained phlebotomist to take blood samples from. Once inserted, a fasting blood sample will be taken.
3. You will be given a carbohydrate snack (Belvita bar – 28g CHO).
4. Resting heart rate will be measured for 10 minutes.
5. You will then complete 45 minutes of treadmill walking up a gradient. The intensity will be ~60 % of the maximum level you reached in the first visit. During the test we will capture your heart rate and oxygen consumption (the amount of air you breathe) using a chest strap and a mask that covers your mouth and nose.
6. Capillary and venous blood samples from the cannula will be taken immediately after completing the exercise and 1 hour after completing the exercise.

The second visit will take 3 hours to complete.

What do I have to do?

It is critical for the study that follow the instructions we will give you for each study date. If the dose of your medications change or you are prescribed a new medication during the study we may need to discuss your participation in the study.

What are the possible disadvantages of taking part in this study?

We will be taking blood from a vein in your arm by inserting a cannula and it is possible you might experience some discomfort when it is being placed in the arm and/or bruising when it is taken out. However, all samples will be collected by appropriately trained personnel to minimize this possibility.

The first exercise test will involve pushing yourself to your maximum level. This can be uncomfortable, although not dissimilar to any time you have pushed yourself hard during exercise. You can choose to terminate the test at any time. There will also be medical cover present throughout your time at the laboratories.

What are the possible benefits of taking part in this study?

We will measure your height and weight and you will learn your maximum fitness level, a test that can be useful for training if you regularly take part in exercise and is a good indicator of future health. If you were to get this test done privately then it would cost £100-150. You will also receive a £50 inTU voucher (Eldon square and the metro centre) in remuneration for taking part in the study.

Will my taking part in the study be kept confidential?

Newcastle Univesity is the sponsor for this study based in the United Kingdom. We will be using information from [you and/or your medical records] in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Newcastle University will keep identifiable information about you for 3 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

The clinical exercise research team will collect information from you for this research study in accordance with our instructions.

The clinical exercise research team will keep your name, NHS number and contact details confidential and will not pass this information to Newcastle University. The clinical exercise research team will use this information as needed, to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Certain individuals from Newcastle University and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Newcastle University will only receive information without any identifying information. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

The clinical exercise research team will keep identifiable information about you from this study for 3 years after the study has finished.

What will happen to the study results?

The overall results of the study may be presented at scientific meetings or published in a scientific journal. You will not be identified in any of these presentations or publications. We will be happy to discuss the results with you when the study is completed and will let you know where you can obtain a copy of the published results.

Will I be compensated for my time?

In recognition of your time commitments to the study and compliance with the clinical investigations, you will be compensated with a £50 voucher for Eldon Square and the Metro Centre.

Ethical Approval

This study was approved by the Faculty of Medical Sciences Research Ethics Committee, part of Newcastle University's Research Ethics Committee. This committee contains members who are internal to the Faculty, as well as one external member. This study was reviewed by members of the committee, who must provide impartial advice and avoid significant conflicts of interests.

Contact for further information

If you have any further questions then please contact Guy Taylor

Telephone: 0191 208 8264 Email: g.taylor3@ncl.ac.uk

And finally…

Thank you for having taken the time to read this information sheet and your interest in the study. If you do decide to take part in the study, you will be given a copy of the information sheets and a signed consent form for you to keep.

8.8. Appendix H. Informed consent form for non-diabetic controls in study 3 (chapter 5)

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP
Tel: 0191 233 6161

Newcastle University

Study Number:1522 Patient Identification Number:___

CONSENT FORM

Title of Project: Factors affecting circulating numbers of Endothelial Progenitor Cells in response to an acute bout of exercise in healthy participants

Name of Researcher: Dr Daniel J West

Please Tick Box and initial below the box:

1	I confirm that I have read and understand the information sheets dated..... for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐ ___
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without prejudice and without my medical care or legal rights being affected.	☐ ___
3	I understand that relevant sections of any of my medical notes and data collected during this study may be looked at by responsible individuals from Newcastle University, from regulatory authorities or from the NHS Trust where it is relevant to my taking part.	☐ ___
4	I give permission for the individuals described above to have access to my records, and I understand that accessible data will not be stored or copied.	☐ ___

5	I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or NHS Trust, where it is relevant to my taking part in this research. I give my permission for these individuals to have access to my records.	☐
6	I agree to take part in this study.	☐

_____	_____	_____
Name of Patient	**Date**	**Signature**
_____	_____	_____
Name of Person Taking Consent	**Date**	**Signature**
_____	_____	_____
Researcher/s	**Date**	**Signature**

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United States

Requestor Information

Name Guy Taylor

Title Dr

Institution Newcastle University

Address 4th Floor William Leech Building, Medical School, Newcastle University, Newcastle

NE2 4HH, UK

City _____ State _____ Zip _____ Country _____

Phone _____ Fax _____

Email g.taylor3@newcastle.ac.uk

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