EXAMINING THE BARRIERS AND BENEFITS TO EXERCISE IN ADULTS WITH NEWLY DIAGNOSED TYPE 1 DIABETES

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ABSTRACT

Type 1 diabetes (T1D) results from autoimmune destruction of pancreatic beta cells. Preservation of beta cell function reduces the risk of the complications of T1D. Regular exercise preserves beta cell function rat models of T1D and patients with other forms of diabetes. We wished to examine whether exercise could preserve beta cell function in T1D and whether this was influenced by adipokine receptor expression and function.

We undertook a qualitative study to explore barriers to exercise in patients newly diagnosed with T1D. This showed that patients lacked confidence managing diabetes for exercise, and were poorly supported by healthcare professionals.

Using these results, we then undertook a pilot clinical trial aiming to determine recruitment and retention, adherence to exercise, and exploring whether exercise preserves beta cell function in patients newly diagnosed with T1D. We show successful recruitment to an unsupervised exercise intervention study. We did not detect a beneficial effect of exercise on beta cell function in this pilot trial, but identified several areas that will need to be addressed in designing a larger scale study.

Finally, we demonstrate improved adiponectin receptor expression and adiponectin mediated suppression of T cell endothelial migration in the months after diagnosis with T1D.

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AdipoR	Adiponectin receptor
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
bpm	Beats per minute
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CFSE	Carboxyfluorescein succinimidyl ester
CI	Confidence interval
CRF	Case report form
DCCT	The Diabetes Control & Complications Trial
DKA	Diabetic ketoacidosis
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FCS	Fetal calf serum
GAD	Glutamic acid decarboxylase
HbA1c	Glycated haemoglobin
HDL(-C)	High density lipoprotein (cholesterol)
H-DMEC	Human Dermal Microvascular Endothelial Cells
HOMA	Homeostasis model assessment
IA-2	Islet antigen-2
INF-γ	Interferon-γ
IQR	Interquartile range
IR	Insulin resistance
MFI	Mean fluorescence intensity
MMTT	Mixed meal tolerance test
mRNA	Messenger ribonucleic acid
NK	Natural killer
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PIC	Patient Identification Centre
PPD	Purified protein derivative to mycobacterium TB
RCT	Randomised controlled trial
RPE	Rating of perceived exertion

RPMI	Roswell Park Memorial Institute
SD	Standard deviation
SOP	Standard operating procedure
T1D	Type 1 diabetes
TNF-α	Tumour necrosis factor-α
UCPCR	Urinary C-peptide:creatinine ratio
UK	United Kingdom
VO2max	Maximal oxygen uptake
WHR	Waist-hip ratio
ZnT8	Zinc transporter 8

1 INTRODUCTION

1.1 Type 1 Diabetes

Type 1 diabetes (T1D) is a metabolic disorder which results from destruction of the pancreatic beta cells causing insulin deficiency and hyperglycaemia (American Diabetes Association 2013). It is thought that autoimmunity to the beta cell is activated in a genetically susceptible individual after exposure to an unknown environmental trigger (Atkinson & Eisenbarth 2001). An affected individual requires life-long insulin treatment to control blood glucose and reduce the risk of complications.

For the individual patient, insulin deficiency eventually will result in diabetic ketoacidosis (DKA). Without insulin replacement, a diagnosis of T1D is inevitably fatal. Hospital admission with DKA still occurs in approximately 3.3% of patients with T1D (Health and Social Care Information Centre 2012), despite modern insulins and regimes. In spite of considerable advances in its management, the life-expectancy of those diagnosed with T1D remains shortened by more than 20 years when compared to healthy individuals (Department of Health 2001). The additional risk of mortality, above that of the general population is 135% in T1D in England and Wales (Health and Social Care Information Centre 2012).

The excess mortality in T1D in the UK is now largely due to cardiovascular disease (Roper et al. 2002) rather than the acute metabolic derangement associated with insulin deficiency. However, considerable morbidity and mortality result from the

microvascular complications; nephropathy, neuropathy and retinopathy. A major goal of T1D management is a reduction in these long-term complications. One way of reducing the risk of developing microvascular complications is intensive glycaemic control (The Diabetes Control and Complications Trial Research Group 1993; The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group 2002); however, in clinical practice optimal glycaemic control is often difficult to achieve. The general principles of treatment are diet and insulin therapy. These are broadly the same treatments have been in use since 1922, when insulin was first used in humans; although there have been considerable developments since, in insulin preparations and regimes.

1.2 Epidemiology of T1D

In 2010, diabetes mellitus was estimated to affect 285 million people worldwide, the vast majority of these will have type 2 diabetes (Shaw et al. 2016). In the UK, approximately 290,000 adults have a diagnosis of T1D and the total cost to health services and the economy in 2010/11 was £1.9 billion (Hex et al. 2012).

T1D presents most commonly in children with incidence rising from birth and peaking between the ages of 10 and 14 years (Diabetes UK 2012). Although the incidence of T1D declines with age, approximately one quarter of people with T1D are diagnosed in adulthood (Maahs et al. 2010).

Large numbers of studies have observed that the prevalence of T1D is increasing (particularly among the young) (The DIAMOND Project Group 2006) and age at diagnosis is decreasing (Patterson et al. 2009). Data collected by the EURODIAB

group, estimates that the prevalence in European children under the age of 15 years will increase by 70% by 2020 (compared to 2005 rates) (Patterson et al. 2009).

Globally there is a great variability in the incidence of T1D. The World Health Organisation reported rates of 0.1/100,000 per year in China and Venezuela, increasing to 40.9/100000 per year in Finland (The DIAMOND Project Group 2006). The discrepancy in incidence rates between countries is intriguing. On the whole, rates are lower in less well developed countries. This may in part be due to poor case ascertainment in countries without comprehensive healthcare systems and registries, as well as, high childhood mortality from other causes, infectious diseases for example. The same group has documented a global increase in T1D incidence.

It is not clear what is driving the increase in rates of T1D, although a number of theories have been postulated. These include altered exposure to environmental triggers; early exposure to cow's milk or enterovirus, or lack of exposure to protective infections; the "hygiene hypothesis" (Gale 2002). In some areas of the world it may be an indication simply of improved case identification. It is also possible that rising rates of obesity, as well as being implicated in increased rates and earlier onset of type 2 diabetes, are responsible for at least some of the rise in T1D incidence. A Finnish study demonstrated that rapid childhood growth was associated with a 20-40% increase in risk of T1D and obesity after the age of three years doubled the risk (Hyppönen et al. 2000).

1.3 Pathophysiology

T1D is an autoimmune process whereby the immune system targets proteins found in the beta cells of the pancreas. Beta cells are insulin producing cells which are located, along with other hormone-producing cells in the islets of Langerhans. A lymphocytic infiltrate, predominantly composed of CD8+ T cells, is seen in the islets of patients with T1D. This histological finding is known as insulitis (Hanafusa & Imagawa 2008).

In normal physiology, insulin is produced in response to a rise in blood glucose. Insulin acts to reduce blood glucose through suppression of glucagon secretion and increasing glucose uptake and storage by tissues such as the liver. These processes are impaired in T1D due to the lack of insulin, and therefore, blood glucose rises (Ozougwu et al. 2013).

Beta-cell function in its simplest sense is the ability of pancreatic beta-cells to produce insulin in response to rising blood glucose and, therefore, maintain normoglycaemia. Cleavage of the prohormone, proinsulin, results in the release of C-peptide alongside insulin and in equimolar amounts. The half-life of insulin in the circulation is short (around four to six minutes) and its significant first-pass hepatic metabolism means that insulin is a less than ideal measure (Palmer et al. 2004). Therefore, in the assessment of beta cell function, C-peptide is measured as a surrogate for insulin production.

1.4 The natural history of T1D

T1D develops after the autoimmune destruction of pancreatic beta cells is triggered in a predisposed individual. The clinical features of diabetes; thirst, polyuria, fatigue and weight loss; are as a result of sustained hyperglycaemia and develop only once considerable beta cell function has been lost. It is estimated that at diagnosis, an individual with T1D has around 25-50% of their beta cell function remaining (Sherry et al. 2005).

With the initiation of insulin therapy and improvement in blood glucose levels, there may be a transient improvement of beta-cell function which results in a period of partial remission of T1D, known as the 'honeymoon'. During this period, insulin requirements are considerably reduced and it is sometimes possible to stop insulin treatment altogether (Abdul-Rasoul et al. 2006). The ongoing autoimmune process results in further loss of pancreatic beta cells, eventually resulting in an absolute insulin deficient state. This process takes a variable length of time, with some patients still having low level but detectable insulin secretion many years after the diagnosis of T1D (Keenan et al. 2010; Scholin et al. 2004).

Significant beta-cell function is seen in the majority of patients with a shorter duration of disease, one study puts the proportion of patients with C-peptide greater than 200pmol/l two years after diagnosis, at around two thirds (Greenbaum et al. 2012). While it is possible to identify some individuals at high risk of developing T1D, through family history and autoantibodies, the majority of people who are diagnosed do not have a relative with T1D. Therefore, an ideal time to study factors potentially affecting beta cell function is soon after diagnosis. The ultimate goal is to find a beta-

cell preserving therapy that could one day be used to prevent T1D in susceptible individuals.

Residual insulin secretion at diagnosis and persistence of beta cell function are recognised to be beneficial to people with T1D. Analysis of data from the Diabetes Control and Complications Trial (DCCT) demonstrated that a cut off level of C-peptide of 200pmol/l conferred glycaemic benefit in fasting glucose and HbA1c (Palmer et al. 2004). Higher initial C-peptide levels and persistence of detectable C-peptide is also associated with a reduction in the complications of diabetes; retinopathy, nephropathy and hypoglycaemia (Lachin et al. 2013; Steffes et al. 2003). This is, therefore, a therapeutic goal endorsed by the Medicines and Healthcare products Regulatory Authority and United States Food and Drug Administration. There are hopes that therapies such as monoclonal antibodies to CD3 and CD20, will preserve beta cell function in early T1D but these have not demonstrated lasting benefit and are not yet in clinical use (Skyler 2015; Ludvigsson 2009; Phillips et al. 2011). The utility of these therapies may be limited by their side effects; mainly, immunosuppression leading to secondary malignancies and infection, and an immunomodulatory therapy with long-lasting effects has yet to be discovered (Ben Nasr et al. 2015).

1.4.1 Immunity in T1D

A diagnosis of T1D is made clinically (National Institute for Clinical Excellence 2015), and is based on the clinical presentation of an individual. It can be difficult however, to differentiate between different types of diabetes, particularly in patients who

present in adulthood, and studies suggest that around 7% of patients diagnosed with type 2 diabetes have evidence of immune driven beta-cell loss (Seissler et al. 1998) and the proportion is higher in younger age groups (Turner et al. 1997).

1.4.1.1 Cellular immunity in T1D

In the Eisenbarth model (Atkinson et al. 2015) of the autoimmune pathogenesis of T1D, auto-antigens from damaged beta cells are processed by antigen presenting cells (APCs). Dendritic cells, B lymphocytes and macrophages may act as APCs. Auto-reactive CD4+ (helper) and CD8+ (cytotoxic) T cells occur due to failures in thymic and peripheral tolerance, and are stimulated by activated APCs in the pancreatic lymph nodes and the autoimmune process is triggered. Autoreactive T cells then migrate to the pancreatic islets where cytotoxic cells destroy cells through Fas-Fas ligand triggered apoptosis or perforin dependent mechanisms (Moriwaki et al. 1999). CD4+ and CD8+ cells produce cytokines, for example, INF-γ which induces Fas expression on beta cells and promotes inflammation through mononuclear cell recruitment, macrophage activation and production of TNF and IL-1β, thus mediating apoptosis (Lehuen et al. 2010). This model is consistent with post mortem studies of the pancreatic tissue of patients with T1D, where an immune cell infiltrate consisting of CD3+ T lymphocytes (predominantly CD8+ T cells), dendritic cells and macrophages has been found (Hanafusa & Imagawa 2008; Bottazzo et al. 1985) along with a reduction of beta cell numbers.

Evidence for the role of autoimmunity and, more specifically, T lymphocytes in the development of T1D comes from a number of other observations.

Relapse of T1D in recipients of pancreatic transplants, is associated with a mononuclear cell infiltrate in biopsies of the transplanted pancreases and ameliorated by immunosuppression (Sibley et al. 1985). Circulating autoreactive T lymphocytes to glutamic acid decarboxylase (a pancreatic protein) have been identified in patients with T1D and their first degree relatives (Honeyman et al. 1993). In the NOD mouse, T1D may be transferred by CD4+ and CD8+ T cells, but only in combination (Phillips et al. 2009), indicating a role for both subsets in T1D pathogenesis. Finally, anti-CD3 monoclonal antibody therapy (targeting T lymphocytes) can suppress beta cell destruction in patients with T1D (Skyler 2013).

1.4.1.2 Autoantibodies in T1D

Autoantibodies that may be identified in the serum of patients with T1D are targeted towards beta cell proteins such as glutamic acid decarboxylase (GADA), tyrosine phosphate-like antigen IA-2 (IA-2A) and the zinc transporter ZnT8 (ZnT8A) (Bonifacio & Bingley 1997; Wenzlau et al. 2007). The same autoantibodies may also be identified in individuals at risk of developing T1D, such as the relatives of people with T1D, where the number and titre relate to the risk of progression to T1D (Bingley et al. 1994; Achenbach et al. 2004). There has been considerable work examining relatives of patients with T1D to identify people at high risk of progression, however, the majority of people have no family history of T1D at presentation (Achenbach et al. 2005).

In adult patients with recently diagnosed T1D antibody positivity has been reported at over 80% (Williams et al. 2012; Vermeulen et al. 2011).

1.4.2 Decline in beta-cell function after diagnosis of T1D

At diagnosis, individuals affected by T1D are estimated to have approximately 25-50% of their beta cell function remaining (Sherry et al. 2005). C-peptide levels fall (before and) after diagnosis, however C-peptide decline is not thought to be linear. The decline in beta-cell function after diagnosis is variable; a biphasic pattern to beta-cell function loss has been described, with slower decline in the second year after diagnosis (Greenbaum et al. 2012). The overwhelming majority of patients with a short duration of T1D will have significant C-peptide levels (i.e greater than 200pmol/l, the level at which clinical benefit in terms of microvascular disease and retinopathy has been demonstrated); 66% at 24months (Greenbaum et al. 2012) and 92% of patients with a diabetes duration of under 5 years (The Diabetes Control and Complications Trial Research Group 1987).

1.4.3 Factors affecting C-peptide levels in T1D

A number of factors are associated with the rate of beta cell loss. In patients with T1D, the presence of islet autoantibodies is associated with a higher rate of beta cell loss after diagnosis (Sherry et al. 2005). Another important factor is glycaemic control, with higher levels of glycaemia associated with accelerated loss of beta cell reserve. Although this phenomenon was first described in the DCCT (The Diabetes Control and Complications Research Group 1998), it has been demonstrated in later

studies as well (Dost et al. 2007; Greenbaum et al. 2009), an indication perhaps that the general tightening of glycaemic control since the DCCT was reported has not eliminated this effect.

The literature on factors affecting beta cell function is rather conflicting and heterogeneous. The majority of the data is from observational studies in children as well as data from the DCCT and the control arms of various intervention studies (predominantly these studies include a majority of children and young adults). Little is known about the factors affecting beta-cell function in patients diagnosed with T1D as adults.

Some studies attempt to determine the effect of various factors on beta-cell function, both at diagnosis and over varying lengths of time (measured by C-peptide release to various stimuli), while others have concentrated on the presence and duration of (partial) remission of diabetes. The definition of partial remission varies in these studies, however, it is usually defined as well controlled diabetes and low insulin requirements (less than 0.3 units/kg (Bonfanti et al. 1998) to less than 0.5units/kg (Dost et al. 2007; Bowden et al. 2008)). Well controlled diabetes may be defined as an HbA1c less than 7% (42mmol/mol) (Dost et al. 2007), 8% (64mmol/mol) (Bowden et al. 2008) or in normal range (Bonfanti et al. 1998). Although the two concepts are not interchangeable (remission is binary and beta-cell function is a continuous variable), the presence of partial remission is associated with higher basal and stimulated C-peptide levels (Bonfanti et al. 1998; Lombardo et al. 2002).

The following have all been reported to affect either baseline C-peptide, rate of decline of c-peptide or presence and duration of honeymoon in T1D:

- Gender
- Age
- Number and titre of autoantibodies.
- Duration of symptoms prior to diagnosis
- Presentation of diabetes (i.e. either in DKA, or hyperglycaemic but not acidotic).
- Blood glucose at presentation
- Smoking status
- HbA1c, and
- Length of time from diagnosis

1.4.3.1 Gender and Age

Clinical remission rates and duration may be influenced by gender. Several studies have demonstrated that males are more likely to experience a remission period (Bober et al. 2001) and experience a longer duration of clinical remission (Dost et al. 2007; Pilacinski et al. 2012). Studies which looked at C-peptide rather than remission, however, reported that male gender was associated with lower baseline C-peptide levels in children (Sochett et al. 1987) and adults (Karjalainen et al. 1989).

Older age at diagnosis confers an advantage in greater rates (Bonfanti et al. 1998; Lombardo et al. 2002; Bowden et al. 2008) and duration (Dost et al. 2007) of remission, as well as baseline C-peptide level (Karjalainen et al. 1989) and

persistence of C-peptide (Sochett et al. 1987; Greenbaum et al. 2009). There is some discrepancy here too, however. One group (Pilacinski et al. 2012) found no relationship with rates/length of partial remission in adult patients and others (Torn et al. 2000; Steele et al. 2004) found that age is not related to decline in beta cell function.

In the DCCT, participants who had diabetes for under five years at study entry were more likely have a stimulated C-peptide >200pmol/l (known as a 'responder') if they were female (The Diabetes Control and Complications Research Group 1998). In addition, participants under the age of 18 were less likely to be a 'responder' at study entry and less likely to have maintained that status after 5 years.

1.4.3.2 Autoantibodies

Presence of GAD antibodies has been associated with lower C-peptide six months post-diagnosis and decreased rates of clinical remission in children and adolescents (Bonfanti et al. 1998) but not in adults (Steele et al. 2004; Pilacinski et al. 2012). There are conflicting results reported with respect to islet cell antibody status/IA-2A, with some groups reporting no effect on C-peptide (Karjalainen et al. 1989; Torn et al. 2000) and others finding an increased insulin secretory response in participants with positive islet cell antibodies (Steele et al. 2004). In general, antibody negative patients have higher levels of C-peptide than those with detectable antibodies (Torn et al. 2000).

1.4.3.3 Presentation of diabetes and duration of symptoms

It has been suggested that the presentation of diabetes (i.e. with or without acidosis) and the length of time that the patient has noticed osmotic symptoms prior to diagnosis is associated with clinical remission of diabetes and persistence of C-peptide. The studies showing this are all of children only. One study (Abdul-Rasoul et al. 2006) showed that lower blood glucose (suggesting earlier presentation to a clinician) and higher pH at diagnosis were associated with a greater chance of remission. In addition, longer duration of symptoms prior to diagnosis was associated with a longer duration of remission (Abdul-Rasoul et al. 2006). In adults, patients not presenting with diabetic ketoacidosis or ketonuria were more likely to have a longer remission period than those who did (Pilacinski et al. 2012). Similarly, lower blood glucose at presentation was associated with higher C-peptide at diagnosis (Sochett et al. 1987) and lower pH at diagnosis with a lower C-peptide three months after diagnosis (Bonfanti et al. 1998) i.e. a faster progression of beta cell decline was seen in those with a greater degree of acidosis at presentation.

1.4.3.4 Cigarette Smoking

The only study to look at factors influencing clinical remission in adults focussed on smoking. The authors (Pilacinski et al. 2012) reported that non-smokers experienced an increased chance of being in remission one year after diagnosis than smokers.

1.4.3.5 Glycaemic control/HbA1c

Lower HbA1c has been demonstrated to be associated with longer duration of remission in children (Dost et al. 2007), with higher initial C-peptide levels (The Diabetes Control and Complications Research Group 1998) and with preservation of beta cell function (Greenbaum et al. 2009).

1.4.3.6 Length of time from diagnosis

Shorter duration of diabetes was associated with higher C-peptide levels in the DCCT (The Diabetes Control and Complications Research Group 1998). Greenbaum et al. 2012 describe a biphasic decline in C-peptide, with a slower rate of fall in the second year after diagnosis than the first.

1.5 Insulin resistance

Hyperglycaemia in T1D is primarily due to insulin deficiency; however, resistance of peripheral tissues to insulin action may also cause elevated circulating blood glucose. Insulin resistance is commonly associated with obesity, where diminished insulin signalling in adipose tissue, skeletal muscle and liver results in impaired glucose metabolism (Kahn & Flier 2000). Levels of obesity are rising both in the UK and worldwide (Health and Social Care Information Centre 2015) and reflect the rising incidence of conditions associated with insulin resistance such as metabolic syndrome, impaired glucose tolerance and type 2 diabetes (Ozougwu et al. 2013). Could the rise in obesity and insulin resistance be contributing to the increase seen in rates of T1D?

This is the premise underpinning the ‘Accelerator’ hypothesis; which argues that both type 1 and type 2 diabetes result from a combination of insulin resistance, beta cell apoptosis and beta cell autoimmunity (Wilkin 2001). The proportions of each of these three factors variably combine to give the different clinical pictures currently known as type 1 or type 2 diabetes. The observation that there is considerable overlap between the two forms of the disease in terms of age of onset (Mølbak et al. 1994; Rosenbloom et al. 1999), presence of ketoacidosis (Aizawa et al. 1997), antibody status (Turner et al. 1997) and eventual beta-cell failure, adds weight to this theory.

Insulin resistance has been implicated in the development and progression of T1D in a number of studies. The importance of insulin resistance in T1D gained recognition in the early 1980’s. Insulin resistant individuals with T1D were found to be less likely to experience clinical remission or “honeymoon” (Yki-Jarvinen & Koivisto 1986). Subsequently, prospective controlled studies have gone on to demonstrate that in an at-risk population, (relatives of patients with T1D with islet autoantibodies), progression to diabetes is associated with greater insulin resistance (Fourlanos et al. 2004; Bingley et al. 2008; Xu et al. 2007).

The gold standard for measuring insulin resistance is the euglycaemic hyperinsulinaemic clamp (DeFronzo et al. 1979). This is a complex and time consuming test, and often surrogate markers of insulin resistance are used, such as waist-to-hip ratio or BMI. The homeostasis model assessment (HOMA) is a mathematical model that quantifies insulin resistance using fasting insulin and glucose. First described in 1985, it correlates well with the euglycaemic clamp

(Matthews et al. 1985) and has since been updated to a more sophisticated computer model (Wallace et al. 2004).

Therapies targeting insulin resistance have been used in the treatment and prevention of type 2 diabetes for many years (UK Prospective Diabetes Study (UKPDS) Group 1998; Diabetes Prevention Program Research Group 2002). In addition, the benefits of lifestyle measures aimed at improving insulin sensitivity such as weight loss and physical activity are well documented in this patient group (Krotkiewski et al. 1985; Umpierre et al. 2011; Andrews et al. 2011; Chimen et al. 2012). Exercise training can also reduce insulin resistance. Exercise training is also associated with improved insulin sensitivity in normal weight (King et al. 1987) and obese subjects (DeFronzo et al. 1987), as well as those with T1D (Yki-Jarvinen et al. 1984). Furthermore, as insulin resistance is also implicated in the development of T1D, it is interesting to consider that these therapeutic interventions may also influence the progression of this disease.

1.6 The effects of exercise

Physical inactivity is a leading risk factor for disease globally, accounting for more than 3 million deaths worldwide in 2010 (Lim et al. 2012). Regular physical activity can reduce the risk of developing cardiovascular disease, type 2 diabetes, cancer, obesity, mental health disorders and musculoskeletal problems (Chief Medical Officers of England 2011).

In patients with type 2 diabetes, exercise training improves glycaemic control, reduces fat mass and visceral adiposity and improves lipid profile (Thomas et al.

2006) and in those with T1D the proven benefits of exercise include weight reduction, reduced insulin requirements and improvements in lipid profile and blood pressure (Chimen et al. 2012). Exercise does improve glycaemic control in patients with type 2 diabetes (Umpierre et al. 2011). The same is not true of patients with T1D, however, with no evidence of exercise training having an effect on glycaemic control (Kennedy et al. 2013)

In view of the undoubted health benefits of regular physical activity, the Chief Medical Officer has produced guidelines for physical activity for adults in the UK (Chief Medical Officers of England 2011), these are given below. Similar recommendations are given by the Association of British Clinical Diabetologists and the American Diabetes Association for people with diabetes (Nagi & Gallen 2010; American Diabetes Association 2010).

Chief Medical Officer's recommendations for adults aged 19-64:

1. Daily activity with at least 150 minutes of moderate intensity physical activity per week, made up of bouts of at least ten minutes (eg 30 mins on at least five days a week).
2. Or, 75 mins vigorous intensity activity spread across the week or an equivalent combination of moderate and vigorous intensity activity.
3. Activity to improve muscle strength on at least two days a week.
4. Minimise sedentary time.

Exercise has been shown to improve beta-cell function in animal studies. These suggest a beneficial effect of exercise training on both beta-cell mass and function in 90% pancreatectomised and streptozotocin-induced diabetic rats (Choi et al. 2005; Choi et al. 2006; Coskun et al. 2004; Park et al. 2007). Exercise also appears to increase the insulin content and secretion of the pancreatic islets in streptozotocin-induced diabetic rats (Huang et al. 2011). In humans, exercise is associated with preservation of beta-cell function in healthy people (Boulé et al. 2004), people with impaired glucose tolerance and patients with type 2 diabetes (Dela et al. 2004; Bloem & Chang 2008)(reviewed in Narendran et al. 2015). There is no published evidence of the effect of physical activity on beta-cell function in T1D in humans.

It is not clear whether there are specific activities that have a greater effect on beta-cell preservation; studies demonstrating improvements in humans have included both cardiovascular (cycle ergometer (Boulé et al. 2004)(Dela et al. 2004) and treadmill walking (Bloem & Chang 2008)), and resistance exercises (isometric muscle strengthening (Grøntved et al. 2013)).

Moreover, there is some evidence that higher levels of physical activity are associated with benefits in prevalence of microvascular complications in T1D. Higher levels of self-reported physical activity in the past are associated with lower rates of nephropathy, neuropathy and retinopathy in humans (Kriska et al. 1991; Wadén et al. 2008). In contrast, a post hoc analysis of data from the DCCT trial did not find any association between baseline leisure time physical activity and rate of subsequent development of retinopathy (Makura et al. 2013), perhaps supporting the suggestion that microvascular complications impair an individual's ability to exercise rather than a direct effect of exercise on complication rates.

1.7 Activity levels and barriers

Despite the recommendations of the Chief Medical Officer and clear evidence of benefit, many people fail to achieve suggested minimum levels of physical activity. Self-reported physical activity data from 2012, demonstrated that only 67% of men and 55% of women achieved this minimum level of activity, with higher proportions in younger age groups and reducing with increasing age (Health and Social Care Information Centre 2015). Objective measures of physical activity, such as data from accelerometer studies, demonstrate a much more sedentary population. In 2008, the Health Survey for England included both self-reported measures of physical activity and accelerometer data and while 39% of men and 29% of women reported undertaking at least 150 mins of exercise each week, accelerometer data (in a sub-group) showed only 6% of men and 4% of women met this target (Aresu et al. 2008). Activity levels tend to be over-estimated by self-reported measures compared to objective measures such as accelerometry, due to recall and social desirability biases (Aresu et al. 2008).

Estimates of physical activity vary for patients with T1D. In a Canadian population only 36.3% of patients met relevant physical activity guidelines (more than or equal to 600 MET.min.wk^{-1}) (Plotnikoff et al. 2006). A similar proportion (30/77 participants, 39%) were classed as 'active' in a Scottish study (Thomas et al. 2004), this included everyone who reported taking part in exercise or sport in the previous two weeks and may therefore represent an overestimate when compared to other studies. Studies in other populations have demonstrated a more active group. A Finnish study reported that only 23% of patients with T1D were sedentary (Wadén et al. 2008), and

in the Diabetes Control and Complications Trial only 19% did not meet the ADA guidelines (Makura et al. 2013). In a large European T1D population, 36% of participants played sports less than once a week (Tielemans et al. 2013). All of these studies relied on self-reported activity levels and, as above, may well be over-estimates of actual activity.

The Health Survey for England, a large scale annual survey, surveyed attitudes and barriers to exercise in 2007 (Chaudhury et al. 2008). In this survey of over 6,800 adults, the most often cited reasons for not meeting physical activity guidelines were work commitments and lack of leisure time for men and women respectively. Other barriers identified in this report included lack of money, caring for children or older people, lack of local facilities, having no-one to exercise with, and not having the right clothes or equipment. More than two thirds of adults stated that they wanted to increase their physical activity levels.

People with chronic disease conceivably may have additional barriers to exercise related to the physical and psychological impact of their diagnosis. When specific patient groups are studied, three classes of barriers are identified;

- General barriers, which are similar to those seen in the general population in the Health Survey for England (particularly lack of time).
- Barriers that may be common to any patient with chronic disease, such as depression and fatigue (Courneya et al. 2005; Korkiakangas et al. 2009).
- Barriers specific to that patient group, such as fear of hypoglycaemia for patients with type 2 diabetes (Korkiakangas et al. 2009).

Table 1-1 below summarises the barriers reported for the general population, patients with type 2 diabetes, chronic pain, stroke and those who have survived colorectal cancer.

General barriers	Work commitments Lack of leisure time Lack of money Caring for children and older people Lack of local facilities Having no-one to exercise with Not having the right clothes or equipment	(Courneya et al. 2005; Chaudhury et al. 2008; Rimmer et al. 2008; Korkiakangas et al. 2009; Slade et al. 2014)
Barriers related to chronic disease	Fatigue Depression Co-morbidity Physician recommendation	(Courneya et al. 2005; Korkiakangas et al. 2009)
Disease specific barriers		
Type 2 diabetes	Being too overweight Unwilling to exercise with people who don't have diabetes Fear of hypoglycaemia Lack of physician advice Lack of personal knowledge	(Korkiakangas et al. 2009)
Chronic pain	Diagnostic uncertainty Fear of movement/pain aggravation	(Slade et al. 2014)
Survivors of colorectal cancer	Non specific treatment side effects Surgical complications Diarrhoea Nausea Mouth sores Blood clot	(Courneya et al. 2005)
Stroke	Exercise won't improve my condition Don't feel trainer in facility is able to help Not comfortable exercising in facility	(Rimmer et al. 2008)

Table 1-1 Summary of barriers to exercise identified in the general population and other disease groups

It is important to examine barriers to exercise in specific populations; as is shown in Table 1-1, patients with one disease may have different barriers to those with another. Even amongst patients with diabetes there may be different barriers; being overweight has been associated with increased barriers to activity in adolescents (Deforche et al. 2006) and while many patients with type 2 diabetes are overweight, this is not true of patients with T1D.

Little is known about the attitudes and barriers to exercise in T1D. Two studies of patients with T1D in Canada (Dube et al. 2006; Brazeau et al. 2008) identified fear of hypoglycaemia as the strongest barrier to regular exercise in T1D. Questionnaire studies, such as these Canadian studies, may identify important barriers to increased physical activity but provide little explanation and cannot identify strategies to overcome them. A qualitative interview study performed in the UK, by our group, suggests that although fear of hypoglycaemia is a factor when patients with T1D consider exercise, external factors , such as lack of time, work pressures and bad weather were greater barriers to physical activity (Lascar et al. 2014). This finding is in common with the studies described above in patients with type 2 diabetes and other chronic diseases where general barriers to exercise were more frequently cited than disease specific ones. No studies have looked at patients with newly diagnosed T1D.

1.8 Potential mechanisms involved in beta-cell preservation in T1D

The effects of exercise on the endocrine system include an increase in levels of growth hormone and glucagon-like peptide 1. These, in conjunction with a rise in

certain interleukins (for example IL-1 and IL-6) are thought to improve beta cell mass (Narendran et al. 2015).

Exercise is recognised to have an anti-inflammatory effect as well as effects on the immune system (Gleeson 2007) and, therefore, may slow the loss of beta cells. Exercise alters levels of adipokines, cytokines secreted by adipose tissue, in particular, it induces an increase in adiponectin concentrations (Ben Ounis et al. 2009). The anti-inflammatory and glucose lowering effects of adiponectin have been well described (Mantzoros et al. 2005; Yamauchi et al. 2002; Berg et al. 2001) and both are mechanisms by which beta-cell function may be preserved in T1D.

We have chosen to examine the effect of exercise on adiponectin receptor expression and T cell immunity in T1D as part of this study. Previously our group (Pang 2010) has demonstrated that adiponectin influences T cell proliferation in health but not in T1D and that adiponectin is involved in suppression of T cell migration across endothelial cells (Chimen et al. 2015). The final part of this study aims to investigate whether exercise up-regulates adiponectin receptor expression, whether this occurs in T1D, and whether this translates into changes in T cell proliferation and endothelial transmigration.

This study is a multi-centre randomised clinical trial designed to investigate effect of a year long exercise programme on beta cell function and autoimmunity in patients newly diagnosed with T1D.

2 HYPOTHESIS AND AIMS

My hypothesis is that exercise preserves beta cell function in adult patients with newly diagnosed T1D.

My aim is to collect the data required to design and undertake a formal trial to determine whether exercise preserves beta cell function in adult patients with newly diagnosed T1D

My specific objectives are:

1. To evaluate the barriers to exercise in patients with newly diagnosed T1D.
2. To determine whether patients newly diagnosed with T1D can be encouraged to take up and adhere to a programme of at least moderate intensity exercise for a period of one year, and monitor the change in exercise behaviour in the non-intervention arm.
3. To determine whether adherence to this programme of exercise preserves beta cell function.
4. To investigate the effect of exercise on immunity in patients newly diagnosed with T1D.

I will achieve these objectives through the EXTOD study which is outlined below.

3 EXTOD (EXERCISE IN TYPE ONE DIABETES) STUDY DESIGN AND PRIMARY OUTCOMES

3.1 Introduction

The Extod (Exercise in type 1 diabetes) study was a mixed methodology pilot study to investigate recruitment, retention and adherence to an intervention aimed at increasing physical activity in adult patients newly diagnosed with T1D. It is intended that the results of this trial will go on to inform a larger study, adequately powered to assess the effect of exercise on beta cell function in adult patients with T1D.

This chapter describes the design of the Extod study and discusses the primary outcomes of recruitment and retention to an exercise intervention study in T1D.

3.2 Methods

3.2.1 Study overview

The study was designed in two parts. Firstly, a qualitative study (Phase 1); to investigate attitudes to exercise, current exercise behaviours and thoughts on trial design with participants potentially eligible for an exercise trial. Subsequently, a randomised controlled trial (Phase 2) with an intervention aimed at increasing exercise levels in a group of participants newly diagnosed with T1D.

3.2.1.1 Phase 1

Phase 1 was designed as a qualitative study to evaluate the attitudes and barriers to exercise in adult patients with newly diagnosed T1D, as well as to discuss trial design and conduct with potential and actual trial participants. This part of the study is discussed in detail in Chapter 5.

3.2.1.2 Phase 2

Phase 2 was designed as a multicentre randomised controlled trial. Participants were randomised in a 1:1 ratio to either an intervention aimed at increasing physical activity or usual care. The primary outcomes were to assess recruitment and drop-out rates as well as uptake and adherence to additional exercise in both the intervention and control groups. The secondary outcome was to evaluate the effect of increased exercise on beta cell function in adults recently diagnosed with T1D.

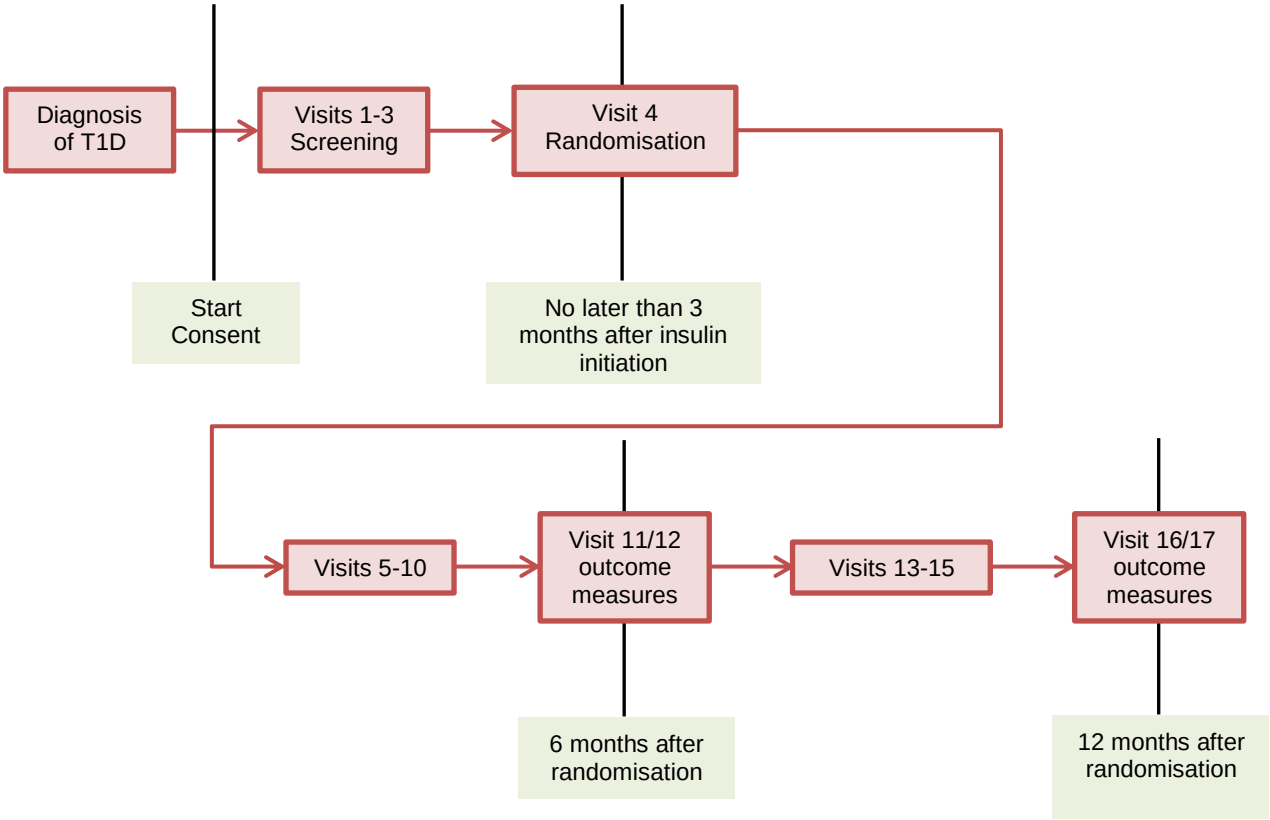

Figure 3-1 - Schematic overview of trial

3.2.2 Phase 2 design

Participants were randomised into an intervention group and a control group in a 1:1 ratio. Randomisation was minimised on the basis of site ("West Midlands" and "Other"), fitness and 90 minute stimulated C-peptide. Randomisation occurred at visit 4 after baseline and screening data had been gathered.

3.2.2.1 Intervention

The intervention consisted of additional support aimed at increasing exercise levels through the use of motivational interviewing techniques, additional contact with the study team to review exercise diaries and goals, and additional support and

information with regards to the management of blood glucose and insulin around exercise. Motivational interviewing is a method of encouraging participants to set their own goals with the aim of increasing their physical activity levels (Miller 1996). Motivational interviewing has been demonstrated to be effective at changing behaviour in many areas of lifestyle including exercise participation (Hettema et al. 2005).

Exercise interventions in clinical trials are often supervised. This allows trial personnel to observe and record the amount of exercise undertaken by each participant and to control/increase the intensity of work during the study in a controlled manner. It is however, labour intensive for study personnel, less amenable to multi-site studies and may restrict participants to certain hours to undertake their exercise programme. In addition, results obtained in this way may not be achievable outside of a clinical trial setting, where individuals must motivate themselves to exercise.

The aim was to use an intervention that could be replicated in clinical practice, setting negotiated and realistic targets. Participants were asked to self-monitor their physical activity levels by recording exercise in a diary and encouraged to discuss the diary with the research nurse at each visit. The exercise diary also recorded exercise intensity which was measured by the use of a wrist worn heart rate monitor (Polar Electro UK) provided by the study. A small number of these monitors had the facility to record this information and be downloaded to allow later correlation with the exercise diaries. Using the heart rate monitors, participants were asked to increase their exercise intensity to a target of 75% estimated maximal oxygen uptake.

All members of the study team involved in delivering the intervention attended a two-day motivational interviewing techniques course (provided by Dr Melvin Hillson, Exeter University) and were given the opportunity to attend face-to-face training on the management of T1D around exercise as well as provided with a written guidance document on this (both developed by the study team).

Participants were encouraged to increase their exercise duration and intensity over twelve weeks according to a graded program (Appendix C). Any activity could be undertaken and all activities took place outside of the hospital setting. The aim of the intervention was to increase and maintain physical activity to at least 150 minutes per week, aiming for 240 minutes per week of vigorous intensity exercise. Those participants who did not reach the goal of 150 minutes exercise per week were offered additional support, for example, assistance with gym membership, supervised exercise classes (through exercise on prescription) or advice from a personal trainer.

3.2.2.2 Usual care

The visit schedule for participants in the usual care arm was arranged to mimic normal diabetes care. Additional visits were necessary to perform the assessments of beta cell reserve (through a mixed meal test) and fitness. This group did not, however, attend the additional visits aimed at increasing activity and did not receive information about insulin and carbohydrate management and exercise routinely (unless this discussion was initiated by the participant). The printed information booklet was only offered to participants randomised to the intervention arm.

3.2.2.3 Trial Procedures

3.2.2.3.1 Dietary advice

All participants received standardised dietary advice at visit 4, prior to randomisation. The study dietitian assessed the patients' current level of understanding against a standardised list of competencies agreed by the trial team. A list of the competencies is given in Appendix D . Further sessions were scheduled with the dietitian if required.

Following randomisation, the intervention group were given further information specifically relating to carbohydrate intake and insulin dose adjustment around exercise. A copy of the written advice given to participants can be found in Appendix E .

3.2.2.3.2 Glycaemic control and insulin regime

Intensive glycaemic control has been demonstrated to protect patients with T1D from microvascular complications (The Diabetes Control and Complications Trial Research Group 1993). In addition, glycaemic control seems to influence beta cell decline (Dost et al. 2007; Greenbaum et al. 2009). Therefore, during the study we aimed to maintain optimal glycaemic control within both study groups (target HbA1c <48mmol/mol where appropriate (as per National Institute of Clinical Excellence Clinical Guidance 15 (National Institute for Clinical Excellence 2004)). All diabetes management was undertaken by the study team to ensure that treatment was standardised across all sites.

All participants had to agree to treatment using a multiple daily injection (MDI) insulin regime or continuous subcutaneous insulin infusion. This was to facilitate both the achievement of optimal glycaemic control and insulin dose adjustment during exercise for those randomised to the intervention group. Those participants not already taking MDI insulin had their insulin regime changed during the screening phase (i.e. prior to randomisation) and maintained on this throughout the study.

Participants both groups were asked to self-monitor their blood glucose using their own glucometer. This information was recorded in diaries and collected by the study team at each visit. The blood glucose diary was reviewed by the research nurse at each visit and advice given on insulin dose adjustment to maintain glycaemic control.

3.2.2.4 Outcomes

3.2.2.4.1 Primary outcomes

The primary outcomes for this study were:

1) the proportion of patients with T1D who started the intervention.

   Data were collected at each site regarding numbers of patients diagnosed with T1D, those approached with information about the study and the reasons for not entering the study (if given).

2) the proportion of participants in the intervention arm who adhered to the required intensity of exercise.

This was discussed at each visit with the participants in the intervention group. It was assessed by means of exercise diaries, heart rate monitoring (including a number which recorded data and were downloaded), accelerometer data, as well as, fitness testing at baseline, six months and twelve months.

3) the proportion that dropped out.

Participants who withdrew or were lost to follow-up were recorded and the reason for withdrawal (if given).

4) the rate of exercise uptake in the non-intervention arm

Participants in the control group were asked to wear an accelerometer for one week every three months during the study. During this week, they also completed a diary, to capture all activities, including swimming, which cannot be recorded on an accelerometer (which is not waterproof). In addition, a fitness test was performed at baseline and after six and twelve months so that VO2max could be estimated.

3.2.2.4.2 Secondary outcome

The secondary outcome for the study was to investigate the rate of loss of beta cell function.

This was assessed by mixed meal test at baseline, after six months and twelve months. The mixed meal test is the recommended test to assess beta cell function in

T1D trials (Greenbaum and Harrison 2003). The protocol for this test was based on that used by the UK islet transplant service (personal communication from S Eckholdt, 2011).

3.2.3 Data collection

3.2.3.1 Recruitment data

All sites (Participant Identification Centres (PICs) and full treatment sites) kept and returned an anonymised log of patients diagnosed with T1D. The logs recorded the outcome of any approach inviting the patient to enter the research study. The logs were collected centrally and collated to produce the Consort diagram.

3.2.3.2 Clinical data

Weight, height, blood pressure, bioimpedance, resting heart rate, waist and hip circumference were measured at baseline and after six and twelve months. These measurements were standardised between study sites by the use of standard operating procedures.

Waist circumference was measured at the midpoint between the lowest rib and the iliac crest and hip circumference at the level of the greater trochanter (World Health Organization 2008). The measurements were taken (to the nearest centimetre) three times and an average of the three readings was used for analysis. Waist-hip ratio (WHR) was calculated from these.

Height was measured to the nearest centimetre using a stadiometer and weight was measured using a calibrated set of scales or stadiometer to the nearest kilogram.

Body fat percentage was measured using a calibrated stadiometer (this was not available in every site).

Body mass index (BMI) is calculated from weight and height measurements (BMI = weight/height^2).

Blood pressure and resting heart rate was measured three times (at each visit) after 5 minutes of rest using a calibrated automated blood pressure monitor. The averages of each of systolic and diastolic blood pressures (SBP and DBP) were used for analysis.

Information about diagnosis, date of transfer to insulin, mode of presentation and family history of diabetes were collected during the screening study visits as recommended (Greenbaum and Harrison 2003).

3.2.3.3 Physical activity measures

It is difficult to accurately assess behaviour, such as physical activity. One concern is that it is altered by observing behaviour, an example of observer bias, when an individual is aware that they are entering a trial of physical activity, consciously or unconsciously, activity levels are altered. Different methods have been used to assess physical activity in trials with exercise interventions. These include subjective measures, such as self-report, activity diaries and questionnaires, and/or objective measures, such as accelerometer data or supervised exercise programmes. Additionally, surrogate measures such as change in fitness (either measured or estimated VO2max) are often used. Each of these has advantages and

disadvantages, subjective measures in particular may be subject to recall bias, however none are ideal and often multiple measures are used.

In order to assess the best way to evaluate physical activity in a trial of patients with T1D, a number of ways of assessing physical activity were used at baseline:

1. Participants were asked to evaluate how much exercise they undertook each week before and after their diagnosis of T1D. The question asked was

   'On average, how many times in a typical week did you participate in moderate intensity exercise and how long were these periods in minutes?'

2. Participants completed a questionnaire to evaluate their current level of activity (International Physical Activity Questionnaire (IPAQ) Long Last 7 Days Self-Administered Format) (Craig et al. 2003).
3. Participants were asked to wear an accelerometer (GT3XE-Plus Triaxial Activity Monitor, Actigraph, USA) for a week before visit 1 and a week after visit 1. During this time they were asked to also keep a diary of any physical activity undertaken.
4. Estimated VO2max was calculated using a cycle ergometer using two methods (Astrand-Ryhming and YMCA/ACSM) (American College of Sports Medicine 2000). The method had to be safe for patients and utilise available equipment in clinical sites, therefore, we chose a submaximal test and estimation of VO2max from heartrate rather than direct measurement of VO2max (as the equipment for this was not available in most centres). A detailed description of the procedure is given in section 3.2.3.6.

3.2.3.4 Monitoring physical activity during the study

Physical activity during the study was assessed in the following ways:

3.2.3.4.1 Self-report

At each visit, participants in the intervention group were asked if they had achieved their target physical activity levels

3.2.3.4.2 Diaries/heart rate monitoring

Participants in the intervention arm only, were asked to complete diaries recording the type and length of exercise they undertook each week. They were provided with a heart rate monitor (FT4/FT7, Polar Electro UK Ltd) and asked to record their average heart rate for each session as well as their rating for perceived exertion (RPE) (Borg 1998).

3.2.3.4.3 Accelerometry

All participants (both in the intervention and control groups) were asked to wear an accelerometer (Actigraph models GT3X and GT3XE-Plus Triaxial Activity Monitor, Actigraph, Actigraph, Pensacola, Florida, USA) during waking hours for one week before visits 8, 11, 14 and 16 (corresponding to 3, 6, 9 and 12 months) (in addition to the baseline measures). During this time they were asked to also keep a diary of any physical activity undertaken. Accelerometer measured activity has been validated with measured oxygen consumption (VO2max), showing excellent correlation (Freedson et al. 1998). Data from the accelerometer downloads is given as the number of minutes spent in moderate-vigorous physical activity (MVPA).

3.2.3.4.4 Fitness testing

Fitness was estimated through VO2max using a cycle ergometer using the same protocol at baseline, 6 months and 12 months (described in section 3.2.3.6).

3.2.3.5 Laboratory data

3.2.3.5.1 Routine laboratory studies

Routine laboratory studies for patients with T1D include HbA1c and lipid profile. HbA1c was measured every three months during the study to aid achievement of optimal glycaemic control. Lipid profile was measured every six months. These samples were analysed in local NHS hospital laboratories as per usual diabetes care.

3.2.3.5.2 Autoantibodies

Serum from blood taken at visit 1 was sent for analysis of three autoantibodies associated with T1D. Measurement of antibodies to glutamate decarboxylase (GADA), the protein tyrosine phosphatase islet antigen (IA-2A) and zinc transporter 8 (ZnT8A) were analysed at the Research Laboratories, School of Clinical Sciences, University of Bristol, UK (Southmead Hospital, Bristol. The methodology of this assay has been previously described (Bingley et al. 1997; Long et al. 2011)

3.2.3.5.3 Mixed meal test

Samples of plasma, serum and urine obtained during the mixed meal test were analysed for C-peptide, glucose and insulin at the Clinical Biochemistry laboratories of the Royal Devon and Exeter NHS Foundation Trust, Exeter, UK. Insulin and C-peptide were measured using a direct electrochemiluminescence immunoassay. The methodology of this assay has been previously described (McDonald et al. 2012).

3.2.3.6 Testing of fitness and beta cell function

3.2.3.6.1 Estimated VO2max

Fitness was assessed by two methods during a single exercise test (Astrand-Ryhming and YMCA/ACSM) (American College of Sports 2000). Heart rate was measured during the test using a Polar heart rate monitor (FT4 model, Polar Electro UK). Calibrated cycle ergometers were used for this test, the make and model used varied between sites, depending on availability, but participants used the same ergometer throughout the study to minimise inter-subject variability.

Method 1

Participants were asked to cycle at a fixed workload for six minutes and the average heartrate in the last two minutes was recorded. If the average heartrate was less than 120bpm the test was repeated at a higher workload, if it was between 120 and 140bpm the participant carried straight on to part 2 of the test and if it was above 140bpm, the test was discontinued.

The estimated VO2 max was then calculated by the use of a nomogram (American College of Sports 2000).

Method 2

In this part of the test the workload was progressively increased at the beginning of three minute stages. In each stage the workload and average heartrate of the last 30seconds were recorded. The increments continued until the participants had reached 85% of their age-predicted maximal heart rate (calculated as 220-age).

These readings were plotted on a graph and the maximum workload predicted at maximal heart rate. Estimated VO2 max was then calculated using the following equation:

VO_2 max ($ml \cdot kg \cdot min^{-1}$) = 3.5 + 3.5 + (1.8 x workload max/body mass)

3.2.3.6.2 Assessment of beta-cell function

The recommended test for measurement of beta-cell function in trials in early T1D is the mixed meal tolerance test (MMTT) (Palmer et al. 2004)(Greenbaum et al. 2008). Classically the oral stimulus (meal) used is liquid Sustacal (Boost), however, due to difficulties with the supply of this, a measured dose of Fortisip (Nutricia, Wiltshire, UK) was used following the protocol used by the UK Islet Transplantation Consortium (personal communication from S Eckholdt, 2011).

Some sources advocate the use of a four-hour rather than a two-hour MMST (Greenbaum & Harrison 2003) as the peak C-peptide release in T1D can occur later. However a two-hour test was chosen in this study to reduce the time burden on participants and as this is the recommended test.

Participants were asked to fast from midnight and to withhold any insulin doses on the morning of the test (basal insulin was not withheld). Capillary blood glucose was checked prior to the start of the test and the test was rescheduled if the reading was not between 3.5 and 15 mmol/l.

A stimulus in the form of 240mls Fortisip was given orally, and blood samples were collected at -10, 0, 15, 30, 60, 90 and 120 minutes. Additional fasting samples were taken for glucose, insulin. The samples were spun and frozen at -80°C for later

analysis. Analysis of all these samples was performed at the Biochemistry Department of the Royal Devon & Exeter Hospital, UK.

The MMTT takes at least two hours to perform and must be undertaken in a setting where venepuncture/cannulation is available as well as facilities to process the samples once taken. More recently there has been interest in development of surrogate markers of beta-cell function which are easier to obtain, fasting C-peptide and urinary C-peptide:creatinine ratio (UCPCR) have both been used. UCPCR has been demonstrated to have a good correlation with meal-stimulated C-peptide in patients with T1D (R. E. J. Besser et al. 2011). At the end of the MMTT, the participant was asked to provide a sample of urine. This sample was frozen and stored for later analysis for UCPCR to compare with the traditional MMTT.

3.2.4 Data and Statistical Analysis

3.2.4.1 Sample size calculations

It was calculated that a minimum of 60 participants (30 in each study arm) were required to provide data to achieve the primary outcomes. We predicted a 30% recruitment rate (95% CI 22% to 39%), a 90% exercise adherence rate (95% CI 76% to 97%) in the intervention arm and a 15% drop out rate (95% CI 5% to 30%).

Our study estimated a confounding effect of 10% increase in exercise in the ‘usual care’ group with a 95% confidence interval of 2 to 27%.

These calculations were performed by Roger Holder at the Birmingham Primary Care Clinical Trials Unit.

3.2.4.2 Calculations and analyses

The majority of data are presented descriptively, for example, mean and standard deviation or 95% confidence interval (95% CI). Where data are not normally distributed, median and range are presented and these are identified in the text. Graphs represent mean ± standard deviation (unless specified otherwise).

To assess for associations, Pearson's correlation coefficient is used. Non-parametric data has been log-transformed prior to analysis or Spearman correlation is used, where this has been done, it is specified within the text of the thesis.

Groups have been compared using Student's t test and Chi-squared analysis or the non-parametric equivalent. Two way repeated measures ANOVA was used for longitudinal data

Mean area under the curve (AUC) was calculated for meal stimulated C-peptide. This is the recommended model for use in clinical trials in T1D (Palmer et al. 2004; Greenbaum & Harrison 2003). AUC was calculated using the trapezoid method (Allison et al. 1995) using GraphPad Prism software (GraphPad Software Inc., USA). Mean AUC was calculated by dividing AUC by the length of the test (120minutes).

Homeostasis Model Assessment (HOMA) was used as an estimate of insulin resistance (HOMA-IR), insulin sensitivity (HOMA-S) and beta cell function (HOMA-B) respectively. The online HOMA2 calculator was used (Wallace et al. 2004) available from https://www.dtu.ox.ac.uk/homacalculator/download.php (last accessed 27th July 2016).

Accelerometer download data was evaluated by Professor Ashley Cooper, Centre for Exercise, Nutrition and Health Sciences at the University of Bristol, UK.

Data were analysed using GraphPad Prism 7.00 (GraphPad Software Inc., USA).

3.2.5 Trial conduct

3.2.5.1 Participant identification

Patients with a diagnosis of T1D within the previous three months were recruited from secondary care providers between April 2011 and February 2014. Nineteen hospital Trusts across the UK were involved either as identification centres or full treatment sites. The centres involved were:

- **University Hospital Birmingham NHS Foundation Trust**
- **University Hospital Bristol NHS Foundation Trust**
- **Taunton & Somerset NHS Foundation Trust**
- **Gloucestershire Hospitals NHS Foundation Trust**
- North Bristol NHS Trust
- Yeovil District Hospital NHS Foundation Trust *
- **East and North Hertfordshire NHS Trust ***
- **Mid Yorkshire Hospitals NHS Trust ***
- **Oxford University Hospitals NHS Trust ***
- **Royal United Hospital Bath NHS Trust ***
- Weston Area Health NHS Trust
- Sandwell and West Birmingham NHS Trust
- The Royal Wolverhampton NHS Trust

- The Dudley Group of Hospitals NHS Foundation Trust
- Worcestershire Acute Hospitals NHS Trust
- George Eliot Hospital NHS Trust
- Heart of England NHS Foundation Trust
- Royal Devon & Exeter NHS Foundation Trust
- Walsall Healthcare NHS Trust

Those sites marked in bold were full treatment sites, the remaining sites were patient identification centres (PICs). Sites indicated with an * joined the study in autumn 2012 as a means of improving recruitment rates.

The study was publicised by Diabetes UK on their website and in their magazine from May 2012, and on the diabetes.co.uk and Juvenile Diabetes Research Foundation website from July 2013.

Prospective participants were also identified through the ADDRESS-2 study from April 2013. This study aimed to recruit patients with recently diagnosed T1D and their relatives to provide a database of potential participants to early intervention trials. The ADDRESS-2 study team contacted participants with details of the Extod study and information on the contact details of their nearest study site.

3.2.5.2 Eligibility criteria

Subjects were eligible for the study if they satisfied the following inclusion and exclusion criteria:

3.2.5.2.1 Inclusion criteria

The inclusion criteria were that participants should be

1. aged between 16 and 60 years.
2. randomised (or interviewed) within twelve weeks of diagnosis.
3. safe to exercise (as determined by the physician).
4. willing to self-monitor blood glucose and record the results.
5. willing and able to take insulin as part of a multiple dose injection regime.
6. sedentary (exercising for no more than 150 minutes a week according to self-report) (later changed to able to increase their current levels of exercise (as judged by the participant)).
7. detectable C-peptide value 90mins after meal stimulation (defined as >200 pmol/l) (not required for interview study)
8. GAD antibody positive (later removed as an inclusion criterion).

3.2.5.2.2 Exclusion criteria

1. uncontrolled blood pressure (greater than 180/100 mmHg)
2. concomitant therapy that affects heart rate (beta blocker, Calcium channel antagonist) as this would affect the ability to estimate VO2max and monitor exercise intensity using heartrate monitors
3. psychological or physical disease that prevents exercise
4. pregnancy or planning pregnancy (as preconception and diabetes in pregnancy care both require specialist input)
5. major surgery or other planned event that would prevent exercise for more than six weeks

3.2.5.3 Randomisation

Participants were randomised in a 1:1 ratio to intervention or usual care groups. Randomisation was stratified by site and minimised on 90 minute stimulated C-peptide level and estimated VO2max. As the expectation was to recruit 60 participants, with approximately half coming through the West Midlands sites and half from the other sites, site was defined in two groups; 'West Midlands' and 'other'. The minimisation criteria were 90minute stimulated C-peptide above or below 600pmol/l and estimated VO2max, criteria set on gender specific levels (above or below 42.8 $ml.kg.min^{-1}$ (males), 34.6 $ml.kg.min^{-1}$ (females)), these were determined by Dr Keith Stokes/Dr Dylan Thompson (Sports Sciences Department, Bath University, personal communication).

Randomisation was organised and supervised through the University of Birmingham Primary Care Clinical Trials Unit, using an on-line randomisation programme with a telephone service used as a back-up. Randomisation was performed by the study dietitian at visit 4, after standardised dietary advice had been given.

3.2.5.4 Blinding

Due to the nature of the study, it was not possible for study participants or trial personnel to be blinded to the allocation.

3.2.5.5 Adverse event reporting

Serious adverse events were notified immediately to the study team and discussed with the Chief Investigator. All adverse events identified at study visits were logged and recorded within the trial database. Hypoglycaemia incidence in the month prior to the visit was specifically identified and recorded in the trial database.

Adverse events were discussed at the trial management and steering committee meetings.

3.2.5.6 Visit Schedule

An overview of the visits is given in Table 3-1. There were a total of seventeen visits. Randomisation occurred at visit 4. Post-randomisation there were eight visits for all participants and a further five for those in the intervention arm only. The additional visits scheduled for participants in the intervention arm could be undertaken over the telephone or in person; other visits had to take place in person. All participants met with a study doctor at baseline, at six months and at the end of the study (after twelve months).

Visit	Action	Time (wks)	Usual care	Intervention
1	Consent and screening		✓	✓
2	Mixed meal test, baseline		✓	✓
3	Fitness test, baseline		✓	✓
4	Randomisation, dietitian visit	0	✓	✓
5	Nurse visit	2	✓	✓
6	Nurse visit or telephone	4		✓
7	Nurse visit or telephone	8		✓
8	3 month clinical assessment by diabetes nurse	12	✓	✓
9	Nurse visit or telephone	16		✓
10	Nurse visit	20	✓	✓
11	Mixed meal test and clinical assessment, mid-point	24	✓	✓
12	Fitness test, mid-point	24	✓	✓
13	Nurse visit or telephone	30		✓
14	9 month clinical assessment by diabetes nurse	36	✓	✓
15	Nurse visit or telephone	42		✓
16	Fitness test, final	48	✓	✓
17	Mixed meal test and clinical assessment, final	48	✓	✓

Table 3-1 Overview of study visits

3.2.5.7 Storage of samples

Samples of serum and plasma were stored at -80°C at the study site immediately after processing, with the exception of two sites that did not have access to a -80°C freezer. These sites (Mid Yorkshire and East & North Hertfordshire) processed the samples according to the SOP and then sent them overnight to the University of

Birmingham where they were stored at -80°C. All unused samples were eventually transferred to the University of Birmingham laboratories for ongoing storage/analysis.

3.2.5.8 Data recording and retention

3.2.5.8.1 Case report forms (CRFs)

Data gathered at each visit was recorded on visit specific CRFs. This enabled inter-person and inter-site standardisation of data gathering and avoided missing data. Each site retained the original CRFs with copies being held centrally. Records will be stored for five years.

3.2.5.8.2 Study database

All visit data were entered by trial personnel onto a database, specifically designed for the study. The database was held on the servers of the University of Birmingham Primary Care Clinical Trials Unit. Access was password protected. The data were monitored throughout the study and missing or invalid data queried with the appropriate site.

3.2.5.8.3 Standard operating procedures (SOPs)

Standard operating procedures for each visit, measurement (blood pressure, waist/hip circumference and bioimpedance) and all tests (mixed meal test, fitness test, venepuncture and sample processing) were used by all sites. These are not provided in the appendix due to space limitation.

3.2.6 Ethical and regulatory matters

A favourable ethical opinion was granted by Birmingham, East, North and Solihull Research Ethics committee in February 2010 (reference number 10/H1206/4). The same committee reviewed and approved an amendment in August 2010; subsequent amendments in 2011-13 were reviewed and approved by National Research Ethics Service Committee West Midlands - Solihull. The final amendment was approved in November 2013 and relates to protocol version 8. The project and amendments were approved by the research and development departments of the participating hospital trusts.

Amendment No.	Date approved	Summary of amendment
4	Oct 2011	Revised patient documentation approved
5	Jan 2012	Continuous glucose monitoring, Yeovil and Bath to full study sites
9	Nov 2012	Alteration to inclusion criteria (GAD antibody and existing exercise levels)
10	Apr 2013	Approaching patients through ADDRESS-2 study, financial incentive to PIC site staff for recruitment
11	Nov 2013	Addition of the option for patients completing the study to enter a follow-up phase

Table 3-2 Summary of amendments with dates

The study is registered with ICRCTN (International Standard Registered Clinical/soCial sTudy Number), registration number ISRCTN91388505.

3.2.7 Study oversight and monitoring

The study was overseen by a management committee and a steering group. The management committee met approximately every six months during the trial period. The management committee was composed of members of the research team, staff from the University of Birmingham Primary Care Clinical Trials Unit, as well as patient representatives. The steering group met once a year to discuss trial conduct and progress.

3.2.8 Sponsorship

The study was sponsored and indemnified by the University of Birmingham.

3.2.9 Funding

Funding for the study was provided by a National Institute for Health Research, Research for Patient Benefit grant.

4 EXTOD TRIAL PROGRESS

4.1 Review of trial

4.1.1 Trial progress

All eligible patients (as defined in section 3.2.5.2), identified at the participating centres, were invited to take part in the study. Recruitment to Phase 1 (qualitative study) opened in April 2011 and to Phase 2 in November 2011. Once recruitment to both was open, those patients unwilling or unable to take part in Phase 2 were offered the opportunity to take part in the qualitative study.

The final participant was recruited to Phase 2 in February 2014 and randomised in March 2014.

4.1.2 Recruitment

Recruitment to Phase 2 commenced in November 2011 and the first patient randomised in January 2012. Progress of recruitment over time until completion of recruitment in February 2014 is shown in Figure 4-1 below.

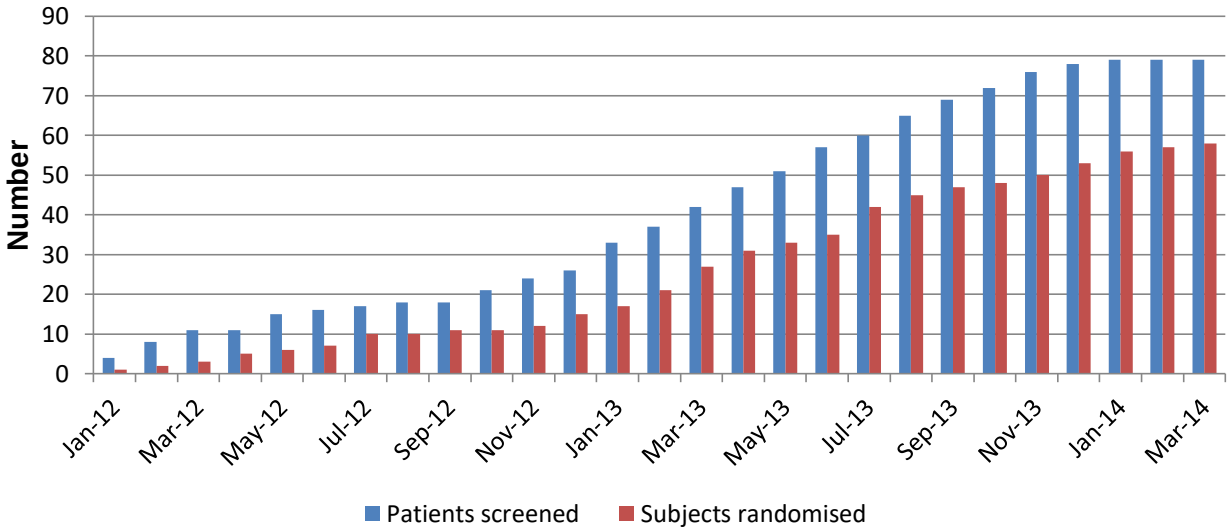

Figure 4-1 - Recruitment to Phase 2 of Extod study over time

Initial recruitment (up to summer 2012) was slower than originally anticipated. Due to this, several changes to the study were implemented at the end of 2012. The effect of these changes can be seen in Figure 4-2, with randomisation rates increasing from approximately one per month in 2012 to three per month in 2013.

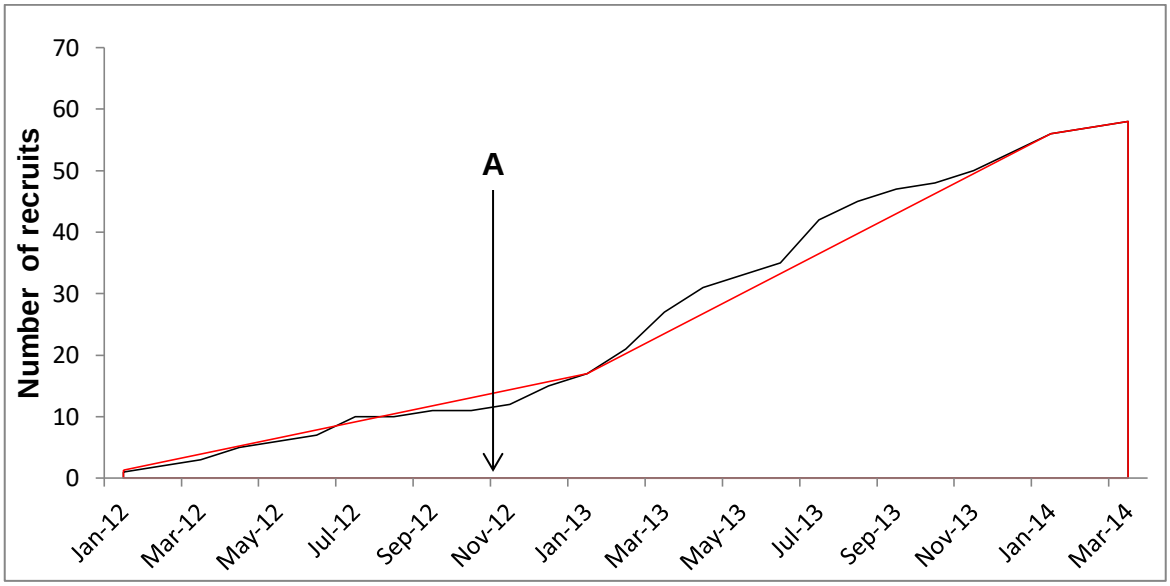

*Figure 4-2 - Recruitment to Extod during study period. The black line indicates cumulative recruitment numbers (to Phase 2). **A** indicates when inclusion criteria were altered. The red line indicates average recruitment rates for each calendar year and demonstrates the increase in recruitment from January 2013 onwards.*

4.1.3 Review of study

Recruitment to Phase 2 was reviewed after 4 months. It was apparent that the recruitment target would not be achieved in the original timeframe. As such, a review of the study was undertaken and several changes to the study were implemented to improve recruitment rates. These are discussed below.

4.1.3.1 Additional sites

Five additional treatment sites were added to the study in September 2012, these were the East & North Hertfordshire NHS Trust, Mid Yorkshire NHS Trust, Oxford

University NHS Trust, Yeovil District Hospital NHS Foundation Trust and Royal United Bath NHS Trust. Bath and Yeovil had been operating as Patient Identification Centres (PICs) until this point in the study.

4.1.3.2 Alteration in the eligibility criteria

Two of the inclusion criteria were altered to reduce the number of people who failed screening. These are described below.

4.1.3.2.1 Diagnosis of T1D

The requirement for participants to have GAD antibodies detectable in their serum was removed; instead, the diagnosis of T1D was based on clinical judgement. There were a number of reasons for this change;

1. Outside of research projects, T1D is predominantly a clinical diagnosis and autoantibodies only measured if there is doubt in the diagnosis. Removing this criterion makes the study more generalisable to the clinic population of patients with T1D.
2. A significant proportion of people with a clinical diagnosis of T1D do not have detectable levels of GAD antibodies in their serum (i.e. GADA negative). The proportion of patients with T1D who are GAD positive has been estimated at around 80% (Long et al. 2012).
3. The two week turnaround time for the GAD antibody analysis proved difficult to fit into a randomisation target of twelve weeks from diagnosis.

4.1.3.2.2 Current levels of physical activity

During the review of the study, it was found that self-reported physical activity time was poorly correlated with the results of either the International Physical Activity Questionnaire (IPAQ) or the measured accelerometer data. Therefore, this criterion was altered to include people who were willing and able to increase their current levels of activity.

4.1.3.3 Use of Internet to advertise study

The study was included on the Diabetes UK and Juvenile Diabetes Research Foundation research webpages with contact details for the investigators in Birmingham. In addition, Diabetes UK featured the study in their "Balance" magazine. Potential participants were linked with their closest site and any travel expenses incurred were reimbursed.

In addition, the study was publicised on the Diabetes.co.uk website. Diabetes.co.uk were payed a small fee for each participant identified in this way and who fulfilled the study entry criteria.

4.1.3.4 Incentives for Patient Identification Centres (PICs)

Referrals from PICs were initially much lower than anticipated (and far lower than potential participants identified at full study sites). For every participant who attended a screening visit or interview, the referring team was sent an Amazon voucher as an incentive.

4.1.4 Impact of changes to study on recruitment

All of these changes were introduced at around the same time (August-October 2012); therefore, it is difficult to assess the individual impact each of these changes had on recruitment. However,

1. The additional sites accounted for 16 of the 89 participants who attended visit 1 and 13 of the 58 randomised participants.
2. Six screened participants contacted the study as a result of the internet/“Balance” magazine advertising.
3. Prior to the PIC incentive scheme (approximately the first year of recruitment) five participants attended a screening visit or interview. In the second year of recruitment (after the Amazon vouchers were introduced), fifteen participants were recruited from PIC sites.

Overall, from the start of recruitment to Phase 2 until the end of recruitment at the end of December 2013, 507 patients were identified as potentially suitable for the study. This takes into account patients identified at all treatment sites, PICs (where data has been made available) and through the ADDRESS-2 database and diabetes.co.uk website. A breakdown of outcome for all of these potential participants is shown in the CONSORT flow chart in Figure 4-3.

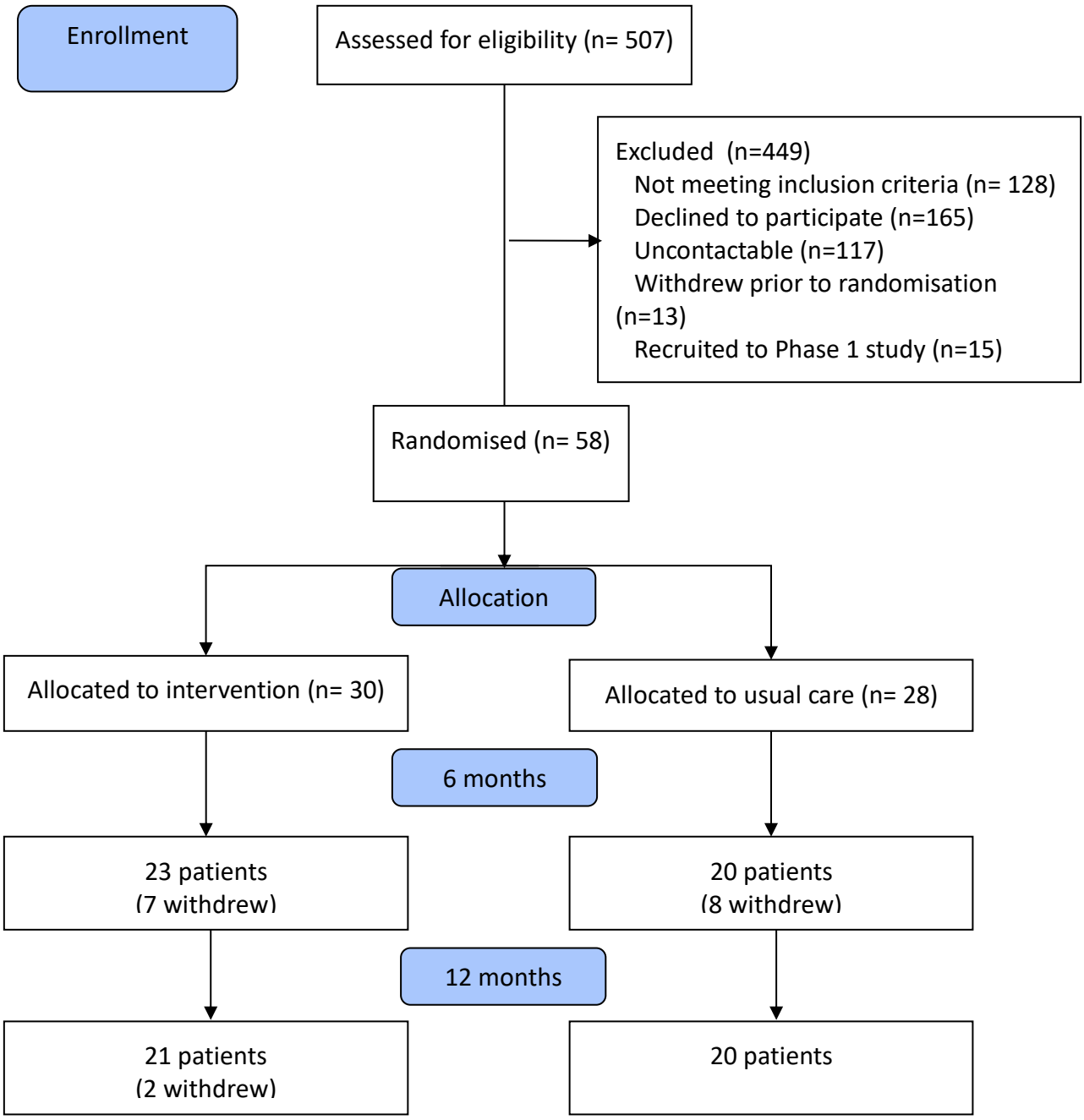

Figure 4-3 - CONSORT Flowchart for Extod Study

The recruitment rate for the study (including Phase 1 and Phase 2) was 14.4%. Once Phase 2 had commenced, potential participants who were ineligible for Phase 2 (for example, because they were leaving the country or GAD antibody negative)

were offered the option of taking part in Phase 1. The uptake rate for the exercise intervention study was lower at 11.4%.

4.1.4.1 Reasons for non-entry into study

The most common reason for potential participants not to continue into the study was that they declined (165/507, 32.5%). Most of these gave no reason, but of those that did give a reason, the most common was lack of time, either to attend study visits or increase their exercise levels.

A further 25% (128/507) were found to not be eligible once contacted by the study team. There were a number of reasons for this (shown in Table 4-1). The most common reasons that potential participants were found to be ineligible for the study was late referral to the study team and that patients did not have T1D (i.e. they actually had a diagnosis of type 2 diabetes or steroid induced diabetes). Before the inclusion criteria were broadened at the end of 2012, a few potential participants were excluded as they had already been found not to have GAD antibodies present or they were already very active. Reasons categorised under 'other' in Table 4-1 included patients who were felt to be clinically unsuitable by the clinical team responsible for their care or who were having difficulty accepting their diagnosis.

Reason for ineligibility	Number
More than 3 months since diagnosis	31
Other	27
Not diagnosed as T1D	23
Doing more than 150mins/week exercise	16
Out of age range (>60 years old)	12
Unable or unwilling to exercise	10
GAD antibody negative	6
Planning pregnancy	3

Table 4-1 - Exclusions based on eligibility criteria

4.1.5 Drop-out

Of the 58 participants randomised, seventeen withdrew from the study or were been lost to follow-up. Fourteen of these participants withdrew soon after randomisation, with three continuing on until the six-month assessments, before withdrawing. The dropout rates at six and twelve months (of those participants who were randomised) were 24% and 29% respectively. There was no difference in the dropout rates between the study arms; a similar number of participants withdrew from both arms (9 from the intervention and 8 from the control group). Most withdrawals (11/17) were within one month of randomisation.

There were several reasons for withdrawal from the study. Three participants lost contact with the study team despite all attempts to contact them (by telephone, email or post). Two participants were no longer eligible for the study, one as subsequent

testing (performed due to family history and antibody status) proved positive for monogenic diabetes (HNF-4α mutation) and another as they subsequently enrolled in another intervention study (and potentially received a medication affecting beta cell function). The other seven participants withdrew.

4.1.5.1 Completion rate

Overall 54 participants completed either Phase 1 or Phase 2 out of 89 who attended for screening. The overall study completion rate of those who consented was 60%. Despite the small numbers, this was similar across most study sites.

Site	Attended	Interviewed	Randomised	Completed Phase 2
QEHB	21	6	14	13
WM PICs	14	2	8	5
Taunton	10	1	8	5
Other entry routes	9	0	6	2
Bristol	8	1	5	3
Wakefield	8	1	6	3
SW PICs	6	2	1	1
Gloucester	5	2	3	1
Welwyn	4	0	4	4
Oxford	4	0	3	2
Total	**89**	**15**	**58**	**39**

Table 4-2 - Study completion rates stratified by treatment site

However, participants who were identified through other routes (self-referrals through the internet, those identified through the ADDRESS-2 study, for example) were less likely to complete; only two of these nine participants did so. Participants identified in Welwyn and at QEHB were more likely to complete (100% and 90% respectively).

4.1.6 Adherence to study arm

Adherence to the exercise program and uptake of exercise in the control arm will be discussed in Chapter 7.

4.1.7 Return rates of participant diaries/activity monitoring

At specific time points during the study the participants were asked to complete and return several diaries. Return rates for the food diaries were 60% at each of the three time points, and for the sleep diaries the rates were 60, 70 and 65%, at baseline, after 6 months and after 12 months.

Adherence to the activity monitoring fell slightly during the study, 86% returned a worn accelerometer at baseline, at 6 months the proportion was 74% and at 12 months, 71%.

4.1.8 Adverse events

4.1.8.1 Hypoglycaemia

Two episodes of severe hypoglycaemia were reported; one each in two participants. One episode occurred in each arm of the study.

There was no significant difference in minor hypoglycaemia events reported between the groups. Participants experienced approximately one episode of minor hypoglycaemia per week. The rates of hypoglycaemia reported are given in Figure 4-4. Rates of hypoglycaemia were highest at entry to the study and fell by three months in both groups. There were small, non-significant increases in hypoglycaemia reported at towards the end of the study in the intervention group (mean number of hypoglycaemia episodes 2.35, 95% CI, 0.015 to 4.54, in the control arm and 3.38, 95% CI, 0.80 to 5.96, in the intervention arm at twelve months).

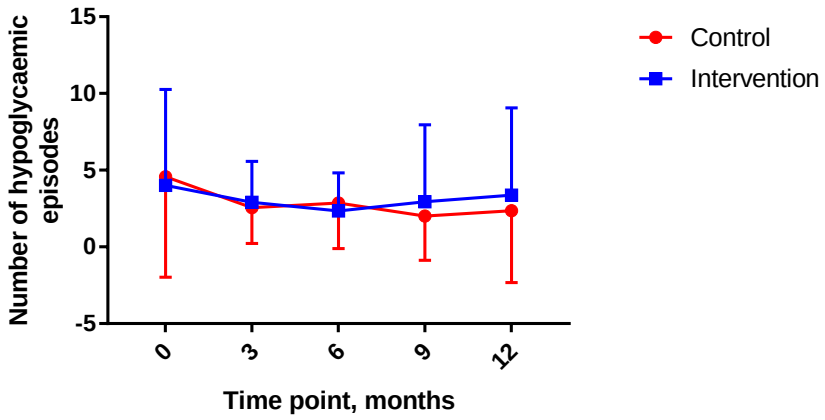

Figure 4-4 - Prevalence of hypoglycaemia during the study. Mean number of hypoglycaemia episodes (capillary blood glucose <4.0mmol/l) reported in the previous month.

4.1.8.2 Other adverse events

4.1.8.2.1 Serious adverse events

Two serious adverse events occurred during the study, both relating to hospital stays. One participant in the intervention arm required an overnight stay unrelated to either diabetes or exercise, the other, in the control arm, was admitted due to hyperglycaemia.

4.1.8.2.2 Adverse events

Overall, adverse events were reported more frequently in the intervention group than the control group (2.9 events per participant in the intervention arm and 0.9 events per participant in the control arm). The majority of the events were minor illness or injuries not requiring any medical advice (e.g. viral illnesses). Difficulties relating to diabetes were also reported more often in the intervention group, particularly hyperglycaemia and missed insulin doses.

Illnesses and injuries requiring medical assistance ranged from dental infections to patella bursitis and skiing accidents. Again, these were more frequently reported in the intervention arm.

4.2 Discussion

This chapter describes the Extod study design, as well as, the primary outcomes of 1) recruitment and 2) study completion rates. The results of Phase 1, other primary outcomes (adherence and exercise uptake in the control arm) and the secondary outcome (the effect of exercise on beta cell function) are described in later chapters.

4.2.1 Primary outcome 1: Recruitment/Uptake

Recruitment to the study did nearly achieve the target of 60 participants randomised, although it took over twice as long as anticipated and required a further three treatment sites to achieve this. In addition, widening of the inclusion criteria and incentivising referrals from PIC sites were necessary. These changes to the study did appear to improve recruitment rates.

In order to randomise 58 participants (and interview 15), over 500 patients were identified. This gives an uptake rate of under 15%. The largest group of people not taking part in the study were those who declined. The study was not designed to pick up all the reasons for this but possible reasons for the low uptake are given below:

- Age of participants. Generally participants were of working age and difficulty fitting visits research around work may have been an issue
- Difficulty adjusting to diagnosis. Participants were in the main previously healthy and had to come to terms with the illness and treatments that were required, to take on research at this time may have been too much.
- Logistical issues due to the limited number of sites.

Suboptimal recruitment of patients to clinical trials is a common problem, with around half of RCTs failing to recruit to target and half of trials who achieve their target numbers, failing to recruit in their originally anticipated time frame (Wilson et al. 2000; Bower et al. 2007; Raftery et al. 2008; Toerien et al. 2009). It is difficult to assess how the Extod trial uptake compares to other T1D trials due to differences in reporting, intervention and study population. For example, one study with a T1D population but with a differing intervention (five-day course) had uptake rates as low as 17% (DAFNE Study Group 2002). Conversely, a recent RCT examining the effect of metformin in adolescents with T1D with a yearlong intervention reported that 163 patients were screened to achieve 140 randomised participants (Libman et al. 2015), giving an uptake rate of 86%. However, this study did not report the total eligible population as we have done here. A study with a similar intervention but different population, the Early Actid study, a trial of lifestyle intervention over 12 months in type 2 diabetes, had a recruitment rate of 36% (593/1634) (Andrews et al. 2011).

Changes made to the study protocol did improve recruitment rates; but as they were all made at the same time, it is not possible to say which changes had the greatest effect. Broadening the inclusion criteria and adding more sites, however, did help identify more potentially eligible participants. Much of the research into identifying successful strategies to improve recruitment to RCTs concentrates on incentives for and characteristics of potential participants (Treweek et al. 2010; McDonald et al. 2006). There is some evidence that factors related to the study design and research team, for example, complexity of the study may also factor in recruitment problems (Fletcher et al. 2010). Therefore, any future study should be designed with this in mind.

4.2.2 Primary outcome 3: Study completion rates

Drop-out rates were high (14/58 by six months and a further three by twelve months). The completion rates were lowest in those participants identified outside of the study sites (via internet or other studies). It is possible that distance from the study site may play a part in retention, (those participants identified at the study site were likely to be local). Other reasons for withdrawal included problems relating to management of diabetes (for example, not wishing to monitor blood glucose and difficulty complying with insulin treatment) and loss to follow-up. The loss to follow-up may in part be due to the fact that our study population was young and more mobile.

In a meta-analysis of thirteen studies involving exercise interventions T1D, we identified completion rates of between 70-100% (Kennedy et al. 2013). None of the studies included in this analysis had a duration of over 6 months. The Extod study completion rate of 70% is at the lower end of this range, however, the duration of follow-up was twice as long as the next longest study.

4.2.3 Adverse events

Hypoglycaemia was not recorded at a higher frequency in the exercise arm. This is reassuring and suggests that the advice given for insulin dose adjustment and carbohydrate intake around exercise was adequate. In addition, episodes of severe hypoglycaemia were rare and occurred only once in each study arm. This population of patients newly diagnosed with T1D are probably relatively protected from severe hypoglycaemia as their counter-regulatory hormone response is affected only later in the disease process.

The apparent increase in non-hypoglycaemia related adverse events in the intervention arm may be due to a number of factors.

Firstly, the study was designed so that the intervention group had more frequent contact with the study team (eleven post randomisation visits compared to six). Actually the number of post randomisation visits that took place and during which data regarding adverse events was recorded (including the absence of adverse events) was nearly double on average (7.8 in the intervention arm and 4.1 in the control group).

There may have been a bias towards reporting adverse events in the intervention group as the study was not blinded. Therefore, particularly musculoskeletal injuries were perhaps more likely to be remembered and reported to the study team.

4.2.4 Recommendations for future studies

It is likely to be necessary to recruit a greater number of participants for a definitive study investigating the effect of exercise on beta cell function in T1D. Consideration to the number and location of sites and more flexibility around the randomisation time (i.e. a longer eligibility period) might help improve recruitment rates from the point of view of the participant. In addition, reducing study design complexity, through careful review of the pilot, may help study teams to recruit more successfully.

Equally, consideration of the factors affecting retention in the study to improve the completion rates will reduce the numbers needed to recruit. Recruiting only from the population local to study sites (rather than nationally via the internet or through distant PICs) may help retention rates.

A further qualitative study involving both the participants who completed and those who did not is planned to investigate the reasons behind both recruitment and retention issues.

5 ATTITUDES AND BARRIERS TO EXERCISE IN PEOPLE WITH A RECENT DIAGNOSIS OF T1D – QUALITATIVE STUDY

5.1 Introduction

The aim of this phase (Phase 1) of the Extod study was to explore the attitudes and barriers to exercise in adults recently diagnosed with T1D using an interview format. The outcomes of this study would inform the design of Phase 2 of the Extod study, exploring the ways to motivate patients with newly diagnosed T1D to increase their activity levels. The currently available knowledge of exercise in patients with T1D and other chronic diseases has been outlined in the introduction section 1.7.

5.2 Methods/Materials

5.2.1 Recruitment

Participants identified as potentially eligible at one of the participating sites (see chapter 3) were approached by a member of the team (doctor, diabetes nurse or dietitian) at their local site and sent a participant information sheet. All participants gave written informed consent.

Prior to November 2011, all eligible participants (satisfying the clinical eligibility criteria) were offered an interview/focus group. After this time, potential participants were offered the option of taking part either in this part of the study or the RCT. In

addition, those participants who were screened out of, or prior to randomisation withdrew from, the RCT were offered the opportunity to participate in the interview study (e.g. as they were found to be negative for GAD antibodies).

5.2.2 Interviews

Participants attended either an individual interview or a focus group. The format varied according to patient numbers/availability and geographical location. Due to the sporadic nature of diagnosis/recruitment, wide geographical recruitment area and the time limitation (i.e. wishing to interview within approximately three months of diagnosis) it proved very difficult to arrange adequate focus groups with all potential participants as initially intended. Therefore, six months into recruitment (during which time only one focus group with three attendees had been arranged), it was decided to offer individual interviews.

Interviews took place either face-to-face or by telephone, depending on patient preference and geographical location. Interviews were semi-structured and lasted between 30 and 60 minutes. Areas of discussion included current and past levels of exercise, understanding of exercise and exercise guidelines, barrier to increasing exercise levels and preferences for monitoring of activity in a trial setting (full interview schedule is given in Appendix H). Prior to commencing the interview, participants were asked to report the amount of moderate-intensity exercise undertaken in a week both before and after their diagnosis of T1D. In addition, they completed a questionnaire to evaluate their current level of activity using the

International Physical Activity Questionnaire (IPAQ) Long Last 7 Days Self-Administered Format.

Participants were interviewed until saturation was achieved.

5.2.3 Analysis

The interviews were recorded and transcribed verbatim by a transcription company. The transcripts were checked and anonymised where necessary. Data analysis was on-going during the collection period to enable full exploration of themes identified in earlier interviews and to identify when saturation had been achieved. Saturation occurs when further data collection does not add any new information to that already gathered (Mason 2010). The data were explored thematically (Ritchie & Lewis 2003).

Themes and a coding frame were developed by reading and re-reading of the interview transcripts and through discussions between members of the research team (Amy Kennedy, Dr Parth Narendran and Dr George Dowswell). Analysis was assisted by the software package N-Vivo 9 (QSR International, Victoria, Australia).

Coding was an inductive process, that is, the codes were generated through reading and re-reading the interview transcripts rather than specified prior to analysis. A combination of coding techniques were utilised, to fit the data. First cycle coding methods used were a mixture of in vivo, initial coding, process coding. For second cycle coding, a combination of axial and pattern coding was used (Saldaña 2008).

Interviews were then analysed using a framework approach to further examine identified themes. The framework method is a type of qualitative content analysis, where the data is summarised into a matrix. The rows of the matrix represent the

cases and the columns the codes. This facilitates constant comparison of the data both within and between subjects. Framework analysis of qualitative interviews is commonly used in the development of themes from semi-structured interviews (Gale et al. 2013).

The use of quantities to represent data in qualitative research is fiercely debated (Maxwell 2010). We have used them in selected instances in this study, however, it is important to avoid putting emphasis on these and recognise that these proportions cannot be generalised beyond the group studied here.

5.3 Results

5.3.1 Participants

Eighteen people expressed an interest in the interview study. Of these, fifteen participants were interviewed. Of the three who did not participate, one person withdrew their consent prior to interview, and the other two did not attend at the scheduled time/weren't available at the scheduled time and it was not possible to contact them or reschedule the interview in the eligibility period.

The demographics of the interviewees are given in Table 5-1 below. The majority were male (11 out of 15), which is a similar proportion to that found later in Phase 2 of the study. The median age of the group was 29 years with a range of 18-53 years. Twelve participants described themselves as of White-British ethnic origin and eight

were recruited through the West Midlands sites. The median length of time from diagnosis to interview was 66 days.

Participant	Age	Gender	Centre	Ethnic origin	Interview Format	Reported exercise, mins/week
A	44	M	Bir	Asian or Asian British – Indian	FG, Face-to-face	60
B	22	F	Bir	White – British	FG, Face-to-face	120
C	53	M	Bir	White – British	FG, Face-to-face	0
D	51	M	Bir	Black or Black British – Caribbean	I, Face-to-face	0
E	24	M	Bir	White – British	I, Face-to-face	60
F	37	M	Tau	White – British	I, Face-to-face	150
G	20	M	Glou	White – British	I, Face-to-face	30
H	23	M	Brist	White – British	I, Telephone	0
I	50	M	Bir	White – British	I, Telephone	40
J	20	F	Wake	White – British	I, Telephone	240
K	47	F	Glou	White – British	I, Telephone	360
L	18	M	Bir	White – British	I, Telephone	90
M	39	M	Tau	Mixed - White and Black African	I, Telephone	
N	29	F	Bir	White – British	I, Telephone	50
O	19	M	Brist	White – British	I, Telephone	240

Table 5-1 - Demographics of individual participants for Qualitative study. (FG = Focus group, I = Individual Interview)

5.3.2 Themes

Five main themes were identified. These were:

1. Exercise context (attitudes to and current and previous exercise behaviour)
2. Diabetes (impact of diagnosis and knowledge)
3. Consequences of exercise
4. Barriers to increasing exercise, and
5. Confidence (in exercising and managing diabetes).

5.3.2.1 Theme 1 - Exercise context

All participants were already doing some form of exercise even those who had reported doing 0 minutes on the initial questionnaire. Most wished to increase their activity levels; given that an inclusion criterion was that they should be willing to increase activity levels it is surprising that three (F, G and L) did not. Five participants reported a reduction in the amount of time they spent exercising, and seven had changed the type or reduced the intensity of activities they were doing since their diagnosis with diabetes (see Table 5-2).

Participant	Previous activities	Current activities
A	Jogging, rope skipping, playing football	Walking while at work (4-5hrs a day)
B	Walking at work, gardening, DIY jobs, gym, squash	Occasional gym session, DIY
C	Physical job, gardening, DIY, repairs	None
D	Regular attendance at the gym (cardiovascular and weight training)	Walking
E	Marshall arts/boxing	Active job 2 days a week
F	Walking while at work	Walking while at work
G	Swimming. Jogging	Swimming. Jogging
H	Combat karate	Jogging, some weights
I	Walking/jogging outside	Walking on treadmill
J	Gym	Gym
K	Gardening	Gardening, walking
L	Walking	Walking
M	running	Running
N	Rugby, football, cycling	Cycling on static bike
O	Badminton/golf	Badminton and golf

Table 5-2 - Description of individual participants current and previous activity

Participants were involved in a number of different activities (see Table 5-2). They defined exercise as anything from ‘anything where you are moving’ (Participant K) to ‘something strenuous, not associated with work’ (Participant E). Activity at work and doing household chores/repairs was classed as exercise by some.

Most participants were either unware that there is guidance on the minimum amount of exercise adults should undertake each week or uncertain as to the amount that it is recommended should be taken. Many were pleasantly surprised that recommendations were not higher and felt that they should be able to achieve this even if they were not already doing so. Some felt that a universal guideline was inappropriate as it could not take in the circumstances of the individual and that a personalised target would be preferable.

> *'Because each person should be done individually. And the doctor should say yes, you're capable of doing this. No, you're not...because he'll have your medical records, …….Not the government telling you, you should do this or you should do that.' (Participant C)*

5.3.2.2 Theme 2 - Diagnosis of diabetes

All participants wanted to talk about the impact that their diagnosis had had on them. The most common way that participants described the sudden nature of the diagnosis of T1D was as a '*shock*' (A, D, H, I, K, M and N).

> *'suddenly it comes like this and it's a shock' Participant A*

> *'if somebody is told they're a diabetic, it's a shock' Participant K*

Other ways of describing their feelings at diagnosis were as being '*hit*' and as a '*kick in the teeth*' (both participant C), and feeling '*stunned*' (participant I). Others emphasised the newness of their diabetes and anticipated that it would become easier to manage with time.

Several participants spoke about their diagnosis as a loss of normality (wanting to get back to a 'normal life'), or of role (uncertainty about being able to work).

'I don't want to make my life any different, because it hasn't changed so I want to keep it that way so that I end up controlling the diabetes, or I am at the moment so and I just want to carry on that way.' Participant G

Participants reported four different fears and anxieties related to their diagnosis with T1D. These were concerns about managing new interactions with healthcare services, concerns about the impact on work, concerns for the future and concerns about blood glucose levels. Some participants reported feeling overwhelmed by the amount of contact that they had with healthcare services since their diagnosis.

'Every other week I'm getting different, another letter through with different things which could be related to it' Participant D

'there's too many things going on at the moment, I think for me.' Participant K

For several participants, the diagnosis of T1D had a negative impact on their work. Some had still not gone back to work and were anxious about their ability to cope. One (Participant N) had lost their job.

'I'm quite concerned about going back to work actually. Because I know that I'm going to be on the go all the time and whether I'm going to be able to cope with doing eight hours worth of walking on a daily basis'. Participant B

'That's the problem, going back into a job now, knowing if you can do it.' Participant C

'Well, they laid off me because I was - I, I hadn't been very well anyway' Participant N

Some participants reported uncertainty about their future health. One participant (Participant D) had discussed this with their GP.

'I goes to him [the GP] 'how long are you going to live on it?' He goes 'if you don't look after yourself, he says, five years'. I thought, what! That's a serious thing.' Participant D

it's just nobody has sort of come out and said like, 'This is exactly like, you know, what's going, what's going to happen and stuff like that.' Participant F

Some participants were concerned about blood glucose levels and many were anxious to get optimal glycaemic control. Participants expected that their blood glucose levels would become 'balanced' with time and that they would then be able to keep them within a tight range.

'I'm finding it hard to get that balance of where I can just stay [with level blood sugars] and treating my levels.' Participant B

'I'm trying to say I want it exactly right, I want to get it right, I want to know what I'm doing' Participant K

Importantly, all said that being diagnosed with diabetes had given them additional motivation to exercise than before diagnosis (even those who didn't plan to increase their activity levels).

'I think it's a reason to exercise a little bit more'. Participant J

'it's changed my ethos of taking time to do some exercise in some, you know, going for walks. It's changed my mind, my what I think.' Participant K

'I mean generally the reason most diabetics start, or people in general start doing more exercise is because the fear. At the end of the day I think it's the fear factor of being afraid that if I don't then my life is going to be worse,' Participant E

Twelve participants (all apart from F, G and L) stated that they wished to increase their activity levels, although some had more concrete plans to do so than others.

'I'll be making a lot more than that target in about a months' time, I will be going to do like a martial arts course, I'm going back to China for a year, I'll be living like at a martial arts camp for a year.' Participant E

'but actually, I could do my 10 minutes [bout of exercise], because we do have a room that nobody ever goes into, erm, so I could do that here, and that's a thought, maybe I could consider.' Participant M

5.3.2.3 Theme 3 - The consequences of exercise

The perceptions of the consequences of exercising were mostly positive. The positive outcomes of exercise cited included; health benefits, improved fitness, enjoyment, a feeling of wellbeing and weight loss. Some participants cited benefits of exercise specifically related to diabetes such as lower blood glucose and insulin requirements.

Although health benefits were commonly mentioned as a motivation to exercise, often participants were vague about the nature of these and unable to give specific

examples. A few mentioned positive effects on blood pressure, cholesterol and risk of heart disease.

'in terms of the actual specific benefits, I'm not… maybe one to … be able to rattle them off.' Participant H

Blood glucose lowering was seen to be a positive effect of exercise by some, however, for others this was a negative result as it was associated with hypoglycaemia. Those participants were particularly concerned about the risk of hypoglycaemia and whether this would in fact counteract the health benefits of exercise, both directly as a consequence of hypoglycaemia and also secondary to the need to increase carbohydrate intake.

Participant C in particular felt there was little point in exercising as although he had previously been active, this had not prevented him developing T1D.

5.3.2.4 Theme 4 - Barriers to exercise

Two main sub-themes emerged, medical barriers and the influence of healthcare practitioners (HCPs). In addition individual barriers to increasing exercise mentioned by participants were noted.

Category	Barrier (number of people mentioning)
Medical	Hypoglycaemia (both actual and fear of) (9)
	Lack of knowledge/confidence in managing diabetes (6)
	Fatigue (4)
	Advice from healthcare professionals to stop exercising (4)
	Planning for diabetes (e.g. checking blood glucose/preparing for hypoglycaemia (4)
	Other physical health problems (e.g. injuries) (3)
	Feeling overwhelmed by diagnosis (1)
Time, work and environmental	Work commitments (9)
	Family and other time commitments (6)
	Availability and location of facilities (4)
	Cost (4)
	Weather/season (3)
	Lifestyle (2)
Social and personal	Lack of fitness (3)
	Lack of motivation (2)
	Lack of enjoyment in certain activities (2)
	Laziness (1)
	Previous negative experience of exercise (1)
Psychological	Feeling uncomfortable exercising (e.g. at a gym) (2)
	Feeling scared of exercising on own (2)
	Feeling daunted at prospect of starting (2)

Table 5-3 - Description of barriers to exercise cited by participants. Barriers grouped into the following categories: medical; time, work and environmental; social and personal; and psychological. Within each category barriers are listed in order of frequency.

5.3.2.4.1 Medical barriers to exercise

Most of the medical factors cited as barriers to exercise were related to diabetes. The most frequently cited barrier in this category was hypoglycaemia (cited by nine participants). For some this related to actual experience of hypoglycaemia during or after exercise, however, others were worried about hypoglycaemia but had not yet experienced this. Six participants cited a lack of knowledge or confidence in managing diabetes around exercise. Four people talked about the need to plan for exercise with diabetes, for example, checking blood glucose before and during activity and preparing for hypoglycaemia, as a discouraging factor. Fatigue (which may be related to hyperglycaemia) was cited by four people. Three people talked about other aspects of physical health being a barrier to exercise, they had all experienced an injury.

5.3.2.4.2 Influence of healthcare practitioners

HCP advice could be either positive or negative. Four participants said that they had been advised not to exercise by HCPs.

> *'They advised me to do no exercise basically at the hospital until they felt like I could.'*
>
> *Participant B*

Some participants (who were successfully exercising) described how helpful and supportive (of exercise) they had found healthcare staff.

'I was a bit cautious, erm, about, erm, doing anything to start [laughs] with, really, but I spoke to the nurses and they were just, you know, within reason, they just said, 'Carry on your life as normal,' really' Participant N

'because when I asked about the fact that I go running, 'Yeah, that's brilliant. That's great,'' Participant M

However, one participant who was general positive about HCP support did comment that this wasn't routinely offered.

'my team have been brilliant with me so far, and it's perhaps something I haven't remembered necessarily to ask when I'm there, but at the same time I'm not sure it's that, sort of, offered that freely [right], erm, about exercise.' Participant N

Other participants, however, had received less helpful advice, with four even being advised not to exercise by healthcare practitioners. This was cited by participants from more than one site.

'Because I was asking in the hospital, I kept going, have you got a gym here? 'oh, you've got diabetes, you can't be going to the gym' and stuff like that.' Participant D

'They advised me to do no exercise basically at the hospital until they felt like I could.' Participant B

'I don't think you should be told not to do anything. I really don't' (Participant B). 'No, I think that's wrong. It's negative.' Participant C

Several participants thought that they had been given conflicting advice about exercise and diabetes, and felt that some HCPs weren't well informed about T1D. Participants found this frustrating .

> *'because it seems like, you know, everybody seems to have slightly different things to say about it, whoever I ask.' Participant H*

> *'I also have a problem though, that you've got doctors in a hospital telling one thing to you, not the diabetic team, another doctor telling you you're type 2.' Participant K*

Importantly, those participants who reported doing most activity (J, K and O) were amongst the group who had had positive experiences. Conversely, those participants who reported doing no exercise at all (C, D, and H) had either been told not to exercise or received conflicting advice.

5.3.2.4.3 Individual barriers to exercise

Twenty-one different barriers to increasing exercise levels were mentioned (Table 5-3) most commonly hypoglycaemia and work commitments (nine participants each). Barriers fell into four categories, either external (medical, time, work and environment) or internal (social and personal, psychological). Participants tended to cite a variety of external factors, with only a few discussing internal barriers.

5.3.2.5 Theme 5 - Confidence

The confidence of a participant both in their ability to perform activities and manage their blood glucose around exercise was a major factor in influencing a participant's determination to increase their exercise levels.

> *'well…is there any way I could improve activity levels? I think probably once I get more confident, give me six months, you know I've not been a diabetic six months, I've got to settle in,' Participant K*

When considering confidence, participants described three areas; managing diabetes, exercise and managing diabetes around exercise.

Some participants felt they had little control over their diabetes, or that something had knocked their confidence, whereas others had developed or maintained confidence in their ability to cope with blood glucose fluctuations.

> *'when I was getting my - my insulin right, I was thinking yeah, this is quite simple to do and then it went off the swan, you know, swanny' Participant L*

> *'because I've had this problem where everything has gone a bit odd, for the last couple of weeks, I think it's set me back a bit and perhaps I want to be more confident, I want to make sure I've got my background insulin right' Participant K*

> *I'm a lot more aware of being out on my- even just being out on my own, especially at the beginning, sort of, if I was asked to babysit and I, kind of, went, 'Oh, are you sure you trust me? What if something happens to me?' Participant N*

Some participants lacked confidence in exercising prior to their diagnosis, others weren't sure if there were any special considerations due to their diagnosis.

'I was never good [at exercise] at school' Participant M

Other participants discussed their confidence in exercising now that they had been diagnosed with diabetes.

'my confidence is, I at the moment, erm I've had a couple of sessions when I've been doing gardening and I've said oh, my legs feel a bit wobbly. Then I go and take a reading and then I've realised I'm like 3.5 reading, [right] and that worried me a little bit,' Participant K

'Now I'm just - I'll get on with it like anything else really, but I'll just take in mind that it's something I need to think about when I'm preparing for a session.' Participant E

'I've been given numbers to aim for at the start of exercise, so check before you start and if it's about that then go ahead. If it's a bit lower then have a little snack of something. I've got quite a lot of information about sport.' Participant O

There was a wide spectrum in the confidence levels amongst participants, from those for whom the anxiety around managing their diabetes during activity prevented most physical activities (e.g. participant C) to those who had confidence in their ability to manage their blood glucose and had concrete plans to increase their exercise levels (e.g. participants E and N).

'Nothing's going to override my ambition to do what I want to do.' Participant E

The biggest influences on participants' determination to improve activity levels were motivation and confidence. Participants broadly fell into three groups; those confidently building up their activity levels or who had concrete plans to do so (CONFIDENT), those keen to increase exercise levels but inhibited by their anxieties (mainly relating to diabetes management) (CONCERNED) and those not particularly interested in currently increasing activity levels (AMBIVALENT). Even the participants with high confidence levels had concerns about some aspects of diabetes management.

Several factors emerged that may contribute to an individual's confidence levels. The most important to the majority of participants was information regarding the management of their diabetes around exercise. In addition, time since diagnosis, experience (both prior experience of exercise and experiences since diagnosis) and confidence in and communication with the healthcare team were also important. Many participants mentioned information and education about the management of blood glucose during exercise in this context.

While many participants felt that they had received inadequate information about the management of diabetes around exercise, some felt that they had got all the information they needed and one felt that they had had more than enough information.

Information people said that they needed ranged from which exercises were suitable for someone with diabetes and which to avoid, to what to expect with blood sugars during exercise, to information on the benefits of exercise to people with T1D.

'I need more explanation of - into things, what you can do and what you can't do.' Participant C

'Erm so yeah, as I say, if I was better informed about what exercise could do to blood sugar levels, then maybe I'd have got back into it quicker.' Participant H

'Yeah I wasn't aware, I thought that, as soon as I did exercise it would happen immediately as well, that my sugars would drop and then I'd go funny - so I'd thought I'd be fine the first time I went to the gym, because I thought oh, feel fine, after I'd done it, and then a couple of hours later I'd had a hypo, as I didn't realise, nobody told me that that would happen as well.' Participant B

'so I assume err that there are, there are some benefits to it.' Participant H

'Educating them that they understand the benefits of exercise; that maybe will encourage them to do it, really.' Participant M

Prior experience of exercise and experiences of exercise since diagnosis with diabetes could have either a positive and negative impact on participants' confidence (see 5.3.4 Case studies in this chapter). For example, participants with previous positive experiences of exercise (e.g. D, E and N) were more confident than those who hadn't (e.g. M) and those who had experience problems with hypoglycaemia or performance since diagnosis (e.g. B) were also less confident.

The relationship of the participant with their healthcare team was important with some participants getting a lot of support and information (e.g. N, O) and others having negative experiences such as being advised not to exercise (B, C, D),

information about activity and blood glucose management not being forthcoming (B) and getting different messages about diabetes from different healthcare practitioners (e.g. generalist verses specialist personnel) (participant K).

Several participants felt that the information/knowledge about how to manage diabetes during exercise was out there but just not accessible.

> *'Information. Because I mean Olympic athletes are doing it, so they must have some kind of regulatory system that they know about that helps you while you're exercising. I mean that would be helpful to disseminate that information' Participant D*

> *'I mean like yeah, if, if there was some like, you know, stuff like perfect rule book for if you do X amount of this type of exercise, you know, your blood sugar might be changing by such amount, or something like that.' Participant H*

It was important to participants that they be seen as an individual and not as a 'person with diabetes'.

> *'because diabetes affects everybody in different ways. It's got to do.' Participant C*

Some participants stressed the importance of being treated as an individual when planning education or giving advice (A, B, C, E, H, N, and O)

> *'Yes, yeah, because I mean I know, I know it's a different case for every person and all that sort of thing' Participant H*

'that's just going to be an average of a load of research of a load of different people, but not everybody would fit into that category.' Participant B

Both the content and the timing of that advice were felt to be important.

'I mean I've - trying to like get a group together of like, you know, different people with all different sorts of backgrounds and lifestyles whatever and then trying to do something that's maybe like acceptable for all of them, when maybe some were quite fit beforehand and others [yeah] weren't at all, then err yeah, erm that could be a bit tough.' Participant H

Group	Participants (number)
CONFIDENT	E, H, J, K, O (5)
AMBIVALENT	F, G, L (3)
CONCERNED	A, B, C, D, I, M, N (7)

Table 5-4 – Identifying participants to confidence group

5.3.3 Suggestions to improve activity levels

Participants made a number of suggestions of ways to improve activity levels. A few felt that they would not need any further encouragement or motivation as they had plans in place. Ideas included additional education, supervised or group activity sessions, a programme of gradually increasing exercise, help with goal setting and a fitness advisor. Although some participants had mentioned cost as a potential barrier to increasing activity levels, no-one felt that assistance with this would be particularly helpful.

5.3.3.1 Educational material

Nearly all participants felt that education about diabetes management was vital in helping improve their exercise levels. Some felt that they needed more than they had already been given, while others felt that they had had all they required but this had been important. The participants who were most confident about increasing their activity levels tended to be happier about the information that they had received.

> *'some kind of health organisation to kind of bring forward a website or pamphlet or whatever about people who want to do sports with diabetes type 1 or even diabetes type 2 now and how to deal with certain things and prepare for them.' Participant E*

Some participants (e.g. participant F) felt overwhelmed by the information they had already been given (although this had not specifically included management of diabetes during exercise) and did not want further information at this time, but thought that it might be useful in the future. Other participants were happy with the timing of their education or would have preferred more information sooner.

5.3.3.2 Supervised or group exercise

Many participants suggested an exercise group, with other people with T1D, or supervised exercise sessions, with staff who training in the management of T1D. Having a trainer with specific expertise in T1D was important to most, as several

participants had experienced ill-informed remarks from members of the public, however, generally it was not felt that it would need to be a healthcare professional. One person suggested that although specific expertise in the trainer was desirable, if there was easy access to advice from the healthcare team, it may not be required. The proposal of group activity sessions was not universally liked and was rejected by some, who preferred to exercise under their own steam.

> *'My dad had a heart attack last year and he got help from the hospital and the hospital gym and he was monitored in a way that he could feel confident with going and doing exercise and helping him - help his heart and diabetics don't get that.' Participant B*

5.3.3.3 Fitness advisor

Regular contact with a fitness advisor, particularly one with knowledge of T1D, was suggested by some as a potential motivator to improve activity levels. Even participants who were happy setting their own programme and targets felt that these regular checks would not be unhelpful. Some participants wanted specific advice on a training programme, while others wanted the regular contact and reassurance of someone with greater experience advising them. For some participants, it was very important that the advisor be able to guide them on diabetes management as well as exercise training.

> *'So you could see a nurse at the hospital or see like a fitness erm - fitness expert at a gym because then you're actually at the place you're going to do it, and you're seeing everybody else doing it, so you might go 'I'll do it'.' Participant D*

5.3.3.4 Gradual introduction of exercise

Help in the form of advice on types of activities, and how to build this up was suggested as potentially helpful by some. Others, generally those with previous experience of exercising successfully, felt that this would be unnecessary. In addition, most welcomed the idea of some-one checking up on their progress and thought that they would find this motivating.

> *'I probably would want advice of how if they say I want you to increase erm from 30 minutes walking to an hour walking, or to doing abs in the gym from half an hour to 30 minutes, yeah,' Participant K*

5.3.3.5 Targets

On a similar note, in general participants felt that setting targets for them to achieve would motivate them to increase their exercise more. In particular, if there was a regular check on progress with an advisor.

> *'I find targets very helpful because I know then - I know what I have to try and get to - I know I have to try and [hmm] reach really. [yeah] It's a bit of competition as well.' Participant J*

5.3.4 Case Studies

5.3.4.1 Case study 1 – CONFIDENT exerciser

Participant O

O is already exercising for at least 2 hours a week. He enjoys competitive sports, for example badminton and golf. His main reasons for exercising are the enjoyment that it gives him and the social aspect of playing with other people. Since being diagnosed with diabetes about a month ago, he has felt more committed to ensuring that he takes regular exercise. He has experienced hypoglycaemia during exercise but has subsequently received advice regarding insulin dose adjustment and carbohydrate intake which he had found really helpful. He has also got a good relationship with his healthcare team and knows who to contact if he runs into any further problems. When he exercises he feels that he is prepared and is confident in managing his blood glucose levels.

'you've just got to be prepared for [hypoglycaemia]'

'if you're supposed to do exercise and you've got [type 1 diabetes] then you need to be taught how to manage it'

5.3.4.2 Case Study 2 – WORRIED about exercising

Participant B

B has always been active, she describes herself as ‘never still’. She has an active job, which requires her to be out walking much of the day and in her leisure time she likes to swim, play squash and go to the gym several times a week. She is keen to keep up her activity levels since being diagnosed with diabetes however, she was initially told not to exercise which she feels has adversely affected her confidence. She hasn’t tried to swim or play squash yet as she is worried about the effect this will have on her blood glucose. She has not yet gone back to work and is concerned that she won’t be able to manage on her return.

She has instigated a discussion about exercise with her healthcare team (she did not feel that it was forthcoming) and has tried to exercise on several occasions. She is frustrated however with regular hypoglycaemia and poor performance. She feels that hypoglycaemia is defeating the object of exercise as she has to eat more and is becoming worried that hypoglycaemia may be causing her some permanent harm that she is not yet aware of.

> *‘that was quite scary, the fact that I don't even feel like I can go to the gym and look after myself and then, just in case I have [a hypo], because I'm going to be on my own’*

5.3.4.3 Case Study 3 – AMBIVALENT about increasing exercise levels

Participant F

F feels that he does enough exercise at present. He estimates that the walking he does during working hours adds up to around 2 hours a week but he doesn't do any leisure time activity. The main reason that he doesn't do more is a lack of motivation. He is struggling with his diagnosis and finding it difficult to manage the appointments he is required to attend with his healthcare team. He feels that he has had more information than he is able to process at the present time and this has not included anything specifically about exercise. He is not worried about hypoglycaemia but this may be because he has not yet experienced it.

> *'at the moment I'm not really thinking about it too much, 'cause I'm trying to get my head round it [diabetes] still.'*

5.4 Discussion

This is the first qualitative interview study to examine the attitudes and barriers to exercise in patients newly diagnosed with T1D. We have identified five themes discussed by patients when they are asked about exercise levels. These are; existing attitudes to exercise, feelings about diagnosis, perceptions about the consequences of exercise, barriers to increasing exercise and, confidence in managing blood glucose.

Around half of participants reported a decline in activity levels around the time of their diagnosis. This is an important finding, as if this is true in the wider population of T1D and it is not addressed, patients may be less willing to be active than the general population and not benefit from the health improvements associated with higher levels of activity. It is reassuring that participants wanted to increase their exercise levels as a way to improve their health after a diagnosis of T1D and this should be encouraged. It is possible that following diagnosis, patients are keen to improve their lifestyle, as is seen in studies of cancer survivors (Demark-Wahnefried et al. 2005; Satia et al. 2004), and HCPs could make use of the 'teachable moment'.

In general, exercise was felt to positively impact on health. Some participants were unsure of the benefits or concerned they may harm themselves through exercise. These concerns could be addressed by the healthcare team during diabetes education.

Many of the barriers identified here have been previously identified in healthy people, as well as people with other chronic diseases including those with longstanding T1D

(Brazeau et al. 2008; Plotnikoff et al. 2006; Chaudhury et al. 2008; Korkiakangas et al. 2009; Courneya et al. 2008; Rimmer et al. 2008; Slade et al. 2014). However, our interviewees placed greater emphasis on fear of hypoglycaemia than previous studies of patients with longstanding T1D (Lascar et al. 2014). Furthermore, the finding that some diabetes patients are being advised not to exercise by HCPs has not previously been identified in T1D qualitative studies and was cited by participants from three different sites. Physician advice has been previously mentioned only in the context of survivors of colorectal cancer (Courneya, Friedenreich et al. 2005).

Increasing exercise uptake in patients with type 1 diabetes has clear health benefits and we should be encouraging all patients to undertake at least 150 minutes a week (the minimum level of activity recommended by the CMO) (Chief Medical Officers of England Wales, and Northern Ireland 2011). Those who are already successfully exercising at diagnosis of T1D should be encouraged to continue to do so. For those whose activity levels are suboptimal, the time of diagnosis might be an ideal opportunity to reinforce the health benefits of exercise and effect behaviour change, a ‘teachable moment’.

This study identifies a number of ways in which improvement in exercise levels might be facilitated in patients with newly diagnosed T1D. In this group particularly, it is critical that confidence in managing diabetes around exercise is addressed.

Some interventions that were identified in this study and that may improve the confidence of people newly diagnosed with T1D and facilitate improved exercise levels were; consistent advice from HCPs, support from diabetes teams for exercise, patient education and time to adjust to the diagnosis.

Participants were frustrated by being given conflicting advice or being told incorrect information (e.g. that you have type 2 diabetes) by HCPs. There was an expectation that healthcare professionals will have a certain level of knowledge about diabetes, and this expectation is not being met.

It may be that HCPs are uncertain about advising patients with T1D regarding blood glucose management during exercise. Further work on knowledge and educational needs of healthcare staff was developed as a result of this study and is ongoing.

It may also be that correct advice about management of diabetes around exercise was seen as contradictory. This is not straightforward, and dependent on both the type of exercise undertaken and the length of time spent exercising, getting this information across to patients in a clearer way may be necessary.

Those participants who were successfully exercising reported getting a lot of support from their diabetes team. It is difficult to say whether this was the reason they were successfully exercising or whether because they were exercising they obtained the information that they required. It was suggested by one participant that although the knowledge and support was there in the team it wasn't forthcoming unless you brought up exercise specifically (which patients may not do if they have been advised not to exercise). Diabetes teams should positively encourage exercise from diagnosis with T1D.

A lack of confidence in managing blood glucose levels around exercise was attributed to a lack of information by most people. Resources for patients about blood glucose management around exercise are scarce and although several participants reported searching for these, only one had actually been given any written information. Information on the health benefits of exercise in T1D would have been valued by the majority of study participants.

A significant number of participants talked about the number of appointments that they had to attend since diagnosis, the fact that they were constantly injecting insulin and checking their blood sugar. Their priority was to 'get their diabetes right' before adding more complexity into the mix. Some patients will need more time than others to adjust to their illness. Others will want to get back to normal as soon as possible and felt that exercise would help them regain 'normality'.

This study describes the attitudes and barriers to exercise in adult patients newly diagnosed with T1D; the first qualitative interview study to do so. Recruitment was from five UK sites, covering both large teaching and district general hospitals, and participants spanned a large age range. It has identified new themes and barriers as well as characterising potential interventions to increase activity levels in patients with T1D.

It is likely however that the study participants were more interested in exercise than those who declined and interest in exercise education and management of diabetes around exercise may be lower in the general clinic population.

5.5 Summary

Many barriers to exercise were identified by this group. Some were not particular to diabetes e.g. time, cost and the weather. Other factors were specific to people with diabetes and some specific to, or more significant for people newly diagnosed with diabetes (such as fear of hypoglycaemia and lack of confidence dealing with diabetes).

Exercise should be encouraged from diagnosis (not discouraged and perhaps at this time they are more amenable lifestyle change.

Advice needs to be made available both to healthcare professionals and patients with T1D. Written guidelines for both healthcare professionals and patients have been developed as a result of this work (see Appendix E for participant booklet) and further study into attitudes and knowledge of both groups is underway. We need to help patients develop confidence managing their diabetes both generally and around exercise, this is happening for some patients but not all.

5.6 Recommendations

1. Further research is required into the knowledge and education of both patients with T1D and healthcare professionals regarding the management of diabetes around exercise.
2. The development of educational materials for patients and healthcare practitioners to aid the management of T1D around exercise. As a result of this study, we have developed a patient information booklet which guides

patients through blood sugar and carbohydrate management around exercise in diabetes (this was used Phase 2 and is shown in Appendix E).

3. Ensure easy access for patients with newly diagnosed T1D to their healthcare team especially when trying to increase exercise levels.
4. An individualised approach to exercise advice and content and timing of education of patients with newly diagnosed T1D.

6 BASELINE CHARACTERISTICS OF PARTICIPANTS

6.1 Introduction

The purpose of this chapter is to discuss the characteristics of a group of adult patients with recently diagnosed T1D who joined an exercise intervention trial (i.e. Phase 2) and to review the inclusion/exclusion criteria and randomisation protocols used in the Extod study in the light of these.

6.2 Methods

Methods and analysis are as described in section 3.2.

6.2.1 Participants

Participants were recruited to the Extod Study as described in Chapter 3. The data in this chapter relate to all patients who attended at least one screening visit.

6.3 Results

77 participants attended at least one screening visit.

Three participants who attended screening visits subsequently entered Phase 1. The reasons for this were

- participant's GAD antibody result was negative (n=1),
- participant changed their mind about progressing (n=1) and
- participant decided to plan for pregnancy (n=1).

Sixteen participants withdrew prior to randomisation or were found to be ineligible, leaving 58 people who were randomised.

6.3.1 Demographics

The characteristics of screened participants are detailed in Table 6-1There was a male preponderance in recruited participants. The majority (67/77) of participants identified themselves as White British. Male and female participants were broadly similar in terms of age, ethnicity, family history and cigarette smoking. Men tended to report a higher alcohol intake than women.

	Male	Female	All participants
Number of patients (%)	49 (64)	28 (36)	77 (100)
Age at diagnosis – yrs ±SD			
mean	31.4	32.8	31.9
median	30	29	30
range	17-55	17-57	17-57
Ethnicity – number of patients (%)			
White British	43 (87)	24 (86)	67 (87)
Other	6	4	10 (13)
Other White Black/Black British – African Asian/Asian British – Indian Mixed – White and Black African Mixed – White and Asian Other Mixed Background Other Ethnic background	3 1 0 1 0 0 1	0 1 1 0 1 1 0	
Family history, n (%)	18 (36)	15 (54)	33 (43)
Smoking – number of patients (%)			
Current	9 (18)	6 (21)	15 (20)
Ex	9 (18)	5 (18)	14 (18)
Never	31 (63)	16 (57)	47 (61)
Alcohol consumption (units/wk)			
Mean	5.8	4.5	5.4
Median	4	1.5	2
Range	0-28	0-30	0-30

Table 6-1 - Demographics of Screened Participants in the Extod study

6.3.2 Diabetes presentation

In this group of adults with newly diagnosed T1D, 16/77 (21%) presented in diabetic ketoacidosis (DKA). Those presenting with DKA had a shorter duration of symptoms prior to diagnosis (5.7 vs 13.4 weeks, $p<0.01$). There was no association between presentation at diagnosis of T1D and any of age, gender, ethnicity, BMI or family history.

	Male (n=49)	**Female (n=28)**	**All participants**	**N**
Presentation of diabetes – number of patients (%)				
Hyperglycaemia without acidosis	38 (78)	23 (82)	60 (78)	77
Diabetic Ketoacidosis	10 (20)	6 (21)	16 (21)	77
uncertain	1 (2)	0	1 (1)	77
Length of symptoms before diagnosis – weeks				
Mean	13.4	8.5	11.6	77
Median	8	6	8	77
Range	0-208	1-52	0-208	77

Table 6-2 - Presentation of diabetes

The onset of T1D is more insidious the older the patient is at presentation with patients reporting many weeks of symptoms. There was no statistically significant

correlation between participant's age and duration of symptoms prior to their diagnosis however, there was one outlier reporting four years of symptoms. If this outlier is disregarded, the association between age and duration of symptoms is statistically significant (Pearson r=0.44, 95%CI 0.2375 to 0.605, r^2=0.19) implying a longer duration of symptoms/onset of T1D with increasing age.

6.3.3 Anthropomorphic and biochemical measures

Mean BMI was slightly lower than that seen in the general population (BMI from the Health Survey for England (HSE) 2013, an annual survey of 8000 adults in England, was 27.4 kg/m^2 men and 26.9 kg/m^2 women (Health and Social Care Information Centre 2014)) as was waist circumference (however, both rose with age in the HSE and the Extod study population was younger).

Percentage body fat was higher in women than men.

Blood pressure was lower in women than men and ten participants had a systolic blood pressure (SBP) greater than 135mmHg.

	Male	Female	All participants	N
Waist circumference (cm), mean	89.3	81.3	86.4	77
Waist-to-Hip Ratio, mean	0.93	0.84	0.90	77
Weight (kg)	81.0	67.1	76.0	77
BMI (kg/m^2), mean	25.2	24.7	25.0	77
SBP (mmHG)	126	117	123	77
DBP	76	73	75	77
Resting HR	71	73	72	77
Body fat, %	18.2	30.0	22.7	59

Table 6-3 - Anthropomorphic measures in screened participants at baseline

Glycaemic control was slightly better in women than men at study entry (although the difference was not statistically significant). Serum total cholesterol was similar in both genders.

	Male	Female	All participants	N
HbA1c at baseline (mmol/mol)	80	71	77	73
Total Cholesterol (mmol/L)	4.48	4.41	4.46	62

Table 6-4 - Biochemical measures in screened participants at baseline

6.3.4 Antibody status

75 patients attended at least one screening visit and had antibody status determined. In total, 56 participants had at least one autoantibody detected (74.3%). 49 (65.3%) were positive for GAD antibody (defined as ≥33 DK units/ml), 32 (43.2%) for IA-2A and 29 (39.2%) for ZnT8A. Of the 26 GAD negative patients, 7 had either IA-2, ZnT8 or both antibodies present (3 had both, 3 had only ZnT8A and 1, only IA-2A). These seven participants would have been excluded under the original inclusion/exclusion criteria, but were included in the study following the changes to the inclusion criteria in November 2012.

	Male (n=49)	**Female (n=26)**	**All participants**	**N**
GAD antibody positive – number of patients (%)	30 (61)	19 (73)	49 (65.3)	75
Average titre (in those with positive GADA), GAD units	367.32	540.26	434.38	49
Number with at least 1 positive antibody	34	22	56 (74.6)	75

Table 6-5 - GADA status of screened participants

Out of the 75 participants with autoantibody results, nineteen did not have any of the three autoantibodies detected (25.3%), 20 had just one (26.7%), 16 had two (21.3%) and 20 had all three (26.7%).

Antibody	No with +ve abs, (%)	Mean titre±SD (in antibody positive)
GADA	49 (66.3)	434.4 ± 300.1 IU
IA-2A	33 (44.6)	205.3±145.4 U
ZnT8A	30 (40.5)	43.88±57.62 U

Table 6-6 - Mean antibody titre in participants with positive autoantibodies

Of the participants who did not identify themselves as White British (n=9), 5 were GAD antibody positive, and 3 had multiple antibodies present.

6.3.4.1 Factors associated with positive antibodies

Participants with at least one positive autoantibody were more likely to have a lower waist circumference (p=0.019), systolic BP (p=0.023), diastolic BP (p=0.011) and HOMA-IR (p=0.005). Other factors, such as gender, ethnicity, presentation of diabetes, baseline mean AUC C-peptide, BMI, weight, and estimated VO2max were not significantly different between those who were antibody positive and those who were not.

6.3.5 Baseline Physical Activity

Baseline physical activity measures in screened participants are outlined in Table 6-7.

	Male	Female	All participants	N
Reported time spent exercising prior to recruitment, mins				
Median	45	100	80	67
Range	0-600	0-450	0-600	67
MVPA, mins/week				
Mean ± SD	288.5±153.8	221.2±126.3	288.5±152.9	69
Median	265	205	250	69
Range	22-689	5-76	22-689	69
Estimated VO2max, mls/kg/min, mean ± SD	35.5±8.3	28.8±5.0	33.2±7.9	61

Table 6-7 - Baseline physical activity measures

We wished to determine whether once participants were recruited to the study activity levels increased. Prior to visit 1, participants were sent an accelerometer to wear for one week. This was repeated after visit one. Paired data were obtained in only 17 subjects, however in these there was no difference in MVPA before and after visit 1 (mean MVPA 270.6 minutes/week (95%CI 173.3 to 368) prior to visit 1 and mean MVPA 270.6 minutes/week (95%CI 188.5 to 368.2) after visit 1).

Overall participants reported a fall in exercise after diagnosis (see Figure 6-1D). 29 participants (39%) reported a decrease in the amount of exercise taken per week after diagnosis whereas only 10 reported an increase (14%).

Participants were on the whole active with 53/68 (78%) exceeding 150 minutes MVPA per week at baseline and 35/68 (51%) already doing in excess of 240 minutes

MVPA per week. When asked to provide a figure for the amount of activity they were undertaking, 47/54 (87%) participants underestimated physical activity.

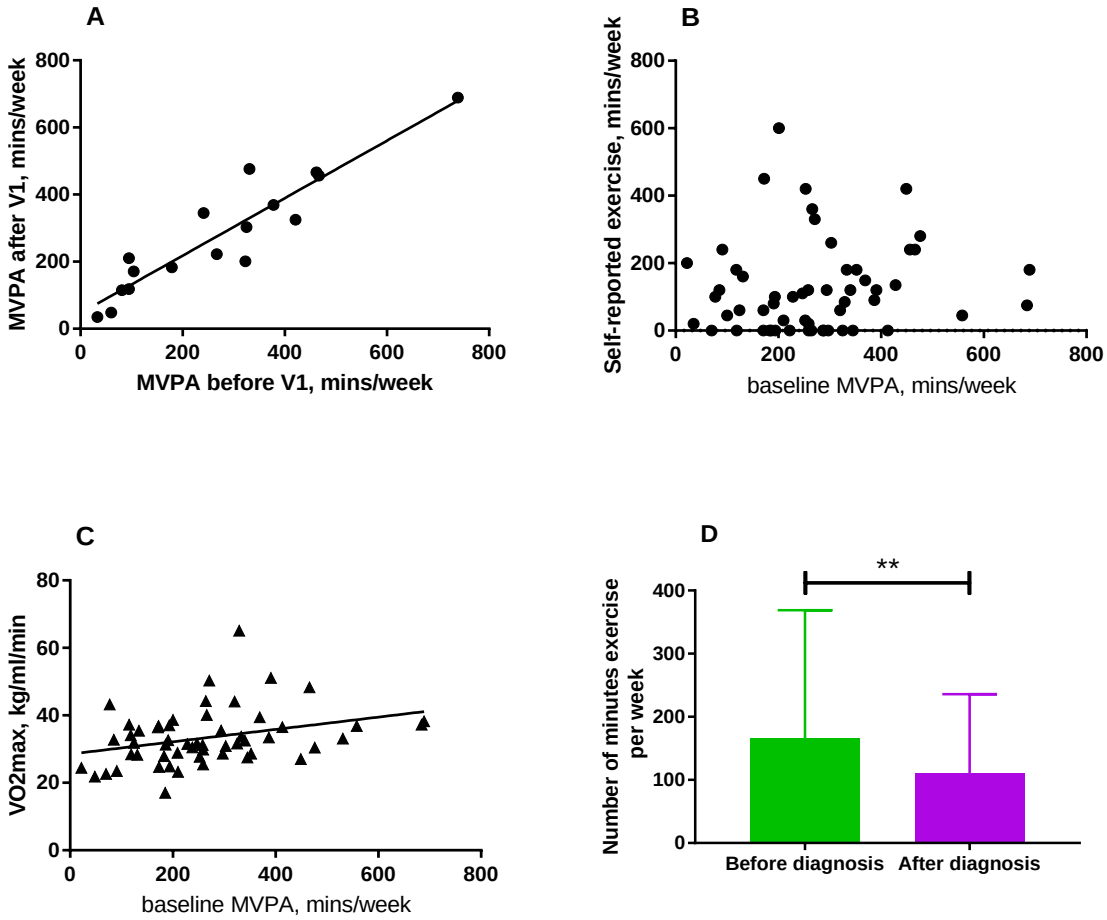

Figure 6-1 – Scatter diagram comparing physical activity measures used in Extod study. (A) MVPA before and after visit 1 (V1) is well correlated (r=0.9279, 95%CI 0.8073 to 0.9741, r^2=0.861 p<0.0001). (B) There is no correlation between MVPA (measured by accelerometer) and reported physical activity. (C) Estimated VO2max and MVPA are correlated (r=0.38, 95%CI 0.1149 to 0.5955, p=0.0049). (D) Participants reported a decline in physical activity after diagnosis with T1D (120 minutes vs 60minutes, median number of minutes exercise per week before and after diagnosis), p=0.0047 (Wilcoxon signed rank test).

6.3.6 Baseline C-peptide

Meal stimulated C-peptide was analysed on 66 participants at baseline. All participants who underwent a MMTT had a stimulated C-peptide more than 200nmol/l at 90 minutes (one of the inclusion criteria).

	Male	**Female**	**All participants**	**N**
Mean 90min C-peptide – pmol/l ± SD	1139±1091	985±385	1086±908	66
Mean AUC C-peptide – pmol/l ± SD	933±775.3	792.4±225	884±659.3	66
Mean urinary C-peptide ± SD	1.69±0.92	2.37±1.14	1.92±1.04	64
Median HOMA2-IR (range)	0.70 (0.16-5.9)	0.58 (0.13-4.2)	0.68 (0.13-5.9)	65

Table 6-8 - Measures of beta-cell function and insulin resistance

One participant had a mean AUC C-peptide greater than 5000 pmol/l (more than twice as high as the next highest participant). This subject was antibody negative and, in retrospect, is likely to have type 2 diabetes.

6.3.7 Randomised patients

The demographic data of randomised participants are given in Table 6-9. There are a number of areas where the two groups are not equal. A lower proportion of antibody positive patients, fewer patients presenting in diabetic ketoacidosis, longer prodromal symptomatic period and fewer smokers have all been suggested to improve the rates of clinical remission, all of these factors were present in the control arm. The higher proportion of female participants in the intervention group, however, is a predisposing factor to slower loss of beta cell function.

It can be difficult to ascertain correctly whether or not a patient has T1D or another form, particularly soon after diagnosis, and particularly in adults who may have an indolent course of diabetes irrespective of which type they have, therefore, the fact that there are more antibody negative, fewer DKA presentations and longer symptomatic prodromal participants in the control arm may represent participants who actually may be better classed as having type 2 diabetes or another form. Indeed one participant, randomised to the control arm, was tested for maturity-onset diabetes of the young (MODY) mutations due to a strong family history and negative autoantibodies, tested positive for the HNF 4α mutation and was withdrawn from the study shortly after randomisation. As demonstrated in section 6.3.6, one participant had a significantly greater mean AUC C-peptide (greater than 5000 pmol/l) than any other participant indicating substantial insulin resistance and likely type 2 diabetes. In addition, three other screened participants who were antibody negative also had a BMI of greater than 35 making type 2 diabetes a possible diagnosis. Two of these were randomised, however, one withdrew shortly after randomisation and therefore, only one completed the study.

	Control (n=28)	Intervention (n=30)
Age – yr		
Mean ± SD	30.81±9.25	33.78±11.44
median	29.84	30.86
range	17-49	17-57
Gender – number of patients (%)		
Male	24 (62)	15 (38)
Female	4 (21)	15 (79)
Ethnicity – number of patients (%)		
White British	28 (56)	22 (44)
Other	2 (29)	5 (71)
GAD antibody positive* – number of patients (%)	14 (35)	26 (65)
Number of patients with at least one autoantibody (%)	17 (61)	27 (90)
Days from diagnosis to randomisation		
mean±SD (days)	84.70±18.71	77.56±20.22
range	45-126	15-115
Weight at baseline (kg)	77.36±15.80	73.5±12.19
BMI	24.69±4.15	24.83±3.40
Mean 90min C-peptide ± SD (pmol/l)	1282±1335	955.1±395.6
HbA1c at baseline (mmol/mol)	75.22±24.77	74.57±24.74
Presentation of diabetes – number of patients (%)		
Hyperglycaemia without acidosis	24 (52)	22 (48)
Diabetic Ketoacidosis	3 (27)	8 (72)
uncertain	1 (100)	0
Length of symptoms pre-diagnosis – weeks (median, range)	8, 0-208	5.5, 1-52
Smoking – number of patients (%)	2 (20)	8 (80)

Table 6-9 - Baseline demographic data for randomised participants

6.4 Discussion

In this chapter I have described the characteristics of adult patients with newly diagnosed T1D who are willing to participate in a trial of an exercise intervention. To obtain fifty eight randomised participants, seventy seven people attended at least one screening visit (approximately 25% were either ineligible or withdrew during the screening visits).

6.4.1 Demographics

There were more male than female recruits. Diagnoses (in children) are approximately equal among both genders in the UK (The DIAMOND Project Group 2006). It is possible that the higher number of male recruits was due to the nature of the intervention, with an exercise intervention study being more appealing to male patients than female patients.

The majority of participants described themselves as of 'white – British' ethnic origin, despite the relatively large ethnic minority populations in some centres (e.g. Birmingham). This is in keeping with the WHO observations in the DIAMOND study that European and North American populations have higher rates of T1D diagnoses than African and Asian populations.

The proportions of patients who smoke were similar to that of the general UK population (Health and Social Care Information Centre 2014).

A similar proportion of participants reported being diagnosed in DKA to that seen in other studies of patients with T1D (21% in Extod vs approximately 25% (Lokulo-

Sodipe et al. 2014; Jefferies et al. 2015). These studies were of children, and did however, report a higher incidence of DKA in very young children, under the age of 4 years.

6.4.2 Anthropomorphic measures

Mean BMI was at the upper end of the normal range in both men and women, however, it was lower than that of the general UK population. There are a number of reasons that this might be the case; prior to diagnosis many patients with T1D lose weight due to insulin deficiency, people willing to enter an exercise trial might be more health conscious than the average population and the age range of the group was lower than that used in the population study.

Mean WHR was in the overweight range for both men and women (World Health Organization 2008).

6.4.3 Antibody status

Antibody positivity in the Extod cohort is lower than has previous been reported. In a population of children and young adults (mean age 10.9 years, range 0.7-20.8 years) the prevalence of GADA, IA-2A and ZnT8 was 79.4%, 78.0% and 70.0% respectively (Long et al. 2012), compared to 65.3%, 44% and 40% in this study. Antibody positivity is more frequent in younger children and a registry study in Belgium has put the proportion of patients over the age of 15 years with one detectable autoantibody at 82%. Ours is comparable to this with 56/74 (76%) with at least one autoantibody detected.

If we were to exclude those cases those with BMI>35 and no detectable autoantibodies (n=4) (who may well be better classed as type 2 diabetes), and one who subsequently tested positive for the HNF 4α mutation, the prevalence in our cohort goes up to 71.0% (GADA), 47.8% (IA-2A) and 43.5% (ZnT8), still lower than that seen in Long et al. 2012. As IA-2A and ZnT8 are generally seen later in the disease process, perhaps this is reflective of the slower progression of T1D usually seen in patients diagnosed at an older age, and they are less likely to be present around the time of diagnosis. The mean age at diagnosis in this study was higher than that in other studies, most of which include children and/or adolescents who often make up the greatest proportion of the cohort.

6.4.4 Physical activity

This study is the first to measure physical activity in patients recently diagnosed with T1D. The most active participant recorded 689 minutes and the least active 22 minutes of MVPA per week at baseline on the accelerometer. Activity levels were high in this group, with 78% exceeding the CMO's recommended target of 150 minutes of exercise per week. Over-reporting of physical activity compared to objective measurement has been reported previously in the Health Survey for England 2008 (Health and Social Care Information Centre 2009), however, we found that participants tended to underestimate physical activity levels. Additionally, participants reported a decrease in the amount of activity they performed since diagnosis with T1D, which corroborates a similar finding among the participants in the qualitative study (Chapter 5).

In those participants who wore an accelerometer before and after consent, there was no difference in measured physical activity. At baseline, there was correlation between MVPA and fitness. Self-report did not correlate with either measure. Low correlation between different measures of physical activity has been demonstrated in healthy people (Ainsworth et al. 2000).

At randomisation, one of the stratification criteria was fitness. The cut off value for estimated VO2max was 42.8ml/kg/min for men and 34.6ml/kg/min for women with the aim of dividing the cohort in two. Above this value participants were judged to be "more fit". However, overall just 9/60 screened participants were in the "more fit" group. The mean estimated VO2max was much lower than this cut off and therefore, use of a lower estimated VO2max for stratification would be appropriate in future.

Compared to other RCTs recruiting adult patients with T1D for exercise interventions, our cohort was slightly less fit, particularly the men. For example, in (Laaksonen et al. 2000) adult males with T1D had a baseline mean VO2max of approximately 43.4 and 42.0mls/kg/min (intervention and control groups) and female patients with T1D (Wallberg-Henriksson et al. 1986), a baseline mean VO2max of 30.2 and 28.0ml/kg/min (also intervention and control groups). Our mean VO2max at baseline was 35.5mls/kg/min in men and 28.8mls/kg/min in women.

6.4.5 Stimulated C-peptide

None of the screened participants had a 90 minute meal-stimulated C-peptide of less than 200pmol/l. This is recommended as an inclusion criterion for intervention studies in newly diagnosed T1D to ensure participants have adequate beta-cell function to preserve at the outset of the study (Greenbaum & Harrison 2003). However, the data presented here would suggest that most adult patients would have adequate beta-cell function within three months of diagnosis. Either this does not need to be an inclusion criterion, or perhaps the eligibility period could be extended in studies of only adult patients with T1D. A larger cohort of patients would be needed to verify this.

During randomisation, one of the stratification parameters was 90 minute C-peptide of 600pmol/l. The aim of this was to have approximately equal spread of participants with higher or lower stimulated C-peptide in both study arms. However, analysis of this baseline data demonstrates that only 10/66 participants had a stimulated C-peptide of less than 600pmol/l. The median C-peptide in all screened patients was 933pmol/l, in those who were antibody positive it was only slightly lower at 878.5pmol/l. To achieve an even split of this cohort into those with greater insulin secretion and those with less insulin secretion, a higher cut off of around 900pmol/l would have been more appropriate.

At baseline meal-stimulated C-peptide was positively associated with anthropomorphic measures associated with insulin resistance, weight, BMI and WHR.

6.5 Recommendations

6.5.1 Antibody measurement - requirement for randomisation

The presence of one or more autoantibodies is recommended as an inclusion criterion for trials of preservation of beta-cell function in T1D (Greenbaum & Harrison 2003). It is not normally used for the diagnosis of T1D in the UK outside of clinical trials (National Institute for Clinical Excellence 2015). Early in the course of the study, the trial team decided to remove this inclusion criterion from the Extod study. The outcome of this was a rate of antibody positivity of just under 75%. Using a clinical diagnosis, rather than antibody positivity, as the criterion for inclusion for the study means that the study population is more comparable to the population of T1D patients seen in clinical practice. However, it does mean that there is more chance of participants entering the study who have another form of diabetes, such as the participant who was subsequently diagnosed with monogenic diabetes. Removal of the antibody positivity criterion also had the advantage of speeding up the screening and randomisation process (as the antibody samples had a two week turnaround time for GADA only) reducing the number of participants who were excluded on the time from diagnosis criterion. In addition, there were 7/75 participants who were GADA negative, but did have other antibodies detected. These participants would have been excluded under the initial inclusion criteria.

Nevertheless, given the imbalance in the study arms with regards to antibody positivity, further consideration of the use of this criterion either for inclusion or as a stratification at randomisation needs to be performed.

6.5.2 Measures of physical activity

Participant's estimation of their activity levels per week is not a reliable alternative to accelerometer measured MVPA or fitness (estimated VO2max). The exercise question is of little use in determining physical activity and therefore should not be used in future studies.

There was no difference in the accelerometer data before and after consent (visit 1). In a future study, this could be measured just once, e.g. after consent to reduce the burden on participants.

Use of a lower estimated VO2max cut off for stratification would result in a more even split of participants (for example, 35mls/kg/min for men and 30 mls/kg for women).

6.5.3 Meal stimulated C-peptide

All participants had a 90 minute meal stimulated C-peptide of greater than 200nmol/l. This was one of the inclusion criteria and analysis of this introduced complexity and delay into the randomisation timescale (which was already tight). Although this is recommended as an inclusion criteria (Greenbaum & Harrison 2003) for trials looking at beta-cell preservation in T1D, it may be that in trials of adults this is not necessary (as the numbers failing on this criteria would be small). Alternatively, trials recruiting only adult participants could consider having a longer eligibility period, which would expand the pool of potential participants and reduce the time pressure on the screening process.

Reassessment of the value of stimulated C-peptide used for stratification during randomisation would be of worth. A level of 900pmol/l (rather than 600pmol/l) would have led to a more equal split of the groups.

7 INCREASING PHYSICAL ACTIVITY

7.1 Introduction

The effect of the intervention on physical activity will be explored in this chapter. This will also explore anthropomorphic and biochemical changes.

7.2 Methods

The timing of measurement of physical activity and fitness, and the methods used for measuring these have been outlined previously (section 3.2.3.4)

Support with statistical analysis for this chapter and chapter 8 was provided by Mohammed Mostazir and Rod Taylor from the Wellcome Trust Biomedical Informatics Hub, College of Life and Environmental Sciences, University of Exeter.

7.3 Results

7.3.1 Motivational Interviewing

Overall, the intervention group had more contact with the study team than the control arm. The mean total time for visits 2-17 was 759.4 minutes (95% CI 633.8 to 884.9) in the intervention arm compared to 652.6 minutes (95% CI 554.8 to 750.5) in the control group. This is not surprising as additional visits were included for the intervention arm to discuss exercise. When adjusted for the number of visits, the mean time per visit was lower in the intervention arm (68.1, 95% CI 61.4 to 74.8 minutes) than the control group (81.7, 95% CI 76.7 to 76.7), this is likely to be due to

the fact that the additional visits tended to be shorter than those that were undertaken by both groups as at each visit the visit time in intervention group tended to be longer (see Figure 7-1C). The mean additional time spent by the intervention group at joint visits was just 5 minutes.

An average of 104 minutes was spent per participant in the intervention group for exercise motivation. A greater amount of time was spent at the start of the intervention (Figure 7-1B).

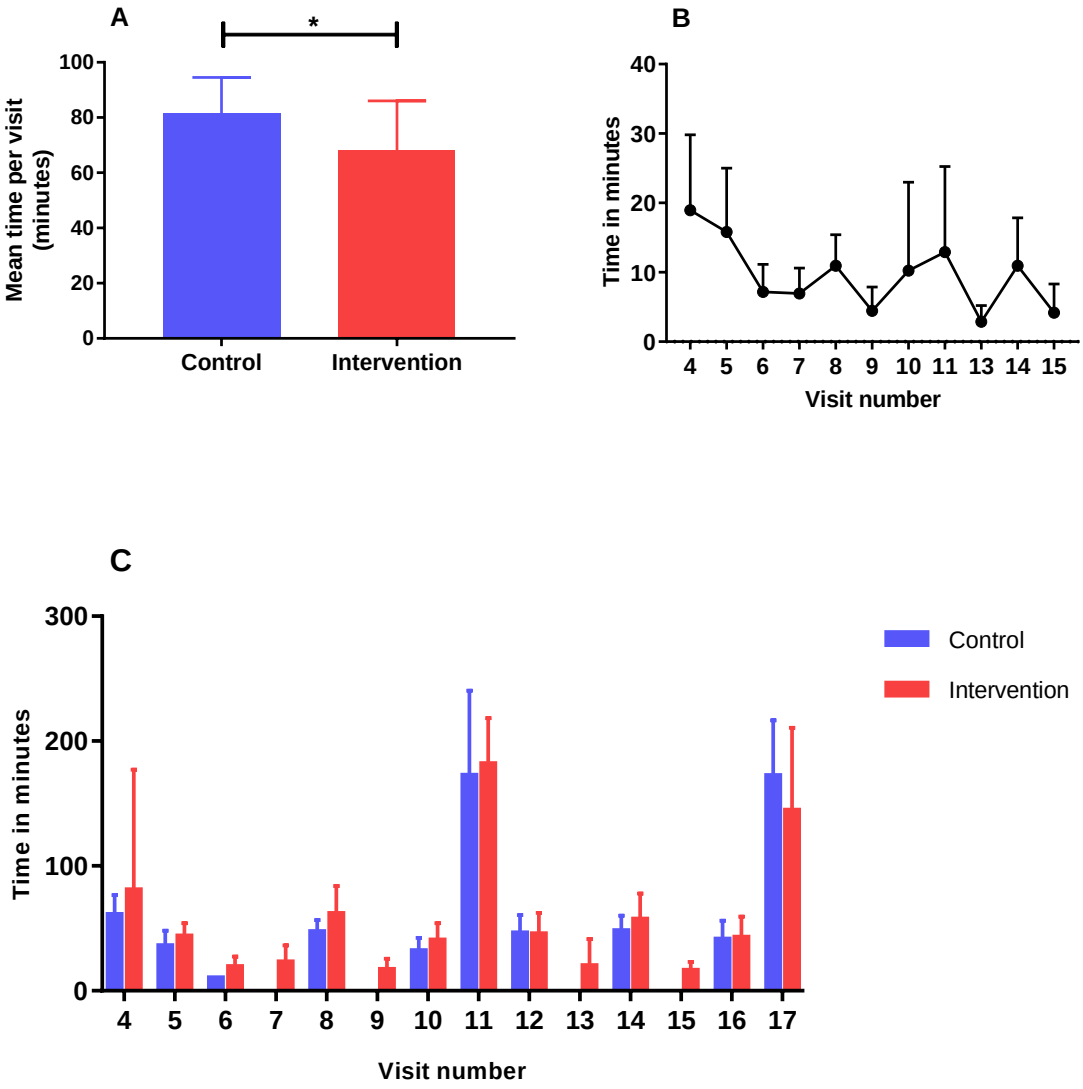

Figure 7-1 - Participant time spent on post-randomisation visits. Mean visit time for each group (A). Amount of time spent on exercise motivation in the intervention arm (B). Breakdown of time spent by visit for each group (C).

7.3.2 Anthropometric data over course of study

There was no difference in waist circumference or WHR between the groups or across the study in either group. Body fat percentage was higher in the intervention

group at all time points, probably due to the greater proportion of female participants in this group.

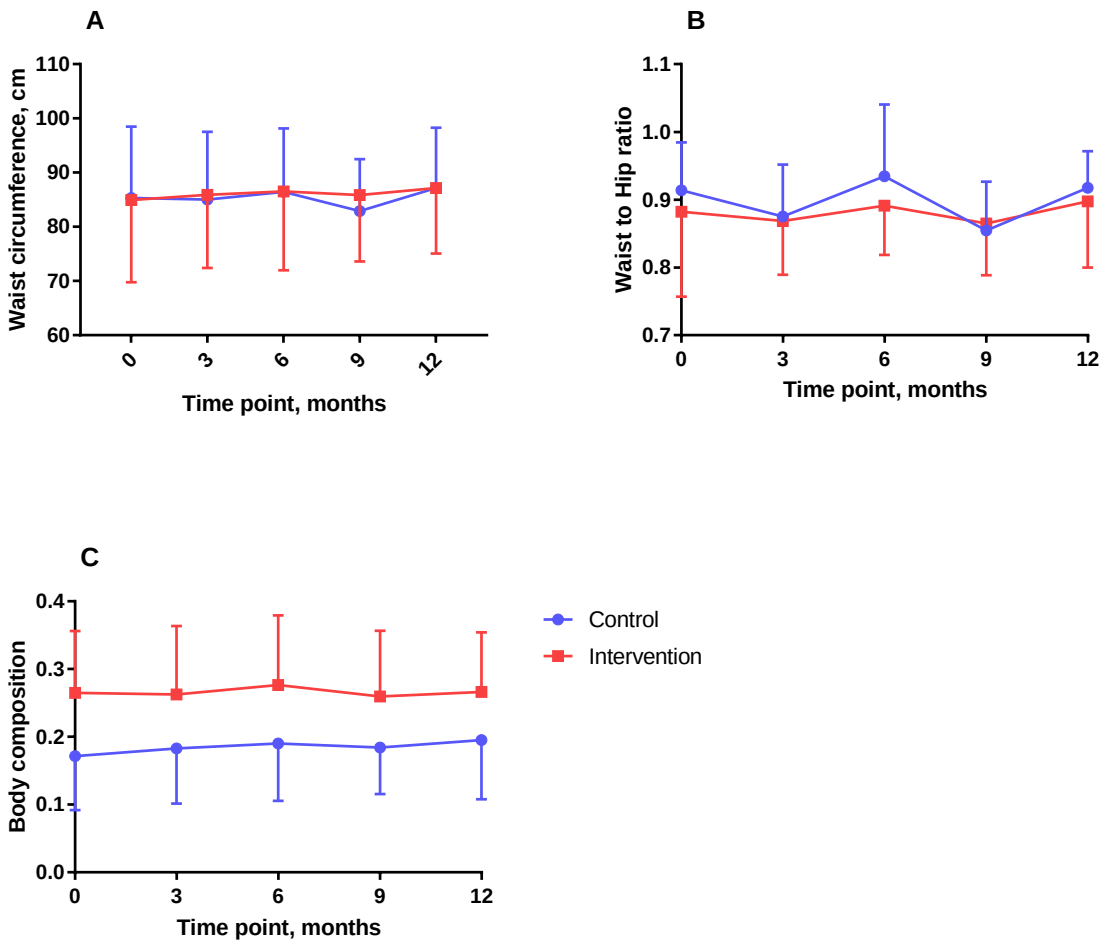

Figure 7-2 - Changes in waist circumference (A), WHR (B) and body composition (C) over the study

Weight and BMI increased slightly in both arms over the course of the study. Weight and BMI were higher in the intervention group at baseline and the difference in BMI

between the two groups was larger at the end of the study. The difference was minimal and not clinically significant.

	Weight, kg (mean ± SD)	BMI (mean ± SD)	N
Control group			
Baseline	77.36±15.80	24.69±4.15	28
6 months	78.98±14.11	25.02±4.22	21
12 months	79.71±14.55	24.21±4.30	20
Intervention group			
Baseline	73.50±12.19	24.83±3.40	30
6 months	74.67±14.18	25.33±4.31	24
12 months	75.14±13.13	25.57±4.18	21

Table 7-1 - Mean weight and BMI at baseline and after 6 and 12 months.

The mean weight gain was 2kg in the first 6 months and then 0.4kg in the second half of the study.

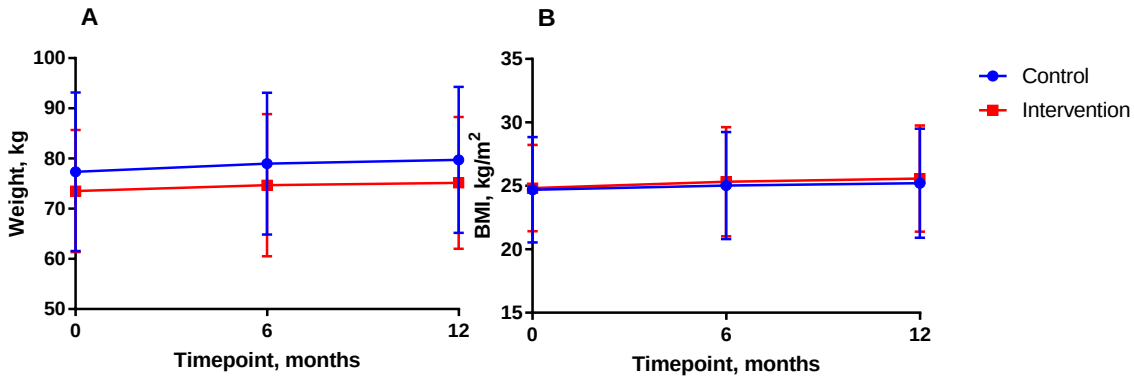

Figure 7-3 - Weight (A) and BMI (B) changes over the course of the study

Systolic blood pressure was slightly lower in the intervention arm at all time points. Diastolic blood pressure and resting heart rate were similar in both groups.

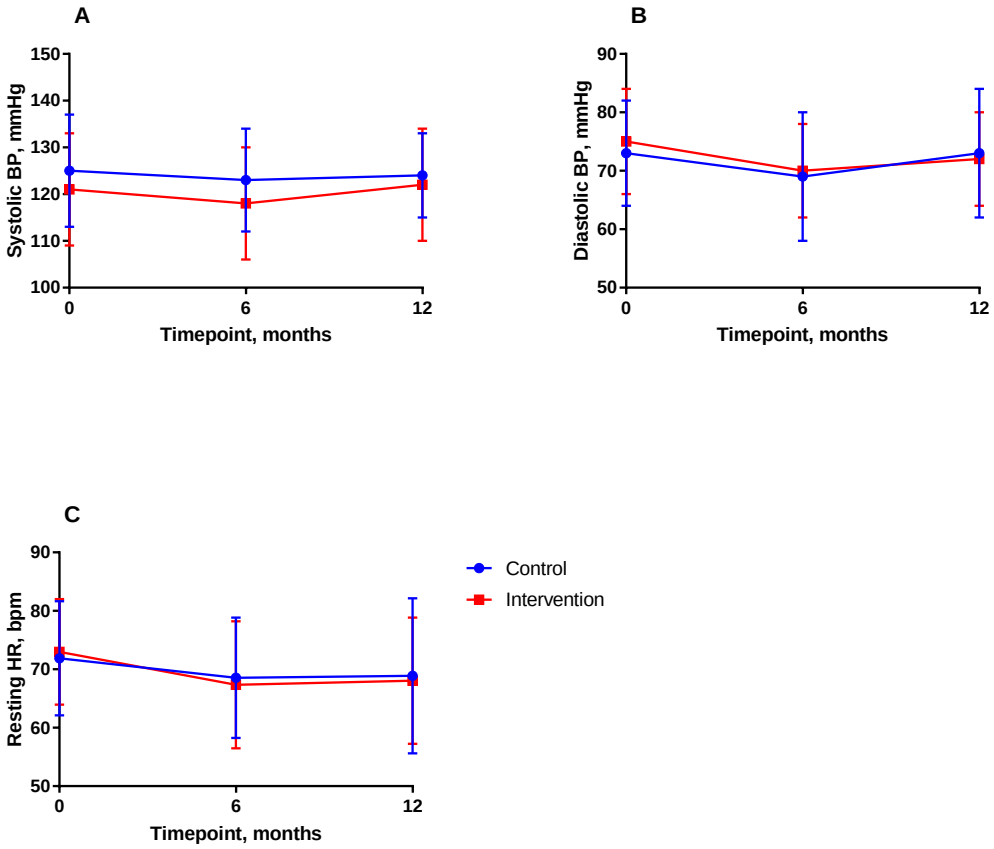

Figure 7-4 - Changes in blood pressure (systolic (A) and diastolic (B)) and resting heart rate (C) during the study

7.3.3 Metabolic parameters

7.3.3.1 Lipids

There was a trend to lower total cholesterol and triglycerides in the intervention group during the study, while both of these parameters tended to increase in the control arm. HDL cholesterol tended to be lower in the control arm at baseline and this

difference was maintained for the duration of the study. The small cohort sizes mean that these trends were not statistically significant.

Glycaemic control as represented by HbA1c was similar in both groups at each time point in the study.

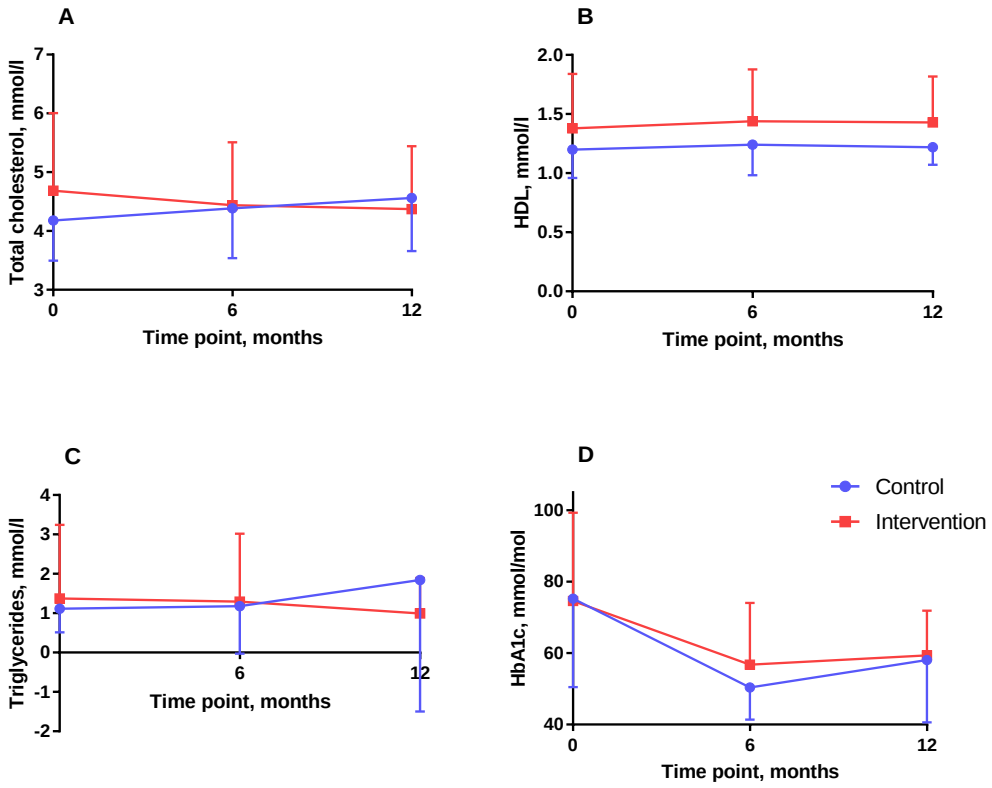

Figure 7-5 - Changes in lipid profile (total cholesterol (A), HDL cholesterol (B), triglycerides (C)) and glycaemia (D) during the study

7.3.4 Activity levels

As discussed in section 3.2.3.4, physical activity was assessed by participants' self-report, accelerometer and fitness.

7.3.4.1 Adherence to exercise targets

Sixty-one percent of participants reported meeting activity targets (at least 150 minutes, aiming for 240 minutes of exercise per week) on average across the study. There was some variation between visits in the proportion of participants reporting meeting the target (range from 42% to 86%), however, overall the proportion did not substantially change with time.

The data for this analysis are incomplete with many missing values (37% of data are missing). Overall, 24% of participants admitted to falling below the activity targets, while 39% felt that they had achieved their target levels.

7.3.4.2 Moderate-Vigorous Physical Activity

Participants in the intervention arm increased MVPA by six months and maintained the increase at 12 months, although this increase was very much smaller than we had aimed to achieve (mean adjusted increase in MVPA amounted to approximately five minutes per day). However, fitness also increased at six months and the increase was maintained for the duration of the study. In contrast, MVPA and fitness was lower at six months in participants in the control arm and although there was a small increase by the end of the study, they remained less active and less fit than the intervention arm.

As discussed in chapter 6, physical activity levels were high at study entry. The proportion achieving the target amount of physical activity remained approximately stable and was similar across both groups (82% control and 79% intervention groups achieved 150 minutes MVPA overall, 57% control and 56% intervention achieved 240 minutes MVPA on average over the whole course of the study).

	Baseline	6 months	12 months
Control group, n	26	15	14
Achieving 150 minutes/week, n (%)	21 (81)	13 (87)	11 (79)
Achieving 240 minutes/week, n (%)	16 (62)	8 (53)	8 (57)
Intervention group, n	28	18	15
Achieving 150 minutes/week, n (%)	22 (79)	13 (72)	13 (87)
Achieving 240 minutes/week, n (%)	13 (46)	11 (61)	9 (60)

Table 7-2 - Proportion of participants achieving activity targets at baseline, 6 months and 12 months. MVPA was also assessed at 3 and 9 months (data not shown).

Figure 7-6 shows the change in MVPA over the course of the study. At baseline the weekly MVPA in the intervention group was lower than that of the control group (by 35 minutes). The absolute (unadjusted) difference in MVPA between the two groups was 28 minutes at 6 months and 32 minutes at 12 months. Due to the wide distribution of MVPA, this difference was not significant. When adjusted for baseline

MVPA, age and gender, the difference in MVPA between the intervention and control groups was just 5 minutes per day.

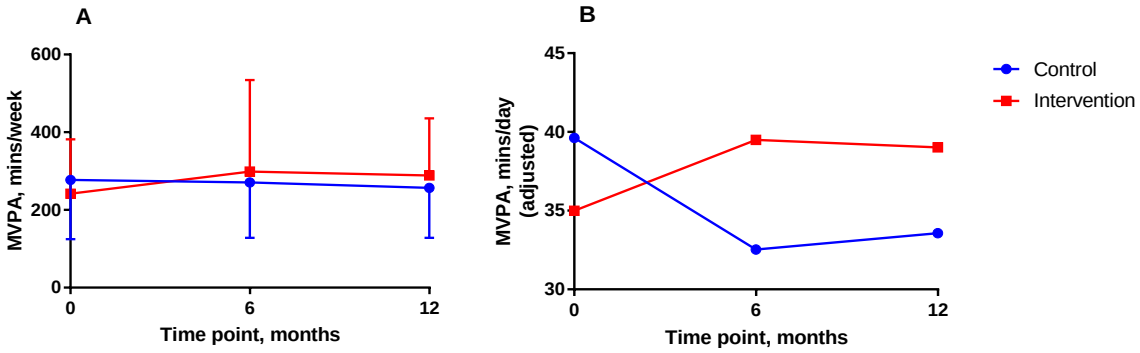

Figure 7-6 – Change in MVPA during the study. Mean MVPA tended to fall in the control arm and rose in the intervention group, although this was not significant. (A) demonstrates the absolute change in MVPA ***per week*** *over the study and (B) the change in MVPA* ***per day*** *adjusted for baseline value, age and gender.*

7.3.4.3 Fitness

A similar trend was seen in estimated VO2max. The intervention group started less fit and fitness increased, whereas the control group became less fit during the study follow-up. This trend is shown in Figure 7-7, again substantial variability in values has led to wide confidence intervals and therefore, these changes are not significant. VO2max has been adjusted for age, gender and baseline value in these analyses.

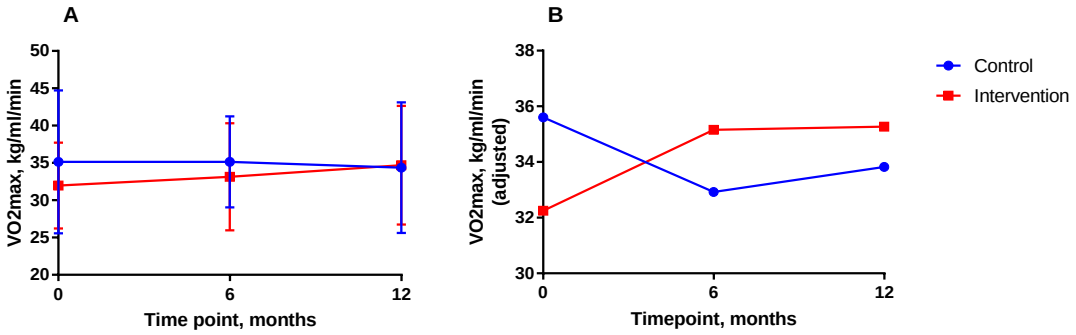

Figure 7-7 – Change in fitness during the study. Mean VO2 max tended to fall in the control group and rise in the intervention group (unadjusted) (A) and adjusted for age, gender and baseline value (B).

7.3.5 Insulin resistance

Measures of insulin resistance (fasting insulin and HOMA2-IR) were similar in the intervention and control arms at baseline. During the study, the intervention group tended to have lower fasting insulin levels and lower HOMA2-IR scores, indicating a reduction in insulin resistance. Conversely, these parameters tended to increase in the control arm during the study. Fasting glucose remained constant in the two groups. Again, the confidence intervals are wide, and the difference is not significant.

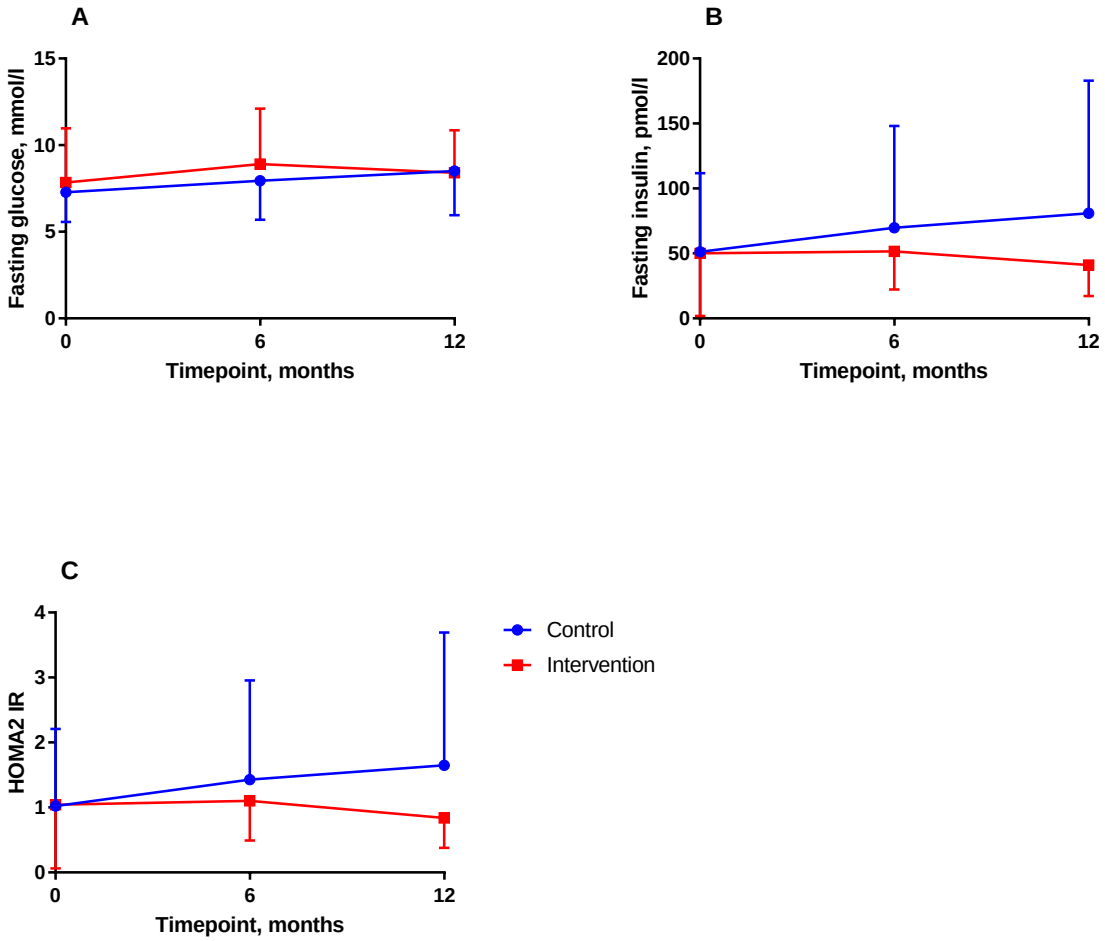

Figure 7-8 – Change in measures of insulin resistance over the study. Fasting glucose remained unchanged (A), whereas fasting insulin fell in the intervention arm (B), HOMA2-IR (C) fell in the intervention arm.

7.4 Discussion

Just under two thirds of participants reported meeting their exercise targets. In fact, data from accelerometer suggests that around 80% were achieving at least 150 minutes MVPA per week. This contrasts with under 10% of the general population (Aresu et al. 2008). Objectively, despite a small increase in MVPA, improvements in fitness and insulin sensitivity were seen in the intervention group. The control arm did not appear to increase activity levels, the overall trend was for a reduction in MVPA, fitness and insulin sensitivity in this group indicating that there was little new exercise uptake.

The increase in MVPA in the intervention arm, in contrast to the decrease in the control arm, resulted in a difference of 5 minutes per day. This is a lower between group difference than we anticipated and is primarily due to the high levels of activity at baseline and in the control group throughout the study (mean MVPA was greater than 240 minutes per week in both groups at baseline, 6 months and 12 months). Activity levels achieved in the Extod intervention group (mean MVPA 42.7 minutes per day at six months and 41.3 minutes per day at twelve months) are comparable to those seen in a group of adolescents with T1D undergoing a 16 week community exercise prescription program where the mean MVPA was 42 minutes per day (Michaliszyn & Faulkner 2010).

Fitness (estimated VO2max) did increase in the intervention arm, by just under 3% at six months and 8% at twelve months. This increase is lower than that seen in other RCTs involving adult T1D patients with supervised exercise interventions. These studies (lasting between three and five months) demonstrated an increase in

VO2max of between 6 and 13% (Laaksonen et al. 2000; Fuchsjager-Mayrl et al. 2002; Wallberg-Henriksson et al. 1986).

The increase in MVPA and fitness did not come at the expense of higher rates of hypoglycaemia (see Chapter 4). In fact, all participants were active and the minor hypoglycaemia rate was less than one per week. This is the first study to demonstrate that exercise can be safely performed by adult patients with recently diagnosed T1D.

There did appear to be a change in insulin resistance. Fasting insulin levels appeared to fall in the intervention group, while increasing in the control arm. This is despite constant fasting blood glucose and similar improvements in glycaemic control. This indicates an improvement in insulin sensitivity in the intervention group and is consistent with previous studies examining the effect of exercise on insulin resistance in T1D (Yki-Jarvinen et al. 1984; Wallberg-Henriksson et al. 1982).

7.5 Recommendations for further work

The initial aims of this study were to maintain a larger difference in physical activity between the control and intervention arms (the original aim was for a difference of 150 minutes per week). The actual difference in MVPA was much smaller and increases in fitness were also small. A future study aiming to increase physical activity in this group will either need to recruit sedentary participants or to increase

physical activity in the intervention arm by a greater amount. Recruiting sedentary participants may prove difficult, as demonstrated in Chapter 4, patients willing to take part in an exercise study may well be already comparatively active. Comparisons of supervised and unsupervised exercise interventions are few. One meta-analysis of the efficacy of interventions to increase physical activity, did find a larger increase in the one included trial of supervised exercise than in the studies of unsupervised motivational techniques (Foster et al. 2005). Another study comparing a six month supervised exercise program with motivational behaviour change techniques found that, although total activity levels in both groups were similar, the supervised exercise groups performed more vigorous and very vigorous exercise (Dunn et al. 1999). A supervised exercise program should, therefore, be considered in a future trial to improve both activity levels and intensity.

8 BETA-CELL FUNCTION

8.1 Introduction

The Extod study was a pilot feasibility study and the primary outcome measures were related to recruitment, adherence and exercise uptake. Decline in beta-cell function was a secondary outcome and we wished to explore this outcome to enable us to design a larger trial including power calculations.

8.2 Methods

8.2.1 Subjects

The subjects and methods used are as described in earlier chapters. The subjects are either all screened participants or those that were randomised. These groups are described in Chapter 3.

8.2.2 C-peptide measurement

Conduct of the MMTT and analysis of the samples is described in Chapter 3.

All of the analyses in this chapter use mean AUC C-peptide, unless otherwise specified.

8.2.3 Statistical analyses

The intention-to-treat analyses (that is the analysis of the effect of group on beta cell function in the randomised Extod patients) were performed by Dr M Mostazir and

Professor R Taylor of the University of Exeter. All other statistical analyses and graphical representation were performed by me.

8.3 Results

8.3.1 Measuring beta-cell function

In this study, we measured C-peptide during the MMTT to obtain mean AUC C-peptide as a marker of beta-cell function. Other markers of beta-cell function that have been reported include; fasting C-peptide, peak C-peptide during the MMTT, 90 minute C-peptide and UCPCR. The peak C-peptide is the value peak value recorded during the 120 minute test.

The relationship between these is shown in Figure 8-1.

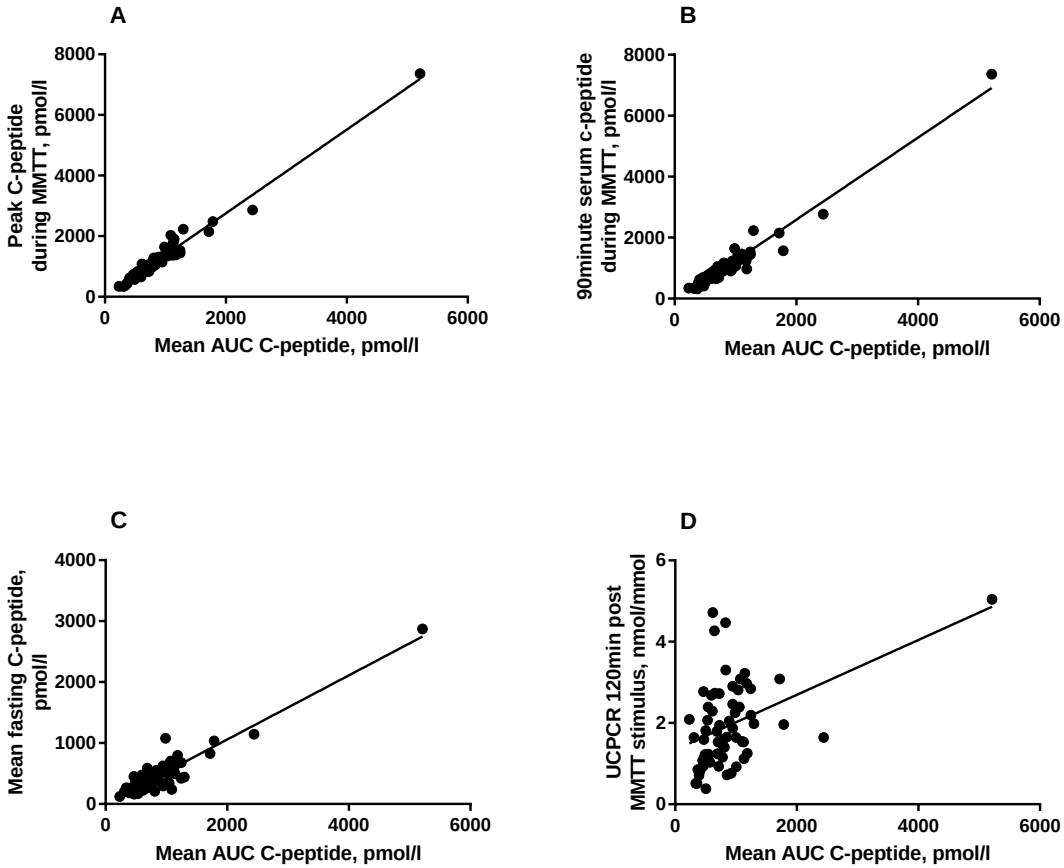

Figure 8-1 - Association between mean AUC C-peptide during the MMTT and peak C-peptide (A), C-peptide at 90minutes (B), fasting C-peptide (C), and UCPCR (D) when measured during the baseline MMTT.

Stimulated C-peptide is the recommended outcome measure for clinical trials of beta cell function in T1D (Palmer et al. 2004). In this study, (log) mean AUC C-peptide was strongly correlated with both (log) peak C-peptide (r=0.9746, 95%CI 0.9587 to 0.9844, r^2=0.9499, p<0.0001) and (log) 90 minute C-peptide (r=0.9562, 95%CI 0.9292 to 0.973, r^2=0.9143, p<0.0001). There was good correlation between AUC C-peptide and both (log) fasting C-peptide (r=0.8437, 95%CI 0.7561 to 0.9016,

r^2=0.7118, p<0.0001) and (log) HOMA2 B (r= 0.628, 95%CI 0.4535 to 0.7561, r^2=0.3943, p<0.0001). Correlation between mean AUC C-peptide and UCPCR was statistically significant (r=0.4373, 95%CI 0.2125 to 0.6181, r^2=0.1912, p=0.0003), however, this relationship was the weakest of all measures. Similar associations were seen between the parameters at later time points.

In Figure 8-1 it can be seen that there is an outlier with mean AUC C-peptide of >5000pmol/l. This individual was antibody negative and it is likely that this participant had type 2 diabetes. Removing this individual from the analysis did not significantly affect the correlation results.

8.3.2 Factors associated with beta-cell function at baseline

8.3.2.1 Age and gender

In our (adult) population, there was no significant difference in baseline C-peptide in males and females, mean AUC C-peptide in men was 346.3pmol/l (95% CI 282.9, 409.6) compared to 369.4pmol/l (95% CI 274.2, 464.6) in women. Age was not associated with baseline C-peptide, Spearman's co-efficient r=-0.0157 (95% CI -0.2780 to 0.2486).

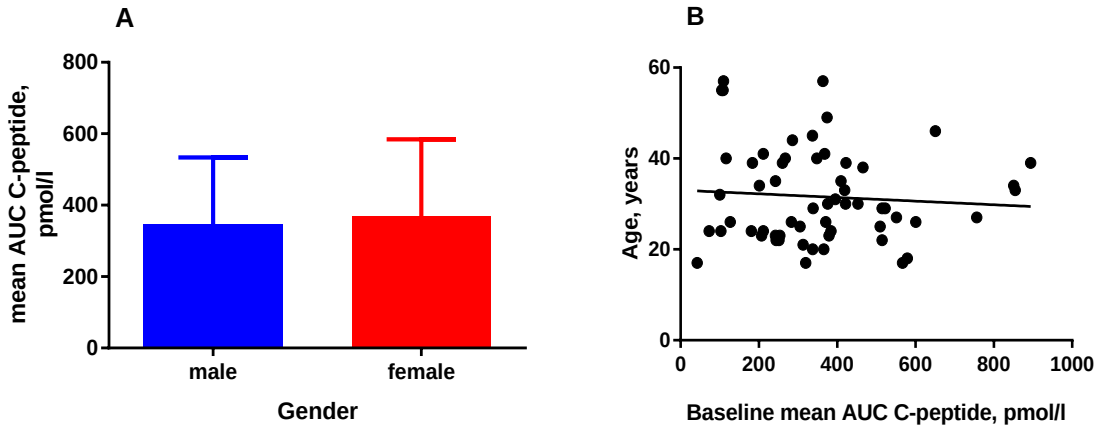

Figure 8-2 – There was no association between baseline mean AUC C-peptide and either gender (A) or age (B).

8.3.2.2 Ethnicity

There does appear to be a lower mean AUC C-peptide at baseline in participants of White British origin and those of other ethnic origin, mean AUC C-peptide 859pmol/l (95%CI 288.5, 1042) compared to 1042pmol/l (95%CI 306.5, 739.5), p=0.02, although there is significant overlap of the confidence intervals. Due to the low number of participants identifying their ethnic background as other than White British this was impracticable to breakdown further into specific ethnicities.

8.3.2.3 Presentation of diabetes

We determined the presentation of diabetes in the Extod cohort (i.e. whether with acidosis or not) by patient notes (where available) and history from the participant. It was not possible to determine the presentation in one participant. There was no difference in baseline C-peptide in those who had ketoacidosis at presentation (mean

AUC c-peptide 898.1pmol/l, 95%CI 656.6 to 1140) and those who presented with hyperglycaemia alone (mean AUC c-peptide 886.5pmol/l, 95%CI 686.8 to 1086).

There does appear to be an association between the duration of symptoms experienced prior to diagnosis and baseline C-peptide levels, with those participants reporting a shorter duration of symptoms appearing to have a higher mean AUC C-peptide at baseline. This was not statistically significant (Spearman r=-0.2365, 95%CI -0.4706, 0.02865, p=0.0713).

8.3.2.4 Lifestyle factors

Within the Extod cohort there was no difference in baseline C-peptide between the groups who smoked (mean AUC C-peptide 366.1 (95%CI 261.5, 470.6)), who had stopped smoking (mean AUC C-peptide 409.1 (95%CI 281.4, 536.9) and those who had never smoked (mean AUC C-peptide 338.8 (95%CI 268.2, 409.4) (shown in Figure 8-3) or with alcohol consumption (data not shown).

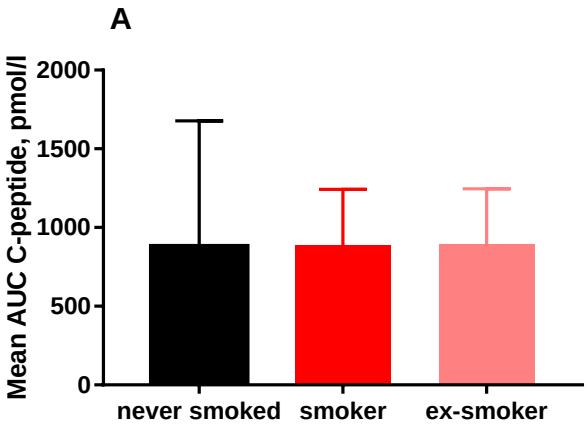

Figure 8-3 - Effect of smoking on baseline mean AUC C-peptide

8.3.2.5 Anthropomorphic measures

Significant association was seen between baseline mean AUC C-peptide and BMI, weight but not WHR.

Log baseline AUC C-peptide (again discounting the prominent outlier) was significantly correlated with with log BMI (r=0.351, 95%CI 0.117 to 0.548, p= 0.0042, r^2=0.123) and log weight (r=0.305, 95%CI 0.066 to 0.511, p= 0.013, r^2= 0.093).

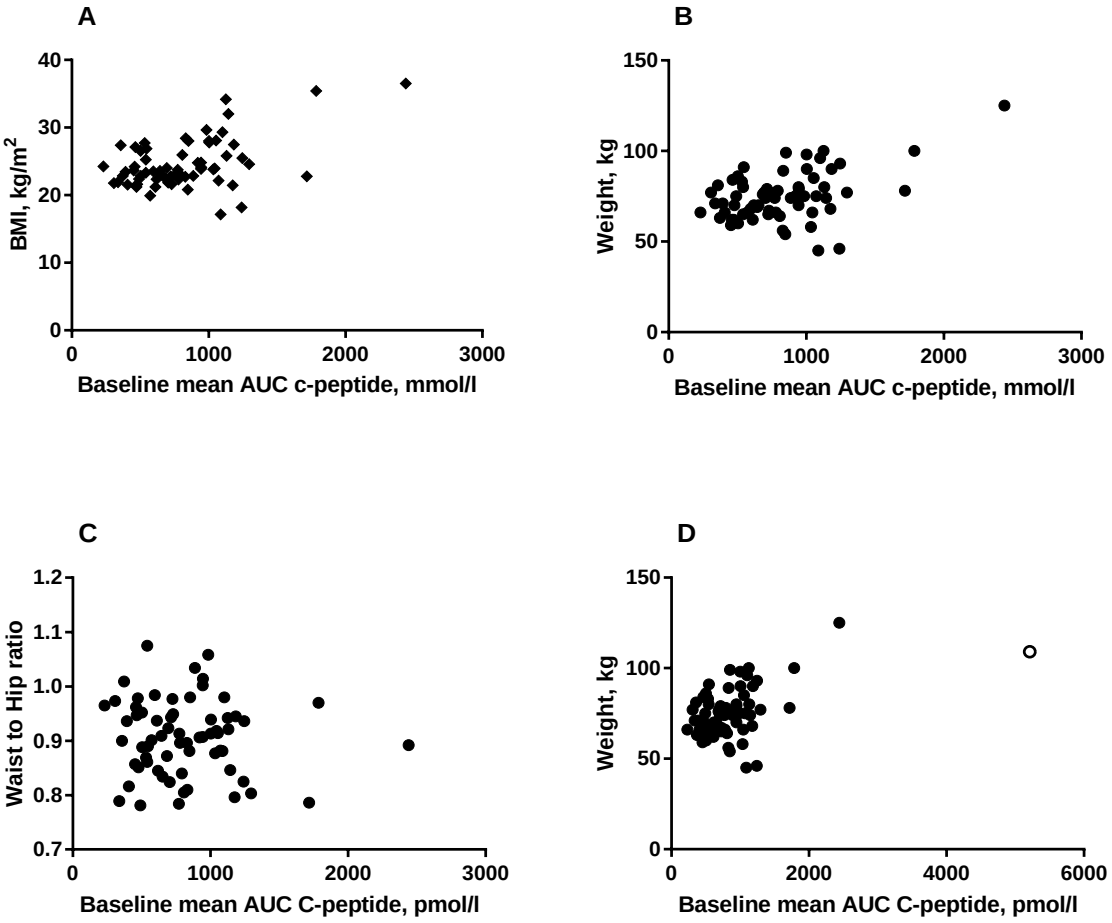

Figure 8-4 – Relationship between mean AUC C-peptide and BMI (A), weight (B) and WHR (C). (D) demonstrates the effect of a prominent outlier (open circle) who is likely to have type 2 diabetes.

8.3.2.6 Glycaemic control

There was no relationship between HbA1c and mean AUC C-peptide at baseline.

8.3.2.7 Autoantibodies

Mean AUC C-peptide at baseline was significantly greater in those participants with no detectable antibodies (1324pmol/l, 95%CI 713.4 to 1935) when compared with those with at least one autoantibody (743.1pmol/l 95%CI 657.5 to 828.6), p=0.0014. Mean AUC C-peptide was very similar in those with one, two or three autoantibodies (*Figure 8-5*A).

Although mean AUC C-peptide (see *Figure 8-5*B) was consistently higher in antibody negative participants for each autoantibody, this was only statistically significant for ZnT8A (mean AUC C-peptide 672.2 vs 1031pmol/l, p=0.0024).

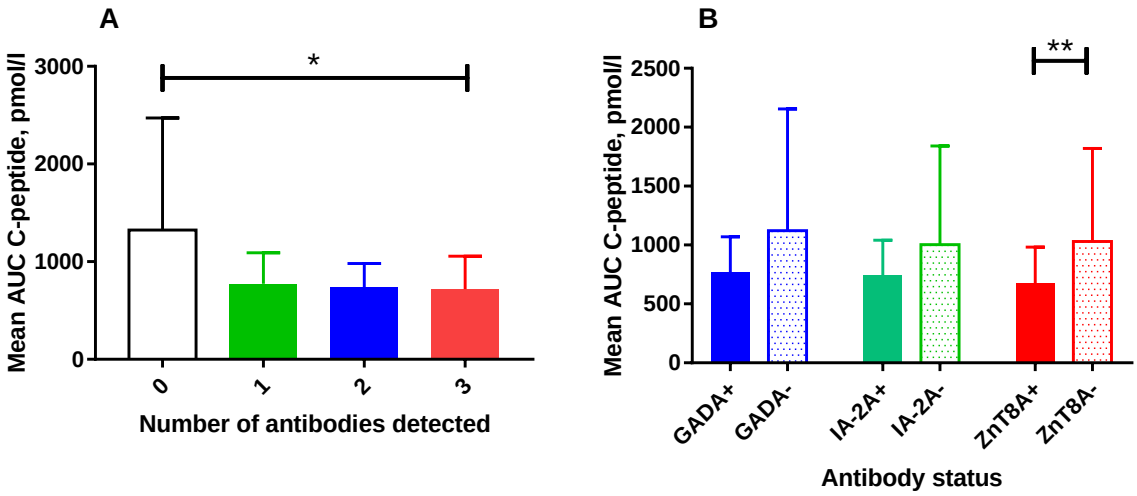

Figure 8-5 - Association between mean AUC C-peptide at baseline in all screened participants and number of detectable autoantibodies (A) and antibody status for each measured autoantibody (B).

Number of antibodies present	N (%)	Mean AUC C-peptide, pmol/l
0	19 (25.3)	1324±1147 (n=16)
1	20 (26.7)	773.6±318.2 (n=17)
2	16 (21.3)	738.9±243.3 (n=15)
3	20 (26.7)	717.7±339.7 (n=18)

Table 8-1 - Mean AUC C-peptide is associated with antibody status. Not every participant who had samples taken for antibody assays attended for the MMTT, hence the different values of N.

8.3.2.8 Insulin Resistance

Both weight-hip ratio (r=0.3961 95%CI 0.1684 to 0.5836, r^2=0.1569, p=0.0011) and HOMA2-IR (r=0.5657, 95%CI 0.3716 to 0.7124, r^2=0.32, p<0.0001) were significantly correlated with mean AUC C-peptide at baseline. As the data were not normally distributed, mean AUC C-peptide and HOMA2-IR were logarithmically transformed prior to analysis.

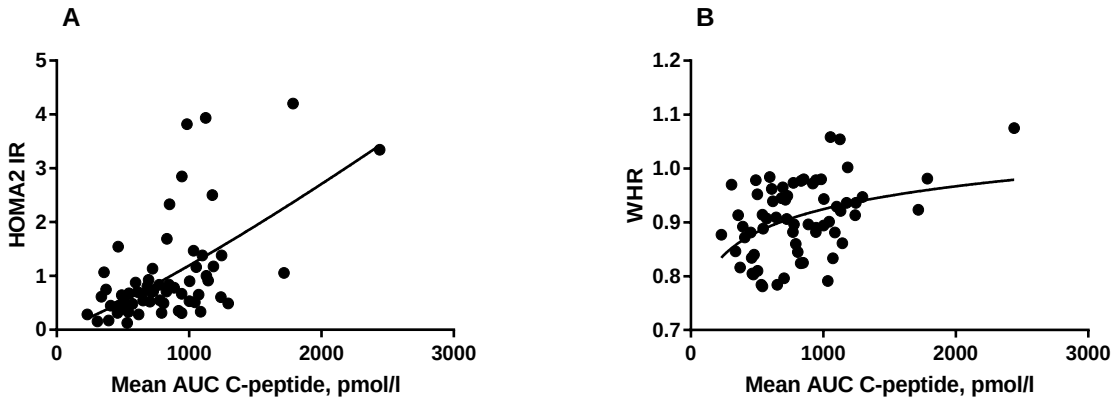

Figure 8-6 - Relationship between mean AUC C-peptide at baseline and HOMA2 IR (A) and WHR (B).

8.3.2.9 Exercise

There was no significant association between MVPA and mean AUC C-peptide at baseline. Log baseline AUC C-peptide (discounting the outlier) was significantly, and negatively, correlated with log VO2max (r=-0.282, 95%CI -0.502 to -0.027, p= 0.0308, r^2= 0.079).

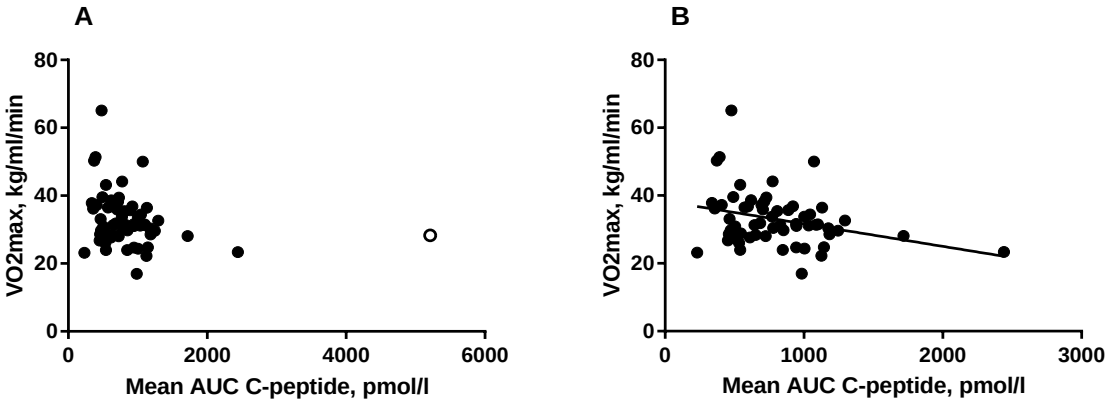

Figure 8-7 - Scatter diagrams showing relationship between mean AUC C-peptide at baseline and estimated VO2max. The whole data set (A) with the prominent outlier represented by an open circle, and a close up of the data with the outlier removed (B).

8.3.3 Change in beta-cell function during the study

8.3.3.1 Beta-cell function overall

Figure 8-8 demonstrates that beta cell function declined overall during the year in those patients who were randomised. In those patients with assessments of beta-cell function at both baseline, six and/or twelve months, mean AUC meal-stimulated C-peptide fell by 0.8% by six months and 19.3% by the end of the study.

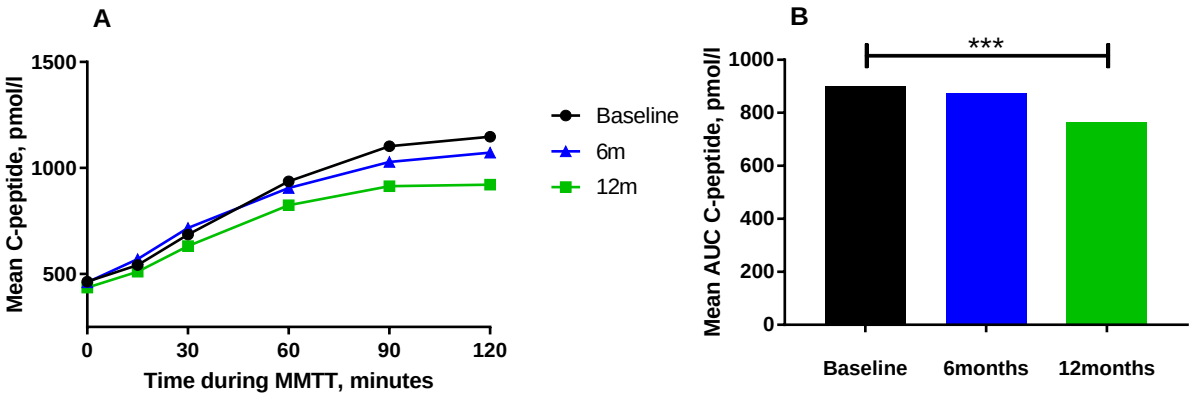

Figure 8-8 – The change in mean C-peptide at each time point during the MMTT (A) (only those with more than one measure are included in this graph). Mean AUC C-peptide at baseline, 6 months and 12 months is shown in (B).

8.3.3.2 Beta-cell function trends during the study

The secondary outcome of the study was to investigate whether exercise had an effect on beta-cell function in patients with a recent diagnosis of T1D.

Mean AUC C-peptide was higher at baseline and remained constant in the control arm through the study. In the intervention arm, there appears to be a downward trend in mean AUC C-peptide as the study progresses. Due to the wide confidence intervals, particularly in the control group, this difference was not statistically significant. Both groups appear to show a transient improvement in mean AUC C-peptide at 6 months which is lost by twelve months when this is adjusted for baseline covariates (Figure 8-9).

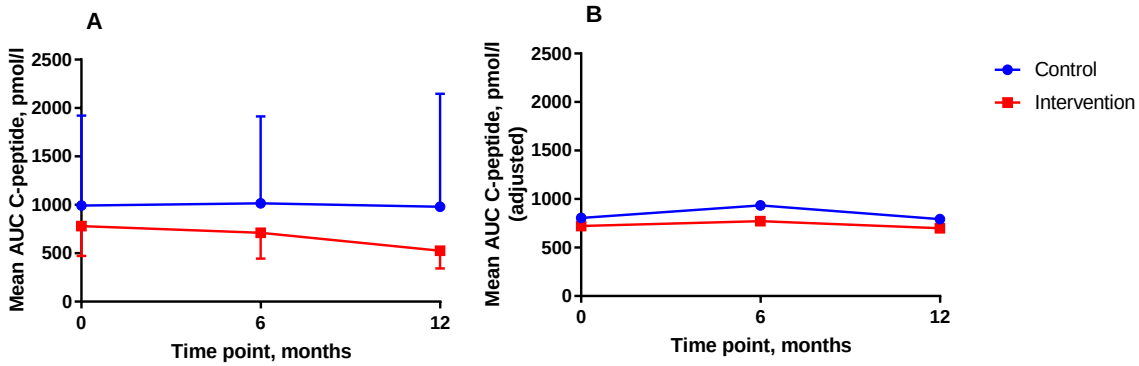

Figure 8-9 - The effect of exercise on beta-cell function (intention to treat analysis). (A) shows the change in unadjusted mean AUC C-peptide over time. In (B) mean AUC C-peptide has been adjusted for baseline value, gender, age, baseline HbA1c, antibody status, baseline MVPA and baseline VO2max.

8.3.3.3 Responders

In the DCCT, 'responders' were defined as participants who maintained a stimulated C-peptide of greater than 200pmol/l. A further group of participants with a stimulated

C-peptide of greater than 500 pmol/l was also identified (The Diabetes Control and Complications Research Group 1998).

In the Extod study, all screened participants had a 90 minute stimulated C-peptide of greater than 200pmol/l. At 6 months, only 2 out of 42 participants (one from each study arm) had lost beta cell function such that 90 minute stimulated C-peptide was less than 500pmol/l, and by 12 months only these same two participants had 90 minute stimulated C-peptide under 200pmol/l.

Maintenance of C-peptide levels has also been used to assess response to beta-cell preserving therapies. Herold et al. 2005 described 'responders' as those who maintained mean AUC C-peptide, that is there was a fall of less than 7.5% in mean AUC C-peptide of from the initial value. There was no significant difference in the proportion of participants who maintained C-peptide by this definition between the two groups.

8.3.3.4 Factors affecting beta-cell function in the Extod cohort

As the exercise intervention did not have a significant effect on beta-cell function in this group, factors other than exercise were examined in the **whole** group.

8.3.3.4.1 Age/Gender

There were significant associations between the change in mean AUC C-peptide and both age and gender (see Figure 8-10). Percentage change in mean AUC C-peptide was significantly correlated with age (r=0.326, 95%CI 0.01996 to 0.5756, p=0.038, r^2=0.106) in the group as a whole, but the relationship was only apparent in the control arm when the groups were analysed separately. There was a trend towards a larger decline in beta-cell function in women over the course of the study, although this was not statistically significant.

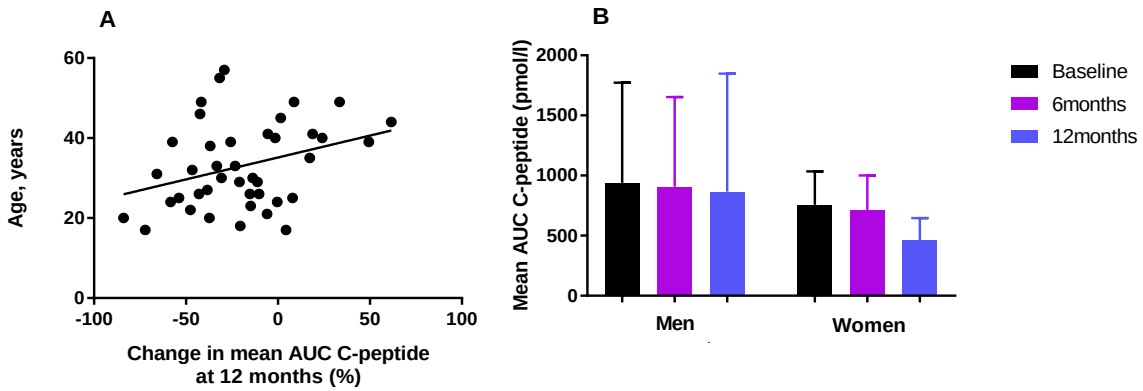

Figure 8-10 - Relationship between beta-cell function and age (A) and gender (B)

8.3.3.4.2 Ethnicity

The number of participants with other ethnicities in whom data was available at more than one time point was very few (n=5) and therefore, analysis of this is limited. There was, however, no apparent difference in decline in beta-cell function in participants who identified themselves as 'White – British' when compared to those who identified themselves with other ethnic backgrounds.

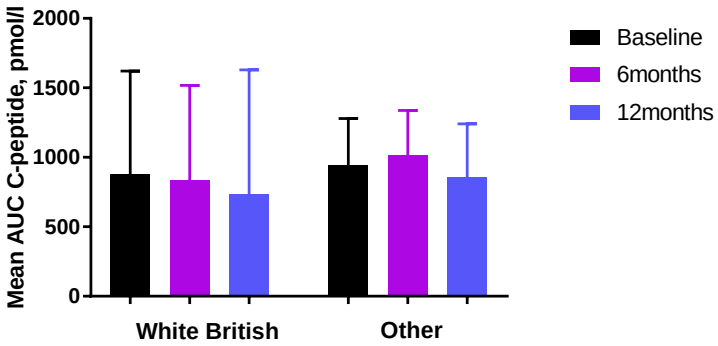

Figure 8-11 - Association between ethnicity and mean AUC C-peptide

8.3.3.4.3 Lifestyle

There did appear to be a trend between percentage change in beta-cell function at the end of the study and smoking status, although this was not statistically significant. There was no significant relationship between alcohol consumption and decline in beta-cell function.

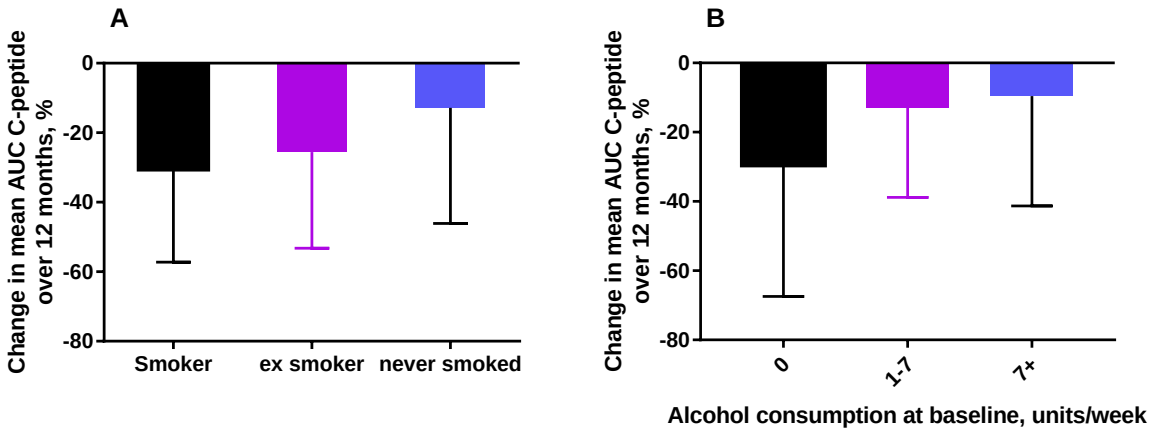

Figure 8-12 - Relationship between change in mean AUC C-peptide, smoking status (A) and alcohol consumption (B).

8.3.3.4.4 Presentation

The change in mean AUC C-peptide was greater in those who presented in DKA than those who presented with hyperglycaemia without acidosis (mean percentage change -47.5% (95% CI -58.37 to -36.63) in those presenting with acidosis compared to -13.43% (95% CI -23.8 to -3.09%) in those presenting with hyperglycaemia, p=0.0175). There was significant correlation between percentage change in mean AUC C-peptide and the duration of symptoms prior to diagnosis (Spearman r=0.432,

95% CI 0.1339 to 0.6581, p=0.0048), but not in either arm individually. This relationship remains significant when the outlying data point (symptoms of 4 years prior to diagnosis, not shown in figure) is removed from the analysis.

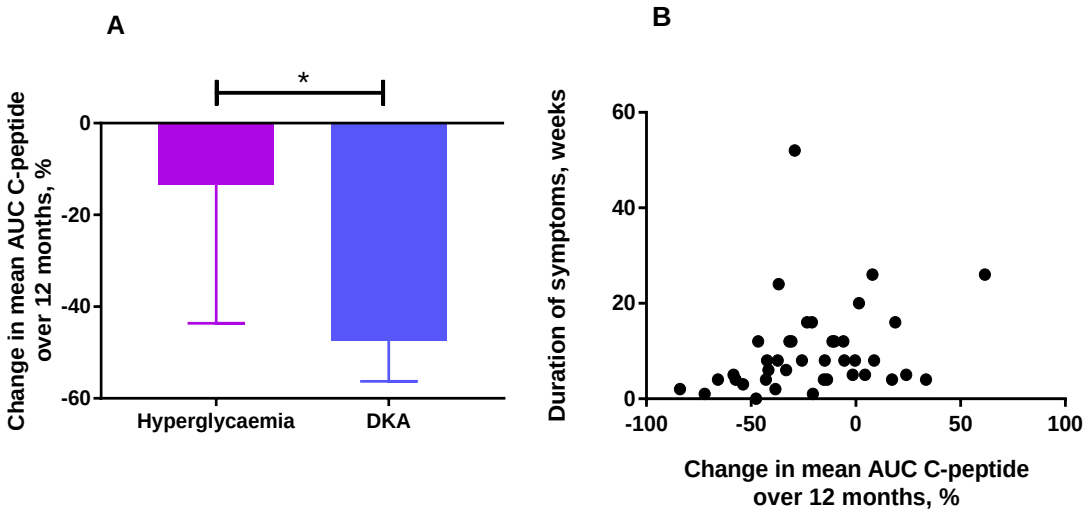

Figure 8-13 - Association between percentage change in mean AUC C-peptide over 12 months and presentation (A) and duration of symptoms prior to presentation with T1D (B).

8.3.3.4.5 Anthropomorphic measures

There were no significant relationships between percentage change in mean AUC C-peptide and baseline weight, BMI, WHR, body composition or HOMA-IR.

8.3.3.4.6 Glycaemic control

There was negative correlation between HbA1c at 6 months and change in mean AUC C-peptide over the 12 months of the study (Spearman r=-0.449, 95%CI -0.6703 to -0.1554, p=0.0032), suggesting that beta-cell function declines more rapidly in those participants with poor glycaemic control (see *Figure 8-14A*). There was no statistically significant association between the change in mean AUC C-peptide at 12 months and HbA1c at the end of the study, however, the numbers are very small and there is one outlier which skews the data (see *Figure 8-14B*).

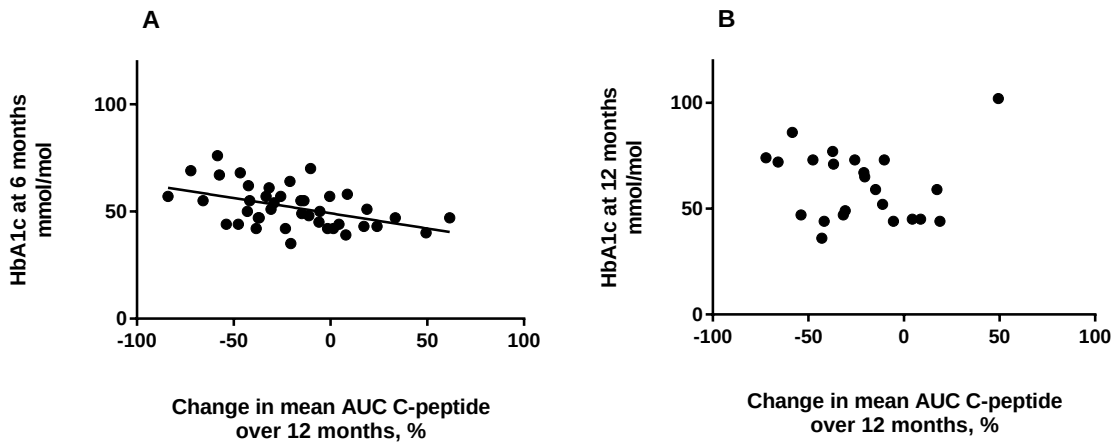

Figure 8-14 - Relationship between percentage change in mean AUC C-peptide over the study and HbA1c at 6 months (A) and 12 months (B)

8.3.3.4.7 Antibody status

There was no significant change in beta-cell function by six or twelve months in those participants with no detectable autoantibodies at study entry. In comparison, those participants with at least one detectable autoantibody had a significant decline in beta-cell function over the twelve months in the study (p=0.0097). If the two groups (intervention and control) are analysed for the effect of antibody status separately, a similar relationship is seen in the control arm to that seen in the whole group (data not shown). In the intervention arm, mean AUC C-peptide declined to a similar extent in both those with detectable autoantibodies and those without. The number of participants in the intervention arm who did not have detectable autoantibodies was, however, small (n=4).

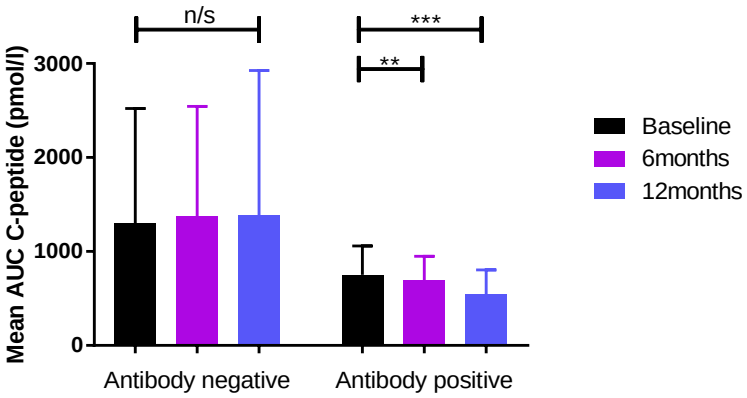

Figure 8-15 - Effect of antibody status on C-peptide decline.

8.3.3.4.8 Physical activity and insulin resistance

There was no association between MVPA at baseline, 6months or 12months and change in mean AUC C-peptide. In addition, there was no significant correlation

between fitness or change in fitness at any time point, and change in mean AUC C-peptide over the 12months of the study.

8.3.3.4.9 Baseline C-peptide

There was no significant correlation between percentage or absolute change in mean AUC C-peptide over the course of the study and the baseline mean AUC C-peptide. Equally, there was no significant difference in baseline mean AUC C-peptide between the ‘responder’ group and the ‘non-responder’ group.

8.4 Discussion

This study did not demonstrate an effect of exercise on beta-cell function in adult patients with recently diagnosed T1D. It does, however, describe the rate of decline of beta-cell function and the factors that have affected decline in beta-cell function in a group of adult patients with recently diagnosed T1D.

Measures of beta cell function showed good correlation particularly mean AUC C-peptide and 90minute or peak C-peptide. These latter measures still require a meal-stimulated test and, therefore, hold little advantage over mean AUC C-peptide in terms of burden on participant or research team. UCPCR would be an easier way to assess beta-cell function, particularly if the sample could be obtained by the participant at home (as described in the study undertaken by a group from Exeter,

UK (R. E. Besser et al. 2011)). In the Extod group there was not such good correlation between UCPCR and mean AUC C-peptide. In the Exeter study, the association between UCPCR and 90minute C-peptide was best in those with least beta-cell function (a 90minute C-peptide of under 500pmol/l), it is therefore possible that UCPCR is less accurate in patients with higher meal-stimulated C-peptide. Hence, this test is less useful in assessing beta-cell function early in the disease process. This is in keeping from the finding in the MonoPepT1De study, where UCPCR was less reliable in the first six months (Tatovic et al. 2016).

Beta-cell function declined more dramatically in the second half of the study (in contrast to the decline seen in the TrialNet studies which showed a constant decline in the first year after diagnosis, with a slower rate of loss thereafter (Greenbaum et al. 2012)). This would suggest that any future study intervention study aimed at slowing the rate of decline of beta-cell function should continue for at least one year.

We chose to stratify randomisation on baseline meal-stimulated C-peptide and fitness. In this cohort, the only factors that were significantly related to greater loss of beta-cell function were age, presentation of diabetes, duration of symptoms prior to presentation and having at least one detectable autoantibody. The caveat here is that this analysis was performed on the whole group (as the numbers involved were too small to analyse the arms separately), and any potential effect of the intervention is a confounding factor not accounted for.

Participants with positive autoantibodies at baseline had a significantly greater decline in beta-cell function over the course of the study. This would suggest that it would be important to aim to randomise equal proportions of antibody positive participants to the usual care and intervention groups. This could be done as originally intended, that is, to exclude antibody negative patients, or to use antibody status as a minimisation criteria on randomisation.

If we refer back the baseline characteristics of the intervention and control groups (see Chapter 6), while the two arms of the study were reasonably equal in terms of age, there were differences in presentation of diabetes, duration of symptoms prior to diagnosis and autoantibody status. The proportions of all of these variables were more favourable to the control arm.

It is important not to conclude that this study conclusively answers the hypothesis that exercise affects beta-cell function in T1D for a number of reasons. Firstly, this study was a pilot study to address issues of feasibility (recruitment, adherence and uptake) and was not sufficiently powered to address the secondary outcome. Secondly, (as discussed in chapter 0) the improvement in exercise level in the intervention group was much lower than expected, although improvements in fitness were of a similar level to that in other studies of T1D. Thirdly, the intervention and control groups were not adequately balanced for important factors that may affect beta-cell function, particularly antibody status, which I have shown here, may have a significant effect on decline in beta-cell function. Finally, the changes in insulin

resistance demonstrated in the intervention group (in Chapter 7) will have a significant effect on mean AUC C-peptide.

The final point to note is that insulin secretion from the beta cell is stimulated by changes in blood glucose. The amount of insulin secreted in response to increasing blood glucose is affected by the secretory capacity of the beta cells (beta-cell function) as well as peripheral insulin resistance. The more insulin resistant an individual, the greater the insulin release for a given glycaemic stimulus. An intervention which improves insulin sensitivity, can also demonstrate a reduction in insulin secretion (as measured, for example, by AUC C-peptide during a MMTT), however, this does not equate to a reduction in beta cell function. In this study, the negative association between fitness and mean AUC C-peptide as well as the fact that mean AUC C-peptide declined irrespective of antibody status in the intervention arm, lends support to this. Ideally, a method for correcting AUC C-peptide for insulin resistance in T1D is needed, as has been used previously in type 2 diabetes (Dela et al. 2004). This is beyond the scope of the Extod study and further research is required to investigate a valid way of doing this.

8.5 Recommendations for future work

Future studies of exercise and beta-cell function in adults with newly diagnosed T1D should:

- Use meal-stimulated AUC C-peptide as the measure of beta-cell function
- Have at least a one year intervention/follow-up time period
- Aim to match intervention and control groups for age, autoantibody status and presentation of diabetes.

Further research is required into methods for adjusting mean AUC C-peptide for measures of insulin resistance.

9 ADIPONECTIN, EXERCISE AND DIABETES

9.1 Introduction

Adiponectin is a 28kDa cytokine secreted predominantly by white adipose tissue and is involved in the regulation of energy homeostasis via lipid and glucose metabolism(Scherer et al. 1995). It exists in three forms; a full-length, high-molecular weight protein, a medium-molecular weight hexamer and a low-molecular weight trimer (Ziemke & Mantzoros 2010). In addition, the high-molecular weight form may be cleaved to form a smaller, globular fragment (Waki et al. 2003). These different isoforms have different affinities for the two adiponectin receptors and appear to induce different metabolic pathways resulting in different actions dependant on receptor site (Yamauchi et al. 2002).

Adiponectin is present in the circulation in humans in the greatest concentration of all the cytokines. The level of circulating adiponectin is inversely associated with the degree of visceral adiposity, as well as affected by age and gender (Cnop et al. 2003).

9.1.1 The adiponectin receptor

Two isoforms of the adiponectin receptor have been identified; known as AdipoR1 and AdipoR2 (Kadowaki & Yamauchi 2005). These are transmembrane G-protein coupled receptors and the binding of adiponectin stimulates several pathways, including the AMP-activated protein kinase and peroxisome-proliferator activated-receptor α. AdipoR1 and AdipoR2 exist in most tissues, including pancreatic beta

cells, adipose tissue, skeletal muscle and peripheral blood mononuclear cells (PBMC) (Ziemke & Mantzoros 2010; Pang & Narendran 2008). AdipoR2 mRNA expression is positively associated with the level of circulating adiponectin and HDL and negatively associated with insulin resistance, obesity and glycaemia in human fat tissue (Bluher et al. 2007).

9.1.2 The role of adiponectin in insulin resistance and glucose metabolism

Reduced adiponectin concentrations are associated with obesity (Hu et al. 1996) and insulin resistance (Yamauchi et al. 2001). Therefore, conditions associated with insulin resistance, including type 2 diabetes, cardiovascular diseases and non-alcoholic fatty liver disease are associated with reduced adiponectin levels. Thiazolidinediones, a class of oral hypoglycaemic agent that ameliorate insulin resistance, increase the levels of adiponectin in patients with type 2 diabetes (Maeda et al. 2001). Furthermore, a causal relationship between higher levels of circulating adiponectin and greater insulin sensitivity, in part mediated by lower adiposity has been demonstrated (Gao et al. 2013).

Administration of adiponectin has been shown to reduce blood glucose levels in wild type mice, mouse models of insulin resistance (*ob/ob* mice) and mouse models of T1D (insulinopaenic non-obese diabetic and streptozotocin-induced diabetic mice) (Berg et al. 2001; Yamauchi et al. 2001). In these experiments, insulin levels remained low, indicating that improvements in insulin sensitivity were responsible for the blood glucose lowering effects of adiponectin rather than an increase in insulin secretion. Adiponectin does, however, influence insulin secretion in mouse models

of insulin resistance and has an anti-apoptotic effect on beta-cells *in vitro* (Turer & Scherer 2012).

Serum adiponectin is increased with physical training (Blüher et al. 2006). Exercise upregulates adiponectin receptor expression in subcutaneous fat and skeletal muscle; indicating a potential role for adiponectin in the improvement in insulin resistance seen with increasing physical activity (Blüher et al. 2006; Bluher et al. 2007).

There is evidence to suggest that adiponectin plays a role in the autoimmune process in T1D. In contrast to type 2 diabetes, serum adiponectin is elevated in T1D; moreover, higher levels of adiponectin in T1D have been associated with increased mortality (Forsblom et al. 2011). Interestingly, adiponectin levels fall prior to the onset of T1D, perhaps mediating insulin resistance which has been identified as a risk factor for T1D (Fourlanos et al. 2004).

9.1.3 The role of adiponectin in immunity

In addition to actions on insulin sensitivity, adiponectin has effects on inflammation; affecting macrophage function and inhibiting the release of pro-inflammatory cytokines (Turer & Scherer 2012).

9.1.3.1 Adiponectin receptor expression on PBMC

AdipoR1 and AdipoR2 are expressed by PBMC (Pang & Narendran 2008). High levels of adiponectin receptor expression are seen on monocytes and B cells; however, expression is also seen on T lymphocytes and natural killer cells (NK cells).

The presence of adiponectin receptors on these immune cells suggests a role for adiponectin in the immune response.

Adiponectin receptor levels on PBMC are upregulated by physical activity in healthy, obese and type 2 diabetic subjects (Pang 2010). In T1D, there is reduced expression of adiponectin receptors on monocytes (Pang et al. 2013) and B cells (Chimen et al. 2015) compared to healthy controls. In monocytes this reduction was correlated with insulin sensitivity; that is, patients with a lower glucose disposal rate (more insulin resistant individuals) had lower adiponectin receptor expression on monocytes.

9.1.3.2 Adiponectin and T cell proliferation

The immune destruction of beta cells in T1D is T cell mediated. Although T cells express adiponectin receptors at only very low levels on their surface, they have high levels intracellularly and surface receptor expression is upregulated on antigen activated T cells (Wilk et al. 2011).

Previously in our group, Dr Terence Pang has demonstrated that adiponectin can alter the expression of co-stimulatory molecules on APCs, affecting the ability of these cells to stimulate T cell proliferation (Pang et al. 2013). In the same work, it was established that reduced adiponectin receptor expression on monocytes (as seen in T1D), is associated with attenuation of adiponectin mediated suppression of T cell proliferation (Pang et al. 2013).

9.1.3.3 Adiponectin and T cell trafficking

Circulating lymphocytes must cross the vascular endothelium to enter inflamed tissue. Leukocyte adhesion and transmigration occurs through a number of steps (Ley et al. 2007); including leukocyte rolling, activation, arrest and migration. These steps are mediated by activation of selectins, chemokine receptors and integrin molecules expressed on the cell surface (Kim 2014). T cell migration is increased in areas of inflammation, stimulated by proinflammatory cytokines (INF-γ and TNF-α) which induce expression of chemokines and integrin molecules (Kim 2014).

Adiponectin is involved in regulation of T cell transmigration in inflamed tissues. Increased endothelial adhesion and rolling of leucocytes has been demonstrated in the adiponectin knock-out mouse and this was ameliorated by the administration of adiponectin (Ouedraogo et al. 2007). Dr Myriam Chimen, from our group, has demonstrated that endothelial transmigration of T cells during inflammation is inhibited by adiponectin. The mechanism by which this occurs is mediated by a peptide from the 14.3.3 protein family (PEPITEM) which is secreted from B cells (Chimen et al. 2015). Circulating adiponectin acts on adiponectin receptors on the B cell surface to stimulate PEPITEM release. In turn, PEPITEM stimulates endothelial cells to release sphingosine-1-phosphate (S1P) which act on adherent T cells to inhibit transmigration.

The reduced expression of AdipoR1 and AdipoR2 on B cells seen in T1D is associated with a reduction PEPITEM secretion from B cells and a release from the inhibition of T cell migration with adiponectin (Chimen et al. 2015). In the same

study, similar findings were demonstrated in rheumatoid arthritis, another T cell mediated disease.

While adiponectin appears to have a marked effect on aspects of the immune response responsible for T1D, other adipokines, such as leptin, have been demonstrated to have no effect on T cell proliferation and limited effect on T-cell transmigration (Chimen 2012).

We hypothesise that serum adiponectin and adiponectin receptor expression on PBMC can be upregulated by exercise training patients with T1D, similar to the effect seen in other tissues (Bluher et al. 2007; Blüher et al. 2006), in healthy people and in response to changes in insulin resistance (Pang 2010). Furthermore, we anticipate that by upregulating AdipoR1 and AdipoR2 expression on PBMC, we will see a functional impact; increased suppression of T cell migration and proliferation with adiponectin. If demonstrated, suppression of migration and proliferation of T lymphocytes by adiponectin in T1D may elucidate a mechanism by which exercise could influence beta-cell function.

9.2 Methods

9.2.1 Subjects

9.2.1.1 Healthy Subjects

Specimens were provided by Sam Shepherd/Professor Anton Wagenmakers of the Department of Sports science, University of Birmingham. The study involved recruiting healthy, but sedentary, volunteers to undergo a 10-week exercise programme. Participants exercised on a bicycle at either moderate intensity 45 minutes, 3 times a week or underwent a programme of low-volume high-intensity interval training. Subjects were all previously healthy and had normal glucose tolerance. Samples were obtained in the resting state, before and after the exercise training period and analysed on the same day (with the exception of the PCR analysis).

9.2.1.2 Subjects with type 1 diabetes

Patients with recently diagnosed T1D were recruited through the Extod study. All participants had venous blood samples taken at baseline and after six months. PBMCs from these samples were isolated and stored for future PCR analysis for adiponectin receptor expression. In addition, fasting serum samples were stored and later used for adiponectin analysis.

In addition those participants recruited to the Birmingham site were asked if they wished to participate in the Immunity study, for which they gave separate written, informed consent. In the patients who gave consent for these additional samples,

whole blood was taken for adiponectin receptor flow cytometry, T cell proliferation and T cell transmigration.

9.2.2 Materials

A list of the reagents used for this part of the study is given in *Table 9-1*.

Reagent	Supplier	Catalogue number	Use
Histopaque 1119	Sigma-Aldrich	11191	PBMC Isolation
Histopaque 1077	Sigma-Aldrich	10771	PBMC Isolation
RPMI 1640	Gibco Invitrogen	21875	PBMC culture
Human Adiponectin (recombinant)	Enzo Life Sciences	ALX-522063	PBMC culture
Adiponectin	Novonordisk		Transmigration
CellGro SCGM	CellGenix	20802	Cell culture
FcR Blocking reagent	Miltenyi Biotec	130-059-901	Flow cytometry
Purified Protein Derivative (PPD) of M. Tuberculosis Tuberculin	NIBSC	PPDT	Proliferation studies
Bovine serum albumin (BSA) solution	Sigma-Aldrich	A8412	Transmigration
EDTA	Sigma-Aldrich	E8008	Transmigration
Tetanus Toxoid Protein	Calbiochem	582231	Proliferation studies
CD14 beads	Miltenyi Biotec	130-050-201	PBL preparation
LS Columns	Miltenyi Biotec	130-042-401	PBL preparation
RNeasy Mini Kit	Qiagen	74104	RNA extraction
M199	Gibco Invitrogen	31150-022	
2X Master Mix	ABI	P02016	qPCR
Random Primers	Promega	C1181	qPCR
Rnaseout. Recombinant Ribonuclease Inhibitor	Invitrogen	10777-019	qPCR
SuperScript II. RNase H- Reverse Transcriptase	Invitrogen	18064-014	qPCR
dNTPs	Promega	U120-3A	qPCR
Human Adiponectin Quantikine Kit	R & D Systems	DRP300	Adiponectin assay

Table 9-1 - List of main reagents

9.2.3 Isolation of PBMC from venous blood

Samples of venous blood were taken in EDTA. PBMC were isolated by layering venous blood onto Histopaque 1119/1017 in equal quantities. After centrifugation at 800g for 35mins, the PBMC were collected and washed twice in RPMI 1640 media. The PBMC were then counted manually.

The isolated PBMC were

a) stored for later PCR analysis of adiponectin receptor expression,

b) analysed for adiponectin receptor expression by flow-cytometry,

c) cultured in various conditions to assess the effect of adiponectin on T cell proliferation, and

d) utilised in assays of T cell transmigration across an endothelium.

9.2.4 PCR for adiponectin receptor expression

Real-time PCR was used to measure adiponectin receptor expression on PBMC.

PBMC isolated from peripheral blood healthy volunteers and those taken from Extod trial subjects were snap frozen in liquid nitrogen and stored at -80°C.

9.2.4.1 RNA extraction

The frozen PBMC were lysed and mRNA extracted using the RNeasy Mini Kit (Qiagen). The manufacturer's protocol was followed. A Nanodrop spectrofluorimeter (LabTech) was used to measure the concentration of mRNA following extraction.

9.2.4.2 Reverse transcription

The RNA (1ug) was heated to 70°C with random primers (Promega) for 5 minutes. Then dNTPs (10mM concentration, Promega), Supercript buffer, Rnasout, Superscript reverse transcriptase (Invitrogen) were added. This was heated to 37°C for one hour, followed by 95°C for 5 minutes to obtain cDNA.

9.2.4.3 Real-rime PCR

Quantitative PCR was performed in duplicate on a RT-PCR machine (ABI 7500, Applied Biosystems).

Taqman gene expression assay kits (Hs00360422_m1 for AdipoR1 and Hs00226105_m1 for AdipoR2) were utilised along with Taqman Master Mix (Applied Biosystems). Relative gene expression was calculated using the ΔΔCT normalised to 18s. Relative expression units (REU) were also calculated using $REU=2^{\Delta CT}$.

9.2.5 Flow cytometry

Flow cytometry was used to measure adiponectin receptor expression on PBMC and PBMC subsets as well as T cell proliferation. The antibodies used for flow cytometry are given in Table 9-2. Samples were assayed using a CyAn FACS analyser (Beckman Coulter) and Summit software. Flowjo V5 (Mac) software was used for analysis. Adiponectin receptor expression is given as percentage of positive cells of mean fluorescent intensity (MFI).

Antibody	Supplier	Fluorochrome	Clone	Catalogue number
Rabbit anti-AdipoR1	Phoenix Pharmaceuticals	Alexa 488/FITC		G-001-44
Rabbit anti-AdipoR2	Phoenix Pharmaceuticals	Alexa 488/FITC		G-001-23
Anti-human CD8a	eBioscience	eFluor 450(PB)	OKT8	48-0086
Anti-human CD4	BD Pharmigen	APC	RPA-T4	555349
Anti-human CD4	eBioscience	eFluor 700	RPA-T4	46-0049
Anti-human CD4	eBioscience	APC-eFluor 780	OKT4	47-0048
Anti-human CD3	eBioscience	Per-CP-Cy5.5	OKT3	45-0037
Anti-human CD19	eBioscience	PE-Cy7	HIB19	25-0199
Anti-human CD14	eBioscience	APC	61D3	17-0149
Anti-human CD56	eBioscience	PE	CMSSB	12-0567

Table 9-2 - List of Antibodies

9.2.6 Quantification of Serum Adiponectin

Serum adiponectin was measured in stored samples using a Quantikine ELISA Human Total Adiponectin/Acrp30 kit (R&D Systems).

9.2.7 T cell transmigration studies

9.2.7.1 Preparation of endothelial cells

Primary HDMEC (Human Dermal Microvascular Endothelial Cells) were obtained from PromoCell (PromoCell GmbH, Heidelberg, Germany) and were cultured in the

manufacturers Endothelial Cell Growth medium. The cells were passaged four times before use.

9.2.7.2 Isolation of Peripheral Blood Lymphocytes (PBLs)

PBL were isolated from the PBMC by subtraction of monocytes. PBMC were labelled with CD14 microbeads and incubated at 4°C for 15 minutes. The cells were then washed in MACS buffer and added to the LS column (Miltenyi Biotech) within a magnetic field. The column was washed three times with MACS buffer. Unlabelled cells (PBL) passed through the column and were collected and counted.

9.2.7.3 Endothelial Transmigration

HDMEC (adult) (PromoCell) were inspected to ensure there was an adequate endothelial coverage and that the cells were suitably primed. Cells were treated with TNFα (100U/ml) and IFNγ (10ng/ml) for 24hours prior to use.

PBL were incubated in three conditions;

1. Control (PBL 0), incubated with media (M199 0.15%BSA) only
2. Adiponectin (PBL AQ), PBL incubated for 1 hour prior to assay with adiponectin (NovoNordisk) at a concentration of 15ug/ml. (These cells were washed and resuspended in fresh media before the experiment as endothelial cells also express AdipoR.)

3. PEPITEM (PBL Pep), PBL incubated with media (M199 0.15% BSA) with 20ng/ml Pepitem added immediately prior to assay.

1million PBL in 1ml of media (+/- adiponectin or PEPITEM) were added to each well (one for each condition, three wells for each individual). The cells were incubated at 37°C for 6minutes. The cells were washed twice with M199 0.15% BSA media to remove non-adherent cells, then fixed with 4% formaldehyde.

Using a phase-contrast microscope six images, randomly acquired from the area of the well covered with endothelium, were acquired.

9.2.7.4 Analysis

The images obtained from the phase contrast microscope were analysed using ImagePro 6.2 (Media Cybernetics) software. Cells were identified as either transmigrated cells (phase dark) or adherent cells (phase bright) see *Figure 9-8*.

The counts were averaged and the results expressed as a percentage of cells migrated and presented as a mean for each condition.

9.2.8 T cell proliferation

To assess responses to various stimuli, PBMC were first labelled with CFSE. Whole PBMC were washed in PBS, counted and suspended at a concentration of $2x10^7$ cells/ml. CFSE at a concentration of 2.5µM was added and the cells were agitated and incubated at room temperature in the dark. After 10minutes an equal volume of fetal calf serum (FCS) was added to quench the reaction. The cells were then

washed three times in RPMI and resuspended in RPMI 10% FCS (treated with Penicillin/Streptomycin) at a concentration of 200,000 cells per 200ul.

Cells were stimulated and then incubated at 37°C in 96 well plates. Stimulation occurred under the following conditions and in triplicate:

- Control (no stimulation)
- Stimulation with OKT3 (concentration 0.5ug/ml)
- Simulation with pancreatic islet lysate (Oxford) (5uL in 50uL media)
- Stimulation with purified protein derivative to M. Tuberculosis (PPD) (National Institute for Biological Standards and Control, UK) (concentration 500iu/ml)

Each of the stimulated conditions was performed with and without adiponectin (Enzo Lifesciences) supplementation (at a concentration of 4µg/ml). After 3 days (for OKT3-stimulated cells) or 7 days (for PPD-stimulated and islet lysate-stimulated cells) the T cells were collected and stained with fluorescent antibodies to CD4 (CD4-APC, BD Biosciences) and CD8 (CD8-eFluor 450, eBioscience) and the proportion of proliferated cells determined using flow-cytometry. Results are expressed as the stimulation index (SI) for the condition. Mean values for triplicates were calculated for each condition. The SI is the proportion of the cell population studied that has proliferated in response to an antigen relative to the unstimulated control (Mannering et al. 2010). A SI of greater than 2 indicates significant proliferation. Percentage inhibition of proliferation with adiponectin was also calculated, this is the percentage change in SI.

9.2.9 Ethics

Ethical approval for the sports science study was given by the University of Birmingham ethical committee in August 2012 (ERN_11-0839).

A favourable ethical opinion for the immunity study was granted by East Birmingham Local Research Ethics committee in June 2006 (reference number 06/Q2703/47).

9.3 Results

9.3.1 Healthy Subjects from the Sports Science Study

9.3.1.1 Description of participants

Samples from 23 participants were obtained. The mean age of these participants was 47 years old (range 31-60 years). Six of the participants were male.

Over the course of the exercise intervention BMI was not significantly changed (mean starting BMI 27.5kg/m^2 and mean final BMI 26.6kg/m^2) , however, fitness (as measured by VO_2max improved from 30.9 ml/min/kg to 33.4ml/min/kg).

In this group, serum adiponectin (results provided by Dr David Bartlett) did not alter (mean starting serum AQ 2.4 ug/ml, mean final 2.5ug/ml).

9.3.1.2 Adiponectin receptor levels by PCR

Baseline adiponectin receptor levels were not statistically significantly correlated with age, BMI, weight or fitness. There was however, a trend towards greater adiponectin receptor expression with increasing fitness.

Analysis of adiponectin receptor levels by PCR did not show a significant change in adiponectin receptor gene expression with exercise training in healthy individuals (*Figure 9-1*). The median fold change in AdipoR1 expression was 1.299, and in AdipoR2 expression was 1.325.

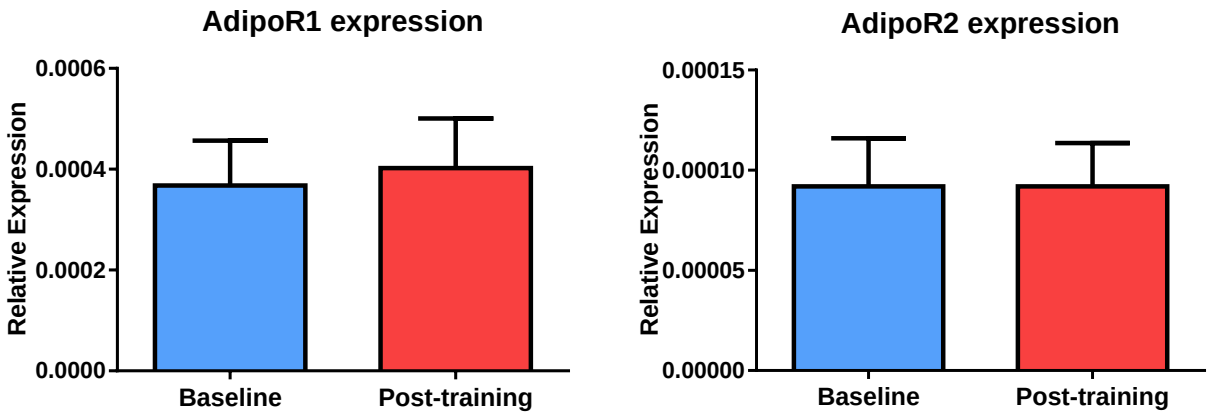

Figure 9-1 – AdipoR expression on PBMC pre and post training in healthy people.

While the change in adiponectin receptor expression after exercise training was not significantly associated with age, BMI or change in serum adiponectin, it was associated with the change in physical fitness (as measured by VO2 max). For AdipoR1 this association was statistically significant (r=-0.453, 95% CI -0.729 to -0.0498, r^2=0.205, p=0.03).

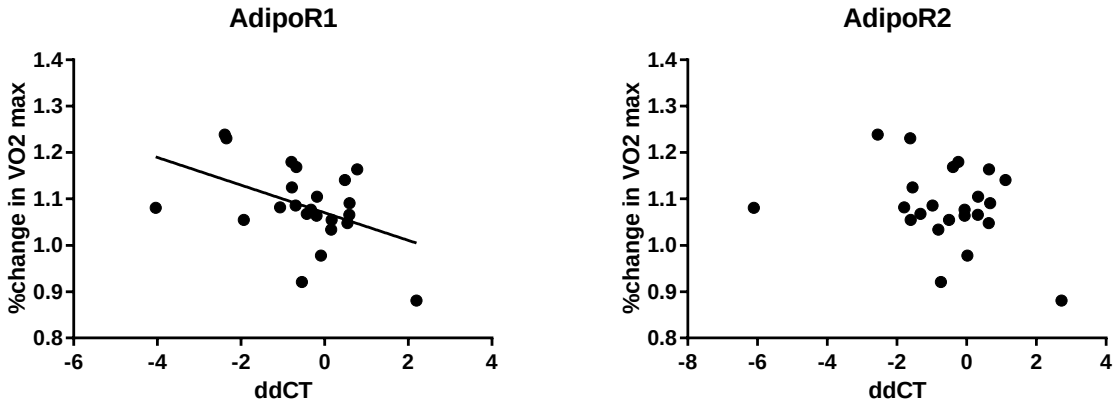

Figure 9-2 – The relationship between change in fitness and change in AdipoR expression on PBMC.

9.3.1.3 Adiponectin receptor levels by flow cytometry

Flow cytometry analysis of adiponectin receptor expression on PBMC was used to ascertain whether there was a differential effect of exercise on PBMC subsets. Cells were gated on forward-side scatter/pulse width and then on phenotyping antibodies (examples are shown in Figure 9-3). Each sample was compared against a negative control (isotype), which had been stained for neither AdipoR1 nor AdipoR2 see Figure 9-3D.

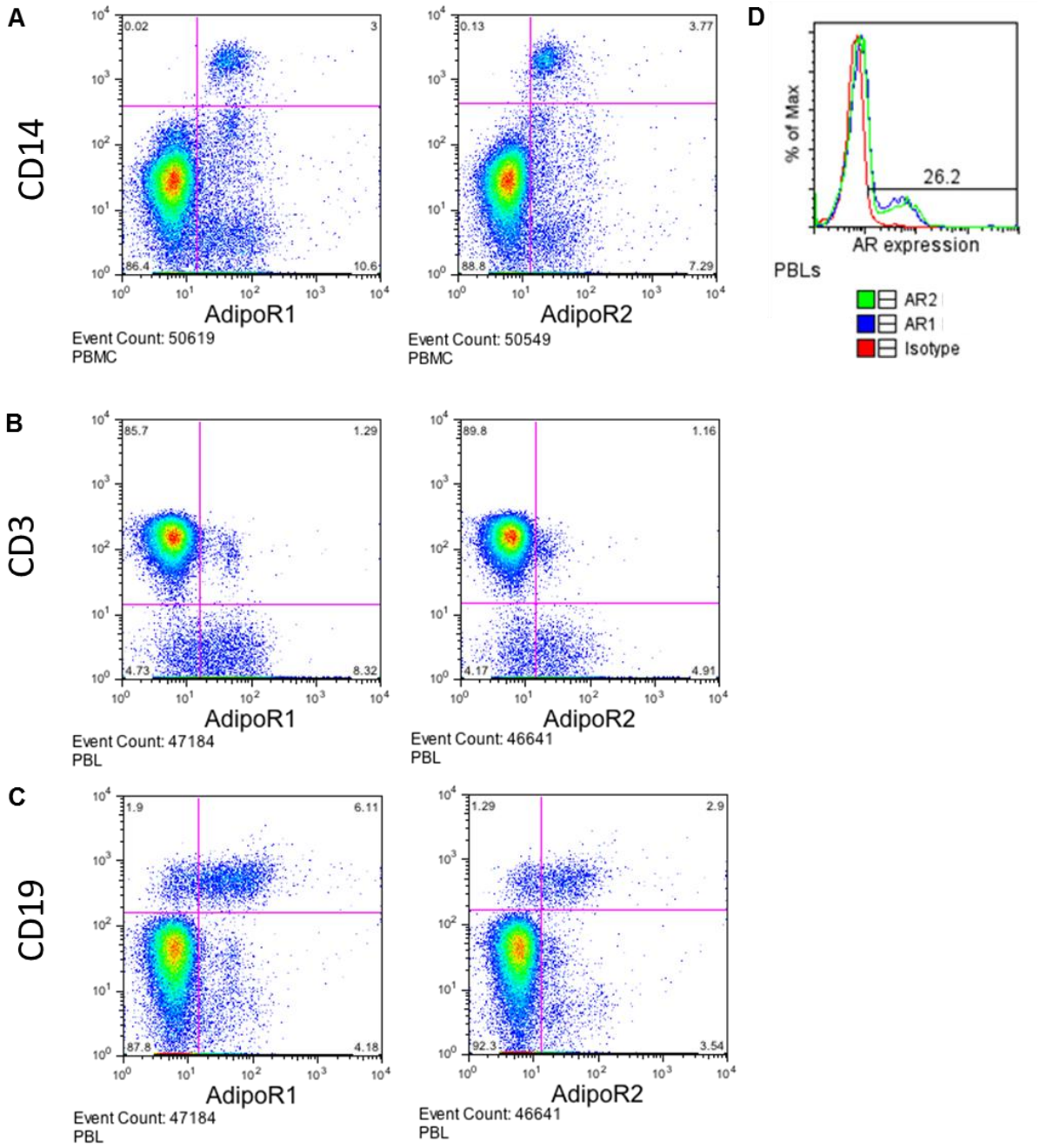

Figure 9-3 - Typical results from flow cytometry for adiponectin receptor expression for CD14+ monocytes (A), CD3+ T cells (B) and CD19+ B cells (C). AdipoR expression was greatest on monocytes. Each subset was compared against an isotype control (which was subtracted) (D).

Analysis of AdipoR expression by flow-cytometry did not show a significant increase in AdipoR on PBMC either in percentage of cells expressing the receptor or mean fluorescent intensity (Figure 9-4). There did appear to be an increase in AdipoR2, however, this was not statistically significant.

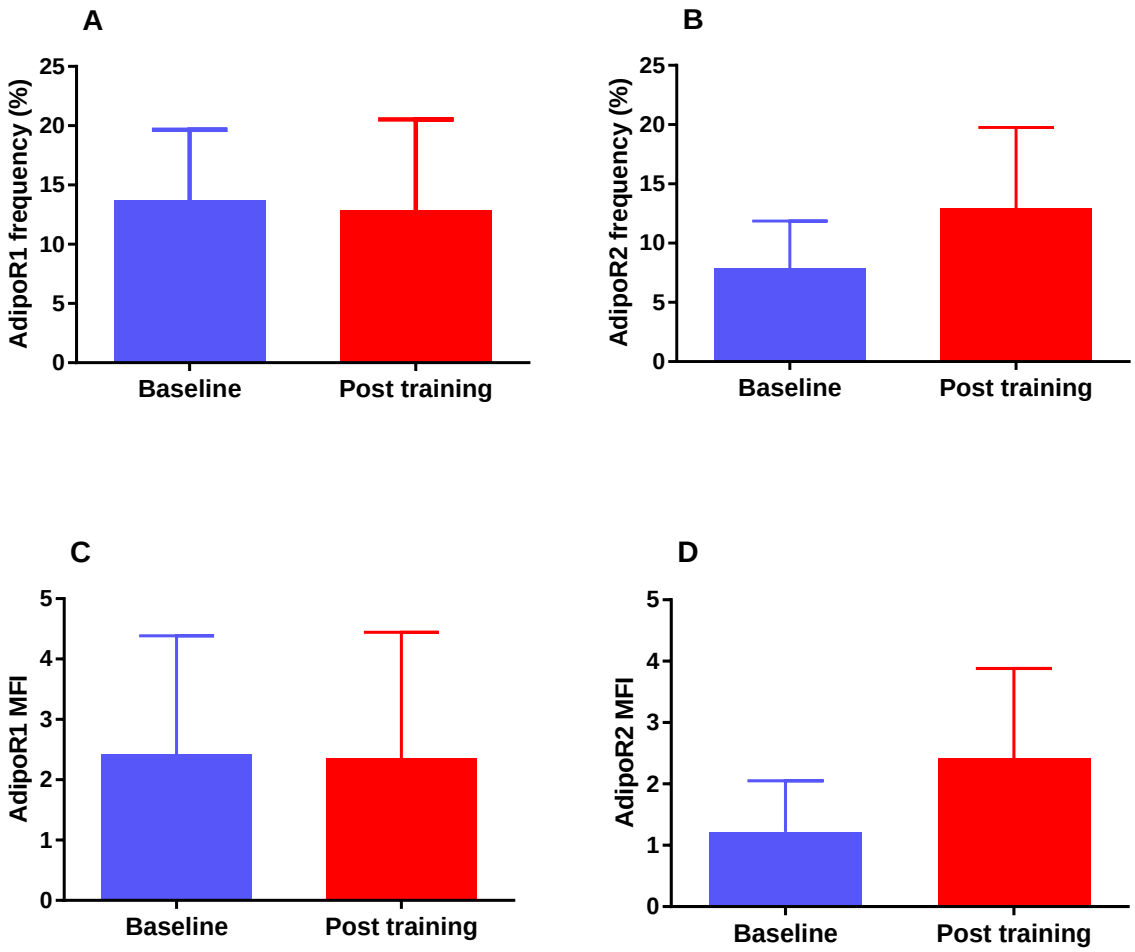

Figure 9-4 - Adiponectin receptor expression on PBMCs before and after exercise training by flow-cytometry. (A) and (B) show percentage of cells expressing AdipoR, (C) and (D) show mean fluorescence intensity (MFI).

AdipoR2 expression on all PBMC subsets appeared to increase with exercise training, however, significant increases were seen only on PBL and NK T cells after exercise training (see Figure 9-5).

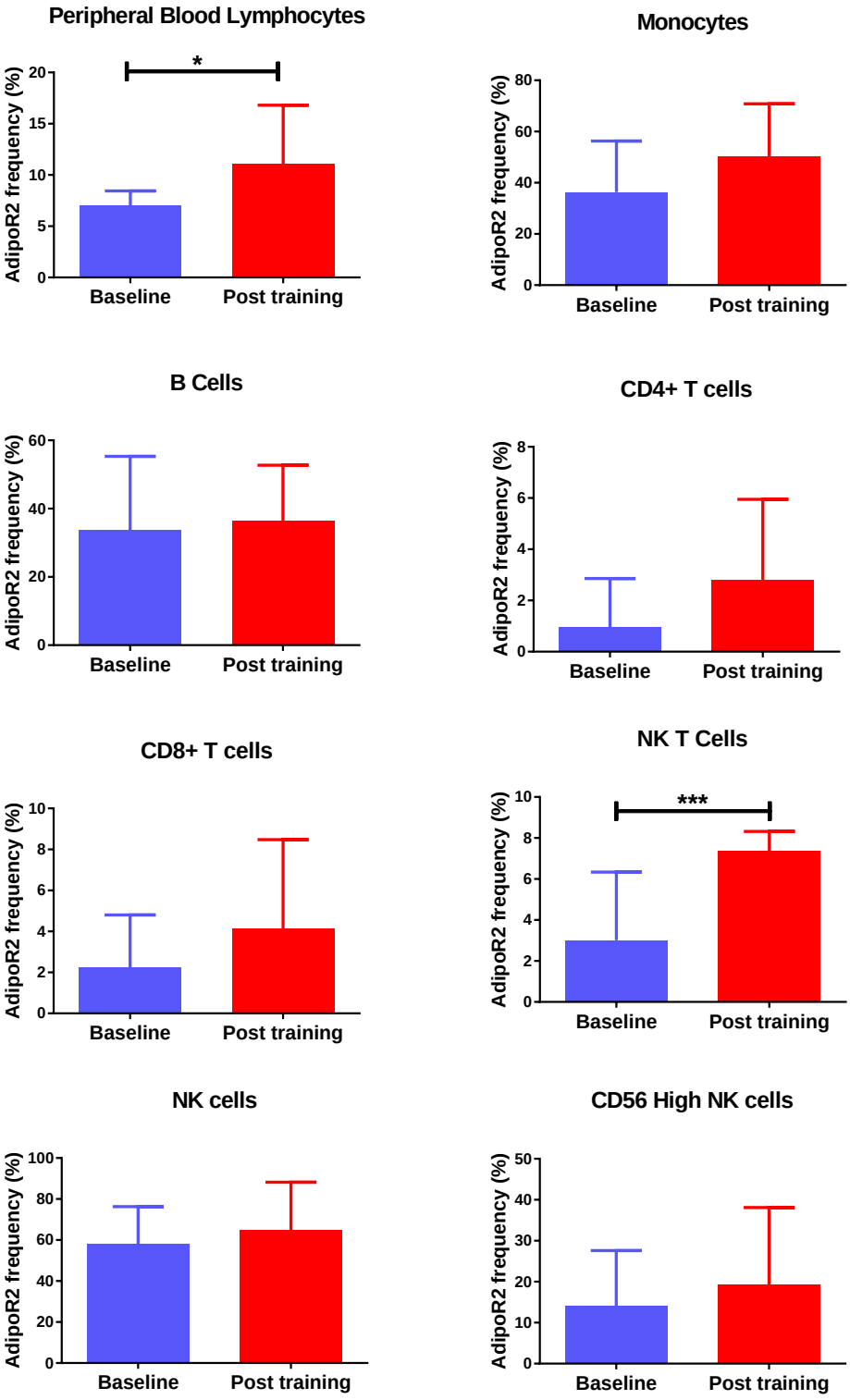

Figure 9-5 – Distribution of AR2 receptors by PBMC subset. Significant increases are seen on PBLs (p=0.027) and NK T cells (p=0.0009) post training.

9.3.1.4 T cell proliferation

Figure 9-6 shows representative pictures of the flow cytometry results for the T cell proliferation studies. Cells were gated on fluorochromes on either CD4 or CD8 antibodies. Stimulation is demonstrated by serial dilution of CFSE staining.

The T cell proliferation studies demonstrate a greater inhibition of proliferation of CD4+ T cells with adiponectin following exercise training (similar results were seen for CD8+ cells, data not shown). This was apparent but not statistically significant when the PBMCs were stimulated with CD3 antibody, but was significant ($p=0.016$) when PPD was used. The mean stimulation index for islet lysate was close to 1 indicating that no proliferation was stimulated, and therefore no inhibition of proliferation was seen with adiponectin. Overall stimulation was less after exercise training (see Figure 9-7A).

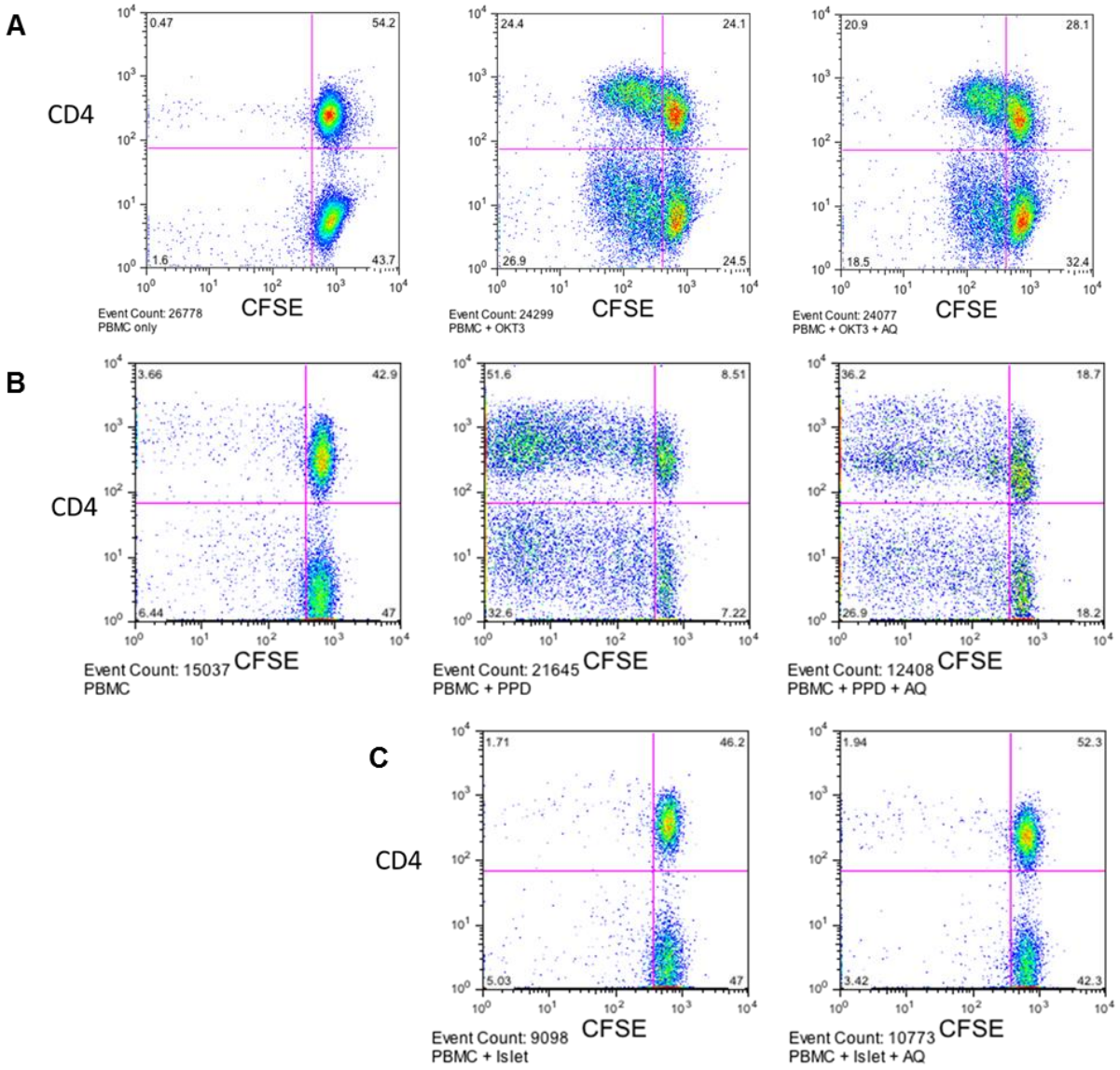

Figure 9-6 – CD4+ T cell proliferation to various stimuli. Stimulation was with CD3 antibody (A), PPD (B) and islet lysate (C). Each condition was compared to an unstimulated control (left).

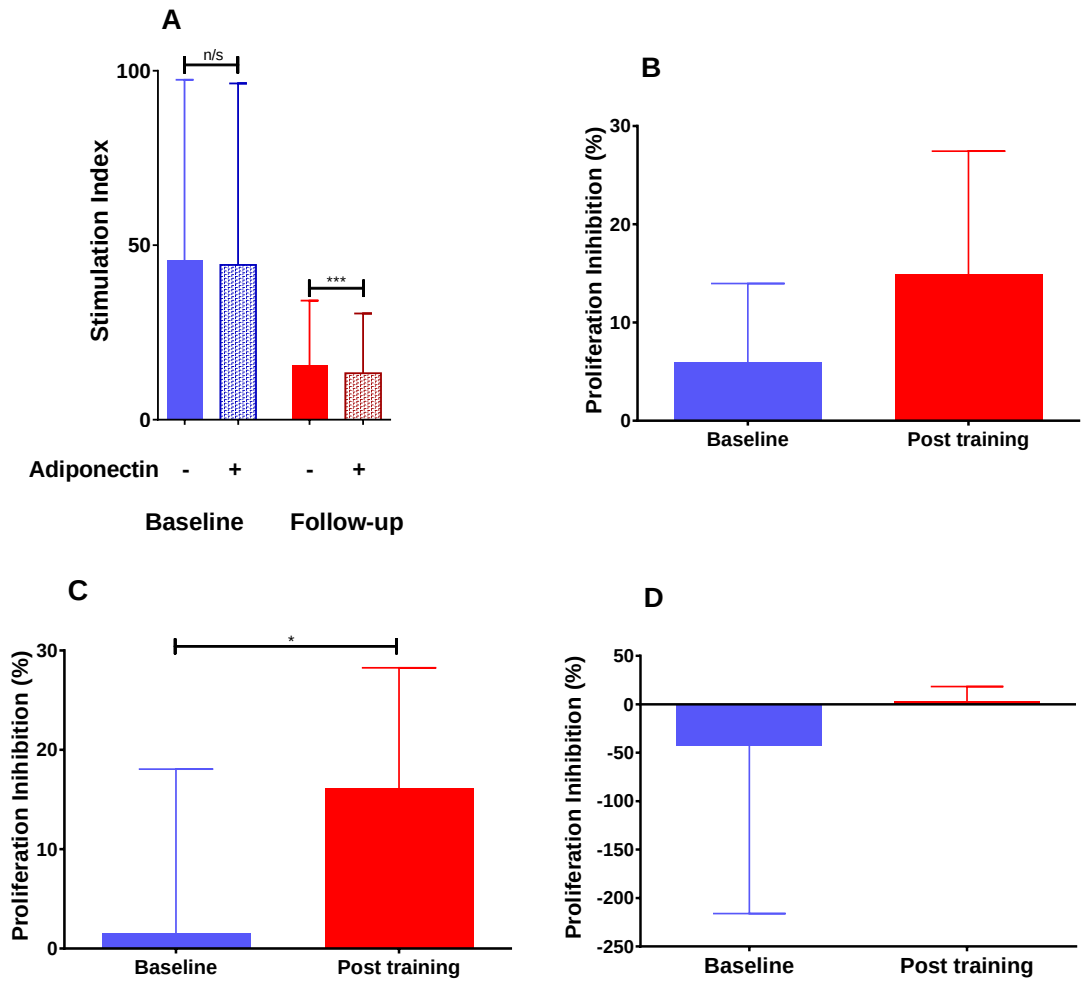

Figure 9-7 Percentage inhibition of proliferation (as referenced to a control) with adiponectin. (A) Stimulation index following stimulation of proliferation with OKT3 in the presence and absence of adiponectin, before and after exercise training. Percentage inhibition of proliferation with adiponectin after stimulation with OKT3 (B), PPD (C) and islet lysate (D)

9.3.1.5 Endothelial transmigration in healthy people

Endothelial transmigration of PBL was performed on only five participants at baseline. However, there was significant suppression of migration with PEPITEM

and adiponectin when compared to the control (p=0.0085, ANOVA) (Figure 9-9). Only one of these participants attended for follow-up and in this participant suppression of migration with both PEPITEM and adiponectin was reduced after training (Figure 9-10). This is consistent with the measured AdipoR in this participant, which fell over the training period.

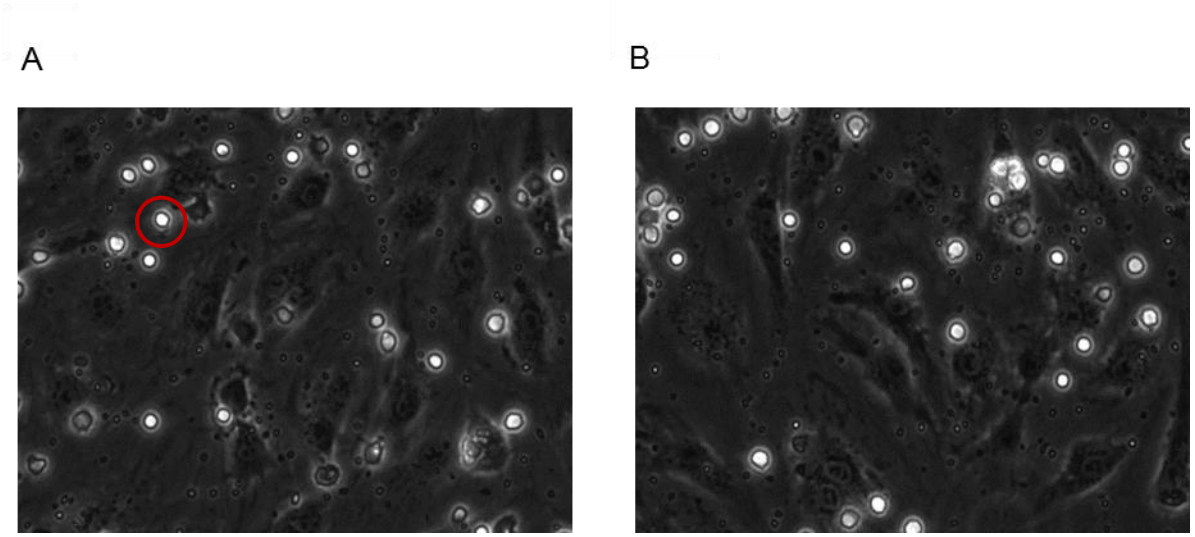

Figure 9-8 – Phase contrast microscopy of HDMEC with adherent (phase bright) PBL circled. (A) control, (B) treated with adiponectin.

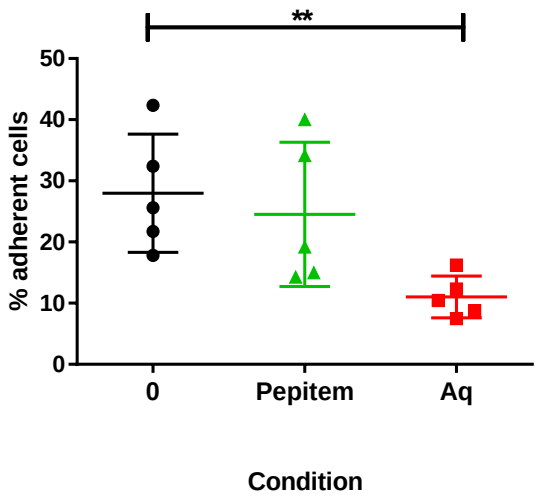

Figure 9-9 - Endothelial transmigration under three conditions (control "0", addition of "Pepitem" and preincubation of T cells with adiponectin "Aq") at baseline (healthy individuals in the sports science study).

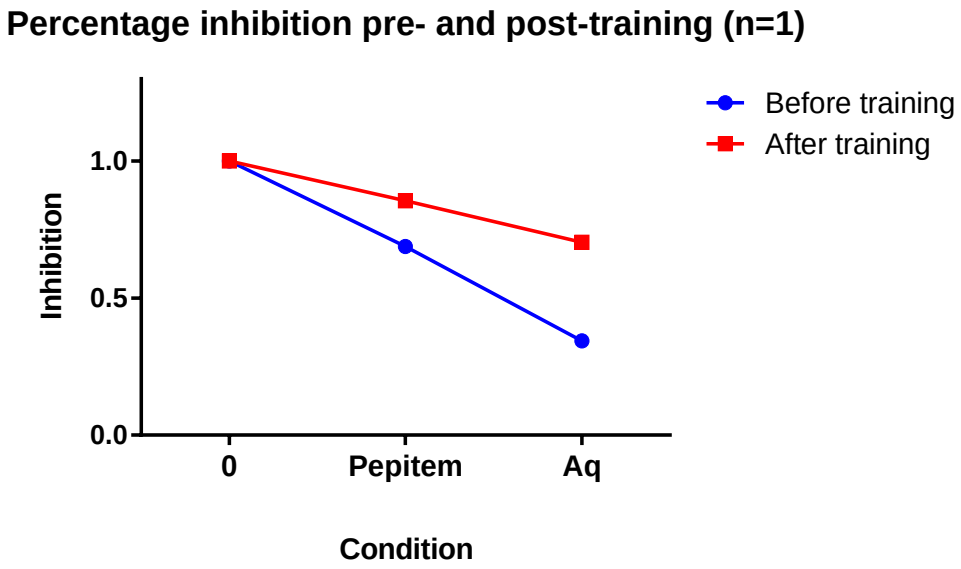

Figure 9-10 - Change in endothelial transmigration after training in one participant (NB adiponectin receptor levels fell over same period of time)

9.3.2 Subjects with type 1 diabetes

9.3.2.1 Serum adiponectin changes with exercise in T1D

The median serum adiponectin in the Extod participants at baseline was 6.6 ug/ml (interquartile range (IQR) 8.73). Baseline serum adiponectin was not correlated with age, BMI, WHR, fitness, activity, HbA1c, or HOMA2-IR. In addition, there was no relationship between smoking status or number of autoantibodies and serum adiponectin at baseline.

Participants in the intervention arm of the Extod study had higher serum adiponectin at baseline (median serum adiponectin 3.99 (IQR 6.25) ug/ml in the control arm compared to 7.42 (IQR 12.27) ug/ml in the intervention arm). In those participants completing 6 months of the study at the time of analysis, serum adiponectin appeared to fall in the intervention group, while the value remained unchanged in the control arm. In those participants completing twelve months at the time of analysis, the fall at six months was also apparent and was sustained over the second half of the study (see Figure 9-11). Serum adiponectin was unchanged in the control arm. The difference in adiponectin between the two groups was significant at baseline, however, there was no difference between the two groups at either of the follow-up time points (p=0.043, two-way ANOVA).

In view of the negligible increase in MVPA in the participants who were in the intervention arm, we went on to examine the effect of increasing physical fitness on serum adiponectin. Those participants who increased their VO2max during the study tended to maintain serum adiponectin and in those whose VO2max declined during

the study, serum adiponectin also declined. These findings were not statistically significant, however (two-way ANOVA).

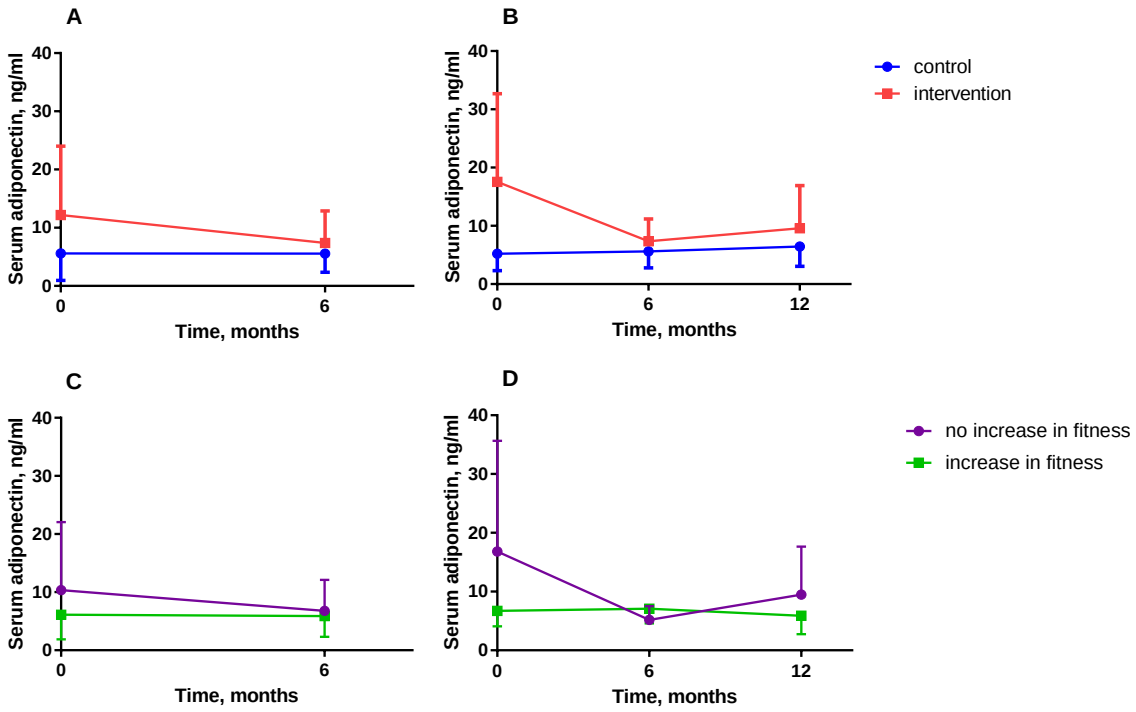

Figure 9-11 – Serum Adiponectin in participants in the Extod trial. Participants up to 6 month time point (A) (control n=14, intervention n=19). Participants up to 12 months (both arms n=8). (C) and (D) compare those participants who increased their fitness levels with those who did not.

9.3.2.2 Adiponectin receptor expression in T1D by PCR

AdipoR1 and AdipoR2 expression were highly correlated (r=0.806, 95%CI 0.6182 to 0.9062, r^2=0.649, p<0.0001). There was no significant relationship between baseline AdipoR expression on PBMC and age, BMI, weight, estimated VO2 max, HbA1c, fasting insulin or HOMA2-IR (Figure 9-12).

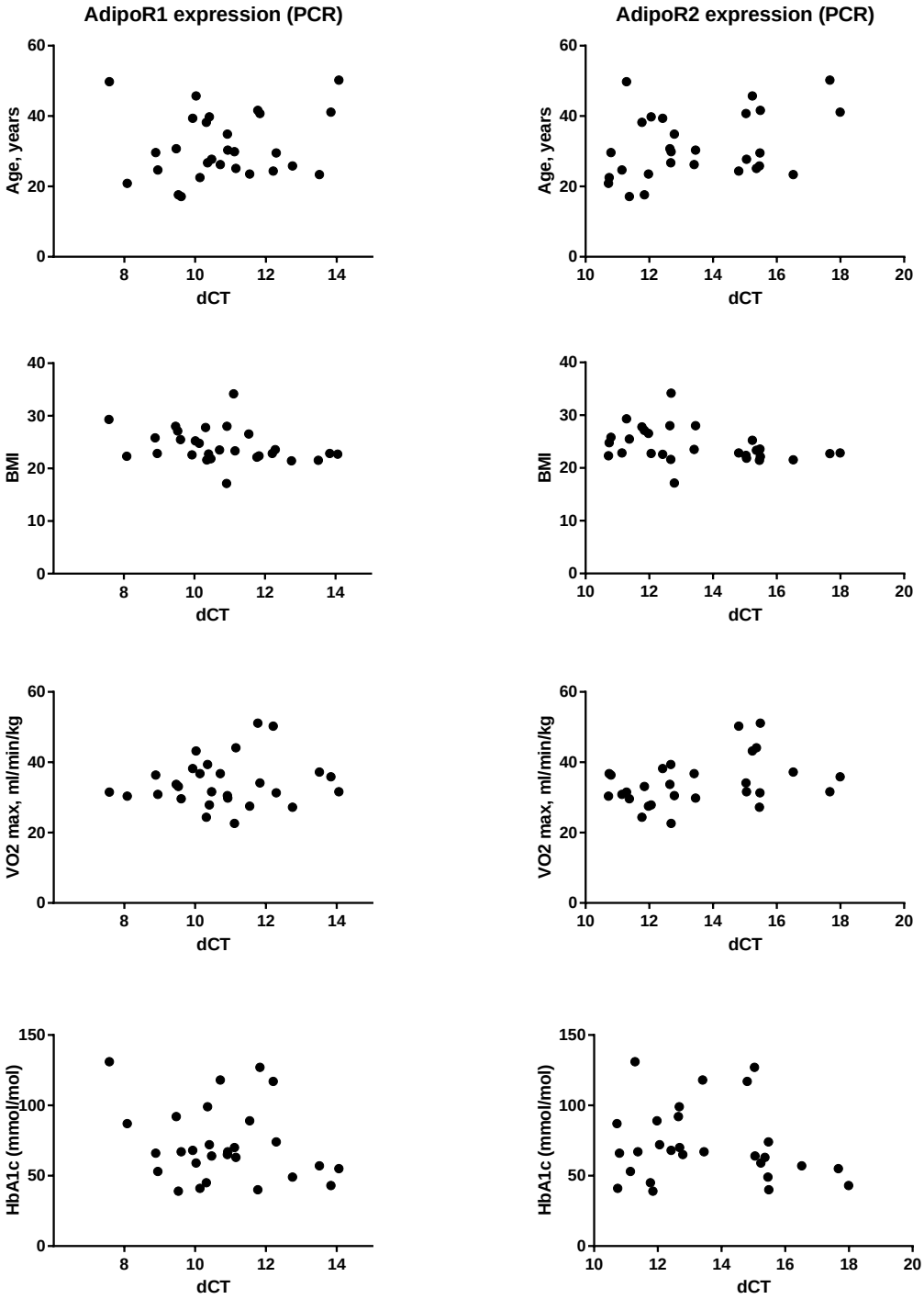

Figure 9-12 – Relationship between AdipoR dCT and age, BMI, fitness and glycaemic control. None of these are statistically significant.

At 6 months adiponectin receptor expression had increased in both the control and in the intervention arms. However, the increase was only statistically significant in the intervention arm. This was true for both AdipoR1 and AdipoR2.

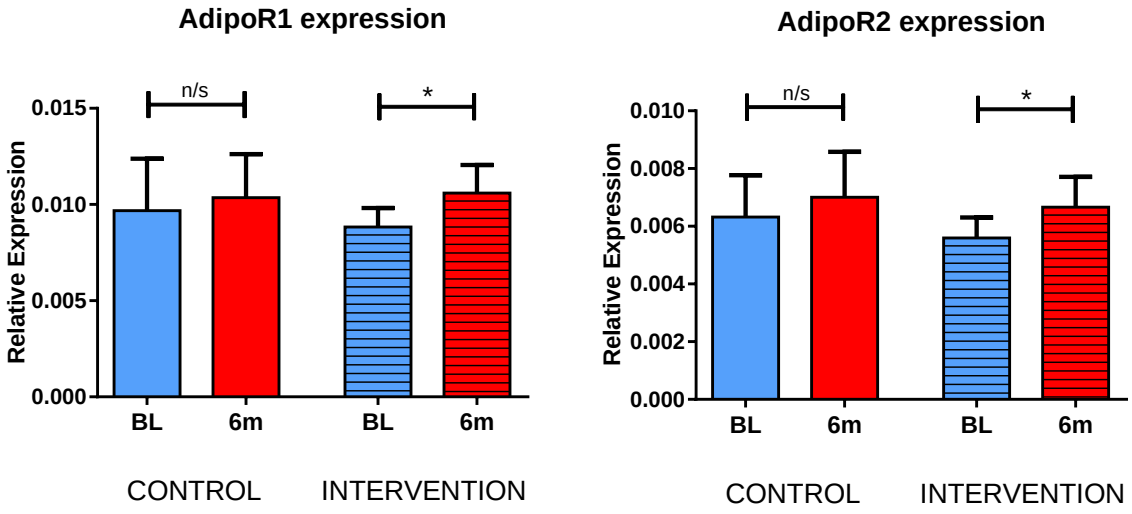

Figure 9-13 – There is a statistically significant increase in AdipoR in patients with T1D after exercise training (ANOVA).

The fold change in adiponectin receptor expression was not significantly different between the 2 groups for either AdipoR1 or AdipoR2 (AdipoR1 control mean fold change 2.437, 95%CI 1.125, 3.749, AdipoR1 intervention mean fold change 2.641, 95%CI 1.466, 3.816) but both groups have a fold change significantly greater than one. This indicates that there is an increase in AdipoR in the first year after diagnosis of T1D.

There was no association between change in adiponectin receptor expression and age, change in any of weight, estimated VO2 max, HOMA-IR, HbA1c or circulating adiponectin.

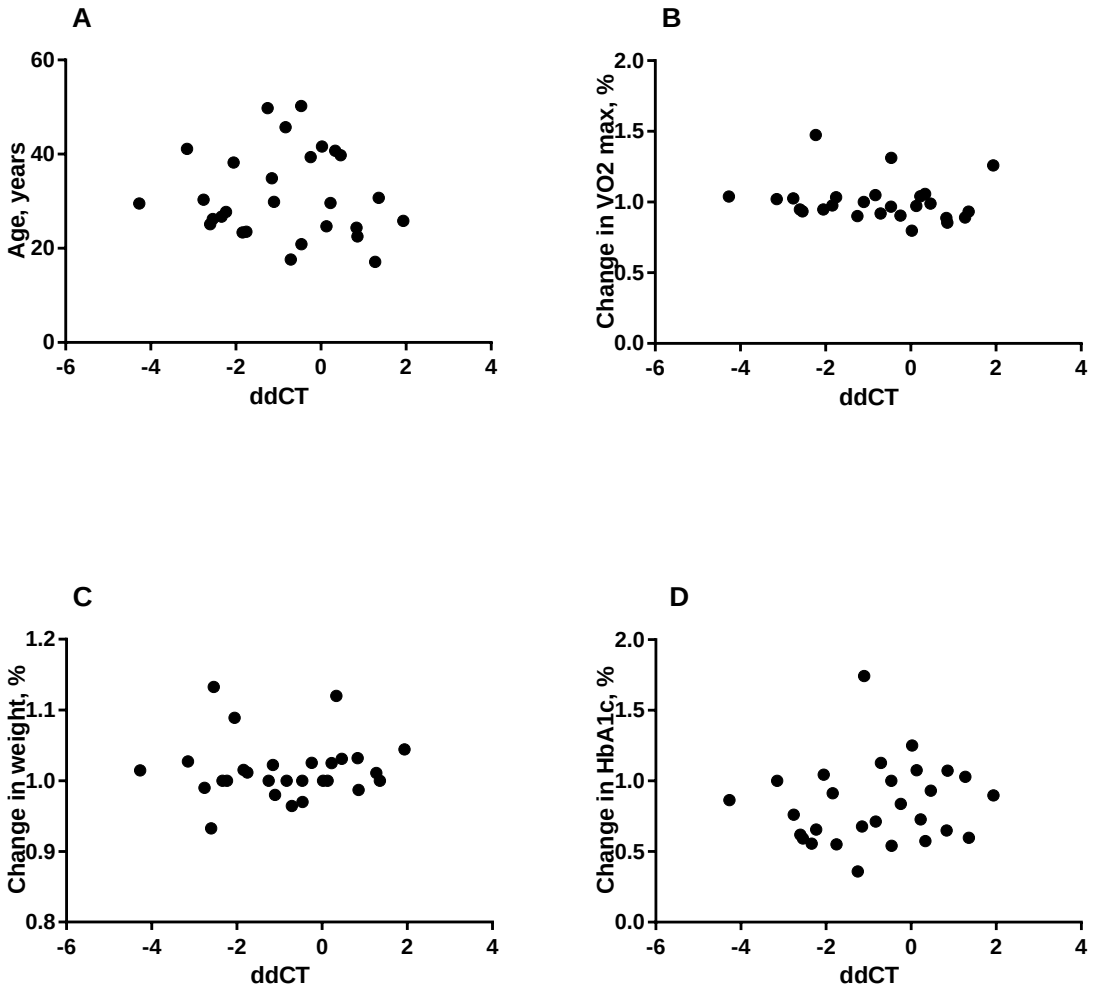

Figure 9-14 – Relationship between change in AdipoR2 and age (A), change in fitness (B), weight (C) and glycaemic control (D). The relationship with these parameters and AdipoR1 is similar (data not shown).

9.3.2.3 Adiponectin receptor expression on PBMC by flow-cytometry

In the subset of participants who have AdipoR determined by flow cytometry, there is no significant difference in AdipoR1 or AdipoR2 frequency before and after exercise.

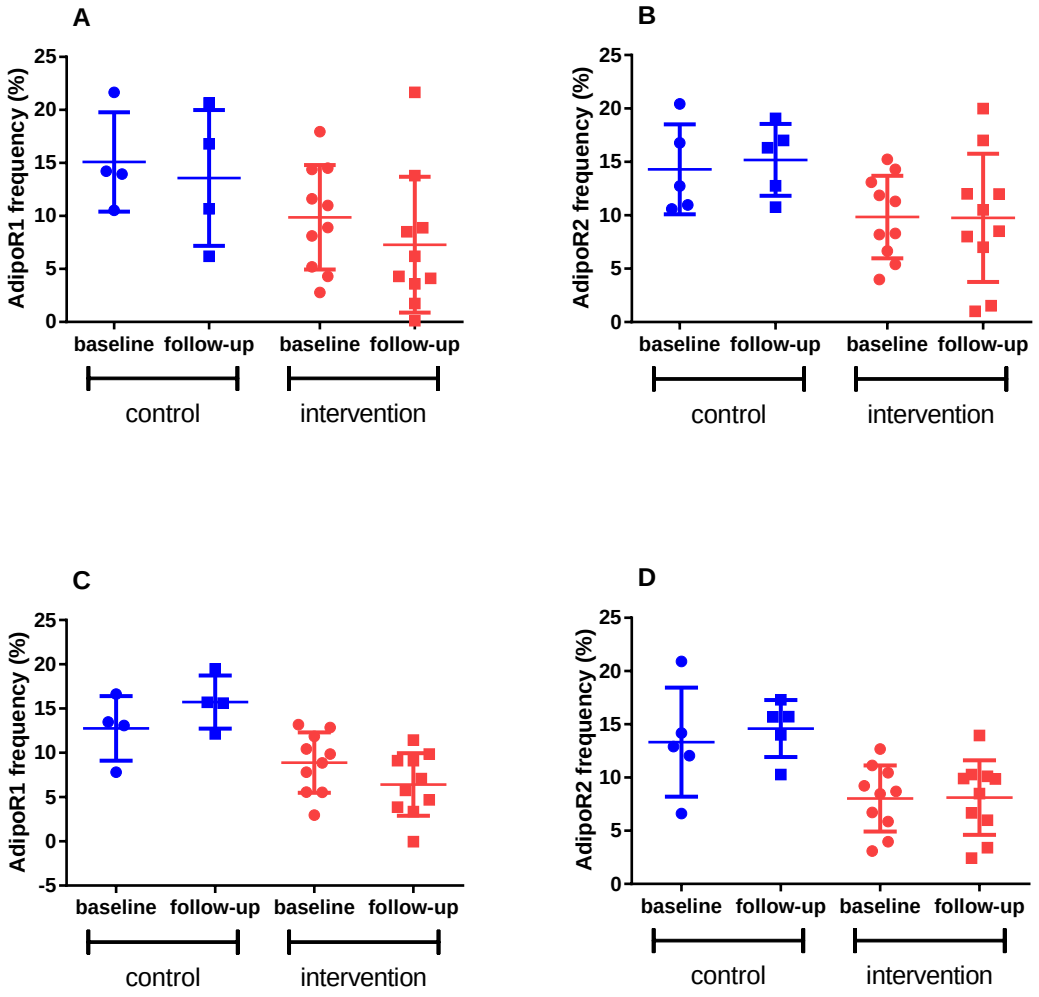

Figure 9-15 - AdipoR expression determined by flow cytometry. (A) and (B) show data for PBMC, (C) and (D) for PBL.

9.3.2.4 T cell Proliferation

Successful analysis of samples for T cell proliferation was achieved in 5 intervention participants and 2 controls (shown in Figure 9-16). There is no clear relationship to group demonstrated.

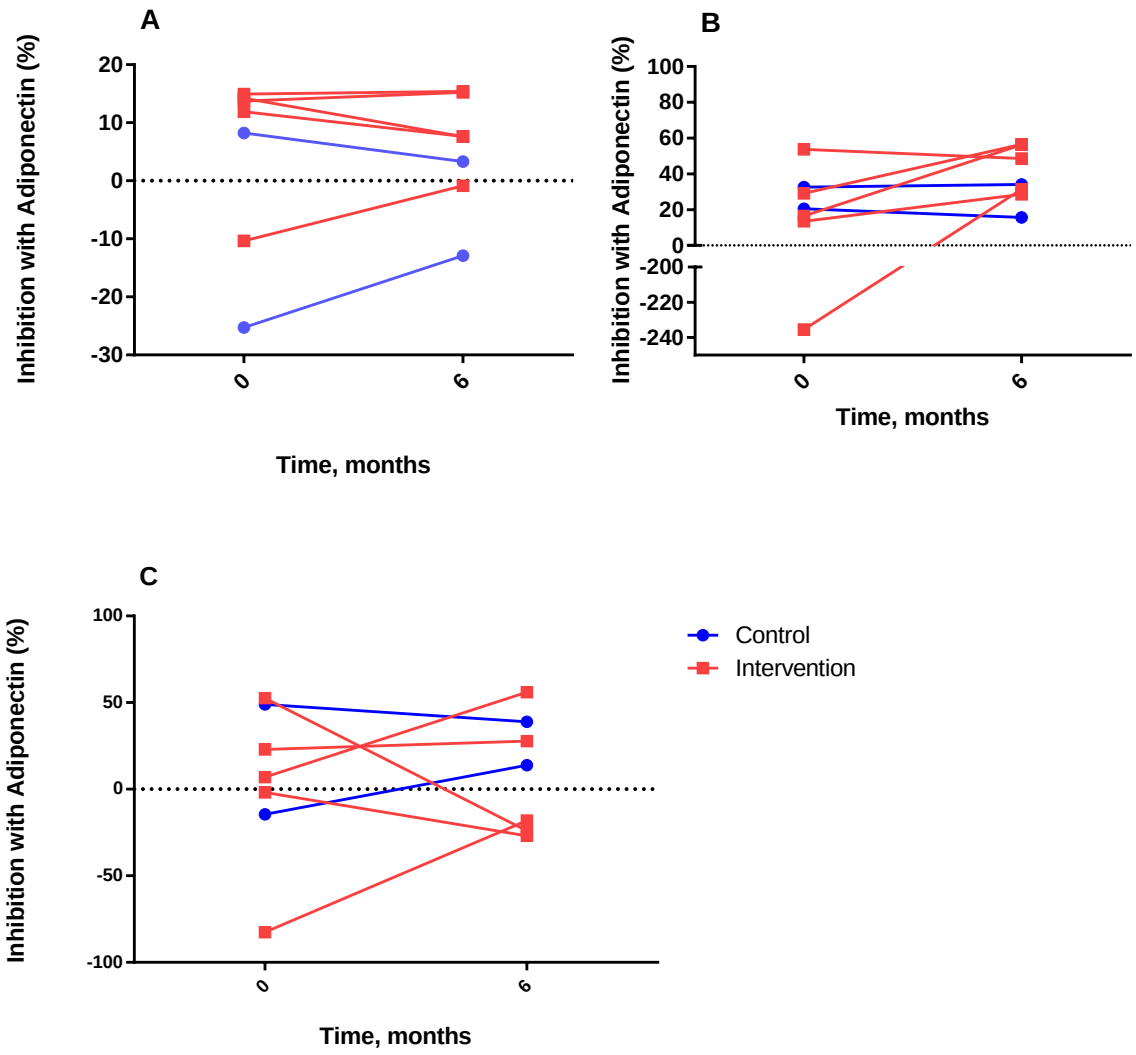

Figure 9-16 – Inhibition of T cell proliferation with adiponectin was not affected by study arm. Each participant is represented by a pair of markers linked by a line.

9.3.2.5 Endothelial Transmigration

Samples were analysed in 12 patients at baseline and eight patients after 6 months in the Extod study.

T cell transmigration was not inhibited by adiponectin in patients with T1D at baseline. This is in contrast to the inhibition seen in healthy participants of the Sports Science Study at baseline (*Figure 9-17*).

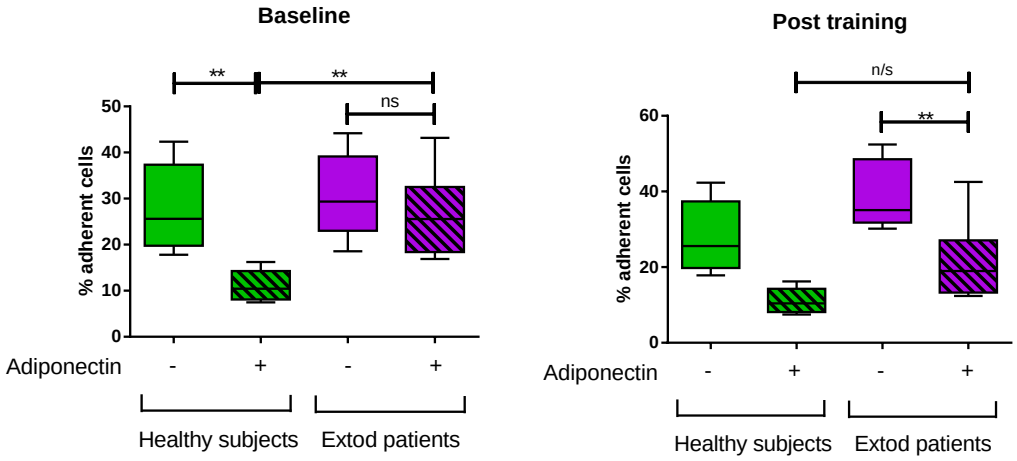

Figure 9-17 – Comparison of endothelial transmigration of PBL in healthy subjects and T1D patients, before and after exercise training.

After six months there was no difference in inhibition of transmigration with adiponectin between those participants in the intervention and control arms. In keeping with the overall increase in adiponectin receptor expression in both groups of the study, there was a significant suppression of endothelial transmigration in the Extod participants as a whole after six months. This was statistically significant ($p=0.0029$, ANOVA).

9.4 Discussion

Here we have shown that supervised exercise training in healthy people did not change adiponectin receptor expression on PBMC. It did, however, result in a greater inhibition of T cell proliferation. In patients with newly diagnosed T1D, there was an increase in adiponectin receptor expression which was seen in both the intervention and control arms of the study. We were unable to demonstrate an effect of this on suppression of T cell proliferation, however, we did see improved suppression of endothelial migration with adiponectin irrespective of treatment group.

9.4.1 The effect of exercise on serum adiponectin in T1D

Serum adiponectin was significantly higher in the intervention arm at baseline and fell to the level seen in the control arm by 6 months. A previous meta-analysis examining the effect of exercise on circulating adiponectin found that moderate to high-intensity exercise increases serum adiponectin, but the effect of low intensity exercise is much less apparent (Simpson & Singh 2008). The studies included in this meta-analysis included healthy and obese participants as well as participants with impaired glucose tolerance or type 2 diabetes. Given the negligible improvement in MVPA seen in the Extod cohort it is unsurprising that we did not see an increase in adiponectin levels in this group. It is somewhat unexpected that our intervention appeared to have had the effect of lowering serum adiponectin.

Previously, in our group, Dr Terence Pang has found that patients with T1D have similar levels of circulating adiponectin to those in healthy controls (Pang 2010), in

contrast to patients with type 2 diabetes who have lower levels. Other studies have shown increasing circulating adiponectin with increasing duration of diabetes (Lindström et al. 2006). Our longitudinal data, suggests that patients with T1D may be able to maintain circulating adiponectin with exercise training, while those whose fitness declines have reduced serum adiponectin levels, however, this finding was not significant and would need to be repeated on a larger sample of patients.

9.4.2 The effect of exercise on adiponectin receptor expression on PBMC

In healthy people, adiponectin receptor expression on PBMC did not alter after exercise training. Our sample size was smaller than that used in work done previously in our group that demonstrated an improvement in AdipoR with exercise in patients with type 2 diabetes (Pang 2010). There was an increase in AdipoR2 expression on PBLs and NK T cells. There was also a trend towards an association between VO2max and change in VO2 max and increased adiponectin receptor expression on PBMC. This was not significant in this sample size and will need to be repeated in a larger cohort.

In contrast, in patients with newly diagnosed T1D, adiponectin receptor expression on PBMC increased by six months in both arms of the study. The increase was only significant in the intervention arm. The change in adiponectin receptor expression was not associated with MVPA, BMI, weight, HbA1c, VO2 max or HOMA2-IR and given the lack of a significant change in activity levels in this group it is not likely to be attributable to exercise training. Type and intensity of exercise has been shown to determine changes in tissue adiponectin receptor expression in rats (Zeng et al.

2007). The most significant metabolic change in the group between baseline and follow-up was the improvement in glycaemic control. It is plausible that this influenced adiponectin receptor expression, although a significant correlation was not demonstrated, perhaps due to an insufficient sample size. Furthermore, neither this study, nor previous work in our group (Pang 2010), have identified a link with adiponectin receptor expression on PBMC and glycaemic control.

Previously, our group have demonstrated that the change in adiponectin receptor expression on PBMC is correlated with change in insulin resistance in patients with type 2 diabetes (Pang 2010). It is possible that the smaller changes in HOMA-IR seen in patients with T1D who are less insulin resistant than those with type 2 diabetes would mean an even larger sample size would be required to demonstrate such an association. Further work on the changes in adiponectin receptor expression around the time of diagnosis of T1D will be needed to elucidate the factors contributing to this change.

9.4.3 The effect of exercise on response of T cell proliferation to adiponectin

In healthy people, there was a greater inhibition of T cell proliferation after exercise training in response to stimulation with CD3 antibody and PPD. In T1D patients, there was no effect of exercise demonstrated on T cell proliferation (but the numbers were small).

9.4.4 The effect of exercise on the suppression of endothelial transmigration to adiponectin

Adiponectin supressed T cell endothelial transmigration to a similar degree to that previously demonstrated by our group (Chimen et al. 2015) and changed in proportion to adiponectin receptor expression.

In patients with T1D, inhibition of endothelial transmigration by adiponectin was not affected by intervention, however, at the six month time point, the effect of adiponectin was superior to baseline in participants in both arms, consistent with the improvement in adiponectin receptor expression seen in the two groups. This significant improvement is notable, but this study was not able to explain the cause of this. Should the reasons underlying the increase in AdipoR expression be identified, this could reveal a mechanism whereby adiponectin-mediated suppression of T cell migration could be enhanced in T1D.

9.4.5 Summary

Exercise training did not upregulate adiponectin receptor expression in these groups of healthy people or patients with T1D. Improvements in adiponectin receptor expression were seen in all patients with T1D and these translated into greater suppression of endothelial migration by T cells with adiponectin. Further work is required to confirm these findings in a larger group and to understand the reasons underlying this result.

9.5 Recommendations for future work

The findings of the study on adiponectin receptor expression in healthy volunteers should be confirmed, ideally in a larger group. It would be interesting to examine whether this related to an improvement in adiponectin-mediated suppression of T cell migration, unfortunately practical difficulties prevented this in this study.

The improvement in adiponectin receptor expression in patients newly diagnosed with T1D is intriguing and research into the factors underlying this finding should be undertaken.

10 CONCLUSIONS

Exercise confers health benefits to healthy individuals and patients with chronic disease, including T1D (Chief Medical Officers of England Wales, and Northern Ireland 2011; Chimen et al. 2012). Preservation of beta-cell function in T1D is associated with a reduced risk of microvascular disease and hypoglycaemia (Steffes et al. 2003; Lachin et al. 2013) and is therefore, a target of significant research interest. Exercise has been demonstrated to preserve beta-cell function in healthy people and people with type 2 diabetes, as well as rodent models of T1D (Narendran et al. 2015). The work presented in this thesis is aimed at examining whether exercise preserves beta-cell function in patients with T1D.

10.1 Evaluating the barriers to exercise in patients with newly diagnosed T1D

In chapter 5, I have described the first qualitative interview study examining the attitudes to exercise in a group of adult patients with newly diagnosed T1D. Five distinct themes were identified: existing attitudes to exercise; feelings about diagnosis; perceptions about the consequences of exercise; barriers to increasing exercise; confidence in managing blood glucose. I demonstrated that this group of patients is keen to increase their activity levels but some lack the necessary confidence in managing their diabetes. Patients with T1D felt that consistent advice from the healthcare team and education in managing their diabetes would help to improve their confidence in taking up physical activities.

Participants in this study reported similar barriers to exercise to those reported by patients with more long-standing T1D (Lascar et al. 2014) and other chronic diseases (Korkiakangas et al. 2009; Courneya et al. 2008; Brazeau et al. 2008; Plotnikoff et al. 2006; Slade et al. 2014; Rimmer et al. 2008), however, more emphasis was placed on HCP advice and fear of hypoglycaemia in the interviewees who had been recently diagnosed.

It is perhaps not possible to generalise the results of the first phase of this study to the clinic population of T1D as participants in this study are likely to have had an interest in exercise prompting them to take part. The results of this study did inform the design of the second phase of the Extod trial, in the form of educational materials for both participants and research team members.

This study has led on to further work, some of which is currently in progress. This involves:

1. Examining the confidence of HCPs in advising patients about diabetes management around exercise.
2. Exploring how and what patients with T1D want to learn about managing their diabetes during exercise.
3. The development of research networks and a conference for HCPs (the EXTOD conference, now in its 4th year) to discuss the area of exercise in T1D.
4. Subsequent NIHR funding (The Extod Education Grant) to develop an education package to aid health care professionals to support patients with T1D to exercise.

10.2 Determining whether patients newly diagnosed with T1D can be encouraged to take up and adhere to a programme of at least moderate intensity exercise for a period of one year, and the rate of exercise uptake in the non-intervention arm.

In chapters 3 and 4, I have described the design, set-up and recruitment of a randomised, controlled pilot study to investigate the effect of exercise on beta-cell function in T1D.

Recruitment to the Extod study was both low (58 participants randomised to the study out of 507 potential participants identified) and slower than anticipated. It is common for randomised controlled trials to fail to recruit to target within the anticipated time frame and reporting of numbers potentially eligible for studies is variable (Toerien et al. 2009). Difficulties in recruitment occur despite evidence that patients view clinical research as important and are willing to take part (IPSOS Mori & Association of Medical Research Charities 2011) and evidence of benefit to the individual taking part in a clinical trial (Braunholtz et al. 2001). Despite the difficulties comparing the Extod study with other studies of patients with T1D (due to differences in population or intervention), some RCTs have reported recruitment rates as low as 17% (DAFNE Study Group 2002). Recruitment in the Extod study was lower than this and it is clear that strategies for addressing this would help any future trials.

Similarly, the completion rate was lower than initially expected. Further qualitative work examining the possible reasons behind this has been undertaken by our group.

Baseline activity levels for our participants were high with 78% already performing more than 150 minutes MVPA per week as recommended by the Chief Medical Officer and diabetes organisations (Chief Medical Officers of England Wales, and Northern Ireland 2011; Nagi & Gallen 2010; Sigal et al. 2006). Physical activity levels in both arms changed little during the course of the study. There was however, an increase in fitness in the intervention arm only slightly lower than that seen in other studies of patients with T1D (Laaksonen et al. 2000; Wallberg-Henriksson et al. 1986; Fuchsjager-Mayrl et al. 2002) with supervised exercise, although of shorter duration. Importantly, despite high levels of activity among participants, hypoglycaemia rates were low, indicating that patients with newly diagnosed T1D can exercise safely.

Future studies will need to address the issues of low recruitment and loss to follow-up as well as design an intervention that achieves a clear difference in activity between the intervention and control arms. There is evidence to suggest that recruitment may be improved with changes to study design to reduce the burden on the clinical research team (Fletcher et al. 2010), therefore, it will be important to review the study design carefully to see where it could be simplified. There was an increase in participants identified from PIC sites following the introduction of an incentive to trial staff. There may be ways to extend this approach to participants, for example, patients may view activity monitoring or glucose monitoring systems as incentives but these would also be useful for trial monitoring.

It is likely that patients interested in taking part in a trial of exercise are already interested in physical activity. As such, the group of participants is likely to be

already active, as seen in this group. This will, therefore, lead to difficulties maintaining a difference in activity levels between the control and intervention arms. In a future trial, a supervised exercise intervention would allow the study team to increase intensity and more accurately monitor exercise duration during the trial. One drawback of this approach is the limitations that it puts on the participant in terms of choice of location and timing of activity sessions, which may in turn negatively affect recruitment.

10.3 Determining whether adherence to this programme of exercise preserves beta cell function.

The work presented in chapter 8 demonstrates that there was no evidence from the Extod study for the beta-cell preserving effect of exercise in T1D. Loss of beta-cell function was similar in both the intervention and control groups over the course of the study. However, there are important reasons why this study does not provide a definitive answer to this hypothesis.

1. The study, as a pilot study, was not powered to test this hypothesis.
2. The difference in MVPA and fitness in the intervention arm was small and is not likely to have been enough to demonstrate a significant effect on beta-cell function.
3. The control arm had advantages in terms of factors favourable to beta-cell function; more patients presenting with acidosis, short median duration of symptoms prior to diagnosis and, a greater proportion of patients with

detectable autoantibodies. All of which, I have suggested had a significant effect on beta-cell function in the group as a whole.

4. The reduction in insulin resistance seen in the intervention arm causes difficulties in the assessment of beta-cell function by mean AUC C-peptide during MMTT. This in itself would appear as a reduction in insulin secretion as measured by AUC C-peptide, therefore, potentially masking any effect of the intervention.

Further studies are required to address the question of the role of exercise in beta-cell preservation in T1D. Consideration of the limitations of this study outlined above will be important in informing these. Either restricting study entry to participants who have detectable autoantibodies or controlling for this at randomisation will be important, as would an intervention more effective at separating the study arms in terms of activity levels.

Finally, some thought and study will need to go into how best to measure beta-cell function in the context of an intervention that reduces insulin resistance as current methods will always appear to demonstrate a loss of beta-cell function in these circumstances.

10.4 Investigating the effect of exercise on immunity in patients newly diagnosed with T1D.

The work presented in chapter 9 examined the effect of exercise on circulating adiponectin levels, adiponectin receptor expression and T cell activity in response to adiponectin in healthy people and patients with T1D.

I found that there was a suggestion that adiponectin receptor levels on PBMC increased in healthy people after exercise training, particularly on PBL subsets. This appeared to translate into increased suppression of T cell proliferation in response to CD-3 antibody and PPD stimulation with adiponectin.

In T1D patients recruited through the Extod study, there was a universal increase in adiponectin receptor expression on PBMC with time. This did not appear to be related to increased activity levels or fitness. The increase in adiponectin receptor expression was associated with greater suppression of T cell endothelial migration with adiponectin.

It is not possible to evaluate the cause for the increase in adiponectin receptor expression in the T1D group from this study. However, over the first few months in the study, glycaemic control improved significantly in the group as a whole. It is plausible that glycaemia influences adiponectin receptor expression.

The limitations of this study are related to sample size and the weaknesses of the Extod study for this specific research (primarily the high baseline levels and minimal increase in activity). Another problem is the lack of a sedentary control group for the healthy volunteers. These studies need confirming in a larger group of subjects with a sedentary control group for comparison. In addition, I would suggest that work examining the effect of glycaemic control on adiponectin receptor expression could yield interesting results.

In summary, I have described the attitudes and barriers to exercise in a group of adult patients recently diagnosed with T1D. This work has enabled me to identify areas where patients can be supported to increase their activity levels. Using the results of this study, I have developed educational materials for patients and healthcare workers to support them to do so in the context of a randomised, controlled trial.

I have discussed the study design and set up of a pilot trial aimed at increasing exercise in T1D patients, examining recruitment, completion, adherence to exercise programme and uptake of exercise in the control group. I also have developed suggestions for how these could be improved in full scale trial.

The work investigating the effect of exercise on beta-cell function in T1D has established the need to compensate for any changes in insulin resistance and further work examining the best way to do this is required.

Finally, I have examined a potential mechanism for exercise to preserve beta-cell function in T1D; through effects on adiponectin receptor levels on PBMC and therefore, immunity. The main finding of this work was that adiponectin receptor levels, and hence, adiponectin-mediated suppression of T cell endothelial migration, are upregulated after the diagnosis of T1D. The cause for this is unclear and will require further study.

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12 APPENDICES

Appendix A EXTOD PATIENT INFORMATION SHEET

Patient Information Sheet
Version 6.
September 2011

Study Title: Benefits of exercise in type 1 diabetes (T1D)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

Study purpose:

The insulin producing beta cells within the pancreas are destroyed by the body's own immune system in patients with T1D. At the time patients are diagnosed with T1D, they can have about a quarter of their beta cells remaining and most of these are gradually destroyed over a number of years. However, even in patients who have had T1D for many years, there is now evidence for some residual beta cell function. Protecting these beta cells from the immune system and preserving their function has been shown to protect the patient from the complications of diabetes. These complications include heart disease, kidney failure and blindness. There is therefore a good evidence to preserve beta cell function in patients with T1D.

There is some evidence that regular and sustained exercise can protect beta cells in patients with other forms of diabetes but we not yet sure is this is the case in T1D. The aim of this study is to find out with confidence if exercise preserves beta cells in T1D.

The study will consist of 2 parts. Firstly we would like to interview patients with T1D to find out why regular exercise is difficult for patients with diabetes. Second, we will exercise patients with diabetes to see if their beta cell function is preserved.

Why have I been Chosen?

You have been chosen because you have type 1 diabetes. We aim to recruit up to 80 patients.

Do I have to take part?

This is entirely up to you to decide. We will explain the study and go through this information sheet that we will then give to you. If you wish to discuss this with others, we can recommend both your GP or the Patient Advice Liaison Service – their telephone number is *local information*, email *local information*. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive. If you agree to participate you may benefit from receiving advice on exercise or supervised exercise training, and also understanding the barriers to taking up exercise in patients with diabetes.

What will happen if I take part?

If you agree to take part in this study, you will be asked to help in one of 2 ways

1) You will be asked to attend a 1-2 hour interview to find out from yourself what the problems are to doing exercise. This may be an individual interview or in a group with other patients with T1D.

2) You will be placed at random in one of 2 groups in an exercise study. The first group will be asked to have usual diabetes care. This will involve written and spoken advice on exercise. This is what we currently give to patients with diabetes in our diabetes clinics. The second group will get usual care for their diabetes but also be encouraged and helped to exercise at a level that is currently recommended by the Department of Health - for 150 minutes a week at moderate intensity. This group will be helped through provision of membership to leisure centres, gyms or swimming pools. They will also be provided advice and guidance to ensure that this exercise does not result in hypoglycaemia (low blood sugar), as well as motivational contact from health advisors and our diabetes nurses. This exercise group will be asked to maintain this level of exercise for 1 year. Though this may sound like a lot of exercise, this is the minimum amount that the Department of Health recommends lifelong, not just for one year. If however it difficult for you to maintain this, you can leave the study at any time.

Both groups (the exercise as well as the 'usual care' groups) will have blood tests taken at 0, 3, 6, 9 months, and after a year. Some of these blood samples will be stored for future testing. There will also be urine tests and tests of your fitness levels (on an exercise cycle) at 0 and 6 months, as well as after a year. This study will finish after this one year. For the duration of the study, all of your diabetes care will happen at *local information* Hospital. This may be different from your usual hospital. One of the blood tests we will request is for analysis of your DNA (this contains your genes). You have the choice whether to give consent for this and your decision will not affect your eligibility to participate in the study.

If for any reason you are unable to finish the exercise, you will be able to leave the study (without giving a reason if you do not wish to), and your diabetes care will not be influenced in any way. Some participants will be invited to be interviewed to give feedback on the study as it progresses. This is so that we can ensure that we give participants the best support.

What are the possible disadvantages and risks of taking part?

Exercise can be associated with injury, however we will ensure that your exercise is started gently and gradually increased to avoid this. In patients with T1D, prolonged exercise can

also be associated with a drop in the blood sugar (hypoglycaemia). We will avoid this by adjusting the insulin doses around the time of exercise and also by providing advice on how to treat low blood sugar levels.

What are the possible benefits of taking part?

Exercise has multiple benefits including well-being, reducing the risks of heart disease and blood pressure. If our study idea is correct, it can also preserve beta cells which means that your diabetes might be easier to control in the long-term.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence.

All the data collected will remain confidential. Your GP will be informed of your participation in the study but will not be informed of any of the data except those that could be important to your health if you have consented to it.

What will happen to the results of the research study?

We will inform you of the results of all the questionnaires and tests that are performed on you personally. The scientific results of the research will be published in medical journals and presented in international conferences.

Who is organising and funding the research?

This study is funded by the Diabetes and Wellness Research Foundation, as well as the National Institute for Health Research, a charity which aims to improve the care of patients with diabetes. The study is sponsored by the University of Birmingham.

Who do I contact for further information?

Local contact details to be inserted here

Appendix B EXTOD CONSENT FORM

CONSENT FORM Version 5
November 2011

Title of Project: Benefits of Exercise in Type 1 Diabetes (T1D)

Name of Researcher: Dr Parth Narendran

Please initial box

1. I confirm that I have read and understand the information sheet 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 my medical care or legal rights being affected. ☐

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

4. I agree to the taking and storage of blood samples for the purposes of this study (not relevant for interview study) ☐

5. I agree to the taking and storage of DNA for the purposes of this study (not relevant for interview study) ☐

6. I agree to my GP being informed of my participation in the study ☐

7. I agree to take part in the above study. ☐

8. I agree to the additional tests of heart function in the study ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

Appendix C GRADED EXERCISE PROGRAMME

Week	Total Minutes per Week	Intensity (% effort)	Heart rate target (bpm)	Borg RPE Scale
1	75	55	(220-age x intensity%)	
2	85	60		Light Exercise
3 & 4	100	60		
5 & 6	110	65		
7	115	70		Moderate Intensity
8 & 9	115	70		
10 & 11	130	75		Heavy breathing, sweating
12 onwards	150 (minimum)	75		

This exercise program is designed to encourage a gradual increase in physical activity over the course of 12 weeks. The starting point on the scale depends on the individual's current fitness level.
From 12 weeks onwards, the aim is to achieve a minimum of 150 minutes per week of vigorous-intensity exercise.

Appendix D DIETARY COMPETENCIES

Within 3 months Dietitian would expect a person with newly diagnosed type 1

diabetes to:

Identify carbohydrate containing foods and describe how these affect blood glucose levels.

DUK Eating well with type 1 diabetes leaflet given

Recognise different carbohydrate foods may have a different effect on blood glucose levels and have a basic understanding of low and high glycaemic index foods.

Introduce fundamental concepts of carbohydrate counting and meal-based insulin dosing.

Build confidence to read and understand food labels.

Identify the role of sugars, alternative sweeteners, and diabetic foods in healthy eating, and identify the safe daily intake of each sweetener.

State that physical activity may cause the blood glucose levels to rise or fall. Hypoglycaemia may occur as a delayed response.

Treat hypoglycaemia with 15-20g quick-acting carbohydrate and list measured amounts of suitable treatments. Follow up with slow-acting carbohydrate if not eating for 1-2 hours.

Understand the effect of alcohol on blood glucose levels.

Discuss snacks, amount, type and frequency and requirement, if any, for insulin.(optional)

Be able to explain reasons for weight loss at diagnosis. Identify strategies for weight control. (optional)

Understand the importance of hydration in relation to exercise and discuss suitable drinks. (optional)

Be aware of support for people with Type 1 diabetes e.g. local diabetes UK support groups, Diabetes UK website as a resource for food and physical activity tips, Runsweet.com website for advice on physical activity and sport.

Appendix E PARTICIPANT INFORMATION BOOKLET ON

MANAGING CARBOHYDRATE AND INSULIN AROUND EXERCISE

Exercising safely with type 1 diabetes
A guide for patients

What do we mean by exercise?

Exercise can be any physical activity.
Usually, it is done for the purpose of improving health or maintaining fitness.

Exercise intensity refers to how much work is being done while exercising. This can be measured in a number of ways but two common ways are:

1. *Percentage of maximum heart rate achieved during exercise*

   Your maximum heart rate is approximately equal to 220 minus your age

2. ***Borg scale** of perceived exertion*

   This is a scale that can be used to describe how hard you feel you are working. It takes into account that the same level of exercise may feel harder or easier at different times.

These both give an idea of the intensity of exercise you are doing and the table on the following page shows how they match up.

My maximum heart rate (220—my age) = ☐ beats per minute.

Table 1-Rating of Perceived Exertion versus Intensity.

Description	Borg Scale (RPE)	% of MHR	My Heart Rate equivalent (220 – my age)	Expected change in blood glucose
Easy	7			↓
	8			
Very Light	9			
	10	50%		↓
Fairly Light	11			
	12	60%		↓
Somewhat Hard	13			
	14			
	15	70%		↓
Hard	16	80%		
	17			
Very Hard	18	90-%		↑*
Very, Very Hard	19			
	20			

* When you push yourself very, very hard, you stress your body and this may cause your blood glucose to increase. (MHR=Maximal Heart Rate)

The intensity of the exercise is important because it influences what fuel the body uses and how the body adapts to the activity.

Exercising at a high intensity, as in sprinting, will train the body to work at a low level of oxygen for short bursts.

Exercising at a medium intensity, as in jogging or swimming, will train the heart, lungs and muscles to work better, for longer, helping to increase endurance.

Many activities, like football or netball will contain periods of high and medium intensity exercise at different times.

What happens when we exercise?

We need fuel in order to supply our heart, lungs and muscles with the energy required for exercise.

The major fuels are carbohydrate (sugar) and fat. At higher intensities of exercise we use more carbohydrate, while at lower intensities we use more fat.

It is very important that the right amounts of the right fuels are available to the body during exercise. As a result, during exercise the body produces a lot of different hormones and substances to control the use of these fuels.

Insulin is a very important hormone in exercise. It ensures that enough carbohydrate is available and can be properly used by the parts of the body which need it.

In people with type 1 diabetes, the body does not make its own insulin and therefore is unable to regulate insulin or carbohydrate during exercise. As a result you may experience symptoms of hypoglycaemia during exercise, in the period immediately after exercise and for several hours after the exercise has finished.

This does not mean that people with type 1 diabetes should not exercise, just that some extra preparation is necessary.

Preparing for exercise

General Advice for safe exercising

1. Wear footwear appropriate to the exercise you plan to do, for example, supportive training shoes for running/jogging. Replace them regularly as they wear out.

2. Choose clothes designed for your type of exercise. Consider high-visibility clothing if you are running or cycling on roads.

3. Ensure you warm-up and cool down for 10 minutes before and after exercise to reduce the chance of injury.

4. Training too hard can cause injury, you will need to build up your training programme steadily.

5. Hold off exercising if you are unwell or very fatigued.

6. Consider the weather conditions. If it is cold, wear layers you can remove as you warm up and in the hot weather ensure you keep well hydrated.

7. Do not exercise alone if you have had hypoglycaemia in the previous 24 hours.

Carbohydrates

It is important to make sure that you eat enough carbohydrate to ensure you have enough 'fuel' to exercise.

For example, if you weigh 70 kg and are doing light training you should aim to eat approximately 280grammes (3-5grams of carbohydrate per kilogram body weight per day). As you increase your exercise intensity, this requirement will increase. You should discuss this with the Extod team.

Before Exercise
It is important to have eaten a meal 3-4 hours before exercising to ensure you have enough carbohydrate to supply the increased demands of your activity. This should contain some "Low GI" foods, such as wholemeal bread, porridge oats, basmati rice etc.

During Exercise
As a general rule, you should take 15g of carbohydrate for every 30mins you are exercising. This can be in the form of a drink or glucose or "high GI" food.

After Exercise:
At the end of your exercise session you should consume more carbohydrate to 're-fuel'. This is best taken with protein e.g. cereal and milk, sandwich, milkshake drink.

Example

You plan to exercise for 60 minutes.....
Check your blood glucose is 7-14 mmol/l. If not take an additional 15-20g carbohydrate before exercising.

You should take:

15g carbohydrate after 20mins

15g carbohydrate after 40mins

and

15g carbohydrate at the end

Remember, everyone is different. You may require more than this.

Review your carbohydrate intake if you have problems during exercising.

Examples of 15g carbohydrate:

The table below gives examples of foods containing approximately 15g and 30g carbohydrate. Take high GI foods immediately before and during exercise.

Carbohydrate	~15g	~30g
High GI		
Jelly babies (large)	3	6
Jelly beans	15g	30g
Cola	150ml (mini can)	300ml
Lucozade Body Fuel Energy Gel	½ X 45g tube	1 x 45g tube
Apple juice	120ml	240ml
Lucozade Sport Body Fuel	250ml	500ml
Powerade Isotonic	200ml	400ml
Gatorade	250ml	500ml
Low-medium GI		
Cereal bar	½-1	1-2 (48g)
Raisins	45g	90g
Cornflakes	3 tablespoons	6 tablespoons
Kit Kat	1 x 2 finger	1 x 4 finger
Semi-skimmed Milk	300ml (½ pint)	600ml (1pint)
Low fat fruit yoghurt	1 x individual (250g)	2x individual (250g)
Potato crisps	1 x 25g bag	2 x 25g bag
Dried apricots	5	10
Weetabix	1·5	3
Shredded Wheat	1	2

Glycaemic index

Glycaemic Index (GI) gives an indication of how quickly a food will raise blood glucose levels.

High GI foods raise blood glucose levels quickly
Low GI foods raise blood glucose levels slowly

High GI (70 and above)	E.g. white bread, mashed potato, white rice, rice cakes, pretzels, water biscuits, water melon, dates, sports drinks (6-8% carbohydrate), sports gels, jelly babies, jelly beans, fruit juice
Medium GI (56 to 90)	E.g., shredded wheat, pitta bread, wholemeal bread, gnocchi, couscous, baked potato, banana, raisins, pineapple, digestive biscuits, Ryvita
Low GI (55 and under)	E.g. porridge oats, basmati rice, rye bread, seeded multigrain bread, spelt bread, sourdough bread, apple, mango, oranges, cherries, low-fat milk, low-fat yoghurt

* Information taken from GI Database, University of Sydney

Check your blood glucose before you start

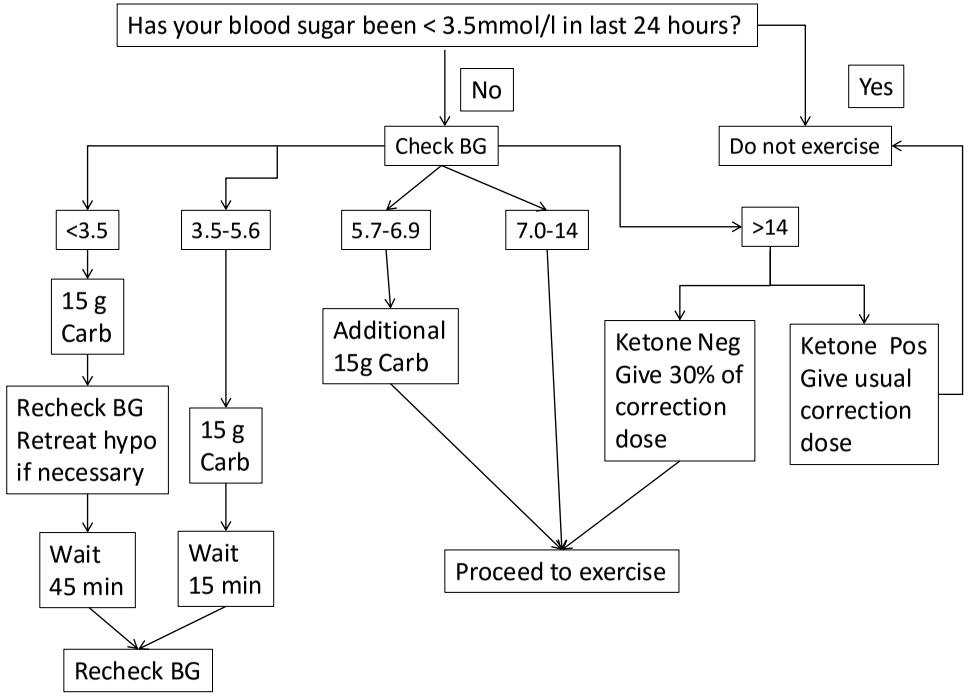
Use this chart to decide if it is safe for you to exercise.

Blood glucose (mmol/l)	Ketones present?	Action	Carbohydrate needed
Less than 3.5	No	**Do not exercise** Treat hypoglycaemia. You must wait at least 45 minutes and check your blood glucose before you start exercise. Take additional carbohydrate before exercising.	Hypoglycaemia treatment PLUS 15-30g carbohydrate for every 30 mins of next exercise session.
3.5—5.6	No	**Exercise with caution** Take 15g carbohydrate and recheck blood glucose after 15 mins.	15-30g carbohydrate for every 30 mins of exercise.
5.7—6.9	No	**OK to Exercise** Take an addition 15g carbohydrate before exercising	15-30g carbohydrate for every 30 mins of exercise.
7.0—14	No	**OK to Exercise**	15-30g carbohydrate for every 30 mins of exercise.
Greater than 14	No	**Exercise with caution** Give 30% of usual correction dose of insulin.	15-30g carbohydrate for every 30 mins of exercise.
Greater than 14	Yes	**Do not exercise** Give usual correction dose of insulin	

Hypoglycaemia

An episode of hypoglycaemia in the 24 hours before exercise will both affect your ability to perform exercise and increase the chance of you having another episode during or after your exercise session. Do not exercise if you have been hypoglycaemic (below 3.5 mmol/l) in the last 24 hours. (See flow chart below.)

It is <u>not</u> recommended to exercise in the 24 hours following an episode of severe hypoglycaemia (one which you are unable to treat on your own).



Blood glucose measurement

Different activities will have different effects on your blood glucose. If you are unsure how to treat hypoglycaemia, please talk to someone from the Extod team.

Type of activity	Change in blood glucose during exercise	Risk of hypoglycaemia after exercise
Continuous	↓	↑
Stop and Start e.g. football	↑ or ↓	↑

You can use this to help you when you are exercising.

If you are at the gym try to do a mixture of activities, like circuit training, or if you are out running try to add in a short sprint every 20-30 mins. This will help you to avoid hypoglycaemia.

You should not exercise if you have high blood sugars and ketones, this indicates that you have not got enough insulin available. Exercise could worsen your ketosis.

It is essential that you wait until your blood sugars are stable and you are free of ketones.

It is important to keep a close eye on your blood glucose around exercise. This will help you to avoid hypoglycaemia and plan your carbohydrate requirement.

As a guide, you should:

1. Measure your blood glucose before you exercise.
2. Measure blood glucose every thirty minutes during exercise.
3. Check your blood glucose after you have finished exercising.
4. Check your blood glucose approximately six hours after your exercise session has finished (if practicable).

Remember: Your blood glucose should be more than 5.0 mmol/l before you start driving and you must wait for 45 minutes after an episode of hypoglycaemia has resolved before getting behind the wheel.

If you are still having problems with your blood sugars, you should get in contact with your Extod team who can suggest other measures.

Fluid intake

As well as using carbohydrate, exercise will also cause the body to lose fluid. This may be through sweat or in the breath. The amount of fluid you lose will vary depending on the type and length of activity, and the temperature. If it is very hot weather, above 30°C, you will need salt replacement.

It is important to make sure that on days when you exercise, you remain well hydrated. You should try to have a drink in the hour before you start and ensure that you have something to sip during your exercise.

Carry a water bottle . Sip cool water and aim to drink 500 - 600 ml each hour. Drink water with all meals. Eating food promotes fluid intake. Try to include fruit and vegetables as these have a high water content. If sweat losses are great the use of salty foods e.g pretzels, added salt to a meal , drinking a sports drink with sodium/salt will help you rehydrate.

You may wish to sip water, or a sports drink that also contains your carbohydrate requirement.

Remember:

Before you exercise

Check you have:

- Your insulin and blood glucose/ketone monitoring kit
- Treatment for hypoglycaemia
- Drinks and snacks

Check your blood glucose: is it safe to exercise?

Do you need any additional carbohydrate before you start?

During exercise

Check your blood glucose every 30 mins.

You will need around 15-30g carbohydrate every 30 mins.

Make sure you drink plenty of fluid.

After exercise

Check your blood glucose as soon as you finish and six hours later (or before you go to bed if this is sooner).

You will need more carbohydrate, about half as much as you consumed during exercise.

Drink plenty of fluid.

Your notes:

Contact details of the Extod team

Useful websites:

Diabetes and exercise	www.runsweet.com
Sports Dietitians UK	www.sportsdietitians.org.uk
UK Sport	www.uksport.gov.uk
Diabetes UK	www.diabetes.org.uk

Appendix F SOP FOR THE MIXED MEAL TOLERANCE TEST

Equipment

Alcohol gel and hand wash

Gloves & apron

Tourniquet

Cannula and fixative dressing

Three way tap/adaptor

Vacutainers (3x SST (gold), 11x EDTA (lavender), 7x sodium fluoride (grey))

240mls Fortisip

24 x 2ml Sarstedt tubes

Sharps container

Cotton wool and plasters

ExTOD Labels

Centrifuge

Prior to Procedure

- Explain procedure to patient
- Ensure patient seated comfortably or lay couch
- Check blood glucose levels (should be between 3.5-15mmol/l)
    - If blood glucose < 3.5 treat for hypoglycaemia and reschedule test
    - If blood glucose > 15 check for ketones and reschedule test

Procedure

1. Wash hands and put on PPE
2. Tighten tourniquet on arm and locate suitable vein
3. Cleanse skin
4. Insert cannula into vein (at least 18 gauge into a large vein in antecubital fossa)
5. Secure cannula with adhesive dressing
6. Withdraw fasting blood samples for lipids, adiponectin and inflammatory factors, and transmigration studies (3x SST tubes, 4x EDTA tubes)

7. Withdraw -10min blood samples (1x EDTA, 1x sodium fluoride)
8. Invert tubes 8-10 times
9. Flush cannula with 2ml 0.9% saline, to ensure patency
10. At 0 mins, withdraw and discard 2mls blood. Then,
11. Withdraw 0min samples (1x EDTA, 1x sodium fluoride)
12. Invert tubes 8-10 times
13. Flush cannula with 2ml 0.9% saline, to ensure patency
14. Ask participant to drink 240mls fortisip
15. Repeat steps 10-13 at 15, 30, 60, 90 and 120 mins.
16. Check blood glucose again at the end of the test
17. Once all samples are collected remove cannula and dispose of immediately in sharps bin
18. Place a piece of cotton wool over bleeding point and ask the patient to press for a minute
19. Check bleeding has stopped and ask if patient allergic to plasters. Apply dressing appropriately

Processing of samples (see also sample processing instruction sheet)

1. Centrifuge samples within 60 min of collection at 25°C, 1100-1300g for 10mins
2. Pipette serum/plasma for each sample into 2 × 2ml sarstedt tubes
3. Label with ExTOD label (see below)
4. Store samples at -80°C

Time	Label	
	EDTA	Sodium fluoride
-10min samples	ex---pm	ex---gm
0min samples	ex---p0	ex---g0
15min samples	ex---p15	ex---g15
30min samples	ex---p30	ex---g30
60min samples	ex---p60	ex---g60
90min samples	ex---p90	ex---g90
120min samples	ex---p2h	ex---g2h

Appendix G SOP FOR THE FITNESS TEST

Sub-maximal test for estimation of VO2 max

Equipment required

Calibrated exercise cycle with the ability to vary workload

Heart rate monitor (chest strap and watch)

Stop watch + spare in case of equipment failure

Blood glucose monitoring equipment

Ketone monitoring facility (capillary or urine)

Hypo kit

Water for the participant

Before the test

Ensure the participant is wearing appropriate clothing (e.g. trainers and jogging trousers).

Ask the participant about alcohol and caffeine intake in the last 12 hours as these may affect the heart rate calculations.

Ask the participant about hypoglycaemia in the last 24 hours. If they report an episode of severe hypoglycaemia, i.e. requiring 3rd party assistance, the test should be rescheduled.

If the participant has overly exerted themselves in the last 24hours, this may cause them to fatigue more easily to normal and give an inaccurate result, in this event, consider rescheduling the test.

Ensure the participant is well hydrated and check their capillary blood glucose (refer to insulin/carbohydrate/fluid and exercise protocol).

Explain the test to the participant and show them the RPE scale.

Weigh the patient and record this on the CRF.

Ask the participant to position the chest strap of the heart rate monitor around their chest. Place the watch somewhere you can take heart rate readings easily (i.e. so you can see the face). Ensure that the watch is picking up a heart rate reading satisfactorily.

Make sure you have the patients target HR for part 2 on the CRF. This is 85% of age-predicted maximum HR, i.e. 85% of (220-age). If not, record and calculate it before you start the test.

The test (*This will depend in part on the bicycle used)*

Position the participant comfortably on the bicycle. The legs should not need to be fully extended to pedal. Record the seat position and handlebar position (if necessary) on the CRF.

Part 1

Depending on size and fitness of the patient set the load at 50 – 100W (1-2kg load at 50rpm). Record the workload on the CRF. Ask the participant to aim to cycle at 50 rpm.

The aim is for the participants heart rate to be between 120 and 170bpm at the end of the six minutes. Check HR and RPE at 2 mins (if HR is <120bpm consider increasing work load and restarting six minute test).

From 4 minutes, record HR every 15 seconds.

At the end of the six minutes, record HR and RPE. Then:

- If the average HR for the last 2 mins < 120bpm, repeat part 1 at a higher workload (if it is clear that the HR will not rise above 120 bpm the six minutes can be restarted earlier).
- If the average HR is between 120 and 170, continue to part 2.
- If the average HR is over 170bpm then finish the test and allow the participant to cool down (cycle against a low resistance for a few minutes).

Part 2

During this part of the test, the workload should be increased incrementally with the aim of the participant reaching their target HR by stage 3 (so that there are 3 recordings of workload and average HR for this stage).

For each stage,

1. Increase the workload by 25-50W depending on participant's fitness level and size.
2. Record the workload for the stage on the CRF.
3. Ask the participant to cycle at 50 rpm.
4. From 2 mins 30 secs, record HR every 15 secs.
5. At the end of three minutes, calculate the average HR for the last 30 secs and the RPE on the CRF.

If after the 3 incline stages the desired heart rate is not achieved repeat this process.

Once target HR has been achieved, finish the test and allow the participant to cool down (cycle against a low resistance for a few minutes).

End of the test

Once the participant has cooled down, check their blood glucose.

If the test had to be terminated early, record this on the CRF along with the reason why.

Check the participant is happy with how the test has gone and allow them to shower/change prior to going home.

Appendix H INTERVIEW TOPIC GUIDE

1) **Moderator's introduction**

2) **About the group**
   - What are we going to talk about/Explain purpose

3) **Activity and exercise behaviour**
   - What exercise do you do?
   - What does the word 'exercise' mean to you? (Focus on activity levels)
   - Why should someone exercise?
   - How active are you on a day to day basis/How do you feel about the amount of exercise you do?
   - Do you think you do enough exercise to keep healthy?
   - Is exercise important in the management of diabetes?
   - What are the recommended guidelines?
   - The DOH recommends 150 mins exercise per week, what do you think of this?
   - Do you think this is achievable?
   - How do you relate to that?

4) **Barriers to exercise**
   - What are the mains reasons for not meeting the guidelines?
   - How do you try to overcome these barriers?
   - If you have a fear of having a hypo, do you make any adjustments?
   - If you had a magic wand what would be the one thing you could overcome in order to allow you to do more exercise?
   - Has the diagnosis of DM changed your attitudes towards exercise?
   - Does education and understanding have a role in the management of your diabetes and therefore your exercise levels?

5) **Encouragement and facilitation of exercise**
    - Can you think of any ways of improving your activity levels?
    - How can small changes be incorporated into your lifestyle?
    - Are there any major themes that would help encourage people to be more active?
    - Would more advice or information help?
    - If you had to choose one intervention that would help your activity levels – which would it be?
    - Has anyone any successful experiences of exercising in the past?

We are thinking about doing a study – if you were to take part how would you like to be **monitored/encouraged?**

- One to one advice from a health and fitness advisor
- Attending an exercise group organised by the hospital or your GP
- Support – someone who keeps in touch to see how you are doing with your exercise programme
- Goal setting/modification/action planning
- HR monitoring
- Chat room with other people from the study to share ideas
- Uploading BMI/weight loss onto website – self monitoring
- If phone calls weren't appropriate, what else could we do to motivate you?

6) **Summary of session,**
    - Outlining main points of discussion and key issues raised
    - Questions
    - Thank everyone for their input

13 PRESENTATIONS – PUBLICATIONS/POSTERS

PUBLICATIONS

Chimen M, McGettrick HM, Apta B, Kuravi SJ, Yates CM, **Kennedy A**, Odedra A, Alassiri M, Harrison M, Martin A, Barone F, Nayar S, Hitchcock JR, Cunningham AF, Raza K, Filer A, Copland DA, Dick AD, Robinson J, Kalia N, Walker LS, Buckley CD, Nash GB, Narendran P, Rainger GE. Homeostatic regulation of T cell trafficking by a B cell-derived peptide is impaired in autoimmune and chronic inflammatory disease. Nat Med. 2015 May; 21(5):467-75

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Lascar N, **Kennedy A**, Jackson N, Daley A, Dowswell G, Thompson D, Stokes K, Greenfield S, Holder R, Andrews R & Narendran P. Exercise to preserve beta cell function in recent-onset type 1 diabetes mellitus (EXTOD) - a study protocol for a pilot randomized controlled trial. Trials 2013 June; 14:180

Kennedy A, Nirantharakumar K, Chimen M, Pang TT, Hemming K, Andrews R & Narendran P. Does exercise improve glycaemic control in type 1 diabetes? A systematic review and meta-analysis. PLoS ONE 2013 Mar; 8(3):e58861

Nirantharakumar K, Marshall T, **Kennedy A**, Narendran P, Hemming K & Coleman JJ. Hypoglycaemia is associated with increased length of stay and mortality in people with diabetes who are hospitalized. Diabetic Medicine 2012 Dec;29(12):e445-8

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PUBLICATIONS IN PREPARATION (TO BE SUBMITTED AS A LINKED SUBMISSION TO DIABETOLOGIA)

Kennedy A, Andrews RC, Greenfield S, Narendran P, Dowswell G. Attitudes and barriers to exercise in adults with a recent diagnosis of type 1 diabetes – A qualitative study

Narendran P, Jackson N, Daley A, Thompson D, Stokes K, Greenfield S, Nouwen A, Mostazir M, Taylor S, **Kennedy A**, Andrews R. Exercise to preserve beta cell function in recent-onset type 1 diabetes mellitus (EXTOD) - a randomized controlled pilot trial

Shneerson C, **Kennedy A**, Andrews R, Stokes K, Thompson D, Daley A , Narendran P, Greenfield S. Barriers to Recruitment in Studies of New Onset Type 1 Diabetes Patients.

POSTER PRESENTATIONS

Kennedy A, Andrews RC, Narendran P, Greenfield S, Dowswell G. Attitudes and barriers to exercise in adults with new onset type 1 diabetes: A qualitative study. Accepted for presentation at IDF 2015

Kennedy A, Sawyer, N, Charlton M, Stokes K, Thompson D, Dowswell G, Greenfield S, Daley A, Cooper A, Andrews R, Narendran P. Physical exercise to preserve beta cell function in patients with new-onset type 1 diabetes (T1D): The EXTOD trial. Accepted for presentation at IDF 2015

Bajwa R, **Kennedy A**, Sawyer N, Andrews R, Narendran P for the Extod Study Group. How Physically Active Are Adults with Newly Diagnosed Type 1 Diabetes? Early Results from the EXTOD Study. Diabetes 2014 June; 63 (Suppl 1):A588 (2317-PO)

Kennedy A, Dowswell G, Andrews R, Greenfields S & Narendran P. What are the barriers to exercise in patients newly diagnosed with type 1 diabetes. Presented at the Diabetes UK APC 2013

Kennedy A & Narendran P. Does exercise preserve the 'honeymoon' period in type 1 diabetes? A presentation of three clinical cases. Presented at the Diabetes UK APC 2012.