The effects of aerobic and resistance exercise on inflammatory markers and metabolic control in healthy individuals and type 1 diabetics using either insulin pump or multiple dose injection

By

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TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

ABSTRACT

Type 1 diabetes (T1D) is characterised by an absolute insulin deficiency resulting from the chronic and progressive destruction of pancreatic β-cells by the immune system cells. Continuous subcutaneous insulin infusion (CSII) is becoming a popular technique for insulin delivery among T1D patients. Exercise is known to exert anti-inflammatory effects and metabolic control. Therefore it was of interest to study this in T1D using CSII. The objectives of this thesis were to further understanding of the effect of exercise on blood glucose, hemoglobin A1c, lipids, insulin and inflammatory markers in healthy and T1D volunteers. Three studies have been investigated where the diabetic volunteers used multi daily injections (MDI) or CSII. Firstly a survey was conducted aimed to investigate the effect of exercise on T1D patients using CSII therapy. The second study examined the acute and chronic effects of resistance and cardio exercise at moderate intensity on inflammatory markers such as IL-6, IL-1β, TNF-α and IFN-γ in healthy and T1D using MDI or CSII. Finally, a study was undertaken to find out the effects of chronic moderate intensity exercise on lipids profile and glycaemic control in healthy and T1D using MDI or CSII.

The statistical analysis of the survey showed that CSII therapy for T1D had a significant reduction on A1c, insulin requirement and improvement of lipids profile compared to MDI. Moreover, majority of CSII users (63%) rarely suffered from hypoglycemia during exercise. The second study demonstrated that acute and chronic exercises have a positive impact on the inflammatory markers among CSII users e.g. in CSII users statistically significant increase in IL-6 and TNF-α levels were observed (P=0.014 and P=0.001 respectively). The last study showed that lipids profile, total daily insulin units were

improved and A1c levels were significantly reduced in CSII as well as MDI groups after 6 weeks of exercise.

T1D affects major organs e.g. heart, kidneys, blood vessels etc. However, good glycaemic control can reduce the risk of diabetes complications. This study suggested that CSII therapy along with exercise can maintain the BG level close to normal, as all 5 participants of the study showed an improvement in their BG levels after exercise.

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DECLARATION

I declare that in this thesis is original work undertaken by me for the degree of Doctor of Philosophy, Faculty of Health and Life Science at De Montfort University. No part of this thesis has been submitted for the award of any other degree or qualification in this or any other university or college of advance education.

Mohamd Abdulrahman Alblihed

ABBREVIATION

Abbreviations	Definition
°C	Degrees Centigrade
1RM	One-Repetition Maximum
Ab	Antibody
ACSM	American College of Sports Medicine
ADA	American Diabetes Association
ADI	ADInstruments Analysis System
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
ApCs	Antigen presenting cells
ATP	Adenosine-5'-triphosphate
BB	Bio-Breeding
BF	Body Fat
BG	Blood Glucose
BHS	British Hypertension Society
BMI	Body Mass Index
BP	Blood Pressure
BSA	Bovine serum albumin
CD	Cluster of Designation/Differentiation
CHO	Carbohydrate
C_{OX}	Carbohydrate Oxidation
CSII	Continuous Subcutaneous Insulin Infusion
CVD	Cardio Vascular Disease
DAFNE	Dose Adjustment for Normal Eating
DC	Dendritic Cells
DCCT	The Diabetes Control and Complications Trial
DESMOND	Diabetes Education and Self-Management for On-going and Newly Diagnosed
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
DMU	De Montfort University
ECG	Electro Cardio Graph
EDTA	Ethylene diaminetetraacetic Acid
ELISA	Enzyme-linked immunosorbent assay
EPL	Exercise Physiology Lab
EURODIAB	Europe Diabetes study
G6P	Glucose-6-Phosphate
GCR	Glucagon Counter Regulation
GLUT	Glucose Transporter

GLUT4	Glucose Transporter Type 4
GP	General Practitioner
GS	Glycogen Synthesis
GSK	Glycogen Synthesis Kinase
GST	Glutathione-S-Transferase
A1c	Hemoglobin A1C (Glycated hemoglobin)
HCT	Normal haematocrit
HDL	High-Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
HLAs	Human Leukocyte Genes
HR	Heart Rate
HR_{max}	Maximum Heart rate
HSPs	Heat Shock Proteins
Hyper	Hyperglycemia
Hypos	Hypoglycemia
IDDM	Insulin Dependent Diabetes Mellitus
IFN	Interferon
IFN-γ	Interferon-γ
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobin gamma
IHE	Institute of Health Economics (Canada)
IL	Interleukin
IL-1β	Interleukin-1β
IL-6	Interleukin-6
INPUT	Insulin Pump and Diabetes Technology UK
IR	Immune Response
IRS	Insulin receptor Substrate
IV	Infused intravenous
LADA	Latent Autoimmune Diabetes of Adults
LDL	Low-Density Lipoprotein Cholesterol
LPS	Lipopolysaccharide
MDI	Multi Daily Injections
MHC	Major-Histocompatibility Complex
MnSOD	Manganese Superoxide Dismutase
N	Number
N-N	The time intervals between consecutive normal beats, reflecting the underlying sinus rhythm
ND	Non Diabetic
NHS	National Health Service (UK)

NIAID	National Institute of Health Autoimmune Disease
NICE	National Institute for Health and Care Excellence
NIDDM	Non- Dependent Diabetes Mellitus
NK Cell	Natural Killer Cell
NOD	Non-Obese Diabetic
NOR	Non-Obese Resistant
NSF	National Service Framework
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCV	Packed Cell Volume
pNN50%	Number of successive difference of intervals which differ by more than 50 ms
PPM	Parts Per Million
R&D Systems	Research and Diagnostics Systems
RE	Resistance Exercise
RER	Respiratory Exchange Ratio
rER	Rough Endoplasmic Reticulum
RM	Repetition Maximum
RMSSD	Square root of the mean of the sum of the squares of differences between adjacent NN intervals
RoS	Reactive Oxygen Species
RPE	Ratings of perceived exertion
RPM	Revolutions Per Minute
SDNN	Standard deviation of all NN intervals
SDSD	Standard deviation of the differences between adjacent N-N intervals
SEM	Standard Error of Mean
SLE	Systemic Lupus Erythematosus
SMBG	Self-Monitoring of Blood Glucose
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Science
sTNF-r	Soluble Cytokine Receptors
T1D	Type1 Diabetes Mellitus
T2D	Type2Diabetes Mellitus
TC	Total Cholesterol
Tc	T-cytotoxic cells
TCR	T-cell receptor
TG	Triglyceride Level
TH	T-helper cells
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor–α
T_{reg}Cells	Regulatory T-Cells

UKPDS	UK Prospective Diabetes Study
USD	US Dollar
VCO_2	Carbon Dioxide Production
VIS	Volunteer Information Sheet
VO_2	Oxygen consumption
WHO	World Health Organisation
μL	Microliter

Chapter 1: Introduction

1. Diabetes Mellitus

1.1 Overview

It has been observed that Diabetes Mellitus (DM) has no geographical boundaries and become a common disease all over the world. In the UK it represents about 5% of the population (see table 1.1)

Table 1.1: Diabetes (Both Types) Prevalence in UK (2011)

	Prevalence	**Number of patients**
Northern Ireland	3.8%	72,693
Scotland	4.3%	223,494
Wales	5.0%	160,533
England	5.5%	2,455,937

Diabetes causes the glucose levels in blood to rise via insulin deficiency, resistance to insulin, or both (Kumar and Kumar 2005). Insulin dependent DM is known as Type1 (T1D) and non-insulin dependent is referred to as type2 (T2D). DM of either type can be treated but it cannot be cured and statistically causes sufferers to die at a younger age than non- sufferers (Kumar and Clark 2002). The number of people who have diabetes (either type) varies from region to region and country to country.

Recently in an international league table compiled and published in Diabetes UK (January 2013) it was stated that the UK (24.5 T1D cases per 1000) and Saudi Arabia (31 cases of T1D per 1000) has one of the highest world's rate of T1D in children aged 14 and below (5^{th} and 3^{rd} highest, respectively in the world). Key statistics on diabetes show that, in UK, in 2012, 10% of adult diabetic patients suffers from T1D and when

children are included, 15% of diabetic patients are diagnosed with T1D (Diabetes April 2012).(Table 1.1 shows prevalence of diabetes both types)

It has been estimated that in UK more than 5%of population is suffering from diabetes, some are diagnosed while others are undiagnosed and are sleep walking into the diabetes trap (Hex, Bartlett et al. 2012)T1D may become apparent at any age; however it is most commonly known to appear before the age of twenty, with children aged 12 years old being most at risk. The average age that T2D appears is 40 years old. However, people from ethnic groups such as Afro Americans are at risk of T2D at a younger age.

1.2 Diagnosing Diabetes

Diagnosis of T1D and T2D is usually triggered by symptoms of frequent urination (polyuria) extreme thirst (polydipsia) and weight loss. These symptoms increase day by day and week by week. Around a quarter of people who have recently developed type 1 diabetes have developed worse symptoms by the time their diabetes is diagnosed. Diagnosis of other forms of diabetes is usually made with other procedures, such as; screening, looking for hyperglycemia during health checks and through the detection of secondary symptoms such as changes in eyesight and extreme tiredness (WHO 2006).

Sometimes diabetes is only noticed when a person experiences a health problem that has been caused by diabetes, such as a heart attack, a stroke, neuropathy, a foot ulcer that will not heal, problems with eyes, fungal diseases, neonatal macrosomia and or

hyperglycemia. A person with diabetes will have relentless hyperglycemia and they will be diagnosed if they are showing any of the following symptoms:

1. A level of fasting plasma glucose at or above 126mg/dL (7.0mmol/l).
2. The WHO states that a person will be diagnoses with diabetes if they have a plasma blood glucose level at or above 200mg/dL (11.11mmol/l) two hours after having a 75mg dose of glucose.
3. If they show symptoms of hyperglycemia and a casual plasma glucose which is at or above 200mg/dL (11.1mmol)

If a person shows a positive result when there is not unequivocal hyperglycemia present then any of the procedures mentioned above should be carried out again on a different day. Most clinicians use the fasting glucose level and glucose tolerance test (which takes two hours) to confirm the diagnosis of diabetes mellitus. Two samples giving fasting glucose measurements above (126mg/dl or 7.0 mmol/l) are considered to be a confirmation of the diagnosis of diabetes mellitus (ADA 2012). People who have a fasting glucose level between 100 and 126 mg/dL (6.1 and 7.0 mmol) are described as having an impaired fasting glucose and may need an oral glucose tolerance test to exclude overt diabetes. Those who have a plasma glucose from 7mmol (126mg/dL) and 11.0mmol/L (200mg/dL) two hours after receiving 75g of glucose are classified as having a low glucose tolerance and they should have an oral glucose tolerance test (ADA 2012). People who have a lowered tolerance to glucose are considered to be at a very high risk of developing overt diabetes (ADA 2012). An increased level of glucose permanently bonded to hemoglobin (glycated or glycosylated hemoglobin or A1c) of 6.0% or more (the 2003 revised U.S. standard) is seen as abnormal by most labs; A1c is

mainly used as a tracking test that reflects average blood glucose levels over the previous 90 days. However, some doctors may order this test at the time of diagnosis of diabetes. The current suggested target for A1c in patients with diabetes is <7.0%, which, although higher than the normal healthy level is an indicator of fairly good glycaemic control in the diabetic subject. Some authorities are stricter (<6.5%) but imposition of this by compliant patients may risk some hypoglycaemic episodes. In the ACCORD and ADVANCE programmes, T2D patients were randomised to intensive or standard regimens but there were excess deaths in the intensive group in which patients were encouraged to aim for A1c levels below 6% but there is dispute to whether this was actually because of hypoglycemia (Bloomgarden 2008). Patients who have diabetes and have A1c levels that fall below 7% have a considerably fewer problems caused by diabetes.

1.3 Impact of diabetes

1.3.1. Epidemiology and Complications of Diabetes

Whiting, Guariguate et al. (2011) reported that in 2011 there were almost 280 million diabetic patients around the globe and this number rose to 371 million in 2012. It is expected that the number will go pass 500 million mark by the end of 2030 (Whiting, Guariguata et al. 2011). While Diabetes UK (Diabetes April 2012) claimed that in 2011 around8.3% of adults (i.e. 366 million) worldwide were suffering from diabetes and it has been projected that by 2030 this figure will rise 552 million. The American Diabetes Association (ADA) has claimed that around 1 in every 3 people born in 21st century (i.e. after 2000), in America, will be diagnosed with diabetes at some point in their lives

(Weiss and Sumpio 2006). In 2011 ADA estimated that the number of people with DM in US were 25.8 million (children and adults) and that means 8.3% of US population live with DM. Diabetes UK 2012 reported that this disease is slightly more common in men (6.3 per cent) than in women (5.3 per cent) in England (Table 1.2).

Table 1.2: Prevalence of diabetes by age group in England (2010)

Age group	Female	Male
16–34	2.1%	1.8%
35–54	6.6%	9.4%
55–64	8.0%	11.1%
65–74	12.2%	15.2%
75+	13.2%	15.9%

Controlling of medical conditions related to diabetes has been shown to reduce the risk of complications (Stratton, Adler et al. 2000; Diabetes April 2012). However, serious complications occur when diabetes is not well managed particularly stroke, cardio vascular disease, renal failure, blindness and amputation of limbs (especially lower limbs) leading to permanent disability and mortality. Microvascular and macrovascular problems can be caused by inefficient glycaemic control. Macrovascular problems are characterised by blocking of the main arteries which in turn can lead to stroke and myocardial infarction. Among diabetic sufferers stroke is the most common cause of death (Diabetes April 2012). In 2013 ADA data shows that, stroke risk and adult heart disease death are 2 to 4 times higher among people with diabetes. NHS data shows that in UK 10% of all hospital admissions are associated with diabetes (Diabetes April 2012).

As mentioned above one of the major causes of mortality and morbidity in patients with diabetes is cardiovascular disease (CVD) (Martins, Fonseca et al. 2013). CVD may increase insulin resistance in T1D patient (Cleland, Fisher et al. 2013). Moreover, Diabetes Control and Complications Trial (DCCT), recruited 1441 T1D sufferers (13 to 39 years old) from 29 medical centres across North America (i.e. US and Canada), concluded the "absolute risk for fatal CVD events per 1,000 person-years to be 1.37 and 2.51 in intensive and conventional diabetes treatment groups, respectively" (Lung, Clarke et al. 2013). In recent research it has been observed that CVD is increased by a minimum of two- to fourfold and in diabetes it is the main reason behind the death rate in both types of diabetes (Martins, Fonseca et al. 2013). It is worth noting that poor glycaemic control and its secondary derangement of biochemistry, is the main cause of CVD in youth with T1D (Dobrovolskiene, Mockeviciene et al. 2013).

CVD is a major cause of death and so a public health concern in the UK (Allender, Peto et al. 2012). It has been reported that in 2010, almost 147,000 deaths i.e. almost third of all deaths were caused by one or other form of CVD. In the US is considered to be the main cause of death and it has been reported that 813,000 deaths were caused by CVD (Allender, Peto et al. 2012). It is also worth noting that in the US around 82.6 million people aged over 20 years are affected by CVD (Roger, Go et al. 2011). There are four different types of CVD:

i. coronary heart disease,
ii. stroke,
iii. peripheral arterial disease; and
iv. aortic disease.

T1D is associated with a high risk of coronary heart disease (Salem, AboElAsrar et al. 2010). In adults, aerobic exercise coupled with a restricted diet regime, is a very efficient way of improving total cholesterol (TC) to high-density lipoprotein cholesterol (HDL) ratio, low-density lipoprotein cholesterol (LDL) and triglycerides (TG) (Kelley, Kelley et al. 2011). Exercise enhances glycemic control and reduces the risk of developing cardiovascular problems (Kelley, Kelley et al. 2011). Aerobic exercise training decreases hepatic and visceral lipids in obese people without weight loss (Johnson, Sachinwalla et al. 2009). Furthermore, resistance training decreased TC, TC/HDL-C ratio, non-HDL, LDL and TG in adults (Kelley and Kelley 2009).

T1D is caused by cellular-mediated obliteration of the pancreatic β-cells that causes a loss of the ability to secrete insulin (Groop and Pociot 2013). Moreover, T1D is a result of a progressive autoimmune process, where β-cells in the islets of Langerhans are destroyed or damaged. Clinical onset and β-cell destruction mechanisms leading to T1D are still unclear (Bonifacio 2013). This process causes insulin deficiency and hyperglycemia. Nonetheless, this condition is potentially treatable by effective interventional therapy (Ahmed, Akirav et al. 2013). In recent years many researchers highlighted the role of T cells as pathogenic effectors cells in T1D. Recently, it has been established that two types of T-Cells are the main contributor to T1D progression.

i. T_{reg} Cells (Regulatory T cells): These are commonly known as suppressor T cells. On one hand these cells control the immune system, while on the other hand these cells abrogate autoimmune disease and maintain tolerance to self-antigens. In summary these cells control the activity of auto reactive and

inflammatory cells, the implication being that either their frequencies or suppressive function are altered in T1D (Brusko and Atkinson 2007).

ii. T_H-17: These cells are a subtype of CD4+ helper T cells. These cells provide protection against extracellular bacteria and mediate pathological responses in autoimmune disease (Ryba-Stanislawowska, Skrzypkowska et al. 2013).

These two types of T cells are responsible for the appropriate function of the immune system and control inflammation. The decrease of T_{regs} and/or increase of T_H-17 may provoke local inflammation and destruction of β cells. It has also been observed that diabetic complications are speeded by the inflammatory effects of the disease which means the complications, as well as the disease, are inflammatory in nature (Ryba-Stanislawowska, Skrzypkowska et al. 2013).

T1D is characterized by the presence of autoantibodies recognizing islet antigens (Diana, Gahzarian et al. 2011). Despite the critical role of antibodies for the diagnosis of the disease in patients, many data suggest that T cells are the key players in the autoimmune attack of β-cells (Bluestone, Herold et al. 2010). Medical research shows that in the development of T1D, infection can have either inhibitory or potentiating role (Lehuen, Diana et al. 2010).

In addition hyperglycemia itself induces inflammation in T1D complications (Jiang, Wei et al. 2012). In the recent past (2010), attempts have been made to study the role of insulin resistance (IR) in T1D and observed that IR plays a greater role in T1D process than commonly known (Dib 2006). Dib (2006) also suggested that "IR may reflect more

aggressive form of autoimmune disease mediated by immune-inflammatory factors that also mediate β-cell destruction (TNF-alpha and IL-6)".

Furthermore, hyperglycemia increases nuclear factor kappa B (NF-κB) p65, interleukin-l beta (IL-1β) and TNF-α levels (Jiang, Wei et al. 2012). Additionally, IFN-γ is playing an important role in the autoimmune pathogenesis in patients with T1D (Lee, Kwon et al. 2012; Yi, Li et al. 2012).

1.3.2. Death Rates of Diabetes

In 2008 the World Health Organisation (WHO) reported diabetes as the ninth cause of death in the world. However, in National Vital Statistics Reports 2012 by Donna, diabetes was the seventh cause of death with 3.4% (Ventura, Curtin et al. 2012).

According to NHS information centre for Health and Social Care, Leeds the mortality analysis 2007-2008 by National Diabetes Audit (2011) it is estimated that 15-16 % of the 460 000 deaths occurring in England in 2009 were attributable to diagnosed diabetes (Duncan and Goldacre 2012).

The number of death certificates, where diabetes is a cause of death or a contributory factor, was increased 5-fold between 1995 and 2010 (Duncan and Goldacre 2012). Furthermore, according to NHS diabetes care in England and Wales, 24,000 people with diabetes deceased earlier than expected. In a report published by NHS on their website it is claimed that the death risk for T1D patients is 2.6 times higher than that of the general population and for T2D it is 1.6 times higher. The report further claimed that in

younger people with T1D the mortality rate is even higher (e.g. 15-34 year old female patients with T1D are 9 times more likely to die than non-T1D females in same age group) (NHS, 2011).

In summary, cardiovascular disease and kidney disease are the main causes of the death among diabetic patients. (de Zeeuw, Remuzzi et al. 2004; Ley, Tsiami et al. 2011) (Roglic, Unwin et al. 2005). A drop in the mortality caused by prolonged diabetes related medical complications was observed in the early onset group. While in the late onset cohort no such effect was observed (Harjutsalo, Forsblom et al. 2011).

1.3.3. Finance and Economic Problem

Globally in 2012, the equivalent to 11% of the total healthcare budget was spent on diabetes i.e. USD 471 billion in 2012 and this may rise to USD 595 billion by 2030 (Guariguata 2012).

According to Diabetes UK records in 2012 it is estimated that above £10 billion were spent by NHS on diabetes which is 10% of NHS budget (with a 2010/2011 total NHS expenditure of approximately £103 billion). This translates to £192 million per week, £27 million daily, £1 million per hour, £17,000 per minute and £286 every second. In UK the cost of diabetes (direct care and indirect costs) stands at £23.7 billion and is estimated to rise to £39.8 billion by 2035; £ 16.9 billion in direct costs (£ 1.8 billion for T1D and £ 15.1 billion for T2D) and £ 22.9 billion in indirect costs (£ 2.4 billion and £ 20.5 billion) (Hex, Bartlett et al. 2012; Diabetes April 2012). In March 2013 ADA

stated that, based on 2012 data, in US total cost of diagnosed diabetes stands at $245 billion, while direct medical cost of DM stands at $176 billion.

1.4. Diabetes Mellitus Classification

In 1979 the National Diabetes Data Group (NDDG) was the first worldwide accepted classification scheme for diabetes. They classified diabetes based on the pharmacologic therapy applied into two major groups (Maraschin Jde 2012). These types are insulin-dependent diabetes mellitus (IDDM) otherwise known as type 1 diabetes (T1D) and non-insulin-dependent diabetes mellitus (NIDDM) which is type 2 diabetes (T2D). While the insulin criterion remains technically true, the fact is that at least 40% of T2D sufferers use insulin (although are not dependent on it), thus the terminology has moved to T1D and T2D. T1D is often known as childhood-onset diabetes (Rother 2007). T2D can be described as adult onset diabetes and obesity-related diabetes (Krentz 2012).

1.4.1. Type 1 Diabetes (Insulin Dependent)

As explained, multiple autoimmune attack underlies the patho-mechanism of T1D (Hermann, Krikovszky et al. 2005). About 80% of β-cell destruction occurs before T1D becomes clinically evident. The islet cell autoantibodies (ICAs) are responsible for this destruction (Shriver 2011).

It has been mentioned before that T1D is characterised by the presence of autoantibodies and the T-cells (T_{regs} &T_H-17) are considered to be responsible for the T1D and the T1D and its complications are inflammatory in nature. In a study of

recently diagnosed children with T1D,insulitis was confirmed to involve the infiltration of CD8+ and CD4+ T-cells, macrophages and β-cells (In't Veld 2011). CD4+ T helper (T_H) cells as well as CD8+ cytotoxic T (Tc) cells are responsible for the T-cell mediated immunity in T1D (Liblau, Wong et al. 2002; Shao, He et al. 2012). These T-Cells release cytokines and chemokines which are responsible for the β-cell destructive functions of these T-Cells. Nonetheless, we are still unclear about how the β-cells are destroyed, which means the immune activity depends upon the structure of the inflammatory proteins. Current theories involve T helper (T_H-1) cells such as lymphocytes which cause the cell-mediated immunity by production of IFNγ alongside TNFα, enhanced activity of cytotoxic T (Tc) cells and initiation of macrophages, together leading to improve the levels of inflammatory cytokines (e.g. IL-6 and IL-1β) (Ryden and Faresjo 2013). IL-1β, and TNF-α were played a central role of β-cells destruction, TNF-α secretion was inhibited by IL-6, and this may have some protecting effects (Hermann, Krikovszky et al. 2005).

It is worth mentioning that the major causes of T2D are insulin resistance and metabolic syndrome (Brooks-Worrell, Narla et al. 2012).

1.5. Management, Lifestyle and Diet of diabetes patients

T1D has a 90 year history of insulin treatment (Hirsch and Skyler 2012) and it is important to support people with diabetes to understand their condition so that they gain optimum benefit from the treatment.

In UK there are two education programmes which are nationally designed to help people with diabetes to gain the skills and confidence to manage their condition. One of is for T1D, a scheme called the Dose Adjustment for Normal Eating (DAFNE) was designed to help people to adjust their insulin injections to fit their lifestyles. The other one is designed for T2D sufferers and is known as The Diabetes Education and Self-Management for On-going and Newly Diagnosed (DESMOND) and not necessarily focused on insulin.

To achieve near normal glycemia, T1D needs intensive diabetes management and the care of T1D patient either child or adult should be under specialists supervision team qualified in the care of diabetes (Care 2011). T1D children are different from T1D adults in many respects such as ability to provide self-care, insulin sensitivity, and unique neurological weakness to hypoglycemia and Diabetic ketoacidosis (DKA)(Care 2011).

In 1980 attempts were made to achieve glycemic control, by developing new techniques and instruments, such as usage of human insulin, CSII therapy, A1c to assess integrated glycemic control and home self-monitoring of blood glucose (SMBG) (Hirsch 2009; Hirsch and Skyler 2012). This aligned with the later DCCT findings that showed a strong relationship between controlled blood glucose and the neuropathic and micro vascular complications in T1D patients(Hirsch and Skyler 2012)

It has been shown that eating healthily, exercising frequently, not smoking and not drinking alcohol are effective in treating both forms of diabetes i.e. T1D and T2D (Hu, Manson et al. 2001).

Diets and reducing energy intake (low in carbohydrate) for twelve weeks cause a reduction in the amount of fasting plasma glucose in the body by 25.7%, decrease blood pressure by 8.1%, cholesterol 9.2% and triglycerides by 26.7% (Anderson, Kendall et al. 2003). The Nutrition Subcommittee of the Diabetes Care Advisory Committee of Diabetes UK (2011) suggests various lifestyle changes, such as having food that has a low glycaemic index and monounsaturated fats instead of foods high in saturated fats and doing exercise (Dyson, Kelly et al. 2011).

Diabetes UK suggests that people with diabetes should have a healthy, balanced diet, as everyone should; a diet that low in fat, sugar and salt, with lots of fruit and vegetables. Meals should contain high levels of starchy foods such as bread, potatoes, cereals, pasta and rice (Anderson, Kendall et al. 2003). Diets that contain 55-60% carbohydrate, 15-20% protein and 20-30% fat have shown to be the most effective in improving glycaemic control and lipid management when used with an optimum insulin regimen.

Regular exercise is considered to play a key role in the management of diabetes, as it improves control of blood glucose, reduce risk of cardiovascular disease, cause weight loss, and enhance overall well-being (American Diabetes Association) (ADA 2012).

1.6. Diabetes and glucose

1.6.1. Overview

Under regular physiological conditions, glucose is the main energy source for the cells. It also acts as a precursor to various metabolites in nearly all tissues. Glucose is

discharged into the blood by the liver in the fasting state due to glycogen being broken down and used (oxidised) for the energy requirements of cells, while the insulin controls the postprandial removal of excess glucose from the blood. Glucose is carried into most cells by specific proteins or transporters that cover the cell membrane and permit the connecting and transferring of glucose across the hydrophobic lipid layer (Scheepers, Joost et al. 2004).

1.6.2. Glucose transport pathways

Glucose is transported around the body by a group of membrane proteins that aid the carrying of glucose over a plasma membrane. Two common types of glucose carriers have been identified in cells are:

i. The facilitative glucose transporters (GLUT) family are a protein family that is found in most mammalian cells (Scheepers, Joost et al. 2004).

ii. Sodium-Glucose linked transporters are found in small intestine and the proximal tubule of the nephron (Scheepers, Joost et al. 2004).

In people without diabetes, the blood glucose remains at a normal level which is 3.5–5.5 mmol/l before meals and less than 8 mmol/l, 2 hours after meals (Diabetes April 2012).

1.6.3. Glucose control

In healthy human beings, when glucose is released during food digestion insulin is released into the portal bloodstream from the pancreatic islet β-cells as will be discussed in detail below (section 1.6). Now due to insulin signalling GLUT 4 glucose transporters move from cytoplasm into the plasma membrane, this movement allows glucose to enter the cell. (1, 2 and 3 are the movements of GLUT4) (see figure 1.1), it starts a cascade ending in the uptake of glucose by recruiting glucose carrier proteins on the cell surface. Within the cell, glucose is metabolised i.e. being utilised as energy or kept in the muscle and liver glycogen (Lin and Accili 2011).

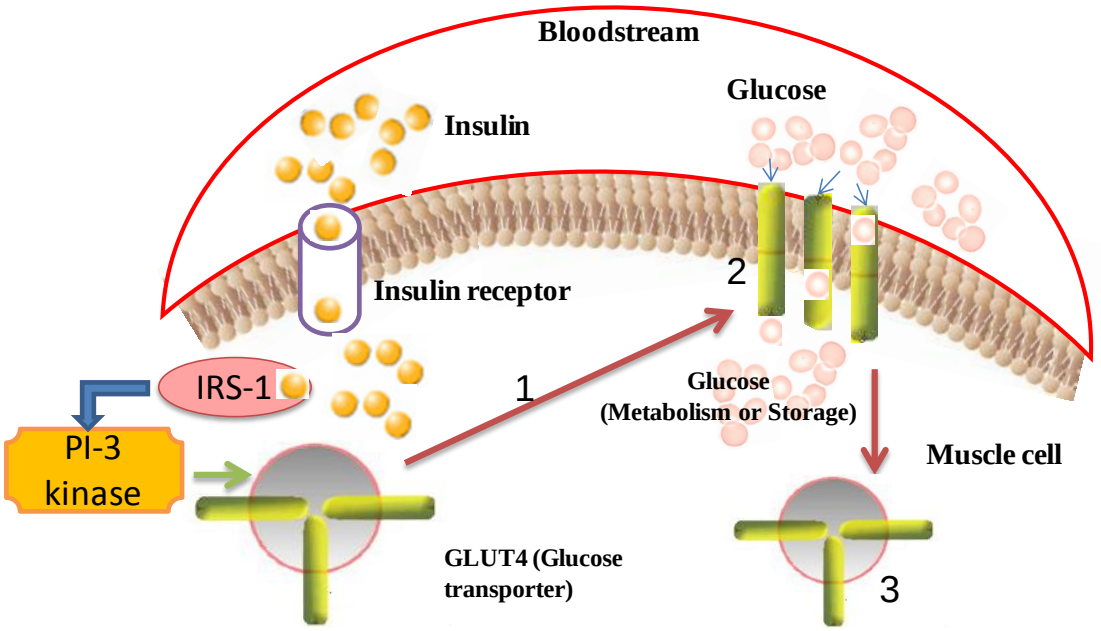


Figure 1.1: How insulin is delivered to cells Insulin receptor substrate 1 (IRS-1), phosphoinositide 3-kinase (PI-3), glucose transporter type 4 (GLUT4) see text..

On the other hand, insulin decreases blood glucose in the fasting state by stopping the production of hepatic glucose from glycogen (i.e. suppressing the gluconeogenesis) (Ramnanan, Edgerton et al. 2010). The exact pathway of gluconeogenesis by insulin in

humans remains a debateable issue (Lin and Accili 2011). However, the brain is an insulin-sensitive organ and uses glucose in a way that is not reliant on insulin (Levin and Sherwin 2011).

Insulin encourages glycogen and lipid creation in the cells of muscles, while stopping lipolysis and gluconeogenesis from muscle amino acids. In summary, the insulin is central to regulating and controlling many metabolic processes which also include the production of carbohydrates from other sources including structural protein (Timmerman, Lee et al. 2010). In healthy tissues this is closely and locally controlled but in T1D patients artificially administered insulin hinders the breakdown of muscle protein (Dunn 2013).

1.6.4. Glycogen metabolism

The body stores glucose for later use by conversion to the branched polymeric form, glycogen. A raised level of blood glucose concentration is required for glycogenesis to be stimulated (Xu, Morgan et al. 2011). Glycogen serves as an accessible storage depot of energy mainly in the liver but also in the muscle and fat cells (Villarroel-Espindola, Maldonado et al. 2013). The liver and muscles change excess glucose into glycogen so that it can be used for glycogenesis in the future. Glycogen breaks down (glycogenolysis) when glucose levels drop, particularly if gaps between meals are lengthy.

There are various enzymes and controlling proteins engaged in the synthesis of glycogen (Xu, Morgan et al. 2011). In the liver, the GLUT-2 transporter controls the

transfer of glucose into hepatocytes (Leturque, Brot-Laroche et al. 2009), whilst in the skeletal muscle; GLUT-4 is the main glucose carrier (Leto and Saltiel 2012).

1.6.5. Effect of diabetes on glycogen stores

As the main places of storage of glycogen are the liver and skeletal muscles, most studies that researched into the effect of diabetes on glycogen stores were carried out with these tissues. Diabetes is considered to be responsible for weakening of glycogen synthesis (GS) in skeletal muscles (Krause, Riddell et al. 2011). In T1D sufferers an inverse correlation between GS and above normal level of glycogen synthase kinase 3 (GSK3) proteins and movement in human skeletal muscles was found (RH 2011; Villarroel-Espindola, Maldonado et al. 2013).

In diabetes sufferers (T2D) weakened glucose transport and raised levels of plasma fatty acids are considered to be responsible of decline in disposal of glycogen (Kleinert, Sylow et al. 2013). In diabetics hyperglycemia is considered to be the result of faults in GS (Pratipanawatr, Cusi et al. 2002; Tanabe, Liu et al. 2011). Impaired glucose transport coupled with increased plasma fatty acids and faulty GS leads to reduction in glycogen synthesis. Furthermore, the decline in the suppression of hepatic glucose production, as seen in diabetes sufferers, is associated with the over expression of a catalytic subunit of the enzyme glucose 6 phosphatase (6GPC) (Marcolongo, Fulceri et al. 2013).

1.7. Insulin

1.7.1. Definition

Insulin is a peptide hormone that is secreted by the β-cells and permits cellular glucose uptake and regulates levels of glucose in the blood stream. Insulin performs its function by controlling the metabolism of lipids, carbohydrate and protein and encouraging cell division (Preza, Pinon et al. 2013; Strachan and Frier 2013).

1.7.2. Synthesis and release of Insulin

Insulin is synthesised as its precursor, proinsulin as in figure 1.2. Proinsulin is created from mRNA as pre-proinsulin in the ribosomes of the rough endoplasmic reticulum (rRERs). The production of pre-proinsulin is a complex process in which the B chain, signal peptide, the connecting (C-) peptide and then the A chain are sequentially synthesised (Lindahl, Nyman et al. 2010). The three dimensional structure of proinsulin is engineered within the endoplasmic reticulum. Proinsulin is carried by secretory vesicles from the rRERs to the Golgi apparatus. The Golgi apparatus contains aqueous zinc and calcium saturated environment which provides perfect conditions for the production of soluble zinc-containing proinsulin hexamers (Lemaire, Chimienti et al. 2012). As under developed storage vesicles are created by the Golgi, enzymes that perform outside the Golgi change proinsulin to insulin and C-peptide hexamers (Haataja, Snapp et al. 2013). When developed granules are delivered into circulation by exocytosis, insulin and an equimolar ratio of C-peptide are released into plasma. Consequently the presence of C-peptide indicates endogenous synthesis of insulin and therefore, in T1D, C-peptide is decreased or absent (Haidet, Cifarelli et al. 2012).

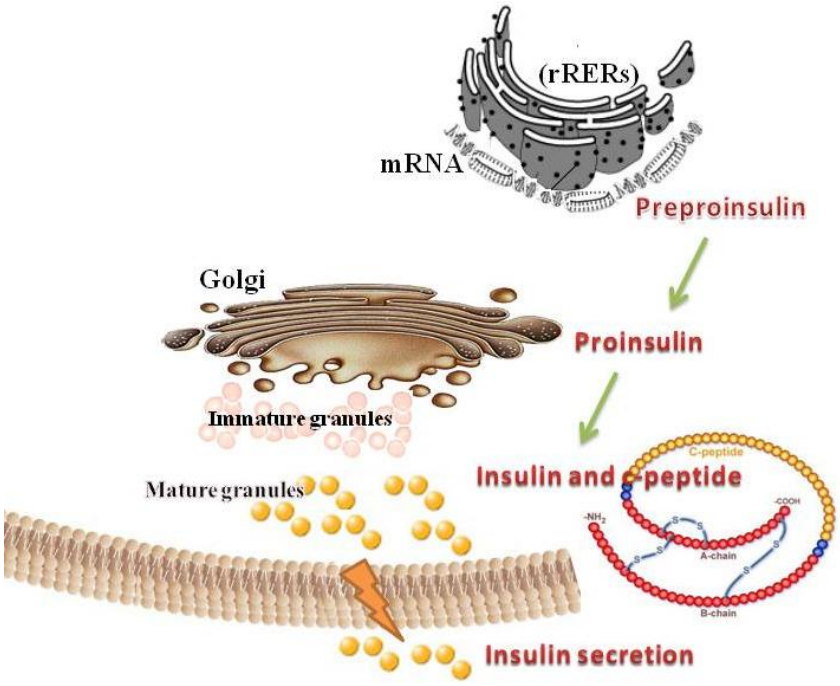


Figure 1.2: Insulin synthesis and secretion process involving the rough endoplasmic reticulum (rRERs) and Golgi complex (see text)

1.7.3. Mechanism of action of insulin secretion

Heightened levels of glucose encourage the first phase of the glucose-mediated insulin secretion by releasing insulin from the secretory granules in the β-cell as in figure 1.2. Glucose entry into the β-cell through Glucose Transporter-2 (GLUT2) triggers glucokinase (GK) (Porat, Weinberg-Corem et al. 2011; Coate, Kraft et al. 2013), which phosphorylates glucose to glucose-6-phosphate (G6P) by GK, producing ATP. Shutting off the K+/-ATP dependent channels (see figure 1.3) causes membranes to depolarize and launching of voltage dependent calcium channels Ca^{2+} causes a rise in intracellular calcium concentrations; this activates pulsatile insulin emission (Porat, Weinberg-Corem et al. 2011). It is evident from figure 1.3 that glucose enters into β-cells through

GLUT2 then phosphorylated to G6P by GK. G6P enters into glycolysis pathway and electrons are transported through the electron transport chain in mitochondria yielding ATP. Increased ATP/ADP ratio and closure of ATP sensitive K+ channels lead to membrane depolarization. Change in membrane potential opens up voltage gated Ca^{2+} channels causing influx of Ca^{2+} into β-cells. Increased cytosolic Ca^{2+} concentration facilitates the synthesis of insulin-containing secretory vesicles with plasma membrane releasing insulin.

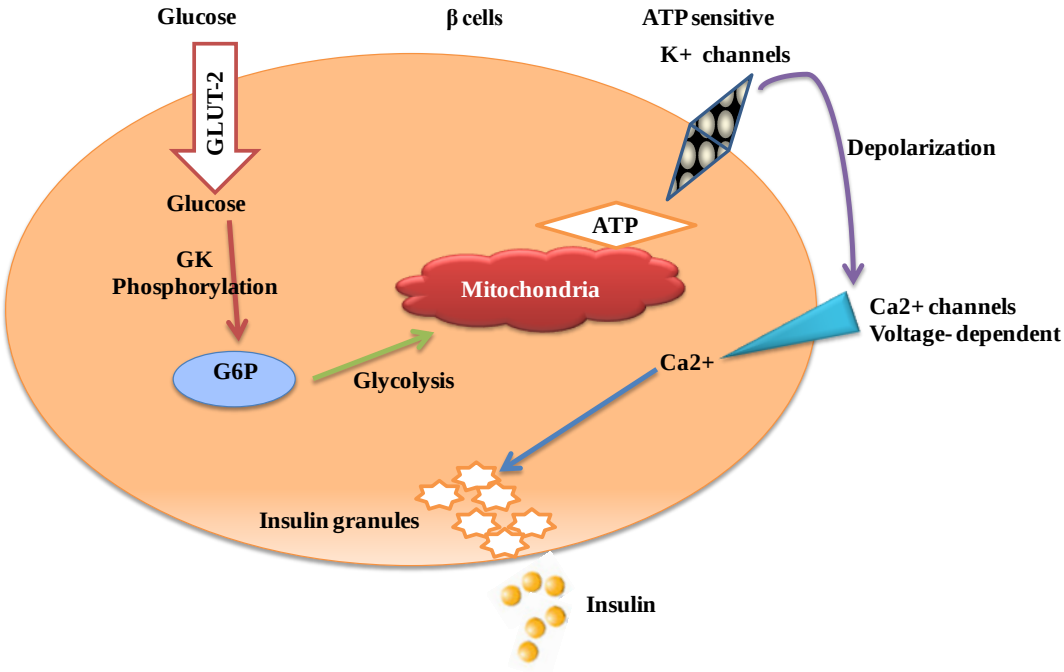


Figure 1.3: Molecular mechanism of glucose induced insulin secretion. Glucose transporter 2 (GLUT2), glucokinase (GK), glucose 6-phosphate (G6P) see text.

Counter-regulation works against insulin action and leads to raised blood glucose (BG) levels in response to hypoglycemia (Szepietowska, Zhu et al. 2012). The glucagon, epinephrine (also known as adrenaline), cortisol, and growth hormone are main counter-regulatory hormones (Chen, Sheng et al. 2012). In diabetes the counter-regulatory

hormones are released in the middle of the night and are responsible for hyperglycemia often mistaken for the superficially similar so-called Dawn Phenomenon i.e. very high level of BG in the early morning (Mandujano, Thomas et al.). Glucagon gene expression is associated with insulin signalling in the alpha cells and regulates both high- and low-glucose conditions (Kawamori, Kurpad et al. 2009).

In healthy people hypoglycemia is countered by the natural defence mechanisms. This defence mechanism, firstly, decreases insulin secretion from pancreas (which causes increase in BG level), and secondly, releases more glucose from the liver by secreting the counterregulatory hormone glucagon by alpha cells of the pancreas. Later, the liver and kidneys produce more glucose under the influence of the sympathetic autonomic nervous system and epinephrine directly secreted from the adrenal glands. Epinephrine is a classical stress hormone, which stimulates α and β adrenergic receptors (Ziegler, Elayan et al. 2012). It controls tissue needs in terms of releasing glucagon to the bloodstream and takes action to reduce insulin secretion and mobilise the conversion of glycogen. When epinephrine and glucagon fail to raise the levels of blood glucose, growth hormones and cortisol are released by the body to increase glucose level in blood stream.

As mentioned above hypoglycemia in healthy people is avoided by the portal insulin-to-glucagon ratio (McCrimmon and Sherwin 2010), but in T1D sufferers this glucagon counter regulation (GCR)is often impaired and may fail to trigger the necessary protection mechanism to avoid hypoglycemia (Farhy, Chan et al. 2012). Insufficient glucagon secretion has been reported in T1D (Karimian, Qin et al. 2013) and is considered to be responsible for the impairment of counter regulation during

hypoglycemia in T1D sufferers. Glucagon raises glutamine uptake and plays an important role to this response. However, in T1D patients, in case of hypoglycemia epinephrine and glucagon fail to response effectively (Battezzati, Benedini et al. 2009).

In people with T2D the impairment of GCR may cause hypoglycemia, particularly in those who inject insulin, but it is less severe and common (Davis, Mann et al. 2009). In T2D patients age plays no role in the counterregulatory responses to the hypoglycemia with older and younger patients showing the same level of GCR (Bremer, Jauch-Chara et al. 2009).

1.7.4. Insulin resistance

Insulin resistance (IR) is a pathologic condition where target cells (in liver and muscle) do not respond to regular amounts of circulating insulin and therefore provide a weakened biological response. Insulin resistance is known as an impaired sensitivity to glucose removal by insulin. It has been reported that T2D sufferers, young and old, develop cardiopulmonary fitness problems, which affect their ability to do exercise and cardiovascular functions. However, IR is not typically considered a contributory factor in cardiovascular dysfunction in T1D sufferers (Nadeau, Regensteiner et al. 2010). In T2D, patients may be managed conservatively with diet, exercise and then subsequently by oral agents that may ameliorate resistance or which may increase pancreatic response (secretogogues). Some type 2 people may receive insulin as a first line treatment, particularly if they have suffered myocardial infarct. However, for type 1, injected insulin must always be instituted immediately to avoid DKA and death. IR may also, in

fact, be applicable to T1D with intensive insulin therapy and bad glycaemic control (Fauci 2008)

1.7.5. Type of therapeutic insulin

1.7.5.1. Injectable insulin

For T1D insulin is needed as a continual replacement therapy. It is a protein that breaks down when taken by mouth and therefore it needs to be injected subcutaneously. At present, most therapeutic insulin is human but bacterially derived and developed through genetic engineering, therefore the negative effects of immunogenic reactions and religious beliefs about beef or pork insulin have been eliminated.

T1D patients can use insulin pump. In which a cartridge stores the insulin as either regular or fast acting insulin analogs; such as insulin lispro (Humalog), insulin as part (NovoLog), or insulin glulisine (Apidra). The analogs have a quicker onset of performance (5 to 15 minutes versus 20 to 30 for Regular) and an earlier peak in performance (90 minutes versus 150 minutes). Table 1.3 lists the insulin actions, peak activity, onset and duration of insulin, while appendix (A) highlights the various insulin types in the market.

Table 1.3: Insulin actions, onset, peak activity and durations

Insulin	Onset begins	Peak activity occurs	Duration after injecting
Analogue rapid	Within 15 min	15 min to 1 hour	3 to 4 hours
Human short	Within 30 min	1 to 3 hours	6 to 8 hours
Animal short	Within 1 hour	2 to 5 hours	6 to 8 hours
Human intermediate	Within 2 hours	2 to 12 hours	18 to 24 hours
Animal intermediate	Within 2 hours	6 to 12 hours	18 to 24 hours
Analogue long	Within 1 hour	No peak as such	18 to 24 hours

Source: diabetes.co.uk

1.7.5.2.Administering insulin by insulin pump

The insulin pumps were first developed in the late 1970s to imitate the way in which a normal pancreas delivers insulin by avoiding multiple doses in favour of continuous administration. In medical term administering insulin by pump is called Continuous Subcutaneous Insulin Infusion (CSII). CSII is an alternative to Multi Daily Insulin injections (MDI) by syringes or insulin pens.

The insulin pump is a device marketed by a range of companies but fundamentally can be used independently by a diabetes patient to administer insulin continuously instead of as discrete volumes during the day. The pump administers insulin solution at an appropriate rate for the patient's blood glucose status and predicted changes for meals. A cannula with insulin-compatible tubing is used to connect the pump and its cartridge of contents to the subcutaneous layer, usually on the abdomen or hip. The patient must change the injecting unit every few days to keep it clean and functioning properly. Sophisticated inserters make this easy. The patient can stop when they have a wash or do exercise. When choosing a pump, several things need to be considered, such as the

size and weight of it, how long the batteries last, model of infusion sets, alarms and special features (Appendix B shows a table of insulin pumps on the market).

1.7.5.2.1. World use of insulin pumps

In March 2012 Canadian Institute of Health Economics (IHE) published a report on the acceptance of CSII among T1D patient. This report claimed that the usage of CSII therapy is gaining worldwide popularity, mainly in US. The report further stated that there are wide spread differences in the acceptance of CSII therapy among medical practitioners around the world.

In 2005, in US it was estimated that 270,000 of T1D sufferers were treated with insulin pump compared with over 180,000 in Europe. Recently, in US, this had grown to more than 375,000 patient using insulin pump to manage their diabetes (McCrea 2013).

In 2006 IHE reported that in US about 8% to 15% of individuals with T1D aged above 12 years are using insulin pump, while the take up of insulin pump among younger people i,e. less than 12 years, vary from 15% to 50%.

In April 2012 Diabetes.co.uk reported that the number of insulin pump users is significantly lower in the UK, it is estimated that only around 1% of people with T1D have an insulin pump. These data were not changed since 2006 according to IHE report "notable low-use countries (such as the UK and Denmark, where about 1% of T1D use an insulin pump)". In the UK the significant low in use of insulin pump is related to the

cost as reported by the Scottish Government when they announced to spend £1.5 million to allow 480 young T1D to have insulin pump therapy.

The financial cost is the main reason behind the discrepancies in the usage of insulin pump, as the insulin pump cost is covered in some countries (e.g. United States, Sweden, and the Netherlands) by insurance companies or state and in some other countries (e.g. United Kingdom and Denmark) individuals have to cover the cost of the pump (Selam 2006). Moreover, training availability for the healthcare professionals and lack of knowledge on part of individual T1Ds may also lead to this variation, (Charles, Sadri et al. 2009).

Finally, the International Society for Pediatric and Adolescent Diabetes, European Society for Pediatric Endocrinology and Lawson Wilkins Podiatric Endocrine Society signed an agreement which was later endorsed by the American Diabetes Association and the European Association for the Study of Diabetes claimed that there is no lower age limit for prescribing CSI therapy and recommended that all T1D diagnosed paediatric patients should be put on CSII insulin therapy. (St Charles, Lynch et al. 2009).

1.7.5.2.2. How insulin pumps work

Nowadays, the pumps are becoming more and more sophisticated, technologically advanced and smaller in size i.e. size of a phone or credit card and can be worn discreetly. These pumps consist of a pump, which is operated by a battery, and insulin cartridge reservoir. Insulin is administered from pump to the body via a cannula, which

is kept in place with the help of adhesive tape. Every 2 to 3 days the cannula is moved to different site on the body to avoid infection or skin irritation, while once a week the tubing is changed (Hindmarsh, Peters et al. 2013).

These pumps are programmed to deliver small amounts of insulin throughout the day to ensure basal blood insulin levels using rapid-acting insulin and delivering it to the patient continuously. Based on the insulin requirements of the user during the day insulin infusion rate could be changed or modified (Pickup 2012). Before taking a meal, pump user need to program the pump manually to deliver a larger amount of insulin as a bolus dose (i.e. supplemental dose) of fast-acting insulin. The amount of insulin in bolus dose depends on the pre-meal BG level and the amount of carbohydrate he is planning to consume.

1.7.5.2.3. Insulin Pump versus Multi Daily Injection

Insulin pump therapy and MDI users both need to measure their blood glucose levels 4-6 times, at a minimum, every day. Nevertheless, insulin pump allows more flexibility precise dose of insulin as compared to MDI (basal bolus regime) and is considered to be a closest replica of the physiologic method of insulin administration (IHE, 2012, p. 83).

The main advantage of insulin pump therapy over MDI is its ability to significantly reducing the risk of hyperglycemia and hypoglycemia. It has been observed that insulin pump users have fewer events of post-exercise hyperglycemia as compared with MDI users (Yardley, Iscoe et al. 2013). Furthermore, T1D sufferers who use insulin pump, children and adults, reported lowering of A1c (Battelino, Phillip et al. 2011).

The insulin pump users invasively insert a delivery needle once every three days while MDI users do this more frequently i.e. not fewer than 3 times in a day. Additionally, with insulin pumps the total insulin requirement during the day is, usually, decreased by 15% to 30%.

The stability of BG level and insulin in the body is better maintained by an insulin pump as compared with the MDI. Moreover, users can adjust the basal and bolus doses more accurately, which means pump user can easily adjust their insulin dose around times requirements such as meals and physical activities.

Comfort and convenience are two very important features to all pump users especially younger children (for example, during school, or day-care time). Delivering very small amounts of insulin is difficult to administer with MDI while the pump can be made to deliver required amount of insulin as and when required. Using CSII in T1D will not significantly change the body weight while increase in MDI patients (Kordonouri, Hartmann et al. 2006).

Despite above mentioned advantages insulin pumps are still slow to gain popularity among the wider public as such pumps are very expensive, especially for patients who do not have health insurance and need to pay from their own pocket. Moreover, techno phobia coupled with pump complications and faults, infections at the cannula site, reactions, and tube blockage are also hindering the widespread usage of these pumps. It is worth noting that insulin pens have made MDI a simplified, flexible and portable alternative to the costly insulin pumps.

The main disadvantages of the insulin pump is the hypoglycemia and DKA events which can occur when the user failed to recognise that the right amount of insulin is not delivered to the body or insulin infusion is hampered. Furthermore, for some patients, such as pregnant, attaching the pump all the time can become a psychological barrier.

Selection of an appropriate insulin pump is very important to meet the individual patient's needs and circumstances, i.e. physiological and financial.

1.8. Exercise

A physical activity which maintains or boosts physical overall health and fitness is called an exercise. People perform physical exercises for different reasons such enhancing cardiovascular system, strengthening muscles and the weight loss or maintaining weight, as well as for the purpose of enjoyment. It is well researched and documented fact that regular physical exercise enhances the immune system, and helps to prevent cardiovascular disease, obesity and T2D (Simpson, Lowder et al. 2012).

It is recommended that a person aged between 18 and 64 years, should spend 30 minutes, at least 5 days a week, to do moderately intense exercise (see table 1.4). However, for diabetics, 150 minutes of exercise per week is recommended (SIGN, 2010; Department of Health, 2010). Following a regular regime of physical exercise a person with T1D can enhance their quality of life and also their psychological well-being (Guelfi, Jones et al. 2005). Moreover, they can decrease the chance of having life-threatening cardiovascular problems (Bracken, West et al. 2011). Diabetes patients

should be encouraged to follow these recommendations and achieve at least 2.5 hours of exercise every week (Kilbride L. 2011).

However, there is not much evidence available that can be used to provide advice to people who have T1D with regards to strategies that they can use to self-manage the illness and maintain appropriate glycaemic control when they carry out exercise (Charlton 2013). It is widely known that hypoglycemia is caused by inappropriate glycaemic control and this stops many diabetics from exercising (Charlton 2013). It is worth saying that one of the objectives of this research is to develop a strategy to fill this gap in the literature.

1.8.1. Resistance and aerobic exercise

Some forms of physical exercises, such as lifting weights, have a significantly different effect on the body than low intensity exercise such as walking. These two types of exercises are called resistance and aerobic exercises respectively (Lucotti, Monti et al. 2011). Under some circumstances, exercise may be anaerobic and this can well be associated with resistance exercise because of its short and intense nature.

Anaerobic exercises are non oxygen consuming high intensity exercises (a brief, infrequent, and intense physical activity) and, after the first few seconds, normally activate lactic acid production. They can cause the secretion of hormones that work against insulin, often resulting in high levels of blood glucose during and after exercise. Conversely, aerobic exercises are generally less intense (termed mild and moderate) exercise performed for longer periods of time and may actually lower blood glucose

levels (Delvecchio, Zecchino et al. 2009). The intention in this project was to include some anaerobic component in the protocol, such that the exercises would differ, not only in type (cardiovascular and resistance) but also in metabolic result. In trained athletes, the anaerobic condition is reached at 80-90% of the VO_{2max} but for untrained individuals such as were planned for this study, it occurs at 50-60% VO_{2max} and it seemed that this was a reachable goal without risking the welfare of individuals undertaking the exercise (Cerretelli, Ambrosoli et al. 1975; Farrell, Wilmore et al. 1979). For diabetic people this threshold is reached at even lower intensity levels. So for example, Komatsu compares trained athletes and found a lower anaerobic threshold in his diabetic cohort. The reasons for this are probably that diabetes is a disease of oxidative stress (Yamagishi, Maeda et al. 2012; Yokota, Kinugawa et al. 2013). The complications of diabetes are related to this via the glycation products but the immediate effects may be that aerobic metabolic processes are compromised and the anaerobic component of exercise may be increased at a given intensity level. This might manifest itself in the lactate levels, depending on conditions (Soultanakis, Mandaloufas et al. 2012). Because it was realised that the anaerobic threshold must have differed in the cohorts being compared and thus the resistance exercise a mixture of aerobic and anaerobic (though likely the latter predominating in these untrained individuals), the term anaerobic has now not been used for this study, but rather resistance.

Table 1.4: Exercise intensity classifications, based on physical activity lasting up to 60 min (Zinman, Ruderman et al. 2003)**.**

Relative Intensity			
Intensity	**VO_2max(%)**	**% Of Maximal Heart Rate****	**RPE***
Very light	<20	<35	<10
Light	20-39	35-54	10-11
Moderate	40-59	55-69	12-13
Hard	60-84	70-89	14-16
Very hard	>85	>90	17-19
Maximal	100	100	20

*Borg rating of perceived exertion(RPE)6-20scale.
**Maximal heart rate = 220 – Age. As exampled below

Age	HR at rest	Intensity	Maximum HR	HR reserve	Target HR for experiment
35	71	55	220-35=185	185-71=114	114×0.55+71=134

When a person has been doing aerobic physical activity two or three times a week their use of blood glucose (insulin sensitivity) will change (Yoshida, Ishikawa et al. 2010). Doing consistent exercise improves the fat use which lessens the need for blood glucose. Nevertheless, for diabetic people, in long lasting aerobic exercise, hypoglycemia may occur and as a result, there is less of a need for alteration to regimen (Kakleas, Kandyla et al. 2009). Moreover, muscle grows as a result of training and a person may become more sensitive to insulin which may decrease the basal and bolus insulin requirements (Heinemann 2009).

Non-diabetic healthy people, who do regular exercise will also become more sensitive to insulin and it can prevent them from developing T2D (Sigal, Kenny et al. 2007). A meta-analysis of diabetic sufferers T2D showed that staying active seems to improve A1c (Boulé, Haddad et al. 2001) and a systemic review of studies that researched mainly dietary advice to patients with T2D also showed the same effect of exercise (Nield, Moore et al. 2007). A range of types of exercises showed benefit (A1c decreased

by 0.8%) for T2D (Snowling and Hopkins 2006). Resistance exercises (RE) are prescribed in diabetes because of the aforementioned increase in insulin sensitive muscle mass that can result in enhanced endurance and muscular strength. These along with improved body composition and flexibility reduce the risk of cardiovascular disease (Cohen, Dunstan et al. 2008).

The American College of Sports Medicine (ACSM) and the American Diabetes Association (ADA) have outlined general clinical practice guidelines for exercise and diabetes for people who use CSII. The guidelines state diabetes sufferers should not exercise if beforehand their fasting blood glucose level is more than 13.8mmol/l and/or if there is a raised level of ketones in their blood or urine. A blood glucose level over 16.6mmol/l with no ketones present should be taken with "caution". A third guideline is to eat carbohydrates if one's blood glucose level before doing exercise is lower than 5.5mmol/l. People with diabetes should therefore assess their pre-exercise blood glucose level. However, in contrast to the ACSM and ADA principles, people who use pumps and have a blood glucose level lower than 5.5mmol/l before doing exercise may not need to eat carbohydrates snacks as they can just lower or stop the basal insulin whilst doing exercise (Colberg 2013). Levels of counterregulatory hormones drop later on in the day and, in most of the users, the amount of insulin that is delivered from the pump will need to be changed 60 to 90 minutes before exercise that is going to last for 30 to 45 minutes. Basal rates may also need to be decreased whilst carrying out the exercise, to lower insulin levels. To maintain control, bolus reductions of insulin will often need to be done immediately after exercise to eliminate the risk of hypoglycemia (Zisser and Riddell 2010). This reduction is dependent on the type, intensity and

duration of exercise. For example, resistance exercise increased BG level after one or two sets/repetitions (Leelarathna, Little et al. 2013). Even when a person has a pump, the metabolic control with exercise can get worse in some circumstances (Hovorka, Allen et al. 2010; Elleri, Dunger et al. 2011). When the blood glucose level is raised (13.8 mmol/L) or more and there are ketones in the blood or urine, it is evident that they do not have enough insulin in the system and are in danger of diabetic ketoacidosis (DKA) which can be fatal. Taking part in high intensity exercise like resistance will raise the blood glucose level in both diabetes sufferers and healthy people (Simpson, Florida-James et al. 2006). In a healthy person natural glycaemic control mechanism will pump more insulin in the system. However, if a T1D sufferer takes part in a resistance activity while his/her BG level is higher than normal i.e. not enough insulin in the blood, the blood glucose level may increase further and as a result risk of DKA is increased (Hanas and Ludvigsson 2006; Wolfsdorf, Craig et al. 2007). In this condition a CSII user may need to raise the level of basal insulin whilst exercising and/or afterwards (Jeandidier, Riveline et al. 2008).

In summary, individuals with T1D should take special care when taking part in physical activity. Although regular and systematic exercise regime is advantageous for all patients, yet high intensity exercise can disturb the BG levels. It has been observed that the glycemic response depends mainly on the type, duration and intensity of the physical activity, along with the insulin in the system and glucose counterregulatory hormone concentrations.

1.8.2. Exercise and Immunity

Though recently voluminous literature has been produced to find a link between exercise and immunity and it can be argued that exercise immunology is a fairly new area of scientific study (Shephard 2010). It has been suggested that exercise may act as a model of numerous clinical stresses as it generates comparable hormonal and immunological alterations (Gillum, Kuennen et al. 2011). These manifest themselves as acute and beneficial inflammatory effects as muscles adapt to exercise and also useful longer term anti-inflammatory changes that benefit the causes and effects of some diseases such as diabetes. The chronic anti-inflammatory effect of exercise has both clinical and public health consequences (Nieman 2012). In a recent study it was observed that acute aerobic exercise enhances insulin sensitivity and cardiovascular fitness in individuals who are insulin-resistant T2D subjects (Musi 2013).

Many studies documented the fact that a modest exercise has a positive and favourable impact on the human body. It has been observed that a six week regime of moderate exercise can elevate activity of NK cells (Nieman, Nehlsen-Cannarella et al. 2008). Furthermore, moderate exercise enhances immunoglobulins, which result in a positive influence on the immune system (Karacabey, Saygin et al. 2005). Frequent moderate exercise has been linked with decreased occurrence of infection (respiratory infection) in comparison to an entirely sedentary state in healthy (Walsh, Gleeson et al. 2011). Moderate aerobic exercise leads to a temporary increase in both innate (monocytes, macrophages, neutrophils, NK cells) and specific (B and T lymphocytes) cells in a person's immune system (Gillum, Kuennen et al. 2011). Light observed that 25 minutes of modest exercise leads to swift upsurges in gene expression for receptors detecting

muscle metabolites and for sympathetic nervous system and immune system in leukocytes in individuals with Chronic Fatigue Syndrome (Light, White et al. 2009) with a decrease in the obliterations of T cell numbers. Similarly, Shephard (2010) showed that levels of both CD4 + and CD45 + cells improved in people who exercise (Shephard 2010). There was also an increase in the latter cell sub-set which was exceptionally significant when taking into consideration its potential to trigger production of CD8 + cytotoxic cells (Shephard 2010).

It is well documented fact that T1D and T2D patients suffer from impaired immune system and aforementioned studies provide enough evidence to suggest that T1D and T2D sufferers can benefit from a moderate exercise regime (Christiansen, Bruun et al. 2013; Ho, Dhaliwal et al. 2013; Kennedy, Nirantharakumar et al. 2013; Musi 2013; Yardley, Kenny et al. 2013).

1.8.3. Effects of Acute exercise on the Immune System

A single session of exercise is considered as acute exercise (Ploeger, Takken et al. 2009). In contrast to a moderate exercise regime, as discussed above, a single, excessive exercise session can have a short-term influence on the immune system (Gleeson 2007). It is also associated with bodily reactions that are very comparable (in many aspects) to those that are brought about by trauma, sepsis or infection. These reactions cause significant upsurge in the level of leukocytes (mainly lymphocytes and neutrophils) in the blood stream. The scale of this upsurge is associated with the length and intensity exercise session (Gleeson 2007).

Cytokines do not only increase during inflammatory disease such as diabetes; acute exercise also affects cytokine reactions and inflammation in healthy people (Ploeger, Takken et al. 2009). Moreover, the plasma concentrations of a range of substances, which are known to have an effect on leukocyte functions (i.e. inflammatory cytokines, e.g. TNF-α, anti-inflammatory cytokines IL-6 and macrophage inflammatory - IL-1β and protein-1) also increases (Gleeson 2007). The heightened levels of plasma IL-6 concentration are seen in people, when they exercise, and this increase is attributed to the release of this cytokine from the contracting muscles (Gleeson 2007). In a study carried out by Christiansen et al. (2013) severe exercise generated an increase in the presence of inflammatory markers (e.g., IL-6, IL-8, and TNF-α) in people who were classed as overweight and obese (Christiansen, Bruun et al. 2013) . Nevertheless, IL-6 generation by monocyte and IFN-γ generation by T lymphocytes are constrained during a period of extended exercise and also for many hours afterwards (Gleeson 2007). The immune system is affected by severe exercise both during and after the exercise session (Pedersen and Hoffman-Goetz 2000). In acute exercise, IL-6 is released by muscles and the levels IL-6 may increase significantly (Petersen and Pedersen 2005). Leukocyte subgroups, by way of neutrophils, lymphocytes subsets NK, B, T cells and monocytes, in addition to plasma concentrations of anti-inflammatory and pro-inflammatory cytokines IL-1, TNF-α and sTNF-r, may increase significantly during a period of exercise (Ploeger, Takken et al. 2009). In 2009, a review of 19 studies were carried out to investigate the effects of acute exercise on inflammatory markers on subjects with a chronic inflammatory disease and found no conclusion evidence of trend from one exercise session (Ploeger, Takken et al. 2009).

1.8.4. Chronic Effects of Exercise on Immune System

Chronic exercise is defined as an exercise regime which lasts for 6 weeks or more (Ploeger, Takken et al. 2009; Al-Nassan, Fujita et al. 2012). It has been reported that the chronic exercise may affect the chronic inflammatory diseases, such as diabetes, by affecting the immune parameters (Petersen and Pedersen 2005).

Exercise is recommended to be used as an anti-inflammatory therapy as it affects basal levels of inflammatory markers in T2D (Bruunsgaard 2005; Petersen and Pedersen 2005). It has been shown that chronic exercise may improve (back to normal) or re-imposed the resting levels of TNF-α and IL-1β in healthy children (Stewart, Flynn et al. 2007). In a study carried out by Ho et al. (2013) a 12-week exercise programme of mixtures of exercise regimes (mainly moderate-intensity resistance and aerobic) lowered TNF-α in obese and overweight people. Thus, combination exercise may help to lower the risk of contracting chronic diseases (Ho, Dhaliwal et al. 2013).

In 2009 six studies were conducted that looked at the impact of chronic exercise in adults, which essentially looked at the impact of exercise on resting levels and basal of inflammatory markers. One of the studies focused on resistance training while the remaining five concentrated on endurance training (Ploeger, Takken et al. 2009). While, to date there is no study conducted to examine the effects of exercise, in children suffering from inflammatory disease, on inflammatory markers. Nevertheless, the impact of chronic exercise on the generation of pro- and anti-inflammatory cytokines from the skeletal muscle has yet to be investigated (Lira, Koyama et al. 2009).

Finally, for individuals with T1D performing resistance before aerobic exercise is suggested to reduce the severity and duration of post-exercise hypoglycemia and improving glycemic stability throughout exercise (Yardley, Kenny et al. 2012; Yardley, Kenny et al. 2013). Moreover, it is recommended to stretch before (warm-up) and after (cool-down) performing any exercise (Petit, Hughes et al. 2010) targeting upper and lower muscles by elongating them to their fullest length (Kluemper, Uhl et al. 2006).

1.9. Overview on the Immune System

1.9.1. Autoimmunity

Autoimmunity is an abnormal response to tissue components. Usually, a system of self-tolerance defends an individual from possible self-reactive lymphocytes. A defect in this regulation can cause the triggering of self-reactive clones of B or T cells and consequently cause a cell or humoral mediated immune reaction against self-antigens. This autoimmune response can seriously harm cells and organs, which can cause autoimmune disease to develop which can be life-threatening.

According to the National Institute of Health Autoimmune Disease (NIAID) on March 2005, autoimmune diseases are a collection of diseases, which up to 8% of the population are affected by. At one end of the spectrum, some of these conditions cause symptoms that are less difficult to manage but in many cases, one individual organ can be severely affected by an autoimmune disease (organ specific autoimmune disease) such as diabetes.

1.9.2. Immunology of Type 1 Diabetes

Before discussing the immunology of T1D it is interesting to note that the biologists use a protocol known as the cluster of designation/differentiation (CD) to identify and investigate the surface of cell molecules which provides targets for the immuno-phenotyping of cells. Physiologically CD molecules perform different functions such as activate a receptor i.e. act as receptors and cell adhesion (Zola, Swart et al. 2007).

Since the discovery of islet cell antibodies in 1974, it has been suggested that T1D is caused by systematic destruction of insulin-producing ß-cells of the pancreas there has been a perception that T1D is autoimmune in nature (Skyler 2011; Hirsch and Skyler 2012). T1D is a chronic autoimmune disease and is caused by the destruction of pancreatic β-cells on a discriminatory basis. The development of T1D comprises complicated collaboration amongst pancreatic β-cells and adaptive immune systems and cells in the innate immune systems (Lehuen, Diana et al. 2010). The T1D is considered to be a T helper 1 (T_H-1) cell-mediated illness that involves both CD8+ T cells and innate immune cells (Lehuen, Diana et al. 2010). Leuhen et al. (2010) further claim that CD4+ and CD8+ T cells along with macrophages play a role in the destruction of β-cells (Lehuen, Diana et al. 2010).

Furthermore, studies that have used animal models, predominantly in non-obese diabetic (NOD) mice, have revealed roles for various different immune cell types in β-cell annihilation. The most widely accepted notion is that antibodies (that characterise the humoral section of the immune system) do not contribute the obliteration of β-cell destruction but instead act as markers of that annihilation, while the cellular section of

the immune system, particularly the T-lymphocytes, facilitate the obliteration of β-cells (Skyler 2011). However, the T-lymphocytes do not act independently; they are aided by antigen-presenting cells such as dendritic cells and macrophages when instigating the reaction, and seem also to be helped by B-lymphocytes (Skyler 2011).

There are some other cell types that exist in the pancreatic infiltrate and also in the pancreatic draining lymph node, where the early appearance of islet antigen by dendritic cells (DCs) to islet antigen-specific T cells arises (Turley, Poirot et al. 2003). These cells comprise B cells and natural killer (NK) cells, in addition to DC subsets, and they may also be responsible for the destruction of β-cells. The generation of chemokines by B cells leads to further recruitment of mononuclear cells to the site, and thus increases inflammation (Eizirik, Colli et al. 2009). Alternatively targeting or controlling the actions of various different immune cell types may also be an effective way of impeding β-cell annihilation (Lehuen, Diana et al. 2010).

Moreover, interferon-gamma (IFN-γ) can trigger macrophages and encourage enhanced pro-inflammatory cytokine generation, such as interleukin-1beta (IL-1β) and tumour necrosis elements (TNF) (Lehuen, Diana et al. 2010).

A high level of IL-1 receptors is expressed by β-cells. β-cells expedite high levels of IL-1 receptor and appear to be more susceptible to IL-1β-induced apoptosis in comparison to other endocrine cells that exist in the islet (Lehuen, Diana et al. 2010). This interaction between T cells and macrophages indisputably aggravates the immune-mediated stress on β-cells and aids in their obliteration. Expression of reactive oxygen species (RoS) such as nitric oxide by β-cells is also induced by interleukin-6 (IL-6)

(Lasota, Penna-Martinez et al. 2013), IFNγ, IL-1β and TNF. While RoS have the capacity to facilitate apoptosis (Lehuen, Diana et al. 2010). IL-6 plays a major role in the development from acute to chronic inflammation which affects clinical expression of T1D (Lasota, Penna-Martinez et al. 2013).

1.9.3. Autoimmune Mechanisms Related to Type 1 Diabetes

Before the symptoms of T1D disease become apparent several unnoticeable immune events will be triggered (Van Belle, Coppieters et al. 2011). T1D is believed to be a chronic immune-mediated illness with a subclinical prodromal period (which is the period during which a process of disease has started but the disease is not yet clinically diagnosable) where the discriminatory loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible individuals. Although auto reactive T cells, both CD4 and CD8 types, play a key role in β-cell obliteration (Knip and Siljander 2008), the sign of autoantibodies is the first obvious sign of emergent β-cell autoimmunity (Knip and Siljander 2008). Thus, autoantibodies are generated and self-reactive lymphocytes are triggered and penetrate the pancreas to obliterate the insulin-producing β-cells in the islets of Langerhans (Van Belle, Coppieters et al. 2011). Various studies have revealed that β-cell autoimmunity may be prompted in the early stages of life (Kimpimäki, Kupila et al. 2001). Results of the Finnish Diabetes Prediction and Prevention (DIPP) (cited in Kukko et al., 2005) reveal that the first autoantibodies may be expressed before a child reaches 3 months; approximately 9% of the children in the study were sampled from the general population based on elevated human leukocyte antigen (HLA) DQB_1 (Kukko, Kimpimäki et al. 2005). Nevertheless, this tenacious, targeted obliteration may go unnoticed for several years, and the initial clinical symptoms only show after most of

the β-cells have been obliterated or have become dysfunctional. This leads to the individual becoming dependent on insulin in order to survive (Van Belle, Coppieters et al. 2011).

Kupila, Keskinen et al. (2012) observed that the spread of humoral autoimmune response from epitope to epitope and from antibody to antibody takes place suddenly (Kupila, Keskinen et al. 2002). If this spread does not occur within a time frame of one year after the first autoantibodies develop, then it is highly unlikely that it will occur at a later date. These, together with other observations suggest that positivity for single autoantibody specificity signifies in most cases, undamaging non-progressive β-cell autoimmunity (Knip and Siljander 2008). The existence of two or more autoantibodies points to a worsening condition (Knip and Siljander 2008; Diana, Gahzarian et al. 2011). In summary in T1D B cells and resulting immunoglobulins i.e. antibodies perform a pathogenic role as antigen-presenting cells and autoantibody secretors which led to autoimmune destruction of insulin-producing β cells mediated by T cell. These findings have generated great interest to develop treatment for T1D by using B cell depletion therapies (Mariño, Silveira et al. 2011).

1.9.4. Animal models of type 1 diabetes

Some studies in animal models (NOD mice, BB rats), have focused on the significant role of dendritic cells (DC), which are main presenters of antigen, in the production of insulitis and diabetes (Lehuen, Diana et al. 2010). The Bio-Breeding (DP-BB) rats and NOD mice develop spontaneous type 1 comparable to those in humans with symptoms such as hyperglycemia and ketoacidosis. These two animal models may add to

knowledge about the causes, issues and medication for of T1D diabetes. The studies based on DP-BB rats and NOD mice are facilitating the understanding of the immuno genetic aspects of disease susceptibility and highlighting the effector cells which play a role in the autoimmune attack on the β-cell.

Bio-Breeding (DP-BB) rats develop autoimmune diabetes with extreme infiltration of the islets and the creation of autoantibodies (Dalberg, Haase et al. 2011). Almost 85% of the DP-BB rats expressed symptoms of diabetes by the time they reached 120 days old (Dalberg, Haase et al. 2011).

NOD mice were first described by Makino and colleagues in 1980 (Makino, Kunimoto et al. 1980). Insulitis in the NOD mouse appears spontaneously around 3 to 4 weeks of age, with progression to overt diabetes in 80% of female mice between 10-30 weeks of age. The insulitis lesions mainly contain T cells and numerous abnormalities have been observed in suppressive T cell of NOD mice (Shultz, Saito et al. 2010).

1.9.5. Cytokines and Diabetes

Gianoukakis and Smith (2004) stated that cytokines are important effectors molecules that play key roles in health and disease as they provide a network via which cells can have an effect on adjoining and distant tissues. The cytokines are membrane-bound and exist in soluble form and operate via attaching themselves to high-affinity receptors on the exterior of target cells. Cytokines also play a part in cell-mediated and humoral immune responses where they should not be perceived as being isolated factors, but rather should be investigated based on their collective affect. For instance, T cells

express T_H-1type; IFN-γ, TNF-α, and Th2-type cytokines IL-4, IL-5, IL-10 and IL-13. It seems that the nature of immune reaction is described by the combination of discharged cytokines and the equilibrium between different cytokine types (Gianoukakis and Smith, 2004). As explained above, cytokines can destroy β-cells through direct cytotoxic effects, potentially acting through the initiation of nitric oxide generation (Weir 2013) and stimulate or impede disease development in T1D. For instance, in patients with some residual β-cell function (as measured by C-peptide), the systemic concentrations of the pro-inflammatory cytokines IL-6 and TNF-α have been shown to be raised in either fasting or meal-stimulated states, while anti-inflammatory agents such as IL-1R, IL-10 are lowered. This suggests the provocative role of the β-cell membrane markers (Pham, Kolb et al. 2013).Specifically, new evidence proposes that the cytokines IL-1β, TNFα and IFNγ which are secreted by macrophages and T cells, play a bigger role in the growth of T1D than previously thought with a range of impacts on β-cells (Vincenz, Szegezdi et al. 2011). Moreover, stress on and obliteration of β-cells certainly involves the secretion of pro-inflammatory cytokines, for example IL-1ß, TNFα and IFNγ (Limbert 2012). However, combinations of IL-1β/IFNγ or TNFα/IFNγ have very powerful, synergistic effects that cause high levels of stress which results in the death of cells (Vincenz, Szegezdi et al. 2011). The initiation phase of T1D takes place in the pancreas, where conventional dendritic cells (cDCs) capture and process β-cell antigens. β-cell damage can occur by 'natural' apoptosis or after viral infections. Invariant natural killer T (iNKT) cells and plasmacytoid DCs (pDCs) control viral replication, preventing subsequent inflammation and T1D (not shown). B cells present β-cell antigen to diabetogenic T cells and secrete autoantibodies (not shown). In the pancreas, β-cells can be killed by diabetogenic T cells and NK cells through the release

of IFNγ, granzymes and perforin, as well as by macrophages through the production of TNF, IL-1β and nitric oxide. Lastly, β-cells can inhibit diabetogenic T cells by expressing PDL1. This complex crosstalk between innate and adaptive immune cells results in the development or the prevention of T1D. APC, antigen-presenting cell; TCR, T cell receptor; dendritic cell (DC); Natural killer cells (NK); Programmed cell death 1 ligand 1 (PD-L1) (see Figure 1.4).

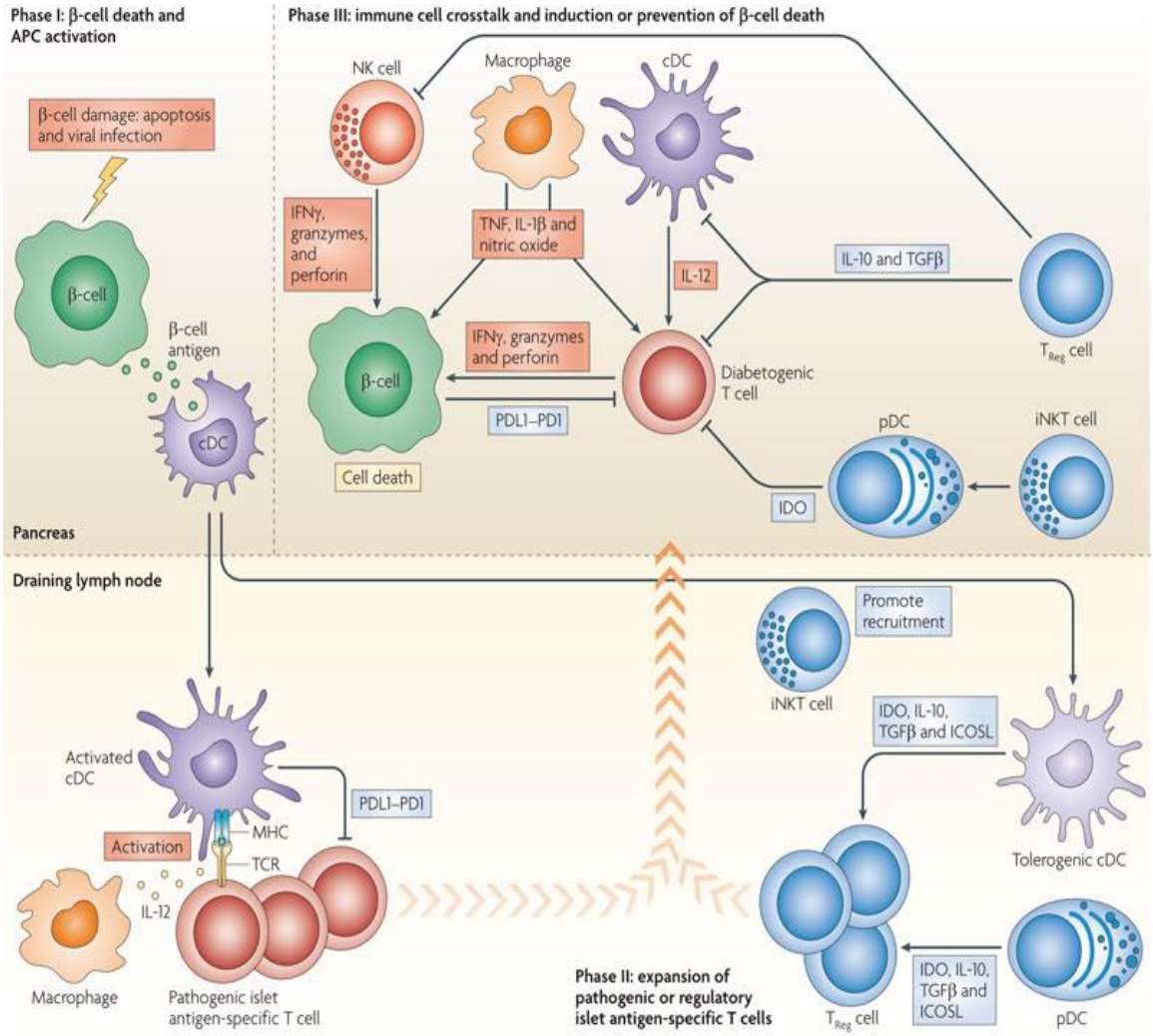


Figure 1.4: Process of T1D initiation. The initiation phase of T1D takes place in the pancreas with a complex signalling that can promote or suppress T1D development. Adapted from (Lehuen, Diana et al. 2010) (see text).

Furthermore, IL-6 plays an key role in the instigation and acceleration of chronic inflammation and may later play a part in the development of microvascular complications in patients with T1D (Wegner, Araszkiewicz et al. 2013).

1.9.5.1.Interleukin-1 beta (IL-1β)

It is believed that IL-1β plays a pathogenic role in T1D (Dinarello 2011; Limbert 2012). Evidence to show that IL-1β is toxic for the insulin-producing β-cell started to appear in 1985 when studies using anti-human IL-1β immuno affinity chromatography emerged (Dinarello 2011). It has been evidenced that the interleukin 1 family (IL-1α, IL-1ß and IL-33), a group of 11 cytokines, causes β-cell dysfunction (Limbert 2012); as a result, therapeutic IL1 receptor blockade has been carried out in humans with T1D and was shown to be successful (Akash, Shen et al. 2012) . IL-1β triggers the death of β-cell in patients with T1D; this occurs through NF-kB activation (Ortis, Miani et al. 2012). In T1D, the chronic hyperglycemia causes the vascular difficulties of the illness. Therefore, if blocking IL-1β-mediated obliteration of β-cell function should lead to better management of glycemia (Dinarello 2011). However, initial data from a short-term pilot study of immunotherapy of T1D showed that the levels of C-peptide were unaffected during this therapy (Sanda, Bollyky et al. 2009). Nevertheless, IL-1β increases in patients who have been recently diagnosed with T1D and it is more than likely that it operates as an early inflammatory signal in T1D development (Grishman, White et al. 2012). Paradoxically, Grishman, White et al. (2012) scrutinized the provoking effects of hyperglycemia itself on elevating the level of IL-1β in peripheral blood mononuclear cells (PBMCs) and islet cells. They failed to achieve consistent results and the mechanisms that cause this increase are still not agreed on (Grishman,

White et al. 2012). It is interesting to note that in T2D sufferers the gene expression for IL-1β was more than 100-fold higher in β-cells as compared to the healthy individuals (Dinarello 2011).

It would appear that results of investigations into pancreatic β-cells suggest that damage to islet cells are caused by IL-1βand involve multiple downstream targets (Grishman, White, Savani, 2012) but the resulting hyperglycemia reinforces the cycle of events. The use of the blockade should elucidate this further.

1.9.5.2.Interleukin-6 (IL-6)

IL-6 is a pleiotropiccytokine, affecting many functions. IL-6 is mostly produced by endothelial, fibroblast and adipocyte cells (Waetzig and Rose-John 2012). IL-6 is involved in infection and inflammation responses of the body. Mihara, Hashizume et al. (2012) stated that there is a consensus amongthe biologists that inflammatory reactions are mainly regulated by IL-6 (Mihara, Hashizume et al. 2012). This cytokine is also considered to be involved in the regulation of neural, regenerative and metabolic processes (Scheller, Chalaris et al. 2011). IL-6 has a key role in the initiation and acceleration of chronic inflammation and may contribute to the progression of microvascular complications in individuals with T1D (Wegner, Araszkiewicz et al. 2013). Neurath and Finotto (2011) found the serum level of IL-6 is low in T1D compared to non-diabetic (Neurath and Finotto 2011).Furthermore, some studies propose that IL-6 plays a part in the instigation and acceleration of the chronic inflammation procedure and may participate in the development of micro- and macrovascular problems in diabetic individuals (Jialal and Kaur 2012; Wegner,

Araszkiewicz et al. 2013).

1.9.5.3.Interferon Gamma (IFN-γ)

Immune cells, which invade the islets and play a role in dysfunction of β-cell and apoptosis, secrete IFN-γ (Eizirik, Colli et al. 2009). The obliteration of pancreatic β-cells in T1D individuals may be possibly mediated by IFN-γ cytokine (Chan, Biden et al. 2012). IFN-γ is an important factor in the induction and maintenance of the autoimmune damage to the islet β-cells, and IFN-γ has been linked with the onset of T1D in humans and animals (Chentoufi, Gaudreau et al. 2011). Nevertheless, the progression of spontaneous β-cell autoimmunity remains unchanged in NOD mice that are deficient in IFN-γ or the IFN-γ receptor (IFNγR) (Yi, Li et al. 2012). IFNγ also triggers the manifestation of reactive oxygen species (RoS) such as nitric oxide by β-cells, and RoS have the ability to arbitrate apoptosis (Lehuen, Diana et al. 2010). NK cells are both cytotoxic and producers of cytokines, especially IFNγ. Therefore, NK cells may have an indirect or direct effect on the obliteration of β-cells (Lehuen, Diana et al. 2010).

IFN-γ has been shown to be a possible constituent of many autoimmune diseases, such as multiple sclerosis and autoimmune diabetes.

1.9.5.4. Tumor Necrosis Factor Alpha (TNF-α)

TNF-α is a pro-inflammatory cytokine that is connected to several autoimmune illnesses. Blocking the TNF-α signalling pathway is an influential approach in the treatment of various auto-inflammatory illnesses like as rheumatoid arthritis and

inflammatory bowel disease. TNFα triggers the death of β-cell death in individuals with T1D individuals; this is done via NF-κB (light-chain-enhancer of activated B cells) activation (Ortis, Miani et al. 2012). In the progression of T1D however, the role of TNF-α is rather abstruse. Whilst the existence of TNF-α appears to quicken the development of the illness early on in the process, it has been shown to reduce the levels of auto reactive T cells after formation of the illness. Therefore, both methods of action may ultimately be targeted in a therapeutic manner (Boettler and von Herrath 2010). People who were diagnosed with T1D showed high level of circulating TNF-α (Arend and Dayer 2005).

Lately, promising data has been produced in a clinical trial using etanercept, a soluble TNF-α receptor fusion protein, in children who had had a recent onset of T1D (Mastrandrea, Yu et al. 2009). In the subjects who were given TNF-α blockade treatment, C-peptide levels appeared to significantly increase, whereas insulin doses reduced from baseline to week 24 in the non-existence of chronic side effects. In the control group C-peptide levels reduced and insulin doses were boosted in the study period. The small sample size of 18 people, and the short follow up period (24 weeks) limit the findings of the study and therefore further trials are needed to confirm the findings (Boettler and von Herrath 2010).

1.10. Study Objectives

The objective of this research exercise is to study the effect of exercise on blood glucose, hemoglobin A1C, lipids, insulin, metabolic control and inflammatory markers in healthy and T1D volunteers. In order to achieve this objective three studies were

undertaken. The first study used survey techniques and the aim was to investigate the effect of exercise on T1D patients using insulin pump therapy, to cover perception, attitudes and opinions about exercise, diet, blood glucose, A1c, lipids and insulin pump satisfaction. The second study examined the acute, i.e. one session, and chronic, i.e. 6 weeks, 2 sessions a week, effect of at moderate intensity resistance exercise and aerobic exercise on inflammatory markers such as IL-6, IL-1β, TNF-α and IFN-γ in healthy and T1D sufferers using MDI or CSII. While the final study was to examine the effects of chronic moderate intensity exercise (resistance and cardio) on lipid profiles and glycaemic control in healthy and T1D using MDI or CSII.

Chapter 2: Materials and Methods

2.1. Exercise study, short-term (acute) and long-term (chronic) effects

The objective of this study was to determine the acute and chronic effects on various immunologic and metabolic parameters of combined exercise program (aerobic, using the recumbent bicycle, and resistance) in Non-Diabetic (ND) and Type 1 Diabetic (T1D) using Multiple Daily Injections (MDI) and using insulin pump as continuous subcutaneous insulin infusion (CSII) patients.

The initial part of the practical research was concerned with investigating the effects of acute exercise (resistance and aerobic) on immunological parameters such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα), interferon gamma (IFNγ) and interleukin-1 beta (IL-1β). In order for researchers to observe the acute effect, volunteers were asked to exercise for one session. Blood samples were collected before commencement of exercise, after resistance exercise and after aerobic exercise.

The second part of this study was concerned with examining the chronic effect of exercise (resistance and aerobic) on the physiological and biochemical variables over a six-week period.

2.2. Insulin Pump Surveys

2.2.1. Surveys Project Aims

An insulin pump survey was designed to investigate the effects of exercise (aerobic and resistance) on Diabetes Mellitus (DM) patients and to discover their attitudes and opinions about their current exercise regimens. Survey results would support and feedback into the practical part of the study.

2.2.2. De Montfort University Insulin Pump Users Survey 2009

A previous survey (referred to as the 2009 survey) designed by this research group asked CSII users about their attitudes and experiences with DM and CSII therapy. That survey was preceded and supported by several focus groups, the members of which read and criticised the questions, as did the consultant diabetologist in the Leicester General Hospital.

The survey was divided into three sections and examined information about current CSII therapy, hypoglycemia and hyperglycemia and respondents' thoughts towards an implantable CSII under development by the research group.

As part of the current study the responses collected from the earlier survey were analysed by researchers to gain an understanding and appreciation of survey analysis, this survey has been published (Appendix C). These responses had been collected by postal mail (100 responses) and electronically (242 responses) by Survey Monkey®.

2.2.3. De Montfort University Insulin Pump Users and Exercise Survey 2011

The second survey (referred to as the 2011 survey) (Appendix D) was compiled as part of the current work and its aim was to determine varying attitudes toward and current trends in exercise among patients with DM. This survey had 74 questions and was divided into five sections: Background Information, Diabetes, Insulin Pump, Exercise and Diet. The survey was designed to collect additional information about exercise and diet for CSII users. During the development of the questionnaire, advice was taken from health professionals and researchers, especially a statistician who has specialist knowledge of questionnaire design. One of the professorial diabetes researchers being consulted was herself a diabetic pump user and sports enthusiast and gave first-hand

account to enhance and improve the questionnaire. This survey was not trialled in focus groups or otherwise piloted because it had been scrutinised in this way

This survey was collected electronically (245 responses) by Survey Monkey® or by postal mail (14 responses).

2.2.4. Survey Distribution

Both surveys (2009 and 2012) were distributed by the research group at the Insulin Pump Clinic (Leicester Royal Infirmary), Balance magazine, the Insulin Pump and Diabetes Technology UK (INPUT) forum and through social network sites such as Twitter® and Facebook®. An advert was also placed in the Diabetes UK bulletin, which produces a quarterly magazine, Stability, which is distributed to all members of the charitable organisation Diabetes UK. Additionally, diabetes exhibitions and events in England, such as Diabetes UK and NHS exhibitions and conferences, were used to distribute these surveys, as well as an advert in the local newspaper (Leicester Mercury).In addition, an internal e-mail and advert were sent to all De Montfort University (DMU) staff and students.

2.2.5. Analysis of survey responses

All postal mail responses were uploaded into Survey Monkey® manually in order to analyse responses in the same way as electronic responses collected in Survey Monkey. All responses were then transferred to a Microsoft Office Excel worksheet specifically designed for analysis of the surveys. Data and open-ended questions were transferred to the IBM Statistical Package for Social Science (SPSS) using standard codification methods. All responses were coded using numbers, e.g. 0 corresponded to 'no response'.

2.3. Practical study -Ethics Approval

Ethics approval of the practical study design and procedures was granted by the DMU Ethics Committee (Appendix E). The following documents were produced and submitted to gain approval of the committee.

2.3.1. Standard Operating Procedure (SOP)

The SOP (Appendix F) provides details about preliminary and main experimental procedures used in the exercise trial.

2.3.2. Volunteer Information Sheet (VIS)

The VIS (Appendix G) describes the purpose of the study for the benefit of volunteers. It contains information about what happens to the volunteer if they decide to take part, an explanation of the experimental trial, expenses and payments, possible benefits of taking part in the trial, dealing with possible problems during the trial, confidentiality, withdrawal time and who has reviewed the study.

2.3.3. Risk Assessment Form

This document is an assessment of all possible risks associated with the experimental study.

2.3.4. Consent Form

This form (Appendix H) confirms that the volunteer has read the VIS and understands the experimental trial. Before being presented with the consent form, the volunteer was made aware that they were free to withdraw at any time. Once the volunteer was comfortable agreeing to all information related to the study, they are asked to sign the consent form indicating their desire to participate.

2.3.5. Volunteers Health Screen

This form (Appendix I) focuses on details about the volunteer's health and medical history. The health screen is used to enter patient details, medication details and healthcare measurements from the past two years before commencement of the practical study. All volunteers were asked to consult their GP before participating in this study to ensure it was suitable for them to participate. Each volunteer had been provided with an information sheet outlining the trial to give to their GP, and a GP approval letter was also sought, although it was optional.

2.3.6. Confidentiality

Confidentiality was recognised and considered at every stage of the research project. Volunteers were made aware that the research team would not disclose any personal information in any report or publication that could identify individual volunteers. All personal data were stored in a lockable filing cabinet accessible only by the research team.

2.3.7. Volunteer Record Sheet

Volunteers who returned a signed consent form and were asked to fill in a Volunteer Record Sheet (Appendix J) which includes volunteer name, contact details, type of diabetes or non-diabetes, GP contact details, history of acute diabetic complications including number of hypoglycemia and hyperglycemia episodes and other medical conditions. Each volunteer was given a unique identification number to preserve confidentiality and facilitate data collection. These were all stored in a lockable filing cabinet.

2.4. Volunteers

2.4.1. Recruitment of Volunteers for Exercise Program

Recruiting for the study was publicised through internal and external adverts. Poster adverts were placed on DMU campus notice boards seeking volunteers, and an internal e-mail was circulated. A display panel on the De Montfort University electronic student portal and an advert in the local newspaper (Leicester Mercury) were also used to attract volunteers. Forums such as Insulin Pump Forums, the Leicestershirediabetes.org.uk website, Input Forums, Royal Infirmary Hospital Leicester (insulin pump clinic) and diabetes events were also used to recruit CSII volunteers.

The target population for volunteers (ND, MDI and CSII) were males aged between 18–55 years who were, ideally, not physically active or engaged in any regular exercise programmes. These volunteers were considered suitable for observing and measuring metabolic and immunological parameters. However, it was not always possible to select volunteers who met the criterion of being physically inactive.

The total number of volunteers who expressed an interest in participating was 49, of whom 18 were excluded for not meeting inclusion criteria, such as being outside the age range, participating in regular exercise or other medical reasons. In addition, 12 participants were unable to attend as a result of the difficulty of making arrangements. The eventual sample was 19 participants.

All volunteers involved in the study were divided into 3 main groups. Group A (N=7) were ND, group B (N=7) were T1D on MDI and group C (N=5) were T1Dusing CSII.

2.4.2. Volunteer Criteria

Inclusion Criteria:

Patients who fulfilled the following criteria were selected to participate in this study

- Males who were aged at admission time between 18 and 55 years old
- Either healthy or diagnosed with T1D of more than 18 month's duration
- Used multiple dose injection or an insulin pump. Followed a stable insulin regimen for at least one month prior involving either use of an insulin pump or multiple daily injections consisting of insulin glargine and insulin lispro or insulin as part or any new or other type of insulin.
- For volunteers using CSII, their system set with the target of maintaining BG between 5 and 6 mmol/l for at least 90 min before the start of exercise.
- A1c ≤10.0% (were measured with the DCA®2000+ (Bayer Diagnostics, Tarrytown, NY).
- Refrain from any form of training or vigorous physical activity for 2 weeks before the beginning of the study.
- Body mass index (BMI) between the 5th and 95th percentile for age and gender.
- Small amounts of intravenous glucose will infused, if necessary.
- Normal thyroid function.
- No medication or anti-inflammatory agents, steroids, antioxidants or vitamin supplements before, during, or after study entry.
- No recent history of infectious, inflammatory or immune diseases.
- Signed information sheet and consent form and returned stamped GP form.

Exclusion criteria:

The following exclusion criteria were applied to patients who:

- had any of heart disease, liver disease, kidney disease, high blood pressure, rheumatic, heart murmur, HIV positive or aids, any allergy, hepatitis, asthma, tuberculosis (TB), stroke, epilepsy, any injury in head, hand or other injury and tumour (cancer) history.
- were currently using glucocorticoids or beta blockers.
- had used pseudoephedrine (used for the temporary relief of stuffy nose and sinus pain/pressure caused by infection such as the common cold, flu) within 48 hours.
- had experienced severe hypoglycemia within prior 2 weeks.
- had an active infection.
- anticipated a significant change in exercise regimen between admissions.
- had another medical condition or were using a medication that in the judgment of the investigator could affect completion of the exercise protocol.
- had blood pressure greater than 160/95 mm Hg.
- had participated in exercise 2 or more times weekly for 20 minutes or longer per session or any resistance training during the previous 6 months .
- showed restriction in physical activity because of disease.
- had recent blood loss or hemolytic anaemia.
- were terminally or mentally ill.
- had any recent surgery.

Inevitably, a study such as this one (and especially as pilot in nature) will be imperfect in terms of matching the characteristics of the participants. This will be dealt with in the discussion in chapters 4 and 5.

2.5. Study design and Description of Exercise Sessions

2.5.1. Exercise Physiology Lab

At the commencement of this study, the Exercise Physiology Lab (EPL) did not exist and had to be built from the very beginning. This included finding a suitable laboratory location with suitable and easy access and with temperature control. Moreover, EPL equipment such as the bicycle ergometer, resistance machine, ADInstruments analyser, ELISA plate reader, ELISA equipment, blood monitors (BP, BG, lipids and A1c) and other equipment. At this phase of the project necessary advice was taken from the Sport Exercise Department at Loughborough University. The specialists were briefed about the research project and they provided technical guidance.

2.5.2. Phlebotomy, First Aid, Defibrillation training and other courses

In order to standardise the blood collection procedure for the study, a phlebotomist carried out all venipuncture. The research team attended and passed a phlebotomy course at the Clinical Department University Hospital, Royal Infirmary Hospital in Leicester so that they were qualified and competent to undertake this task.

The Advanced First Aid at Work course provided by the East Midlands Ambulance Service NHS, Leicester and a defibrillation course provided by HeartSine Technologies were also attended and passed.

Other courses for doctoral training, compulsory (18) and optional (11) were attended. These courses were provided by School of Pharmacy, Graduate School and DMU library.

2.5.3. Initial EPL Visit

Before enrolling in the study, all interested volunteers attended a screening visit to discuss and complete questionnaires regarding their health, family history and current physical activity levels.

At this visit, all interested volunteers were asked about current treatment of their diabetes and other medications they were taking. When a volunteer was not prevented from taking part by the exclusion criteria based on information collected on this initial visit, an appointment was made for pre-exercise assessment.

2.5.4. Pre-exercise Visit and Familiarisation with exercise

All participants were asked to visit to familiarise themselves with the laboratory and exercise equipment, testing procedures and exercise protocol.

During this session, volunteers were instructed on how to perform the exercises using the correct technique for resistance routines using the multi gym machine and for aerobic exercise with recumbent ergometer bike. Volunteers were then given the opportunity to practise each exercise and to ask questions. Additionally volunteers were instructed to follow their normal diet, to try to eat very similarly the day before and during each study event and to not engage in any extra exercise activities different from their normal routines.

2.5.5. Determination of intensity levels for aerobic exercise

As stated earlier, each volunteer would be required to perform each exercise at 50- 60% of their estimated maximum heart rate (HR_{max}). After reviewing of literature related to the research, it was decided to use the Karvonen formula method to determine HR reserve (Goldberg, Elliot et al. 1988; Shnayderman and Katz-Leurer 2013) (see table 2.1) as follows

Karvonen formula: [(220-age)-(resting HR) × intensity] + resting HR

Table 2.1: Karvonen formula calculations methods

Age	**HR at rest**	**Intensity**	**Maximum HR**	**HR reserve**	**Target HR for experiment**
		0.5	220-Age	Maximum HR-HR at rest (BPM)	(HR reserve ×intensity (as fraction))+ HR at rest (BPM)
Example					
35	71	0.5	220-35=185	185-71=114	(114×0.5)+71=128

In order to record heart rate at rest the subjects were given heart rate monitors to take three readings when they are in complete rest (sitting or lying down) in the late evening. The justification for this will be discussed in chapter 4.

2.5.6. Determination of One Repetition Maximum (1RM) for RE Exercise

One repetition maximum (1RM) is the maximum amount of weight that can be lifted in a single repetition for resistance exercise. Since this might not be without hazard if attempted directly, methods have been devised so that it can be inferred from a lower weight that is limiting for the subject if attempted through a stated number of repetitions. There are several methods of calculation that converge for some conditions but in this case, the value was determined according to the Brzycki (Brzycki 2000) formula [1RM=100×repweight/ (102.78 – (2.78×reps))] (see table 2.2) where repweight

is the workload value of repetitions performed and reps is the number of repetitions performed. Prior to performing a 1RM, a warm-up was required.

Table 2.2: Example of using Brzycki formula to determine the 1RM for RE exercise. The weight for back, triceps and bicep target was converted from pounds to kilograms (lbs to kg).

RE exercise types	**Repetitions weight** (kg) (repweight)	**Number of repetitions** (reps)	**1RM** 100×repweight (kg)/(102.78-2.78×reps	**Intensity level** (50 or 60%) 1RM× 0.5 or 0.6
Squat	60	10	100×60/(102.78- (2.78×10)) = 80.02 kg	80.02×0.5= **40.0**1**kg**
Chest	40	12	57.62	**28.81kg**
Back	36.28	15	59.4	**29.70 kg**
Triceps	22.67	15	37.13	**18.56 kg**
Bicep	27.21	7	32.66	**16.33 kg**

Later, volunteers were asked to perform each RE exercise, during the weeks they participated in this study, at 50 to 60% of their 1RM, this was considered as a safe level for diabetic people who are non-trained to do such exercise and who might be at additional risk of covert cardiovascular disease. Though some authorities recommend to continual assessment of this level, simply for safety reasons in their pilot study, yet in this study this level was not continually assessed.

2.5.7. Warm-up

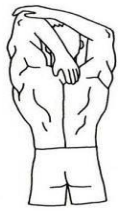
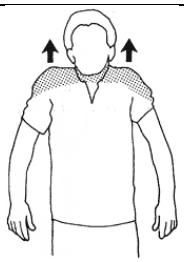
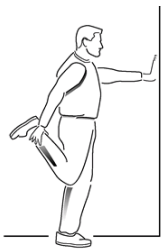
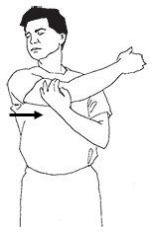
Warm-up is an essential part of exercise sessions and designed to prepare the body for exercise, increase body temperature and reduce the potential for post-exercise injury or pain, especially muscle stiffness.

In each session, all volunteers were asked to relax for 5–7 min before any measurements were taken. The objective of this step was to get baseline measurements of blood pressure, blood glucose and respiratory exchange ratio (RER).

After relaxing, volunteers stretched lower and upper muscle groups left and right for a total of 5–7 min, as shown in figure 2.1. Stretching included loosening front and back of upper arms, chest, shoulders, middle back, lower arms, wrists, hands, fingers, back, tops of shoulders, neck, triceps, waist, quadriceps, inner thighs and groin, as well as hip flexor stretches and chest stretches for the pectoral muscles.

After stretching, volunteers performed 5 min on a recumbent bike as part of the warm-up session.

Figure 2.1: Stretching left and right for lower and upper muscle groups

<table>
<tr>
<td> 
loosens upper arm and chest muscles</td>
<td> 
Stretching shoulder, middle back, arms, hands, fingers, wrist</td>
<td> 
Stretching the back and shoulder muscles</td>
<td> 
Stretching the chest, top of shoulder and lower arm muscles</td>
</tr>
<tr>
<td> 
Stretching the shoulders and neck</td>
<td> 
Stretches triceps, top of shoulders, waist</td>
<td> 
Stretching the quadriceps</td>
<td> 
Hip flexor stretches</td>
</tr>
<tr>
<td> 
Chest stretch for pectoral muscle</td>
<td> 
Stretches inner thigh, groin</td>
<td> 
Stretches side of shoulder and back of upper arm</td>
</tr>
</table>

Source:http://www.womensheart.org/content/exercise/stretching_exercise.asp

2.5.8. Resistance exercise (RE)

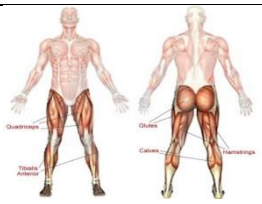
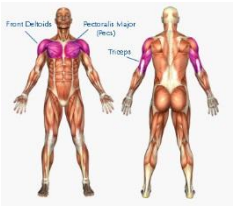
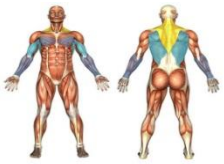
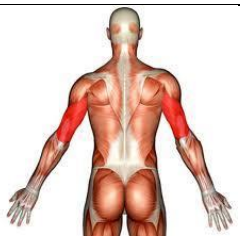
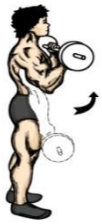
At 50 to 60 % of the volunteer's1RM as described above in section 2.5.6, they were asked to perform five different types of RE exercises on the multigym machine (Bodycraft, F610.F602.5046.5049B, US). This phase included 3 sets of 10 repetitions with 40 seconds of rest between each set. A Polar heart-rate chest sensor (Polar, FT1-TRA/BLK) was attached to volunteer's chest during the session. After each set, the HR was recorded and the rate of perceived exertion (RPE) on a6-20 scale (table 2.3). The

target muscles and how volunteers were performing the RE exercises in this session are explained in figure 2.2.

Table 2.3: Rated Perceived Exertion chart (RPE)

Rating of Perceived Exertion (RPE) Scale	
6	Very, very light
7	
8	Very light
9	
10	Fairly light
11 ↑	
12 ↕	Somewhat hard
13 ↕	
14 ↓	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	Maximum exertion

Figure 2.2: Five types of resistance (RE) exercise (3 sets, 10 repetitions each set)

Type of RE exercises	Description	Muscles targeted
Squat		
Incline bench press		
lat curl pull-down		
Triceps		
Biceps		

URLS source:http://www.muscle-fitness-tips.net/biceps-training.html

2.5.9. Cycling

Both the volunteer and the bike were attached to an ADInstruments Analysis System (ADInstruments) (PL3516/P ADInstruments, 16 channels, Australia). ADI produces an instant graphical representation of performance linked to work done and various metabolic parameters such as oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER), heart rate (HR), bike revolutions per minute (RPM) and breath flow and temperature.

Physiological signals were converted from analog to digital during aerobic exercise by a PowerLab data acquisition unit LabChart (LabChart v6.1.3, ADInstruments, springs, Colorado) modules provided acquisition and analysis features specific to specialised applications.

Volunteers were attached to the ADI via a breathing mask (breath-by-breath) system, Polar chest heart-rate monitor, pulse rate and blood pressure. A 3-lead electrocardiograph (ECG) system was linked by electrodes to the ADI during the experiment.

Volunteers were asked to cycle for 20 min at moderate intensity at 50 to 60% of predetermined maximum heart rate (HR_{max}) on a recumbent ergometer bike (Lode, Corival Recumbent, US), as described previously in section 2.5.5.

After 10 min, blood glucose was monitored and volunteers were asked to rate their RPE.

2.5.10. Warm-down

Finally, all volunteers were asked to stretch the lower and upper muscle groups for 5–7 min as described previously in section 2.5.7.

2.5.11. Long-term (chronic) exercise

This exercise programme involved 2 visits (48 hours apart) for a total of 150 min each week for a 6-week period. All exercise sessions included stretching, warm-up on the bike (5 min), five types of RE exercise (3 sets of 10 repetitions), as described previously in section 2.5.8 followed by 5 min rest. After that, volunteers exercised aerobically for 20 min at 50-60% of predetermined maximum heart rate (HR_{max}), as shown in section 2.5.5, then cooled down and rested before final observation of volunteers for hypoglycemia (BG level). In the event of low BG after final exercise, volunteer was asked to stay in the exercise lab and not to move out for final observation for 45min to 2 hours.

In order to control the lab temperature, the exercise room was fitted with cold and hot air conditioning system. The lab temperature was adjusted at 20°C by a weather station (Oregon Scientific, Hong Kong).

Blood samples were taken after the 1^{st}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} sessions to determine the effect of chronic exercise on immunologic and metabolic parameters.

2.5.12. Acute exercise

In this phase, volunteers were asked to do the same as in the chronic phase described above. However, in this session, three venous blood samples were collected: before exercise (baseline), after RE exercise and after aerobic exercise.

2.6. Visit measurements

On each visit, the Measurements form (Appendix K) was filled out. This form was designed to measure volunteer health during each exercise session.

2.6.1. Body fat percentage, weight, height, body mass index (BMI)

Body composition according to volunteer age was analysed by a bioelectrical technique using SECA scales. The scale sends a small, harmless electrical current through the volunteer's body when they step on the scale plate.

A triple SECA scale (SECA model 808) was used to determine volunteer weight (in kilograms) and BMI. Height (in centimetres) was measured using SECA (SECA Stadiometer model 217). BMI was calculated as weight (kg)/height $(m)^2$.

Body fat (BF%), weight, height and BMI were measured 3 times: at the beginning of the trial, after 6 visits and at the end of the trial (visit12).Table 2.4shows standard BMI percentage as used in this study.

Table 2.4: Standard BMI index

	Description	BMI
1	Underweight	<18.5%
2	Normal weight	=18.5-24.9%
3	Overweight	=25%-29.9%
4	Obesity	>30%

2.6.2. Lipid profile

High-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol (TC) and triglyceride level (TG) were measured three times: before the trial, after 6 visits and at the end of the trial. HDL, LDL, TC and TG were measured by a CardioChek analyser (PA Bundle, Health Check Systems, Germany). It is recognised that this desk top equipment is not able to produce the accuracy of the bench top instrument such as the BioRad, Beckman etc as used in hospitals etc. The kits come with calibration solutions, against which the precision can be measured over the time period for which the kit is in date. Thus the precision is not in doubt, but the exact level

or accuracy can be guaranteed, rather references only in terms of the calibration solution which is supplied within a defined range but not an actual value. To test this, we asked the supplier, BHR, to cross-test our instrument and strips against their demo instrument and strip batch and the differences were small. We were therefore confident that readings were close to actual and that significant changes could be picked up. A limitation of this study was that many of the volunteers would have been taking lipid moderating medication as standard diabetes protective therapy and therefore large changes were not expected.

2.6.3. Blood glucose (BG) and hemoglobin A1c (A1c)

Blood glucose was measured specifically 4 times for each volunteer: at rest before exercise, after RE exercise, after 10 min of aerobic exercise and at the end of each trial. BG was measured using a blood glucose kit (Contour, BAYER, Switzerland). Fast-acting sugar sources were given if required.

A1c level was measured at the beginning of the trial and after the last visit for ND and diabetic volunteers. A1c was measured by A1c Now® and Quo-Test A1c reagent kit which we had already tested for reasonable precision and accuracy, although since the study it has been replaced. Sample results are available within five minutes and are reported in IFCC and DCCT.

2.6.4. Venous blood sampling

Volunteers were asked to sit on a comfortable chair in order to collect blood samples. An alcohol swab and a disposable latex-free tourniquet (Fisher Thermo Scientific- Cat no. 22-040-225) were used to avoid any infection or contamination. Venous blood samples from the median basilica or cephalic veins were collected using S-Monovette blood collection system needle and 10 ml tube containing EDTA anticoagulant

(ethylenediaminetetraacetic acid) (Monovette, SARSTEDT) syringe system. Twenty-four samples, one for each volunteer, were collected during successive visits and labelled with volunteers' reference numbers and the date.

In order to collect blood plasma, labelled samples were centrifuged at 3000 rpm for 15 min at 4°C using a Fisher Thermo Scientific centrifuge (23RSorvall Legend, Thermo). Plasma was then separated into labelled 1.5 ml Eppendorf tubes (Eppendorf, Germany) and stored in a -80°C freezer until analysis.

2.6.5. Assessment of caloric intake

It was important that all volunteers weighed and recorded everything that they ate and drank for the day prior to each experimental exercise session, although as explained above this approach is imperfect. Food inventory sheets were provided to volunteers (Appendix L). Volunteers were asked to try to keep the same food regimen for the 24 hours before each exercise session and to refrain from alcohol and smoking 24 hours before an exercise session if possible.

2.7. Calibrating, filtering and sterilising the ADInstruments analyser

At the beginning of each trial, the ADInstruments PowerLab was calibrated with 4% CO_2 and 16% oxygen/nitrogen (BOC cat no 226927-v-c). To prevent moisture damage to the sensors and to remove any possible damaging particulates, an In-line Filter (0.45µ hydrophobic membrane) was changed every 2–3 weeks.

A face mask, nose clips and drying tube were cleaned and sterilised before each volunteer commenced exercise.

2.8 Statistical analyses

2.8.1 Survey

All data were analysed using Statistical Package for the Social Sciences (SPSS) (version 20 “IBM”, Chicago, IL, US).Microsoft Excel and SPSS were used to for statistical analysis. To examine sample characteristics, frequencies were calculated for variables. Descriptive statistics were calculated for all patient demographics. Variables were described using main values as well as total numbers and relative frequencies. Responses and variables were compared using chi-square test. All data were filtered and missing data were excluded from the analyses and only valid percentages were considered. Cross tabulations were used to explore the relationship between the sections of the questionnaire. Data distributions were checked for normality, for normally distributed data paired t-test were used while for non-normal distribute data Wilcoxon test was used to determine the significant. All test of significance were conducted using $\alpha = 0.05$ i.e. level of significance.

2.8.2 Practical study

All data are presented as mean ± standard error of the mean (SEM) unless otherwise stated. T-tests were used in practical study to calculate the P value significance were conducted to study various relationships among the data and to conduct meaningful analysis.

The data for the acute test were entered into Excel to detect differences in the same exercise session (pre, during and post-exercise) and between (acute and chronic) exercise sessions. Analysis was carried out using the IBM Statistical Package for Social

Science (SPSS), Version 19 software for Windows to determine the significance of difference between the mean values.

A p value less than 0.05 was considered statistically significant. When a significant difference was found, comparison was carried out using the T-test.

2.9 Cytokine detection

One of the main objectives of this study was to determine the effect of acute and chronic exercise on selected inflammatory cytokines on T1D either MDI or CSII users and to compare these with ND. Cytokines selected to be examination were IFN-γ, TNFα, IL-6 and IL-1β. To determine the level of these cytokines, the Sandwich Enzyme-linked Immunosorbent Assay (ELISA) test was used.

2.9.1 Enzyme-linked immunosorbent assay (ELISA)

The ELISA assay was developed using commercial kits (DuoSet ELISA Development Kit, R&D systems, US).

2.9.1.1Capture antibody

Capture antibody from R&D Systems were diluted to a working concentration according to the R&D Catalogue, as shown in table 2.5.

Table 2.5: Catalog number and dilutions for capture antibody to working concentration, PBS (phosphate buffered saline). Dilution e.g. (360/2) = 5000/180=27.77 µL

Capture Antibody	Mouse anti-human	Dilute to working concentration	Working concentration
IL-6 (part 840113, 1 via)	360 µg/mL	2.0 µg/mL	27.77 µL of IL-6 into 5 ml of PBS
IL-1β (part 840168, 1 via)	720 µg/mL	4.0 µg/mL	27.77 µL of IL-1β into 5ml of PBS
TNF-α (part 840119, 1 via)	720 µg/mL	4.0 µg/mL	27.77 µL of TNF-α into 5 ml of PBS
IFN-γ (part 840101, 1 via)	720 µg/mL	4.0 µg/mL	27.77 µL of IFN-γ into 5ml of PBS

2.9.1.2 Detection antibody

Detection antibody was diluted to a working concentration according to R&D Systems Catalogue, as shown in table 2.6.

Table 2.6: Catalog numbers and dilutions of detection antibody, RD (Reagent Diluent). Dilution e.g. (9 µg=9000ng); for IL-6 (9000/50) = 5000/180=27.77 µL.

Detection Antibody	Biotinylated goat anti-human	Dilute to working concentration	Working concentration
IL-6 (part 840114, 1 via)	9 µg/mL	50 ng/mL	27.77 µL of IL-6 into 5ml of RD
IL-1β (part 840169, 1 via)	36 µg/mL	200 ng/mL	27.77 µL of IL-1β into 5ml of RD
TNF-α (part 840120, 1 via)	45 µg/mL	250 ng/mL	27.77µL of TNF-α into5ml of RD
IFN-γ (part 840102, 1 via)	9 µg/mL	50 ng/mL	27.77 µL of IFN-γ into 5ml of RD

2.9.1.3 Standard Antibody

A standard curve was conducted with each assay for quantification, as shown in table 2.7.

Table 2.7: Dilutions and catalogue numbers of standard antibody.

Standard	Recombinant human	Dilute to working concentration	Working concentration
IL-6 (part 840115, 1 via)	120 ng/mL	600 pg/mL	5 µL of stand to 995 µL of distilled water
IL-1β (part 840170, 1 via)	75 ng/mL	250 pg/mL	3.33 µL of stand to 996.66 µL of distilled water
TNF-α (part 840121, 1 via)	340 ng/mL	1000 pg/mL	3 µL of stand to 997 µL RD
IFN-γ (part 840103, 1 via)	55 ng/mL	1000 pg/mL	18.18 µL of stand to 981.81 µL of RD

2.9.1.4 Plate preparation for ELISA

2.9.1.4.1 Preparation

ELISA preparation involved several steps:

1. Capture antibody was diluted to the working concentration as in table 2.5in phosphate-buffered saline (PBS) without carrier protein. Immediately, a 96-well microplate was coated with 100 µL per well of the diluted capture antibody. The plate was sealed and incubated overnight at room temperature.

2. Each well was aspirated and washed with wash buffer, repeating the process twice, for a total of 3washes. Washing was by filling each well with wash buffer (400 µL) using a squirt bottle, manifold dispenser or auto washer. Liquid was completely removed at each step. After the last wash, any remaining wash buffer was removed by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Plates were blocked by adding 300 µL of reagent diluent to each well. The plates were then incubated at room temperature for a minimum of 1 hour.

4. The plate was washed as in step 2. The plates were now ready for sample addition.

2.9.1.4.2 Assay procedure

1. 100 µL of sample or standards were added per well. Then the plates were covered with an adhesive strip and incubated for 2 hours at room temperature.

2. The plate was washed as in step 2.

3. 100 µL of the detection antibody was added, diluted in reagent diluent, to each well. Then the plates were covered with an adhesive strip and incubate 2 hours at room temperature

4. The plate was washed as in step 2.

5. 100 µL of the working dilution of Streptavidin-HRP were added to each well. Then the plates were covered with an adhesive strip and incubate 20 minutes at room temperature.

6. Plate was washed as in step 2.

7. 100 µL of substrate solution were added to each well then incubated for 20 min at room temperature avoiding placing the plate in direct light.
8. 50 µL of stop solution were added to each well then gently tapped the plate to ensure thorough mixing.
9. Immediately the optical densities of each well were determined, using a microplate reader set to 450 nm (no correction subtraction needed for high concentrations in this case). This determination was determined by microplate absorbance readers with Manta PC analysis software (Labtech LT-4000)

2.10 Materials

❖ ELISA solutions

- **Phosphate-buffered saline (PBS)**

137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4. Filtered (0.2 µm pore size).

- **Wash buffer**

0.05% Tween 20 in PBS, pH7.4. (R&D Systems cat. WA126)

- **Block buffer**

1% BSA, 50% Sucrose in PBS with 0.05% NaN3.

- **Reagent diluent**

0.1% BSA, in PBS, pH 7.2–7.4, 0.2 µm filtered. (R&D Systems cat. DY995)

- **Substrate solution**

1:1 mixture of colour reagent A (H_2O_2) and colour reagent B (Tetramethylbenzidine). (R&D Systems cat. DY999)

- **Stop solution**

2 NH_2SO_4.(R&D Systems cat. DY994)

- **Streptavidin-HRP (Part 890803, 1 vial)**

1.0 ml of Streptavidin conjugated to horseradish-peroxidase, stored at 2–8°C for up to 6 months. Diluted to the specified working concentration by using reagent diluent.

Chapter 3: Insulin Pump Survey

De Montfort University Insulin Pump Users' Diet and Exercise Survey 2012

3.1. Introduction

Diabetes mellitus (DM) is a metabolic disorder and recognised since 2000 B.C. Type1 diabetes (T1D) is known as a permanent lack of insulin production from the pancreas due to the destruction of the β cells of the Islets of Langerhans. This results in raising the blood glucose concentration in the blood, for the reason that with no insulin, the cells will not exchange carbohydrates such as sugars or other foods into energy usable by the body.

T1D is an autoimmune condition and where insulin producing beta cells in pancreas are destroyed and body lacks necessary insulin to maintain blood glucose level and maintaining the insulin level in the body becomes a difficult challenge. Diabetes Control and Complications Trial (DCCT 1993) and the UK Prospective Diabetes Study (UKPDS) were established the significance of intensive management in achieving tight metabolic control and improving long-term health in patients with diabetes (Turner, Holman et al. 1998). Intensive insulin therapy can be implemented using either continuous subcutaneous insulin infusion (CSII) with insulin pump therapy or multiple daily insulin injection (MDI) regimens. Insulin pumps deliver fast-acting insulin continually at a rate that is pre-set according to patients' needs throughout the day and night. After a meal patient can press a button on the pump to get an additional dose of the insulin. This capability of delivering insulin as and when required makes the pump a replica of pancreas. Recent advancements in sensors and pumps made it possible to automate the management of insulin within the given parameters. Further advances of insulin pumps are to make them wearable and programmable.

Intensive insulin therapy helps to counter the rise in blood glucose and as a result can reduce the risk of developing advanced complications, such as cardiovascular disease, chronic renal failure, retinal damage, nerve damage, and microvascular damage. Though exercise is an important contributor to healthy outcome in intensive (or any) diabetes yet managing T1D requires balancing diet, physical activity and medication to keep blood glucose as close to normal as possible.

Physical activities have acute and chronic effects on BG, lipid and immunological parameters (cytokines). Regular aerobic exercises reduces visceral fat mass and body weight without decreasing lean body mass, improve insulin sensitivity, glucose and blood pressure control, lipid profile and reduces the cardiovascular risk. For these reasons, regular physical activity must be considered an essential component of the treatment of DM. Nonetheless, during physical activity insulin demands of body change and T1D may need careful monitoring of their BG levels during and after the exercise.

Although researchers have conflicting views on effects of exercise on blood glucose control in people with T1D yet, given its other numerous benefits, exercise is still an important part of living well with T1D. Furthermore, a carefully planned exercise regime can help people with T1D better use insulin and reduce their risk of heart disease.

On the other hand in T1D the lack of the physiological inhibition of insulin secretion during exercise results in a potential risk of hypoglycemia. While exercise induced activation of counterregulatory hormones might also trigger an acute hyperglycaemic metabolic derangement in severe insulin-deficient subjects.

In summary, T1D patient must discuss their physical exercise plans with the health care professional to understand the consequences of physical activity on their blood glucose and the appropriate modifications of diet and insulin therapy.

The aim of this survey was to assess glycemic control on T1D using the insulin pump, to assess the satisfaction with this therapy option and to gauge the frequency of treatment-related complications among a large group of people with diabetes around the world under conditions of routine daily life including diet and exercise.

3.2. Study Objectives

T1D is a condition without any cure in sight yet there are efforts made to develop strategies to help T1D to better manage their life. The main aim of this research is to study the effectiveness of automated device which pumps insulin into the body as and when required to keep a balance in the body and avoids the risks of hyperglycemia and hypoglycemia. As mentioned above a balanced diet and a suitable regular exercise regime also play an important role in managing the condition. Hence this study further aims to study the effects of diet and exercise on T1D especially who relies on CSII for insulin.

This study was designed to investigate the effect of exercise on T1D patients using insulin pump therapy, to cover attitudes and opinions about exercise, to support and to combine with practical study in chapter 4 and 5 and to gain more information about insulin pump and it complications. The ethical approval for the study was obtained from the Ethical Review Committee, Faculty of Health and Life Science, De Montfort University (DMU), Leicester.

The respondents were volunteered to take part in this research and were free to withdraw from the survey, if they wished to do so. Although no personal information was collected in this survey every effort was made to keep the collected information strictly confidential. Over 245 insulin pump users responded to the Insulin Pump Users' Diet and Exercise Survey from January 2012 till July 2013; and the method to deliver these responses were through SurveyMonkey™ and free post service.

3.3. Materials and Methods

An international survey of diabetic patients using insulin pump therapy was carried out in 2012 and 2013. This survey was distributed as explained in section 2.2.4 to the insulin pump volunteers. In total, there were 74 structured questions with five sections.

3.3.1. Survey Design, Layout and Measures

In order to achieve the research goals a survey was conducted using a structured questionnaire. The questionnaire was divided into following five sections:

1. **Identity:** This section helped to understand the demographics of the respondents and collect background information such age, gender, weight and height, level of education, time of first diagnosed with DM, ethnic group, pump resources and country of residence.

2. **Diabetes history:** This section was aimed to collect information about the diabetes related medical history. This section asked questions about type of diabetes, diagnosis details, family history of DM, A1c levels (diagnosed time, before using insulin pump, after using insulin pump), and other health check (blood pressure, cholesterol, LDL, HDL, eye test, bare feet, kidney disease or dialysis, heart attack, angina, stroke).

3. **Medication** section asked questions about insulin pump therapy including type of insulin pump, number of years with insulin pump, type of insulin infused into insulin pump, basal rates throughout an exercise and non-exercise day, bolus for meals on an exercise and non-exercise day, total amount of insulin on an exercise and non-exercise day, pre-exercise bolus, BG before and after exercise, hypoglycemia after exercise and level of BG pre-exercises.

4. **Exercise section** focused on the exercise and lifestyle. The questions were aimed to study the effects of exercise on BG, usage of insulin pump (on or off) during exercise, level of intensity, type of exercise, exercise amount (times, sessions and days), hypoglycemia and hyperglycemia events (decrease or increase), symptoms with exercise and ketoacidosis events.

5. **Diet section** collected information about number of calories and carbohydrates, diabetic food and drink, dietician consultative, medically advised dietary programme, diet approach, eating habits, smoking, drink alcohol and symptoms after exercise.

At the end of this survey the respondents were asked for any comments they would like to add.

All responses were administered and completed by the subject themselves.

3.3.2. Target audience

As mentioned above the objective of this study was to explore the impact of exercise on T1D sufferers who relied on insulin pump to administer required dosage of insulin. Hence, the target audience of this study were T1D patients who were using insulin pump therapy.

3.3.3. Study population and sampling

Insulin users were invited to represent the wider population. The distribution and uptake cannot be claimed as random because the respondents selected themselves and thus had certain characteristics such as reading ability, adequate eyesight or other means to complete the survey, English language, motivation, understanding, access to selection points etc. A total of 245 T1D volunteers who used CSII, male and female, aged between 1-80 years old from different ethnic groups and different levels of education took part in the survey.

3.3.4. Survey Distribution and response collection

In order to collect sufficient responses; this survey was distributed by different channels such as Insulin Pump Clinic (Leicester Royal Infirmary Hospital), the Insulin Pump and Diabetes Technology UK (INPUT) forums and through social network sites such as Twitter® and Facebook®. Furthermore, an advert was also placed in the Diabetes UK bulletin, which produces a quarterly magazine, Balance, which is distributed to all members of the charitable organisation Diabetes UK. Additionally, diabetes exhibitions and events in England, such as Diabetes UK and NHS exhibitions and conferences, were used to distribute these surveys, as well as an advert in the local newspaper (Leicester Mercury). An advert with this survey link was placed on the main home page of DMU. In addition, an internal e-mail and advert were sent to all DMU staff and students.

3.3.5. Data collection

Survey responses were mainly collected by Survey Monkey®. However, all postal mail responses were uploaded into Survey Monkey® manually in order to analyse responses in the same way as electronic responses collected in Survey Monkey. Information

collected was gleaned to conduct a statistical analysis. All responses were coded using appropriate numeric codes, e.g. number 1corresponded to ‘no response’.

3.3.6. Statistical analyses (Analysis of responses)

All data were analysed using Statistical Package for the Social Sciences (SPSS) (version 20 “IBM”, Chicago, IL, US).Microsoft Excel and SPSS were used to for statistical analysis. To examine sample characteristics, frequencies were calculated for variables. Descriptive statistics were calculated for all patient demographics. Variables were described using main values as well as total numbers and relative frequencies. Responses and variables were compared using chi-square test. All data were filtered and missing data were excluded from the analyses and only valid percentages were considered. Cross tabulations were used to explore the relationship between the sections of the questionnaire. Data distributions were checked for normality, for normally distributed data paired t-test were used while for non-normal distribute data Wilcoxon test was used to determine the significant. All test of significance were conducted using $\alpha = 0.05$ i.e. level of significance.

3.4. Results

Following sections describe and discuss the data collected from the survey and analysed using SPSS.

3.4.1. Background information Section

This section discusses the demographics of the respondents such as country of origin; ethnic group, gender and T1D diagnosis (see table 3.1).

Almost 89% of the respondents live in UK or US and the majority of respondents (i.e. 83.96%) are white. Approximately 52% of the respondents were female while 48%

were male. The data showed that 54.67% of the respondents did not have a family history of T1D while 58% of respondents were living with T1D from 1 to 20 years, which means they have clear idea of the T1D related health issues and complications.

Table 3.1: Survey background information regarding participants in the exercise survey

Background information	Responses %
Countries:	
UK	44.64
US	44.21
Canada	6.01
Australia	2.15
Others (France, Italy, New Zealand and Denmark)	2.99
Ethnic groups:	
White British	41.98
Other white background	41.98
White Irish	5.35
Others ethnic (Asian, Asian British, Indian, Black or Black British, African)	10.69
Gender:	
Female	52.05
Male	47.95
Diagnosis time (years) with T1D:	
1-10	30.33
11-20	27.46
21-30	18.03
31-40	9.84
41-50	10.66
51-60	3.28
61-80	0.41

The data show that most of the respondents (i.e. 42.86%) had normal weight as describes by body mass index (BMI) (see figure 3.1) while 4.59% of respondents were under weight, 27.04% were overweight, with20.92% were classed as obese.

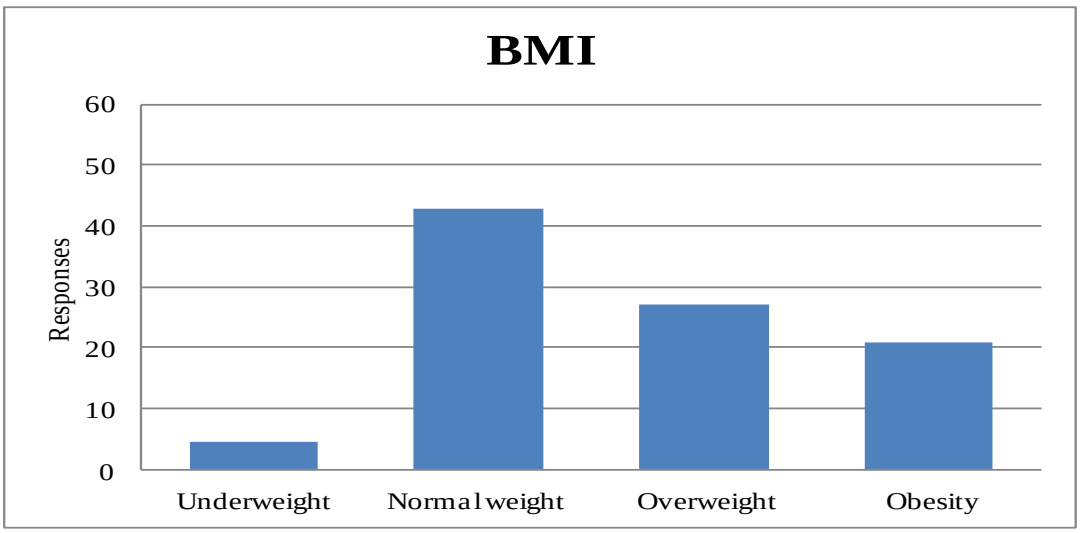


Figure 3.1: Frequencies of BMI values in the survey. Underweight = <18.5 %, Normal weight = 18.5–24.9 %, Overweight = 25–29.9 %, Obesity = BMI of 30 % or greater.

The lipid level guidelines as set out by NICE (2010) (Table 3.2 A and B) were used to analyse the lipid levels in the respondents. Figure 3.2 shows that 75% respondents reported "desirable level of total cholesterol" while 52% respondents reported "desirable level of LDL and HDL". Around 10% of respondents reported "Tc and HDL in the undesirable region" while less than 5% respondents had an "undesirable level of LDL".

Table 3.2A: Lipids level (absolute values mmol/l) according to (NICE, 2010)

	Normal	**Abnormal**	**Highly abnormal**
Total Cholesterol (Tc)	Less than 4.0	4.1-6.0	Greater than 6
LDL-Cholesterol	Less than2.0	2.1-4.0	4.1-4.8
HDL-Cholesterol	>1.4	1.0-1.4	Less than 1.0

Table 3.2B: Lipids ratio level (mmol/l)

Total Cholesterol to HDL ratio	
Risk	**Men**
Very low (1/2 average)	<3.4
Low risk	4.0
Average risk	5.0
Moderate risk (2x average)	9.5
High risk (3x risk)	>23
LDL to HDL ratio	
Very low (1/2 average)	1
Average risk	3.6
Moderate risk (2x average)	6.3
High risk (3x risk)	8
Very low (1/2 average)	1

It is worth noting that for some authorities such as the Mayo clinic these figures vary a little.

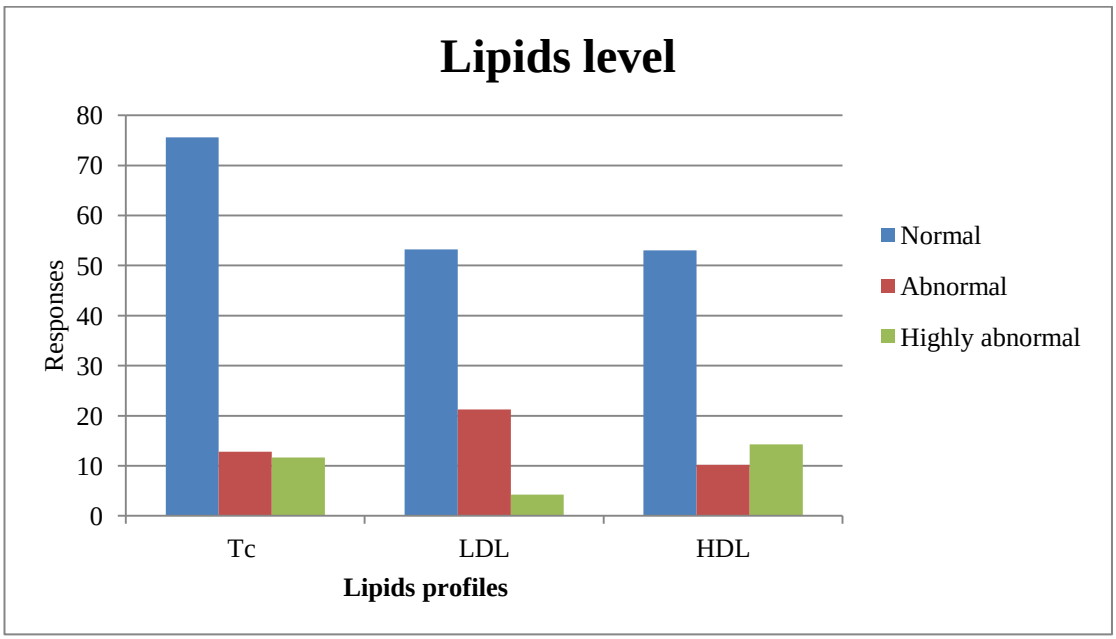


Figure 3.2: Survey respondents' lipids levels

In this survey 90.43% respondents who are CSII users were highly educated while only 9.57% had not had any formal education. This fact was further supported by the fact that approximately 63% of CSII users were professionals or skilled workers, while

11.98% were retired, 5.21% student, 4.5% were semi-skilled or manual labourer and 5% were unemployed.

3.4.2. Diabetes section

This survey was focused on T1D yet some respondents (only 6.44%) turned out to be diagnosed with T2D. The vast majority of the respondents (i.e. 89.60%) were diagnosed by T1D, while 3.95%were diagnosed with LADA (1.7%) and type 1.5 (2.25%). Only the T1D people were included in the results.

50% of T1D respondents, who were using CSII therapy, reported to be diagnosed by General Practitioners or medical centres, while 21% were diagnosed by hospital clinics, 10.30% were diagnosed in A&E (accident and emergency) and 2.48% during routine medical check-ups. In summary the data shows that almost 85% of the T1D cases were diagnosed by formal medical diagnostic techniques while 15% reported to be diagnosed by friend and family who picked some signs of the disease and led the patient to the medical professional.

Table 3.3 shows 81.70% respondents with T1D reported that at the time of diagnosis their A1c was above 10 (DCCT-%) (96% reported A1c levels higher than 7 at the time of diagnosis). However, 81% of such respondents reported that before using CSII therapy their A1c was above 7, which is considered to be high according to health guidelines. It is worth noting that only 39% of these respondents reported A1c level higher that 7 after using CSII. The further analysis of the data show that approximately 52% of such respondents reported A1c level higher than 8 before starting CSII therapy as compared to 12% after using CSII. This shows that CSII is a very effective therapy to control and manage the A1c level in T1D sufferers (see figure 3.3).

Table 3.3: A1c levels reported by respondents

	A1c levels (responses %)						
	<5	**5.1 - 6**	**6.1 - 7**	**7.1 - 8**	**8.1 - 9**	**9.1 - 10**	**>10**
A1c at time of diagnosis	0.9%*	0.5%*	2.7%	4.6%	2.3%	7.3%	81.70%
A1c before using CSII	0.9%	6%	12%	29.2%	23.6%	12.5%	15.8%
A1c after using CSII	1.35%	18.83%	36.32%	26.91%	5.38%	2.24%	4.04%

* It seems that 4 out of 245 respondents failed to understand the question or clicked in the wrong box. This makes 1.4% of the total sample, which would not statistically significantly to alter the results.

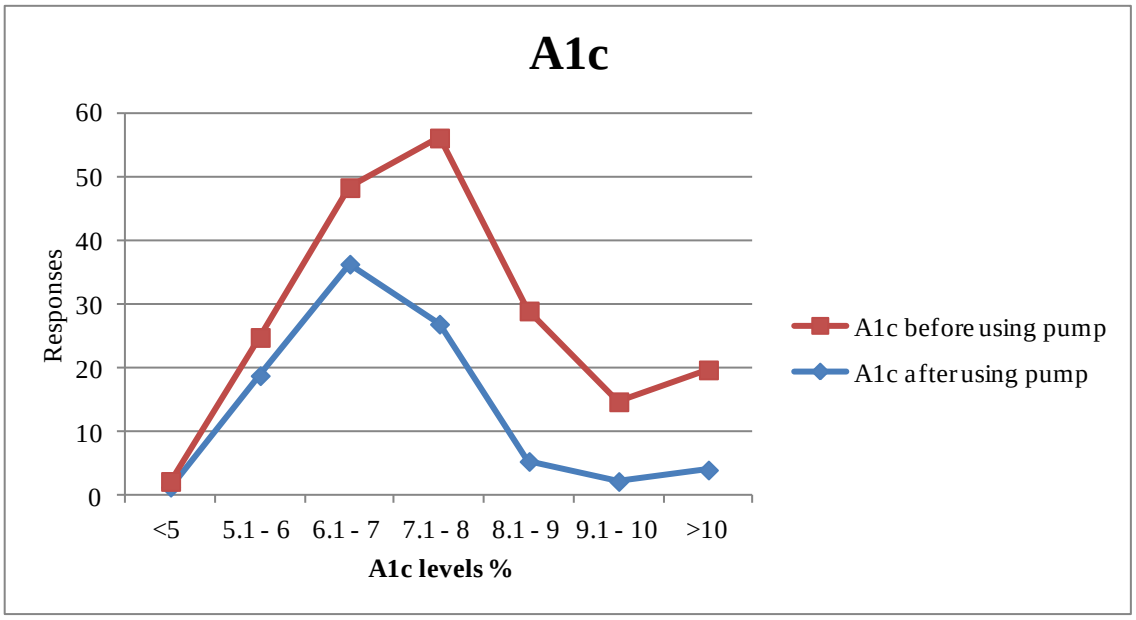


Figure 3.3: A1c level before and after insulin pump therapy as reported by the respondents.

As mentioned above the majority of CSII users were highly educated and professionals so it is not surprising that they checked their BG level before every exercise session. 59% of T1D sufferers who were using CSII reported that they start exercise when their BG level was ≥7mmol/L and 88% reported that BG level dropped significantly after exercise. 77.30% reported that they took carbohydrates if their BG level was ≤4mmol/L

before exercise and 89% reported that they took carbohydrates in the event of hypoglycemia after exercise.

3.4.3. Insulin Pump Section

Almost 50% of the insulin pump user in this survey had been on CSII therapy for more than 3 years while 26% for less than 1 year. Respondents gave varying reasons for selecting CSII therapy. However, 80% of the insulin pump and the infusion sets were provided by NHS or health insurance providers.

When asked about the type of pump being used the respondents mentioned five different makes of pumps (Medtronic Paradigm, Animas, Accu-Chek, Omnipod and Roche). Medtronic Paradigm pump was among the most popular (55.21%) then Animas (22.51%), Accu-Chek Spirit and Roche (14.57%), Omnipod (5.9%) and Smiths Medical–Cozmo (1.81%) as in figure 3.4.

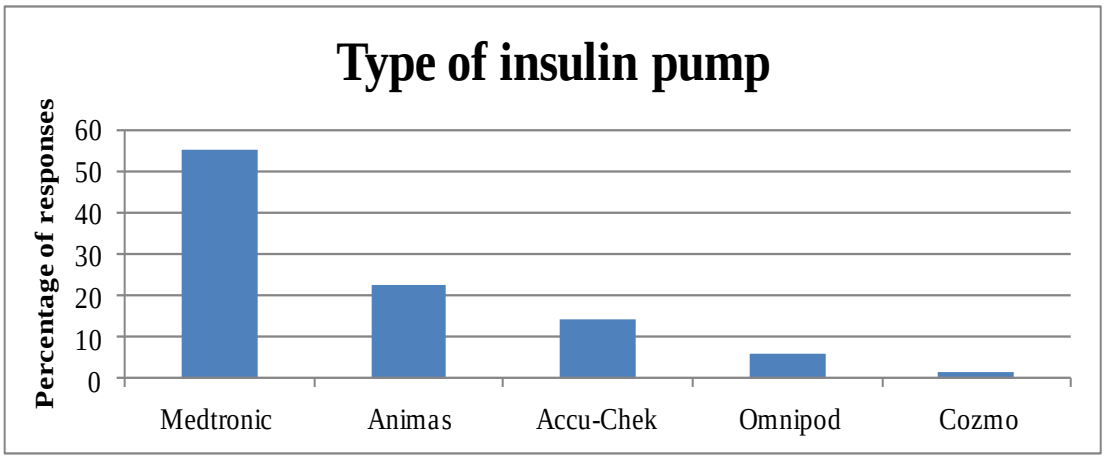


Figure 3.4: Insulin pumps that had been selected by respondents or their providers

The respondents used six different types of insulin. Novolog® or Novorapid® (Aspart) which are considered to be fast acting insulin is most commonly used i.e. by 56.28%,

while 31.43% used Humalog® (Lispro),9% used Apidra,2.29% used Actrapid® (regular or soluble insulin) and 1% animal insulin (figure 3.5).

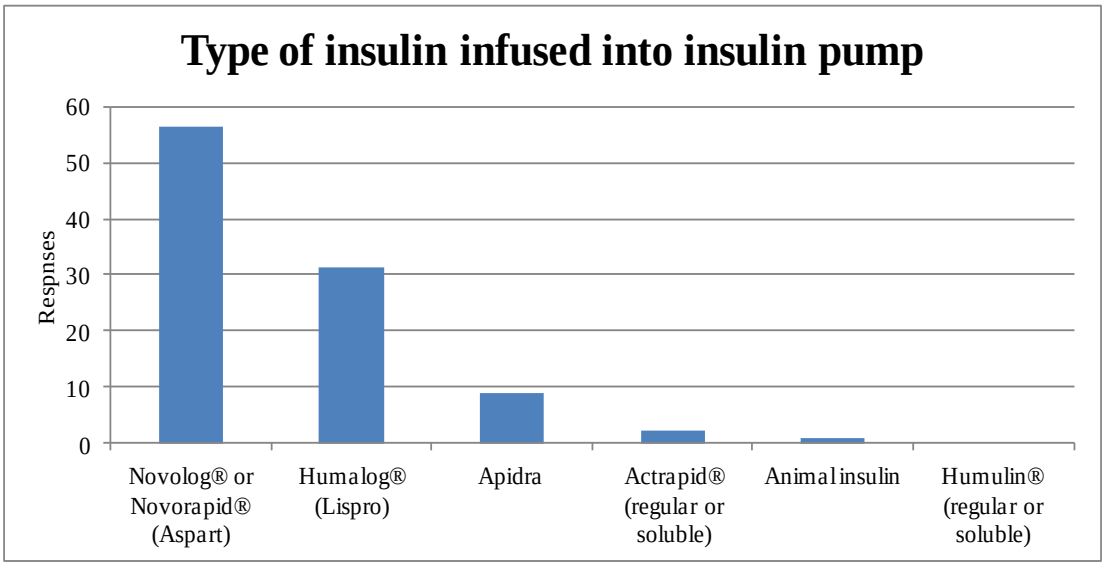


Figure 3.5: Types of insulin stated by respondents to be infused into their insulin pumps being used

It seems that patients were highly satisfied with CSII treatment when exercising because 86.71% did not face any problems during and after their exercise sessions while using pumps. Furthermore, 77.65% said that they did not change their exercise routine after switching from MDI to CSII therapy.

Almost 83% of the respondents, who were using CSII, reported that they switched on their pumps during the exercise sessions while remaining 17% preferred to keep it switched off. 10% of the respondents who switched off their pumps did so on a medical advice while 50% did it due to fear of hypoglycemia and 20% reported that pump interfered with their exercise regime (20% of such respondents did not give any reason).

41.21% of the respondents, T1D sufferers using CSII, reported that on an exercise day their basal rate was 0.5-1 units/hour daily, while 48.95% reported the same basal rate on

a non-exercise rate. In 91.44% of cases, the bolus was not used before exercise and a standard bolus for a meal was used on an exercise and non-exercise day (55.68% and 63.16%, respectively).

Between 21 and 40 units of insulin (total daily) were used on non-exercise day (48.68%) and on exercise day (47.8%).

The data analysis show that in case of elevated BG level (i.e. over 10mmol/L) 95.21% of respondents would increase the insulin bolus, while 4.19% would not intervene and would wait for the BG level to come down and 0.60% relied on water or any drink with low calories.

A small number of serious adverse events such as hypoglycemia or elevated BG level at fasting were recorded. 32 % of participants reported to have more than 3 episodes of hypoglycemia per week while 37% reported 2-3 such episodes per week. Almost 41% reported that their BG level at fasting was above 10mmol/L more than twice a week. It is worth noting that 63.25% very rarely suffered from hypoglycemia during an exercise session. However, 52.41% reported to have more than usual episodes of hypoglycemia after exercise while 45.18% witnessed no effect on the occurrence of hypoglycemia after exercise.

3.4.4. Exercise Section

67.35% of respondents, who were using CSII therapy, considered physical activities as an important aspect of their life while 25% were indifferent and 7.7% gave exercise no importance. Furthermore, 74.17% of respondents indicated that they always lived actively. Almost 35% of the participants considered reducing A1c as the main motivation behind participating in exercise, while 18% considered weight reduction and

16% considered staying motivated as the main reasons (30% gave other reasons e.g. burning fat, healthy life style etc.). It is worth noting that only 5.52% of CSII users frequently missed an exercise session due to feeling unwell (29% sometimes and 62% rarely).

A majority of pump users i.e. 61.59% were not a regular member of a sport centre or physical activity group and only 13.50% were doing exercise with a sport team (e.g. football, netball, aerobics, dance, etc.). 46.63% preferred to exercise in open areas and 20% indoor i.e. at home or office, while only 18% liked to go to gym. When asked about the intensity level of exercise 75% were doing moderate exercise while remaining 25% were on the extreme ends of the spectrum (i.e. 10% high and 15% low) figure 3.6.

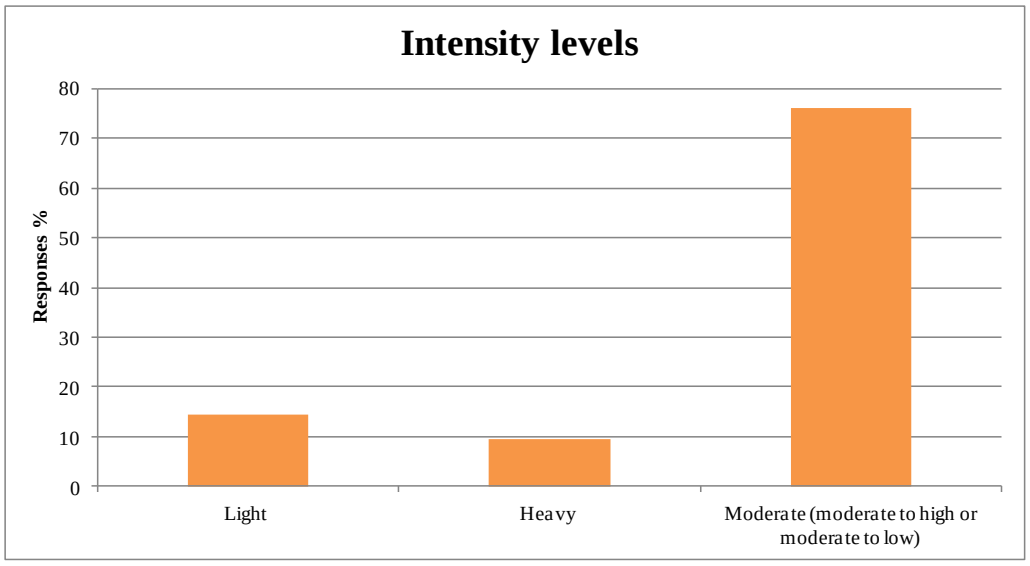


Figure 3.6: Intensity level during exercise sessions as reported by respondents

40% of the respondents, who were using CSII, were doing a combination of aerobic and resistance exercise regime (i.e. walking, cycling, weight lifting, swimming, running and team sport), while 55% were relying on aerobic only (see figure 3.7 A and B).

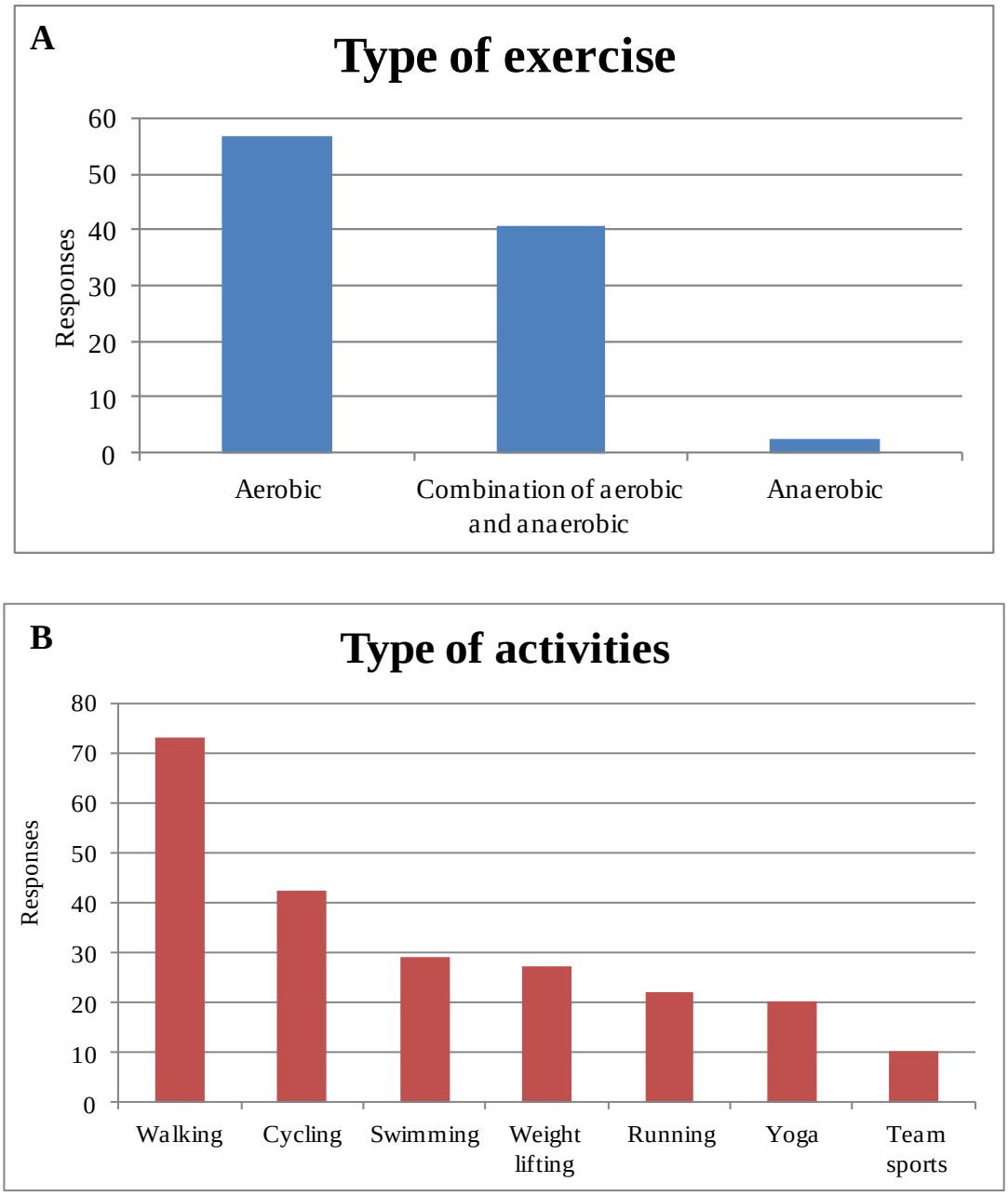


Figure 3.7: Types of physical activity reported by respondents. **A** are the main types of exercise and **B** is the subtype of activities.

It was observed that the majority of the CSII users were following a regular exercise regime with 17% doing exercise more than 5 times every week, almost 45% doing exercise 3-5 times every week and almost 38% 1-2 times per-week. Respondents said

they exercised once a day (80.25%), twice a day (15.92%), three times or more daily (3.83%). A typical exercise session comprised less than 30 minutes (13.58%), more than 30 min to 1 hour (42.59%), from 1 to 2 hours (33.33%) and more than 2 hours (10.49%). Exercising time was mainly stated in the morning (58.18%), afternoon (48.48%), evening (49.09%), after meal (32.73%) and before meal (22.42%).

Despite the fact that exercise has many health benefits, especially for diabetes mellitus (DM), yet it was observed that majority of DM in this survey did not follow a regular exercise regime due to fear of negative effects such as muscle cramps, flushing etc. 46.05% of CSII users in this survey reported that they never witnessed any ill effects of exercise. 54% of the respondents muscle cramps (20.39%), breathing shortness (11.18%), flushing (7.24%), red face (7.89%), chafing (1.97%) and others (5.26%) ill effects of the exercise were observed. On the other hand, when asked about Diabetic Ketoacidosis (DKA), 91% of the pump users reported that they had never experienced of DKA since they started to exercise.

3.4.5. Diet Section

Almost 89% of the responders said that they did not follow any medically approved dietary programme and 95.21% did not consume any special diabetic food or drink. This is further confirmed by the fact that 64% of the CSII users never consulted a dietician, while 28% did so regularly and a small percentage 8% had regular consultations with a dietician. However, 99.40% of insulin pump users who participated in this survey regularly counted their carbohydrate intake. Furthermore, 70% of such respondents reported to eating healthy as a priority, while 25% try to eat healthy food. Only a tiny percentage i.e. 2% said that they were too busy to search for healthy food options. And 3% described their eating habits as poor. It was observed in this survey

that most of the CSII users were conscious of their A1c level (this fact is supported by the previously mentioned stats that vast majority of CSII users were highly educated professionals). 35% of such respondents follow healthy eating regime to control their A1c level.91.62% of CSII users did not smoke while 51.5% consume alcohol.

As identified in this survey there were other factors that affected their eating habits such as trying to eat a healthy diet (63.69%), parents and family (4.46%), school and work (3.18%), socialising live (12.10%), not to try to change eating habits (3.18%) and others factor (13.38%). The average of calories number was intake in a typical day as listed in the table 3.4.

Table 3.4: The average number of calories intake in a typical day by CSII users as reported by respondents

Average of calories number/ daily	Responses %
Less than 1500	12.57
Over 1500 up to 2000	34.13
Over 2000 up to 2500	19.16
Over 2500 up to 3000	13.17
Over 3000 up to 3500	1.20
More than 3500	0.60

3.4.6. Diabetes complications

97% of CSII users among the respondents considered themselves as in good health and they confirm that they have regular medical check-ups. Table 3.5 provides a brief summary of some of the findings:

Table 3.5: Medical check-ups (responses %) as reflected in survey responses

Medical check list	Yes	No
Have had your weight checked by a doctor or nurse?	98.21	1.79
Blood pressure taken by a doctor or nurse?	96.89	2.67
Is your blood pressure usually normal?	78.67	20.44

Do you take any medication to control your blood pressure?	32.59	67.41
A cholesterol test by a doctor or nurse	90.67	9.33
Are you on lipid lowering medication (for high cholesterol or triglycerides)?	33.78	65.33
An eye test where a photograph of the back of your eyes was taken?	81.25	17.86
Has your eyesight suffered as a consequence of your diabetes?	33.33	65.78
Bare feet were examined	78.22	21.33
Do you have diabetic kidney disease?	7.11	92.89
Have you had a kidney transplant?	0.90	99.10
Do you require dialysis?	0	100
Have you ever had, or suspected that you had a stroke?	1.79	97.77
Have you ever had a heart attack?	2.67	96.89
Do you ever have chest pain due to angina?	1.79	97.77
Have you ever had heart bypass surgery (coronary artery bypass)?	0.44	99.11
Have you ever had a balloon angioplasty or a coronary stent placed?	3.11	96.44

3.4.7. Bivariate Analysis (Crosstab Analysis)

In statistics bivariate data analysis is a technique to study how two variables or questions relate to each other. In SPSS, bivariate analysis is called crosstab analysis and performed by tabulating them in a two way table.

CSII therapy relies on a portable pump which constantly delivers insulin throughout the day based on a pre-set basal rate. The basal rate can be programmed and changed at different times of day according to the need of the patients (figure 3.8) i.e. how much background insulin patient received each hour by pressing few buttons on the device.

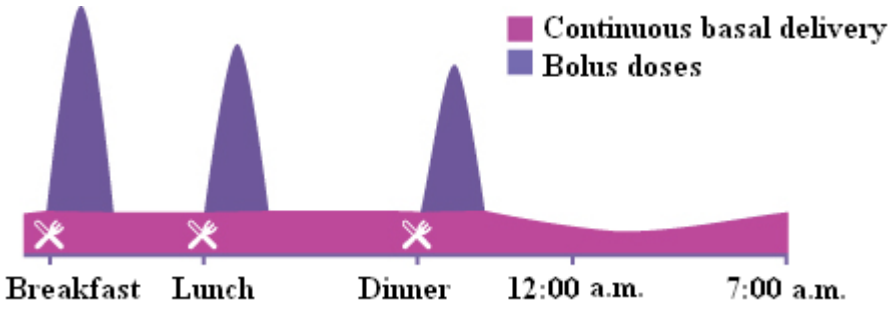


Figure 3.8: Basal and Bolus delivery by insulin pump**.** (Adapted from Accu-chek website)

In this study the CSII respondents stated that they have a lower basal rate in an exercise day compare to a non-exercise day (see table 3.6).

Table 3.6: Basal rate cross-table on an exercise day and on non-exercise day as reported by survey respondents

Basal rates (Unit/hr)		An exercise day			
		<0.5	**0.5 - 1**	**1 - 2**	**>2**
Non-exercise day	**< 0.5**	100%	0%	0%	0%
	0.5 – 1	28%	72%	0%	0%
	1 – 2	5%	37%	55%	3%
	>2	0%	0%	31%	69%

For example: when insulin pump users use 1-2 Unit/hr in non-exercise days then 55% of them will use the same amount in an exercise day while 37% of them will use less than 1 unit/hr and 5% of them will use less than 0.5 unit/hr.

The insulin pump can also deliver a shot of insulin (bolus), to control the BG levels following a meal (see figure 3.8). Insulin pumps are programmable and flexible devices which can be set to deliver half doses of required insulin before meal and delivering the second half of the required insulin dose one hour later or so.

In this study the respondents stated that if their bolus types for meal were of the following type (standard bolus (spike), combination bolus (spike and square wave) they

were slightly adjusted on exercise days compared to non-exercise days on which they preferred standard bolus (spike), extended bolus (square wave) and combination bolus (spike and square wave). However, extended bolus (square wave) and super bolus (increased spike) were not changed on exercise and non-exercise days (see table 3.7).

Table 3.7: Bolus types on an exercise and non-exercise day (responses %)

Bolus types for meal		An exercise day			
		Standard bolus (Spike)	Extended bolus (Square wave)	Combination bolus (Spike and Square wave)	Super bolus (Increased spike)
Non-exercise day	Standard bolus (Spike)	85%	6%	9%	0%
	Extended bolus (Square wave)	0%	100%	0%	0%
	Combination bolus (Spike and Square wave)	14%	8%	78%	0%
	Super bolus (Increased spike)	0%	0%	0%	100%

Table 3.8 shows the total daily amount of insulin (Units/day) infused into the pump on the exercise and non-exercise day. Pump users reported that the total daily amount of insulin infused into the pump was reduced in an exercise day as compared to non-exercise day when they use more than 20 units of insulin. However, the total amount of daily insulin did not changed in exercise and non-exercise day when pump users used less than 20 units of total daily insulin.

Table 3.8: Total insulin through an exercise and non-exercise day (responses %). *Responders reported sometime when they exercise they eat more than on non-exercise day.

Total amount of insulin (Units)		An exercise day					
		<20	**21 -30**	**31 - 40**	**41- 50**	**51- 60**	**>61**
Non-exercise day	**<20**	100%	0%	0%	0%	0%	0%
	21 -30	29%	69%	0%	0%	0%	2%*
	31 - 40	0%	37%	63%	0%	0%	0%
	41- 50	0%	11%	36%	53%	0%	0%
	51- 60	0%	0%	14%	38%	43%	5%*
	>61	0%	0%	0%	4%	21%	75%

Data analysis shows that the CSII users who were doing BG tests more than 6 times day did not make any adjustments for exercise or non-exercise day. However, 78% of CSII users conducted more frequent BG tests on exercise days (49% from 5±1 to more than six times a day and 29% from 2±1 to 5±1 times a day) (Table 3.9).

Table 3.9: Number of BG tests on exercise and non-exercise day

Blood glucose test times		An exercise day (% of responses)		
		1-3	**4-6**	**>6**
Non- exercise day	**1-3**	71%	29%	0%
	4-6	0%	51%	49%
	>6	0%	0%	100%

As mentioned previously most of the respondents (i.e. approximately 88%) in the survey reported that exercise session reduced their BG level. Table 3.10 summarise the data to highlight the effect of exercise on CSII users. It is clear from the data in table 3.10 that all the respondents who started exercise with BG level higher than 8 mmol/l witnessed a significant drop in their BG level.

Table 3.10: BG level before exercise and the change after exercise

Blood glucose (mmol/l)		Effect of exercise on BG level (% of responses)		
		Increase in BG level	**Decrease in BG level**	**No change in BG level**
Pre-exercise BG level (mmol/l)	**<5**	25%	50%	25%
	5.1 - 6	0%	94%	6%
	6.1 - 7 l	11%	86%	3%
	7.1 - 8	7%	84%	9%
	8.1 - 9	0%	100%	0%
	9.1 - 10	0%	100%	0%
	>10	0%	100%	0%

Most of the respondents of this survey were based in UK or US (44.64 and 44.21% respectively) and a comparison of data from respondents from these countries reveals that:

- In UK, 71.11 % of insulin pump users are female in contrast to US, 64.38 % are male.
- More than 80% in both countries underwent some form of educational training (e.g. vocational or college) or have had a higher education (e.g. university)
- This research is mainly focused in T1D patients and Type 2 diabetes patients who are using insulin pump were excluded from the data analysis. However, there were only 2.38% from UK compare to 14.29% from US who are diagnosed with T2D and using CSII therapy.
- In UK 66.67 % did not have a family history of diabetes in contrast to 65.22% in US who have a family history of diabetes.
- 26.19% T1D sufferers in UK discovered that they are diabetic when their friend or member of family picked some symptoms, while in US 4.29% cases for discovered this way.

- In UK almost 38% were diagnosed at the age less that 10 while in US 38% were diagnosed bet 1-20 years of age (table 3.11).

  Table 3.11: Insulin pump users at diagnosed age.

First diagnosed age	UK	US
1-10	37.78%	17.81%
11-20	28.89%	30.14%
21-30	20%	19.18%
31-40	6.67%	12.33%
41-50	4.44%	16.44%
51-60	2.22%	4.11%

- 39% of the respondents from US are using CSII for more than 5 years, while only 22.5% in UK are using it for more than 5 years (table 3.12).

  Table 3.12: Insulin pump users on the pump (years).

Years on pump	UK	US
Less than 1 year	15%	20.31%
1-3 years	32.50%	17.19%
3-5 years	30%	23.44%
More than 5 years	22.50%	39.06%

- In UK most of the CSII pumps are sourced by NHS (i.e. 97.73%) and a very small number i.e. 2.27% are paid for by the patients. While in US the main provider for the insulin pump and the infusion set is the capital health plan insurance or other insurance (in some cases patient pay 20% of the total cost and the insurance cover the rest of the cost) and 14.29% of insulin pump users reported that they paid for the pump infusion sets.
- 6.67% of CSII users in UK are retired while in US 19.18% of respondents reported to be retired (table 3.13).

Table 3.13: Insulin pump users age (years).

Age years	UK	US
1-10	4.44%	1.37%
11-20	6.67%	6.85%
21-30	11.11%	4.11%
31-40	24.44%	10.96%
41-50	31.11%	21.92%
51-60	13.33%	41.10%
61-80	6.67%	13.70%

- In the UK 29% of the responders reported their A1c at the time of diagnosis was above 9% and almost same of number of respondents from US had the same experience (table 3.14).

Table 3.14: A1c level.

A1c	UK	US
At diagnosed time		
7.1-8	4.76%	7.14%
Above 9	28.57%	30%
Before using pump		
6.1-7	9.52	14.49%
7.1-8	26.19%	31.88%
8.1-9	30.95%	18.84%
9.1-10	9.52%	14.49%
Over 10	19.05%	1.45%
After using pump		
5.1-6	4.76%	28.57%
6.1-7	35.71%	40%
7.1-8	38.10%	15.71%
8.1-9	7.14%	5.17%
9.1-10	2.38%	1.43%
Over 10	7.14	2.86%

- In UK NHS is offering different types of insulin pumps and Medtronic Paradigm (34.15%), Animas (21.95%) and Accu-chek (21.95%) are the most commonly supplied devices. While in US the insurance companies are also following the same pattern and offering Medtronic Paradigm (62.50%), Animas (15.63%) and Accu-chek (4.69%). Moreover, in both countries patients are using almost the same type of insulin in their pumps (table 3.15).

Table 3.15: Type of insulin.

Insulin infused into pump	UK	US
Humalog® (Lispro)	32.50%	29.82%
Novorapid® or Novolog® (Aspart)	47.50%	50.88%

- Basal rate on a non-exercise day for UK based respondents on CSII therapy is less than same respondents from US. In UK insulin pump users mainly use less than one unit of insulin on non exercise day and less than half unit on an exercise day as in the table 3.16. In UK insulin pump users show that they use less total daily insulin in exercise and non-exercise day as compared to US based respondents.

Table 3.16: Basal and total amount of insulin daily.

Basal rates:	**UK**	**US**
On non-exercise day		
0.5-1 unit	57.50%	32.81%
1-2 units	20%	43.75%
On a an exercise day		
Less than 0.5 unit	39.47%	15.8%
0.5-1 unit	34.21%	39.68%
1-2 units	10.53%	25.40%
The total amount of daily insulin:		
On non-exercise day		
21-30 units	25%	18.75%
31-40 units	27.50%	25%
51-60 units	7.50%	18.75%
More than 61 units	7.50%	21.88%
On a an exercise day		
Less than 20 units	20.51%	9.68%
21-30 units	28.21%	29.03%
31-40 units	28.21%	17.74%
41-50 units	10.26%	9.68%
51-60 units	7.69%)	19.35%
More than 61 units	5.13%	14.52%

- 75% of responders from UK and 50% from US reported the acceptable BG level before exercise is to be above 7mmol/L. However, same percentages in both countries reported the decreased in BG level after exercise. In UK 73% of

insulin pump users did not report hypoglycemia during exercise whereas 50% from US report hypoglycemia during exercise.

- In the UK and US (85% and 80% respectively) were switched on their pump during exercise and 88% of such respondents from both countries have no pump problems during exercise.

- The exercise hours, intensity and types are almost same in UK and US as show in the table 3.17. Importantly, more than 88% in both countries insulin pump users did not report any DKA events since they start exercising.

Table 3.17: Exercise duration, level and types.

Exercise hours/week:	**UK**	**US**
1-3 hours	23.68%	30.36%
More than 3 hours	39.47%	48.21%
Level of intensity:		
Light	20%	14.04%
Moderate	28.57%	38.60%
Type of exercise:		
Aerobic exercise	47.22%	57.14%
Resistance exercise	2.78%	0.0%
Mixture	36.11%	28.57%

- In the UK 100% and 90% from US of T1D using CSII did not take any special diabetic food or drink and did not follow a medically approved dietary programme while 100% of pump users in US counted intake carbohydrates compare to 87% from UK do so as in the table 3.18.

Table 3.18: Calories Number.

Number of calories in a typical day:	UK	US
Less than 1500	5.26%	14.29%
1500-2000	36.84%	35.71%
2000-2500	28.95%	12.50%
2500-3000	10.53%	19.64%
3000-3500	0.0%	1.79%
More than 3500	2.63%	0.0%

The above analysis shows that T1D sufferers in both countries had almost same experience with the CSII therapy and found CSII therapy and exercise helpful to manage their BG level and over all quality of life.

3.5. Discussion

Diabetes is becoming more and more common across the world. It has been observed by different researchers that although insulin is vital for T1D, exercise and diet play an important role to manage both types of the condition. For some time now, CSII therapy has been an alternative to MDI to manage and control the insulin in T1D (Wu, Graves et al. 2010). CSII therapy was first introduced in 1970 and the past few years have witnessed significant improvement in the design, ease of use and effectiveness of this device (Fuld, Conrad et al. 2010). The aim of this survey was to study the attitude and perception of T1D sufferers about the effectiveness of CSII therapy, exercise and diet. A questionnaire for this study was published on SurveyMonkey® and different diabetes related publications were used to advertise it.

In total, 273 people completed the survey but only 245 responses were considered in this study for analysis. Others were disregarded for the present purpose because some of the respondents failed to fully complete the questionnaire while some others were not T1D patients rather diagnosed as T2D or LADA. It may be a shortcoming that this

survey was not piloted in focus groups or to small recipient audience to trial it. However, it had been scrutinised and edited by professionals, one of whom was a diabetic sportswoman apart from being a professor of diabetology. One or two questions were inadequately posed including asking for lipid level information, although it is hard to see how information can be elicited that is not in the respondent's possession. This did blunt the power of the lipid data but inferences could be gained and we did not find any obvious contradictions in the data. A survey is just a snapshot, however, and is open to many biases such as we discovered in the first survey mentioned earlier. In that study, the responses included a large majority of T1D despite being open to T2D subjects as well, of whom there are in fact ten times the number. This anomaly turned out to be a question of enthusiasm for surveys, access to PCs and the web and the ability to read the small print in the Balance magazine advertisement.

The findings of the current study are discussed in details as below:

- In this study almost 89% of respondents lived in the UK or US and the majority (i.e. 83.96%) were white. Pickup (2011) and Renard (2010) reported that the US is the leader in CSII therapy, with 40% of T1D patients on insulin pump (Renard 2010). Moreover, (Pickup 2011) also stated that in UK less than 5% of T1D are using CSII therapy. Only 3% respondents were from mainland Europe (excluding UK). This small response could be associated with survey being in English and also the lack of formal interest of medical professionals and limited research in the field of CSII in large parts of this region. Furthermore, as reported by NICE, there are very small numbers of European medical professionals who are trained to advise patients about CSII therapy in these

countries, as the manufacturers of insulin pump have neglected to invest there in research and training, probably due to health insurance in these countries failing to routinely underwrite insulin pump use.

- The data showed that 54.67% of the respondents did not have family history of T1D. In the UK 66.67% did not have a family history of diabetes in contrast to 65.22% in US who did. These findings should be compared with those of Steck and Rewers (2011) who reported that in US more than 85% of T1D patients with T1D did not have a family history of T1D (Steck and Rewers 2011). However, American Diabetes Association (ADA) in a report entitled "Definition and Description of Diabetes Mellitus" considered T1D has genetic factors, and this claim is supported by some other studies as well (Diabetes Care 2011; ADA 2013). However, T1D is a complicated disease as the genes which affect T1D susceptibility can be a function of 1) β-cell function 2) insulin expression 3) immune function (Noble and Erlich 2012)
- It is a fact that onset of T1D usually occurs in children and young adults. This claim was supported by that fact that 58% of respondents in this pump study were living with T1D for 1 to 20 years. This result is in line with Renard (2010) who reported a sharp rise in CSII usage among children and teenagers (Renard 2010).
- The data show that most of the respondents (i.e. 42.86%) have normal weight as described by body mass index (BMI) while 4.59% of respondents were under weight, 27.04% were overweight and 20.92% were classed as obese. Further analysis of data in this respect showed that the female respondents have the higher percentages of overweight and obesity as compared to the males. This

result is supported by other studies such as Łuczyński, Szypowska et al (2011) they compared the BMI of male and female CSII users and found that more females are classed as overweight/obese with higher BMI values as compared to the boys (Łuczyński, Szypowska et al. 2011). On the other hand, DiMeglio, Pottorff et al. (2004) in their study found that CSII therapy had no effect on patients' BMI. They further argued that increased level of insulin to control A1c may result in increased BMI in youth with T1D (DiMeglio, Pottorff et al. 2004).

- In the survey, it was found that 70% of respondents had desired levels of total cholesterol while almost 65% respondents reported a desirable level of LDL and/or HDL. While it has become widespread practice to use ratios of TC/HDL and LDL/HDL, it should be noted that some authorities, such as the Mayo Clinic, prefer ratios only for the purposes of diagnosis and not for treatment basis. In this survey, the question asked for numbers, giving no prompts and not specifying that all values were needed. Many respondents gave insufficient information to calculate these ratios e.g. missed one or other value. It is worth noting that 65.33% (see table 3.5) respondents were not taking any lipid lowering medication which means the majority of the ratio values may well have been maintained at normal/acceptable values for the purpose of this study. This could well apply to the un-medicated and to the 35% who took statins or their alternatives (assuming appropriate prescribing and compliance). The greater efficiency of pumped insulin might well affect lipid control. Maahs, Nadeau et al. (2011) reported that reduced HDL is associated with lower insulin resistance in youths with T1D (rather than T2D resistance and as assessed by lower glucose infusion rates in clamp experiments) (Maahs, Nadeau et al. 2011).

Meanwhile, Feitosa, Feitosa-Filho et al (2013) showed that intensive insulin therapy even with poor glycaemic control may lead to lower LDL than in controls, arguing that insulin may control lipids and glucose with different ratios of efficiency. Ho, Dhaliwal et al. (2012) found in their study that exercise plays an important role to lower the lipids and is therefore likely to have an augmented effect for insulin pumpers. This may be associated with weight control and thus Guo, Kawano et al. (2011) argued that for non-diabetic Japanese subjects, a drop in BMI may cause a drop in LDL level and elevate the HDL level.

- In this survey 90.43% of CSII users are highly educated, while 63% of CSII users are professionals or skilled workers. These findings are in line with Valla (2013) claim who suggested that insulin pump users should be able to respond to education regarding the functions of the pump to use it effectively and control their glycemia
- In this study it was found that insulin pump therapy is an effective means to lower the A1c in T1D (statistically significant with P=0.001) which is consistent with other studies such as (Pickup and Sutton 2008; Bergenstal, Tamborlane et al. 2010; Misso, Egberts et al. 2010; Hermanides, Nørgaard et al. 2011).This study also found that T1D using insulin pump therapy reached the target A1c of 7.1% or less, and this was achieved by reducing hyperglycemia without increasing hypoglycemia these results are in line with Levy-Shraga, Lerner-Geva et al. (2013). These results are further supported by NICE guidelines where CSII therapy is recommended for T1D to improve their diabetes control and hypoglycemia and to achieve and maintain target A1c (Hitman 2012).Moreover, Aberle, Scholz et al. (2009) reported that the A1c is

significantly lower in T1D using insulin pump as copmapred to MDI users (Aberle, Scholz et al. 2009) as well as Knight, Northam et al. (2009) reported that CSII is effective to control A1c (from 8.2% to 7.5%) (Knight, Northam et al. 2009). de Vries, Grushka et al. (2011) examined 530 patients with T1D who had had insulin pump therapy for 8 years (2000 and 2008), and found that A1c was significantly higher in patients discontinuing pump therapy than in the controls ($p = 0.02$) (de Vries, Grushka et al. 2011). It is worth noting that in the US, CSII is also prescribed for T2D people who fail to control their A1c (Bode 2010). Pickup (2011) argued that most of the T2D patients poorly control their glycaemic while CSII improved their glycaemic control (Pickup 2011). Hence it could be concluded that both types of diabetes could improve their glycaemic control after using CSII therapy.

- In this study 88% reported that BG level was dropped significantly after exercise and no hypoglycemia events were recorded. Yardley, Iscoe et al. (2013) also reported that, CSII users have less hyperglycemia post-exercise than MDI (Yardley, Iscoe et al. 2013).
- Our study indicates that more than half of T1D on Medtronic Paradigm insulin pump. Though the type of pump is mainly selected by NHS or the insurance company yet Agrawal, Welsh et al. (2011) study found Medtronic Paradigm insulin pump easy to use and the most effective on their criteria (Agrawal, Welsh et al. 2011).
- 56.28% of respondents used Novolog® or Novorapid® (Aspart) insulin (figure 3.5). Garg, Ampudia-Blasco et al. (2010) supporting the choice of this type of insulin as rapid-acting insulin analog, lowering incidence of hypoglycemia as

well as providing improvements in glycemic control (Garg, Ampudia-Blasco et al. 2010). Moreover, this insulin is safe and effective for use with insulin pump in T1D patient's (children or adult) (Weinzimer, Ternand et al. 2008).

- In this study patients were highly satisfied with CSII treatment because 86.71% did not face any problems during and after their exercise sessions while using pumps. Furthermore, 77.65% said that they did not change their exercise routine after switching from MDI to CSII therapy.
- In this study it was found that users who take less that 0.5 insulin units/hour did not need to change their basal rates. Interestingly, when insulin pump users injected more than 0.5 insulin units/hour only about a third reduced the dose for exercise. Tildesley, Chan et al. (2012) reported having multiple basal pumped rates did not appear to affect A1c reduction (Tildesley, Chan et al. 2012). Delivery consistency in basal rates 24/7 days is one of the advantages of using CSII to control BG levels, injections being variable for reasons connected with skin sites and technique. However, elsewhere it has been reported that the variable basal rates and low insulin depots also contribute to pumps' reported clinical superiority (Valla 2013). This makes sense because the variation is aimed to control identified times when glucose might be expected to change or has changed.
- 63.16% of cases, standard bolus for a meal were used on an exercise and non-exercise day. The use of standard bolus was supported by Maahs, Horton et al. (2010) to correct elevated BG levels after meals (Maahs, Horton et al. 2010) and Danne et al. (2008) demonstrated that patients taking more of their daily insulin as boluses had significantly lower A1c levels than patients taking fewer boluses

(Danne, Battelino et al. 2008). Moreover, Cukierman-Yaffe, Konvalina et al. (2011) recommended to use this bolus method to achieve better glucose control (Cukierman-Yaffe, Konvalina et al. 2011). The strategic use of bolus dosing is associated with better glucose control and improved estimates of carbohydrate ingestion.

- 64% of respondents said they very rarely suffered from hypoglycemia during an exercise session. However, 52.41% reported to have more than usual episodes of hypoglycemia after exercise. Yardley, Iscoe et al. (2013) are supporting this statement when they compare the post aerobic exercise (moderate-to-heavy) hyperglycemia on CSII and MDI and CSII group reported limited the post-exercise hyperglycemia but this is not associated with increased risk for post-exercise late-onset hypoglycemia (Yardley, Iscoe et al. 2013). One of the greatest advantages of CSII therapy is to reduce the number of hypoglycemia during and after exercise.
- 75% of the respondents preferred to exercise with moderate intensity. Yardley, Iscoe et al. (2013) supported this level of intensity for T1D using CSII (Yardley, Iscoe et al. 2013). This may further support the finding that majority of the insulin pump users are educated and are following the latest American College of Sports Medicine (ACSM) guidelines.
- 91%of responders in this study reported no DKA since they started to exercise. This is similar to Levy-Shraga, Lerner-Geva et al. (2013) when they reported the DKA events were not reported with CSII therapy (Levy-Shraga, Lerner-Geva et al. 2013). These results also in line with previously discussed finding of this

study and Fredheim, Johannesen et al. (2013) that T1D CSII users experience a significant drop in A1c level (Fredheim, Johannesen et al. 2013).

- ❖ 99.40% of insulin pump users who participated in this survey regularly counted their carbohydrate intake in order to calculate bolus and basal insulin doses. Enander, Gundevall et al. (2012) and Cukierman-Yaffe, Konvalina et al. (2011) reported that majority of CSII users are conscious of their diet and keep a record of their carbohydrate intake (Enander, Gundevall et al. 2012).

3.6. Conclusion

- For this survey, managing diabetes with a pump is apparently associated with low incidence of cardiovascular and other complications. This may represent the advantages of CSII but may also reflect the age, condition and enthusiasm of responders, as well as to the biases relating to language, the means of access to the survey and other factors discussed above.
- CSII therapy using an external insulin pump enables the T1D responders to enjoy a more flexible lifestyle with everyday activities and to reduce the A1c level.
- CSII is known to reduce hypoglycaemic and DKA events, improves glycemic and metabolic control and this study supports that finding.
- As a result of better control, CSII should decrease out-patient consultations and hospital admissions and does not appear to be associated with significant adverse outcomes compared to MDI. This is reflected in this survey.
- Moreover, CSII therapy is shown here to reduce the insulin requirement significantly which brings in other health advantages such as lowered BMI and lipids, as well as less likelihood of iatrogenic obesity.

- This study in line with many previous studies showed that CSII therapy provides multi facet benefits and minimal problems for users. Most recently 39 different efforts were made to study the potential disadvantages of CSII usage (for example site infections, pump malfunction, DKA and hypoglycemia). However, these studies failed to provide any conclusive evidence on the risks or ill effects of CSII therapy.

CHAPTER 4: The Immunological Effect of Exercise

The immunological effect of exercise on Type 1 diabetic (MDI or CSII) and non-diabetic volunteers.

4.1. Introduction

A complex interaction between innate and adaptive immune system cells and pancreatic β-cells is considered to be behind the development of type 1 diabetes (T1D) (Lehuen, Diana et al. 2010). A similar statement could be made for the effects of exercise, since these are inflammatory. The mechanism and processes of inflammation are the key players in the development of T1D (Kristiansen and Mandrup-Poulsen 2005). This chapter will outline some immunological parameters for T1D, such as tumor necrosis factor (TNFα), interferon gamma (IFNγ), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β).

With respect to T1D, research studies have shown that tumour necrosis factor-alpha (TNFα), interleukin 1beta (IL-β), interleukin 6 (IL-6), and certain other chemokines, which are mediators of inflammation, are involved in the pathogenesis (Kristiansen and Mandrup-Poulsen 2005). These immunological markers can be detected in the blood at and before disease onset.

Various silent immune occurrences take place prior to the appearance of the clinical symptoms of T1D. Lehuen, Diana et al. (2010) argued that autoantibodies are generated and self-reactive lymphocytes are triggered, penetrating the pancreas in order to obliterate the insulin-producing beta cells in the islets of Langerhans (Lehuen, Diana et al. 2010). This on-going and targeted destruction remains undetected for several years

and the first clinical symptoms may only become obvious after most of the beta-cells (possibly 80%) have been destroyed or have become non-functional. This will make the sufferer become reliant on insulin supplementation in order to survive (Lehuen, Diana et al. 2010).

The natural process of cytokine interplay in the immune system is a complex phenomenon and crucial parts of this phenomenon are still unaccounted for; which is often associated with pathology. Most of the cytokine manipulations cause a dual effect, which is reliant on timing of medication, dose and channel of administration (see table 4.1).

Cytokine-induced destruction of β-cell is principally arbitrated by apoptosis in human islets (Eizirik and Mandrup-Poulsen 2001; Hoorens, Stange et al. 2001). Even though this has not yet been confirmed, the proximal mechanisms in cytokine signal transduction has been widely studied. Nevertheless, the precise distal intracellular molecular activities that are accountable for the death of β-cell are not yet very well understood. Therefore, it is more than probable that determining the cytokine-mediated changes in gene and protein expression profiles will generate useful information in relation to future treatment and prevention approaches for T1D (Rewers and Gottlieb 2009).

When β-cells and islets are exposed to cytokines many different alterations take place in the expression profiles of mRNA and proteins. These alterations are demonstrated by up- and down-regulation in addition to de novo synthesis of various groups of genes. Regulating these genes causes further loss of differentiated β-cell roles and stimulates both pro- and anti-apoptotic methods in the β-cell.

Moreover, cytokines reduce the expression of various genes concerned with the mitochondrial respiratory chain (Cardozo, Kruhoffer et al. 2001; Larsen, Fey et al. 2001; Sparre, Christensen et al. 2002), which causes decreased energy production in reaction to cytokine disclosure. Furthermore, cytokines reduce the expression of various genes linked with differentiated β-cell functions and protection of β-cell mass (Cardozo, Heimberg et al. 2001; Cardozo, Kruhoffer et al. 2001; Larsen, Fey et al. 2001; Kutlu, Cardozo et al. 2003). These alterations bring about a reduction in insulin generation (Southern, Schulster et al. 1990; Welsh, Eizirik et al. 1991) and a reduction in the growth capability of the cytokine-exposed β-cells or islets (Sjoholm 1991).

Cytokines (IL-1β and IFN-γ) also encourage production of free radicals such as hydrogen peroxide (H2O2) and superoxide (O2-) (Nerup, Mandrup-Poulsen et al. 1994; Mandrup-Poulsen 1996) that could be toxic to the β-cell. After this exposure of β-cells' to cytokine several "protective" proteins like heat glutathione-S-transferase (GST), metallothionin, shock proteins (HSPs) and manganese superoxide (MnSOD) are up regulated (Rieneck, Bovin et al. 2000; Cardozo, Kruhoffer et al. 2001).

Table 4.1: Cytokines associated with T1D (Bergholdt, Heding et al. 2004).

Cytokine	Function	Production	References
IL-1β	IL-1β Involved in the inflammatory response and contributes to inflammatory pain hypersensitivity; IL-1β induces a systemic and local response to pathogenic invasion or injury of tissues by inducing transcription or enhancing mRNA stability of a large range of pro-inflammatory genes.	Produced by activated macrophages as a proprotein	(Pociot, Mølvig et al. 1992; Krikovszky, Vasarhelyi et al. 2002; Schmid, Haslinger et al. 2012)
TNFα	TNFα regulates a wide spectrum of biological processes including stimulating cell proliferation, induction of cell differentiation, apoptotic cell death, involvement in lipid metabolism and response to sepsis via IL1 & IL6 production.	Mainly secreted by macrophages and T cells	(Pociot, Briant et al. 1993; Obayashi, Nakamura et al. 1999; Obayashi, Hasegawa et al. 2000)
IL-6	IL-6 is a cytokine which response to muscle contraction and fatty tissue during exercises (IL-6 stimulates energy mobilization which leads to increased body temperature) and stimulates immune response. As well as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1.	Secreted by T cells, macrophages and produced from muscle	(Jahromi, Millward et al. 2000; Kristiansen, Nolsoe et al. 2003; Febbraio and Pedersen 2005; Kristiansen and Mandrup-Poulsen 2005)
IFNγ	IFNγ promotes NK cell activity. It has antiviral activity, important immuno regulatory functions and a potent activator of macrophages. Furthermore, it has anti-proliferative effects on transformed cells.	Produced mainly by lymphocytes (NK, T_H1)	(Pociot, Mølvig et al. 1992; Awata, Matsumoto et al. 1994; POCIOT, VEIJOLA et al. 1997; Jahromi, Millward et al. 2000; Pravica, Asderakis et al. 2002; Tegoshi, Hasegawa et al. 2002)

In T1D, it was revealed that treating patients with etanercept (a soluble TNF-receptor) reduced A1c and increased the production of endogenous insulin, symptomatic of the safeguarding of the beta-cell function (Mastrandrea, Yu et al. 2009). This is not however a simple procedure; TNFα may have a dual role in T1D. For example, in animal models, TNFα boost the diabetogenic reaction too soon or reduce the diabetogenic reaction too late in the T1D procedure (Lee, Xu et al. 2005). Furthermore, some clinical reports have revealed the development of T1D in arthritis sufferers after receiving etanercept treatment (Christen, Wolfe et al. 2001; Bouwens and Rooman 2005; Tack, Kleijwegt et al. 2009), but also the resolution of T1D in patient that needed anti-TNFα therapy to treat arthritis (Arif, Cox et al. 2010).

Other studies have proposed that autoimmunity arises from a lack of type 1 IFN. Type 1 IFNs can offset type 2 IFN which is presumably a key factor in autoimmune inflammation (Brod 1999). Nevertheless, this proposal is in fact controversial. Other academics propose that stimulating Toll-like receptors (TLRs) by double-stranded RNA or poly I: C (viral mimic) via introducing IFNγ may in fact trigger or accelerate immune mediated β-cell annihilation (Devendra and Eisenbarth 2004) (see figure 4.1).

It has been observed in different studies (for more details see table 4.2) that in normal people (i.e. non-diabetic) regular moderate exercise causes release of some cytokines which enhance immune function and attenuates immune disturbances associated with acute exercise (e.g., a single bout of vigorous exercise) (Nieman, Nehlsen-Cannarella et al. 2008).

Acute exercise is defined as a sustained moderate for single bout (50 to 65% intensity) (Ploeger, Takken et al. 2009) (see section 1.7.3 for details). Acute exercise session has a short-term depressive effect on immune function. When a person engages in a strenuous exercise session, there are bodily responses that are very similar in many aspects, to those that are bought about from infection, sepsis, or trauma (Northoff, Berg et al. 1998). Plasma concentrations will increase in numerous substances that are known to affect the functioning of leukocyte, for example inflammatory cytokines like TNFα, macrophage inflammatory protein-1, and IL-1β; anti-inflammatory cytokines IL-6, and IL-1-receptor antagonist (IL-1ra). Contracting muscle fibres during exercise cause release of cytokine which gives rise to the level of plasma IL-6 concentration (Steensberg, van Hall et al. 2000; Wallberg, Mikael Mattsson et al. 2011). Nevertheless, IL-6 production from monocyte (Starkie, Rolland et al. 2001; Dickie, Church et al. 2010)and production of IFNγ produced by T lymphocytes is reduced after exercise (Lancaster, Halson et al. 2004). Steensber, Fisher et a. (2003) showed that moderate increase in plasma levels of IL-6 provoke the two anti-inflammatory cytokines IL-1ra and IL-10 (Steensberg, Fischer et al. 2003).

In line with the increase of the following IL-6, and IL-1ra, exercises lowers the percentage of type 1 T cells in this flow (Lancaster, Halson et al. 2004). Another significant activity of the IL-6 is that it holds back the production of TNFα, which is a powerful activator of inflammation (Starkie, Ostrowski et al. 2003).

Inflammation has been associated with the pathology of various chronic diseases; diabetes. For example, blood markers of inflammation are very much linked to

cardiovascular and metabolic disease in T1D middle aged people and the elderly (Schram, Chaturvedi et al. 2005). Thus, raised systematic levels of IL-6 during exercise sessions may be one of the means through which exercise protects individuals against developing chronic illnesses. However, it could be disputed that it is probable that the comparative significance of IL-6 in these circumstances is probably quite small, as noteworthy health benefits derived from taking frequent exercise are apparent even when the exercise is only light or moderate in intensity (Wallberg, Mikael Mattsson et al. 2011).

Frequent moderate exercise (50-60%) has been shown to provide support for the immune system (Cannon, Fiatarone et al. 1994; Kenney and Ho 1995; Mackinnon 1997; Gleeson, Nieman et al. 2004; Nieman, Henson et al. 2005). Various studies have claimed that frequent moderate exercise is advantageous for cytokines and immunoglobulin parameters in some people (Cannon, Fiatarone et al. 1994; Kenney and Ho 1995; Mackinnon 1997; Furusawa, Tajima et al. 1998; Mackinnon 1999; MacKinnon 2000; Bauer and Weisser 2002; Gleeson, Nieman et al. 2004) (see also table 4.2). As moderate exercise can reinforce the immune system, extreme exercise can suppress the immune system (Fitzgerald 1988; Liesen and Baum 1997; Gleeson 2007)

The inflammatory pathway to T1D is understood to have a cytokine pathway and the blocking agents are known. However, the effects are incompletely understood and the interplay is so complex that responses to experimental therapy have been paradoxical. Figure 4.1 explains cytokine pathway and the blocking agents in T1D (Agnès, Julien et al. 2010). Figure 4.1 explains that important pathways changed in expression profiling studies after cytokine-exposure, Toll-like receptors (TLRs) are expressed by pancreatic

β-cells to detect danger from microbial products and can trigger T1D, during a localized virus infection in the pancreas. Cytokines, such IL-1β, IFNγ and TNF-α, secreted by immune cells, such as macrophages, could cause direct damage (such as apoptosis) of β-cells but could also induce self-defence mechanisms; for example, IFNγ induces indoleamine 2,3-dioxygenase (IDO) expression by β-cells. Programmed cell death 1 ligand 1 (PD-L1)TNFα is a "master regulator" of the inflammatory reaction in several organ systems (Feldmann and Maini 2003).

Here where the blockers work

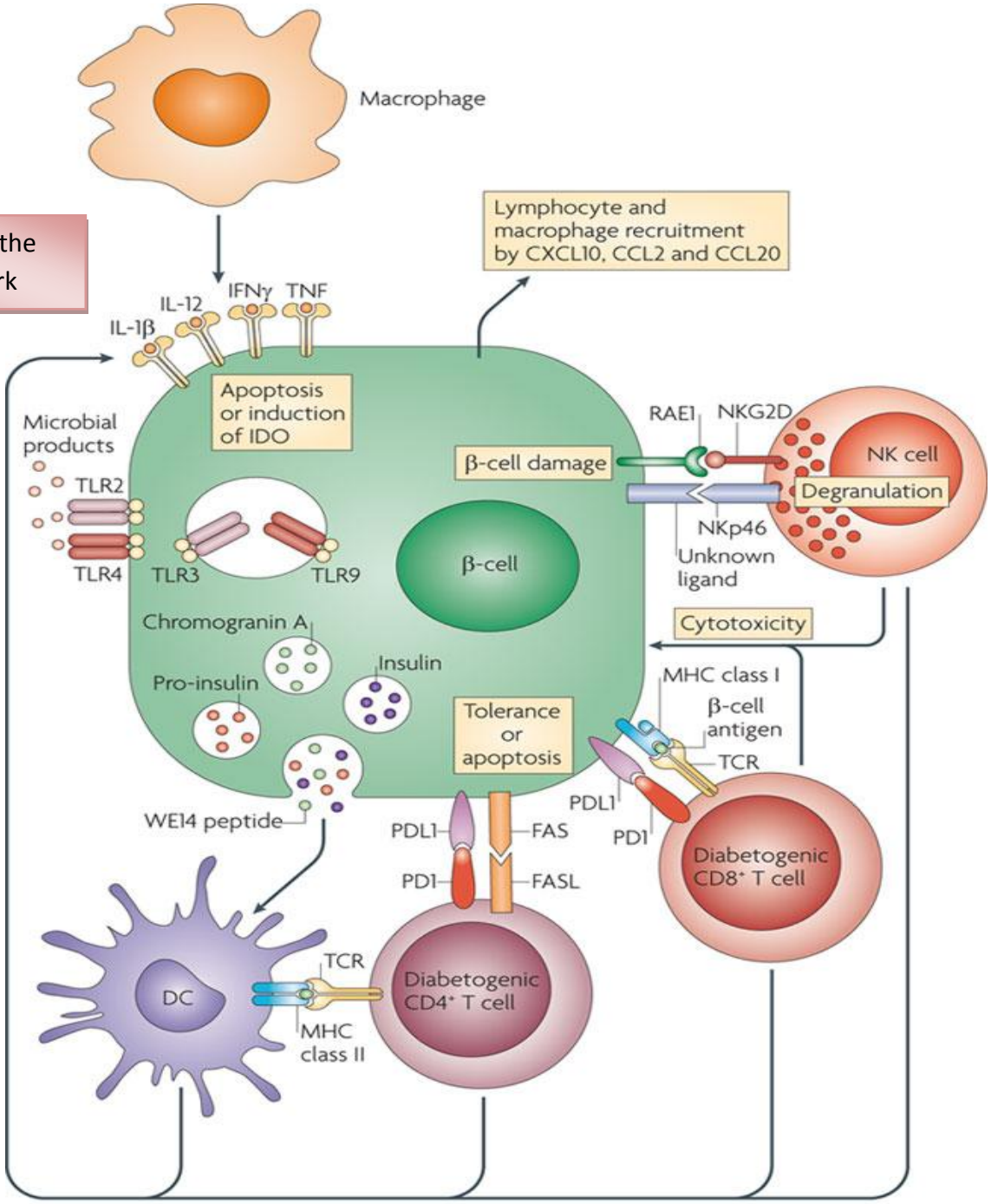


Figure 4.1: Cytokine pathway and the blocking agents in T1D (Agnès, Julien et al. 2010).

Table 4.2: Selected studies evaluating the acute effects of exercise bouts on inflammatory markers

Reference	No of Subjects	Exercise protocol	Cell Type	Result	Volunteers
Bente (1999)	Randomise	Acute exercise	Neutrophil	Increases during and after exercise	Healthy
			Lymphocyte	increases during exercise and falls below pre-exercise values	
Smith (2000)	6 active men (24 ± 3 yr) (untrained)	Bench press and leg curl eccentric actions 4X12Rep100%1RM 2rest	Plasma Pre-, Post-1.5, 6, 12 h and 1-6d after the session	IL-1β was reduced at 6, 24 and 120 h after exercise. IL-6 was elevated at 12, 24, and 72 h after Ex. No effect on TNF-α	Healthy
MacIntyre (2000)	12 healthy recreationally active men (20-29 yr)	30X10Rep of eccentric actions of the right leg (quadriceps muscles) using the continuous eccentric mode on an exercise machine	Plasma Pre-, Post-2, 4, 6, 20 h and 1,2, 3, 6 and 9 d after the exercise session	IL-6 increased at 6 h compared to Pre-Ex. By 20 h post-exercise IL-6 levels return to baseline values and continued the same for the rest of the time points, except for a higher value at 24 h compared to Pre-Ex	Healthy
Hirose (2004)	10 healthy men untrained (20 ± 2 yr) performed 2 bouts of eccentric action of the elbow flexor using the same non dominant arm	6X5Rep40%1RM 2'rest for each exercise bout	Plasma pre-, immediately post-1,3 h and 1-4 d after each test exercise session	IL-1β,IL-6, and TNF-α After the 1st bout there was an unexpected decrease in plasma pro-inflammatory TNF-α (1, 3 h and 1 d) Post-ex. There were no changes in the rest of the cytokines between bout or Pre-ex	Healthy

	separated by 4wks				
Peake (2006)	10 healthy untrained men (23 ± 5 yr)completed a sub maximal followed by a maximal exercise protocol vs non-dominant arm randomized and counterbalanced	**Submaximal**: 10X60rep10%1RM elbow flexor of one arm **Maximal**: 10X3rep100%1RM elbow flexor of the opposite arm	Serum Pre-,Immediately Post-1,3 h and 1-4 d after each test exercise session	IL-1ra, IL-6,TNF-α IL-6 was elevated 3 h Post-ex after Submaximal but not for Maximal protocol. The rest of the cytokines remain unchanged after the exercise protocols, with a trend for an increase in IL-1ra	Healthy
Uchida (2009)	35 male Brazilian soldiers (19 ± 2 yr)physically trained but not involved in RT for at least 1 yr	A **control** (n = 6) plus 4 groups follow 1 single bench press **exercise**: 4X20Reps 50%1RM (n = 8) 5X11Reps 75%1RM (n = 7) 3X10Reps 90%1RM (n = 7) 3X10Reps 110%1RM	Plasma Pre-exercise and 24, 48 and 72 h after the test exercise session	IL-1β and IL-6 No changes in any cytokine compared with pre-exercise. No differences in cytokine responses among the different intensity protocols. Cytokines levels were not even detectable for some of the participants.	Healthy

		(n = 7) 2’ rest Same total load volume for all groups			
Phillips (2010)	14recreationally active men (untrained for RT)(22 ± 2 yr) followed 2different exercise protocols and a rest condition in a randomized order	8 Exercises working major muscle groups of upper and lower body. Low intensity: 2X12Reps 65%1RM High intensity: 2X8Reps 85%1RM and 3rd set until exhaustion for both protocols. 2’ rest	Plasma Pre-,Immediately Post and6 h after each test exercise session	IL-6 Increased immediately Post-exercise compared to control for both exercises and went back to baseline levels at 6 h. The Low intensity protocol resulted in the highest total volume load and greater circulating IL-6 compared to the High intensity protocol at the Post-Ex time point.	Healthy
Buford (2009)	24 recreationally active women (untrained for RT) (54 ± 4 yr)	3 exercises: squat, leg press and leg extension. 3X10Reps 80%1RM for each exercise	Serum and muscle leg biopsies at baseline and 3 h post-exercise to determine cytokines mRNA expression	IL-1β, IL-6 and TNF-α No changes in serum cytokines after the exercise session. But there was mRNA up-regulation for TNF-α, IL-1β and IL-6 Post-exercise.	Healthy
Bente (2000)	Resistance		TNF-α, IL-6 and IFN-γ	the expression of a broad spectrum of cytokines in response to exercise is possible	Healthy
Pietro Galassett	6 male /6 female	30-min exercise challenge @ 80%	Plasma	IL-6↑	T1D

(2006)		VO_{2max}			
Belotto MF (2010)	Rats	3-week moderate exercise on a treadmill (60% of VO_{2max}	Serum	TNF-α (6%) (decreased) IL-1β (34%) (decreased) IL-6 (86%) (decreased)	Diabeticrats
Jenni S (2010)	7 male	Moderate aerobic exercise	Plasma	IL-6 (increased)	T1D
Rosa JS (2010)	47 children	30 min of cycling exercise @ 80% of VO_{2max}	Plasma	IL-6 (increased)	T1D
Ho SS (2013)	----------	12 weeks of moderate-intensity aerobic, resistance, or combination exercise		TNF-α (decreased) with aerobic exercise group resistance group and in the combination group	Healthy
Galassetti et al (30)	12 children	30-min exercise challenge @ 80% VO_{2max}	Plasma	IL-6 (increased)	T1D
Nikolao (2007)	28 exercise group and 26 control group.	16-week,45– 60 min sessions per week aerobic exercise walking or running on treadmill, cycling	Plasma	IL-6 decreases resistant and inflammatory cytokines	Healthy
Kyle (2008)	15 physically active PA 15 inactive group PI	12 weeks (3 days/week) of endurance PI: (20 min at 70–80% heart-	Plasma	TNF-α ↑ IL1-β ↑ CD14 ↓	Healthy

		rate reserve) and resistance exercise training (eight exercises, two sets at 70–80% of one repetition maximum).		CD16 ↓ Monocytes ↓	
Nienam (2004)	30 strengths trained male athletes (21 ± 1 yr)	Already strength trained 10 different exercise of upper and lower body, (lasted 2 h) 1X10Reps 40%1RM +3X10Reps 60%1RM 2' and 3' rest between sets and exercises respectively	Muscle tissue (vastus lateralis) and plasma Pre-Ex and Post-Ex.	Muscle (mRNA): IL-1β, IL-6 and TNF-α Muscle mRNA was detected Pre-Ex for IL-1β, IL-6and TNF-α and of these, IL-1β, IL-6 and TNF-α mRNA were increased Post-Ex. Plasma: Plasma IL-6were modestly but significantly increased Post-Ex.	Healthy
Stewart (2007)	Young (25 ± 5 yr) and old (71 ± 4 yr) men and women were divided in YPA(15) or YPI(14) and OPA(14) or OPI(17)	12 wk (3 d/wk) The physically inactive trained for 12 wk and the physically active participants serve as Controls 20 min warm up (walking or jogging) and 2 sets x 8 exercises at 70-80%1RM (1st set) and muscular failure (2nd set),	Plasma baseline and after 12 wk training at rest	IL-1β, IL-6 and TNF-α No age or physical activity differences in IL-6 or IL-1β among groups at baseline or after training. TNF-α levels were higher in the young group (PA and PI) compared to the old group. No effects on TNF-α with training	Healthy

		stretching and cooling period			
Izquierdo (2009)	12 physically active men untrained (33 ± 4 yr)	7 wk (2 d/wk 45-60 m/session) Non-linear undulating multi set progressive program Pre training and Post training test 5X10RM leg press. The Post training test using the same absolute load (kg) or another day using the same relative load (%RM) than pre-training. 2’ rest between sets	Plasma baseline and after 7 wk training: Pre-ex, middle-ex, Post ex 0, 15 and 45 min	IL-1β, IL-1ra and IL-6 Post-training: there was a greater released of IL-1β and IL-1ra after both exercise tests (absolute and relative load). However the increases in IL-6 and Intensity plays a role in anti-inflammatory cytokine responses	Healthy

4.2. Objectives of the Study

The purpose of the present study is to examine the effects of one exercise session (acute exercise) and 12 sessions (chronic exercise) of combination, (resistance) and aerobic exercise (cycling) on selected immunological parameters such as IL-6, TNFα, IFNγ and IL-1β on typ1 diabetic (T1D) either MDI or CSII and nod diabetic subject (ND).The study was approved by the Ethics Committee of De Montfort University, Leicester (DMU).

4.3. Materials and Methods

4.3.1. Study Volunteers

Physically inactive male, aged 18–55 years, either healthy (non-diabetic ND) or diagnosed with T1D and using MDI or CSII were recruited for this study. All subjects were apparently healthy and free of disease other than diabetes.

Study recruitment was publicised through internal and external adverts as described in section 2.4.1.Majority of volunteers were from DMU either staff or students.

Exclusions criteria were playing an important role. The total number of volunteers who expressed an interest in this study was 49 individuals (ND= 24, MDI= 14, CSII= 11), 14 were excluded as they did not meet the inclusion criteria. In addition, 16 participants were unable to attend as a result of the difficulty of making arrangements. The eventual sample was 19 participants. Volunteers were divided into three main groups; ND group (N=7), T1D using CSII group (N=5) and T1D using MDI (N=7).

4.3.2. Screening and preliminary testing

All subjects were underwent a preliminary health screening and were required to obtain approval from their personal GP before being enrolled in the study. Subjects involved in this study were read and signed the exclusions and inclusion criteria forms. Furthermore, others forms were provided and signed by the volunteers themselves such as; Standard Operation Procedure (SOP), Volunteer Information Sheet (VIS), Consent Form, Health Screen and Record forms were provided (see section 2.4.2).

After read and signed consent form, subjects reported to the laboratory and completed a health screen questionnaire, and a record sheet.

After preliminary screening, a one-repetition maximum (1RM) was measured for resistance exercises (see section 2.5.6).

4.3.3. Exercise Training

All subjects attended the demonstration and practice sessions prior to beginning the exercise training program. On 1RM day, subjects were instructed in proper lifting technique and use of exercise equipment. The 1RMwas assessing each of the following resistance exercises: Squat, incline bench press, lat curl pull-down, triceps and biceps

In the acute exercise time, all volunteer groups were asked to exercise for only one session (3 sets 10 rep each) at moderate intensity while the chronic stage involved 12 sessions (48 hours between each session). As described in figure 4.2 and section 2.5, each session was including stretching, warm up and then resistance exercise at 50% of

volunteer 1RM then rest for 5min after that cycle for 20 min at moderate intensity at 50-60% of heart-rate reserve on a recumbent ergometer bike then cool down.

Heart rates (HR) were checked regularly at rest and after each set of resistance exercise. HR was also checked during all aerobic exercise to ensure that they were still exercising at the specified intensity level.

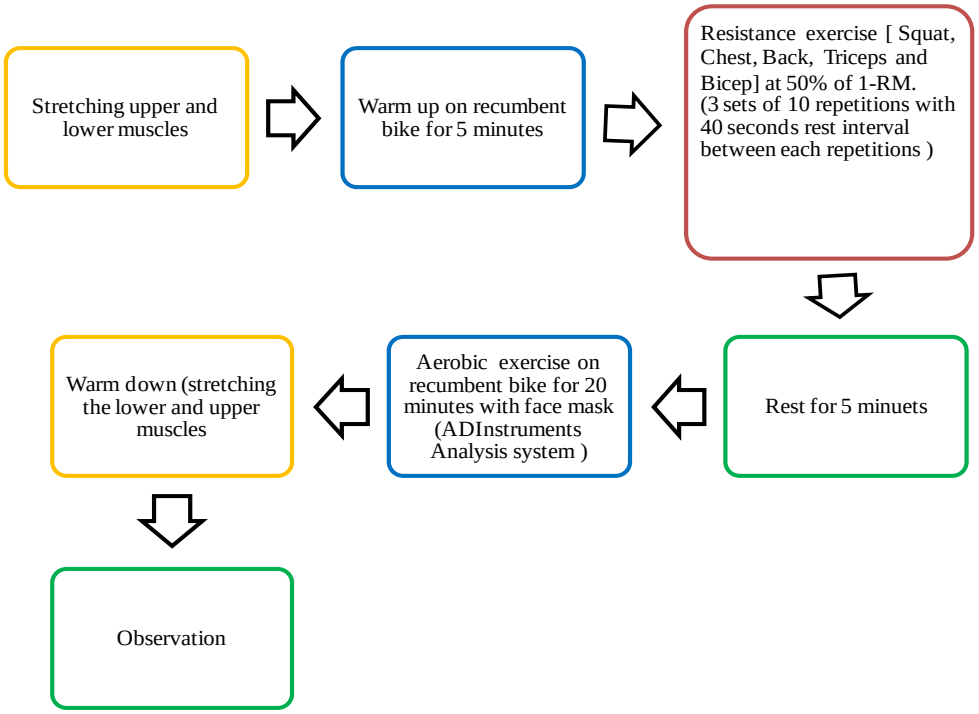


Figure 4.2: Division of the exercise sessions throughout the study

4.3.4. Blood collection

All the blood samples were collected and analysed at DMU, School of Pharmacy, Leicester, UK. From each subject, 10 ml of blood was collected in EDTA tube from antecubital vein then centrifuged at speed of 3000rpm for 15min at 4°C. All sera were aliquoted and stored at -80°C until the time of analysis.

During this study, three blood samples were collected from each subject at baseline (before any exercise), after resistance and after cardio exercise for all groups (figure 4.3). A total of 21 blood samples were collected from MDI group, 15 from CSII group and another 21blood samples were collected from ND group i.e. a blood sample from each volunteer.

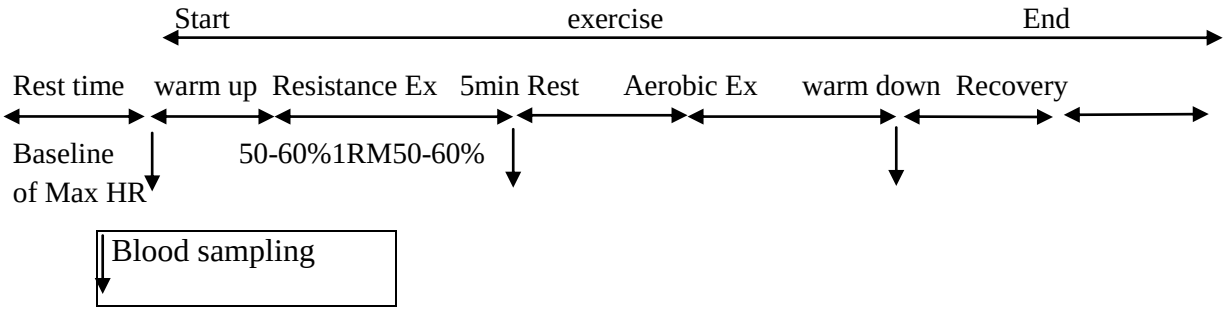


Figure 4.3: Exercise protocol and blood sampling scheme for acute exercise (one session).

In the chronic exercise, seven blood samples were collected from each subject at baseline, then after 2nd, 4th, 6th, 8th, 10thand after 12th sessions (figure 4.4). A total of 42 samples were collected from MDI group and 35 from CSII group. Another 42blood samples were collected from ND group.

A total of 171 blood samples; 63 were collected from MDI group, 45 from CSII group and 63 blood samples were collected from ND group.

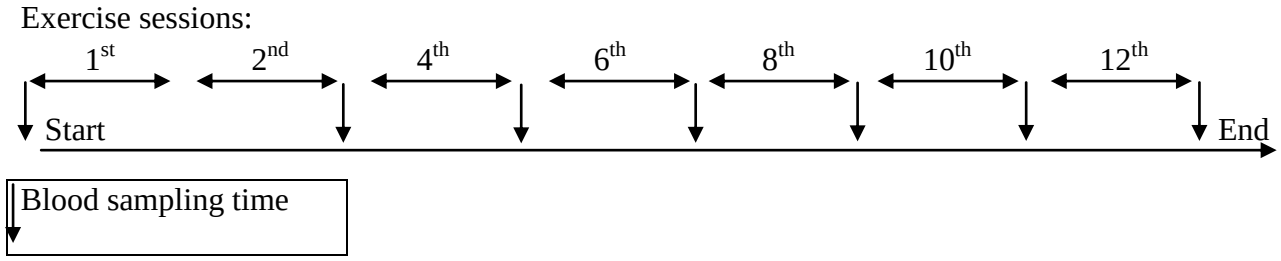


Figure 4.4: Blood sampling scheme for chronic exercise.

4.3.5. Statistical Analyses

Descriptive statistics e.g. mean, variance and standard deviation were used to express the data from all cytokines concentrations unless otherwise stated. Test of statistical significance based on two tailed t-test and p-value were conducted using 0.05 level of significance (i.e. α=0.05).All data were analysed using the Statistical Package for Social Sciences (IBM SPSS, v.19, Chicago, IL).

4.3.6. Cytokine Measurements

Determining the effect of acute and chronic exercise on selected inflammatory cytokines on T1D either MDI or CSII users and to compare these with ND was one of the main objectives of this study. Selected cytokines were examined; IFN-γ, TNFα, IL-6 and IL-1β. To determine the level of these cytokines, Sandwich Enzyme-linked Immunosorbent Assay (ELISA) was used. ELISA assay was developed using commercial kits (DuoSet ELISA Development Kit, R&D Systems).

Briefly, capture, detecting and standard antibodies were diluted to the recommended working concentration as in section 2.10.1. The 96-well plate was coated with 100 µL per well with diluted capture antibody and incubated at room temperature for overnight. Each well was aspirate and wash by filling with wash buffer (400 µL), for a total of three washes. After the last wash, remaining wash buffer were removed by aspirating or by inverting the plate and blotting it against clean paper towels. This was followed by blocking the plate by adding 300 µL of reagent diluent to each well and incubated at room temperature for 1 hour then washed as above. Then 100 µL of sample or standards were added and incubated for 2 hours at room temperature then washed. 100 µL of

detection antibody were added and incubated for 2 hours at room temperature and washed.100 µL of the working dilution of streptavidin-HRP was added to each well and incubate for 20 minutes at room temperature and washed. Substrate Solution100 µL were added to each well and incubated for 20 minutes at room temperature. It was important to avoid placing the plate in direct light. After that 50 µL of Stop Solution were added to each well. Finally determine the optical density of each well. Readings made directly at 450 nm without correction.

4.4. Results

4.4.1. Acute exercise (one session)

Pre-exercise plasma concentrations of IL-6 were lower in the CSII group compared with MDI and ND group. The IL-6 concentration was significantly higher in MDI group than in CSII and ND groups ($P<0.001$).In CSII and ND a statistically significant increase in IL-6 level was observed after resistance and cardio exercise ($p = 0.025$). The CSII volunteer group showed elevated levels of IL-6 after resistance and cardio exercise and at both stages this rise was statistically significant ($P=0.033$ and $P=0.014$ respectively). After resistance and cardio exercise MDI group showed elevated IL-6 levels but observed changes were not statistically significant as it failed t-test (figure 4.5). The statistical significance values for effect of exercise on IL-6 are detailed in table 4.3.

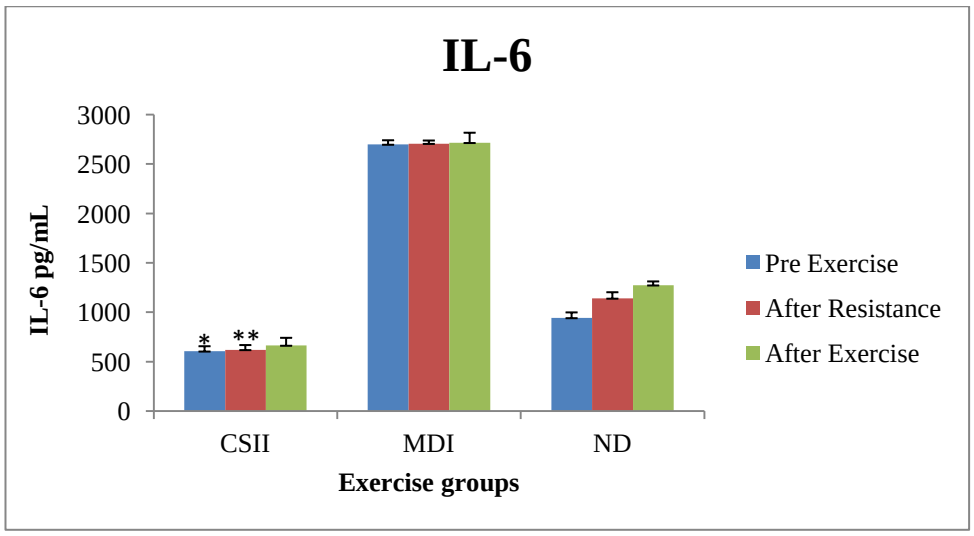


Figure 4.5: Acute plasma levels of inflammatory cytokines IL-6.

Table 4.3: Description of p-value (t-test) for the effects on IL-6 of the acute exercise phase in MDI and pump users, comparing before and after, with respect to the resistance (Re) and cardiovascular (aerobic).

Acute exercise for IL-6			
	Before vs. after exercise	Before vs. after Resistance exercise	After Resistance vs. after cardio exercise
ND	0.34	0.71	0.42
MDI	0.89	0.87	0.94
CSII	0.033	0.26	0.014

It was observed that volunteers in CSII groups have the lowest level of TNF-α. TNF-α was decreased in all exercise groups compared to the pre-exercise condition. In CSII group this reduction was statistically significant especially between pre exercise and after resistance (P=0.0002). But in MDI and ND groups this drop n TNF-α was not statistically significant (Figure 4.6).The statistical significance values for effect of exercise on TNF-α are shown in table 4.4.

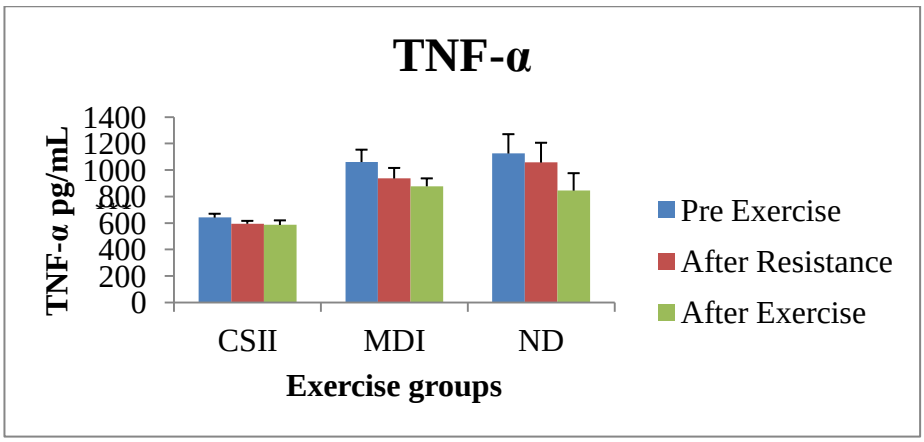


Figure 4.6: Acute plasma levels of inflammatory cytokines TNF-α. CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. T-test P-value was for before and after exercise, after RE and after exercise, The error bars show the Standard Deviation (SD).

Table 4.4: Description of p-value (t-test) for the effects on TNF-α of the acute exercise phase in MDI and pump users, comparing before and after, with respect to the resistance and cardiovascular (aerobic).

Acute exercise for TNF-α			
	Before vs. after exercise	Before vs. after Resistance exercise	After Resistance vs. after cardio exercise
ND	0.14	0.70	0.26
MDI	0.14	0.36	0.12
CSII	0.09	0.0002	0.71

Figure 4.7 is showing a significant reduction in plasma levels of IL-1β in CSII group with MDI and ND groups (P=0.0081, P=0.026, respectively). Moreover, there was significant increase in IL-1β level in CSII group (P=0.006) and (P=0.0011), (pre and after resistance, after resistance and at the end, respectively) after one session. In ND group IL-1β level was decreased after exercise (i.e. resistance and cardio). While, in the MDI group, IL-1β was decreased after resistance exercise and slightly raised after aerobic exercise, however, post exercise session the IL-1β level was still less than the pre-exercise levels. The statistical significance values for effect of exercise on IL-1β are displayed in table 4.5.

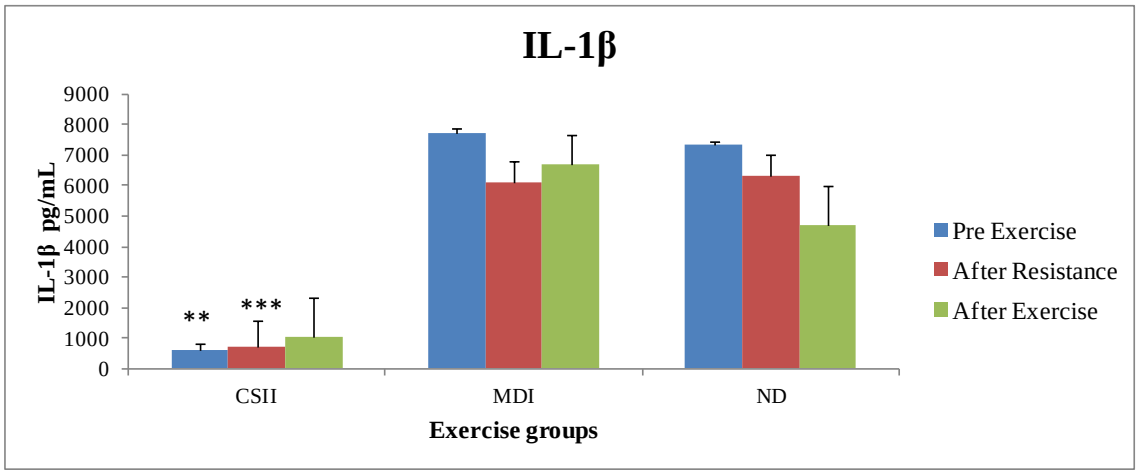


Figure 4.7: Acute plasma levels of inflammatory cytokines IL-1β. CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. T-test P-value was for before and after exercise, after RE and after exercise, and bars showing SD

Table 4.5: Description of p-value (t-test) for the effects on IL-1β of the acute exercise phase in MDI and pump users, comparing before and after, with respect to the resistance and cardiovascular (aerobic).

Acute exercise for IL-1β			
	Before vs. after exercise	Before vs. after Resistance exercise	After Resistance vs. after cardio exercise
ND	0.14	0.45	0.31
MDI	0.56	0.30	0.22
CSII	0.006	0.34	0.0011

The acute plasma level of IFN-γ was significant lower in CSII group compared to MDI and ND groups (P=0.018, P=0.0045, respectively). In CSII and ND after resistance exercise IFN-γ was dropped and increased slightly after cardio exercise but was still lower than its initial level (figure 4.8).The statistical significance values for effect of exercise on IFN-γ are given in table 4.6.

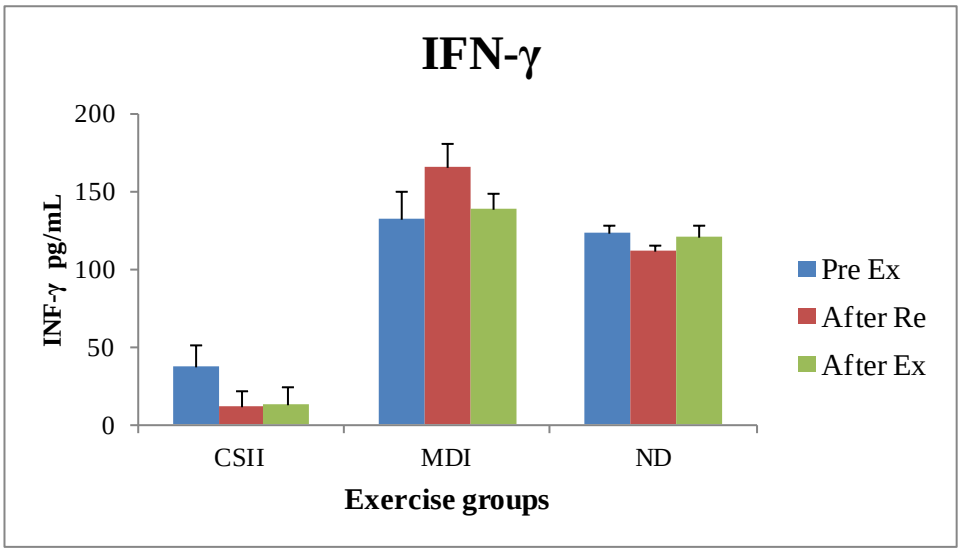


Figure 4.8: Acute plasma levels of inflammatory cytokines IFN-γ . Ex, exercise; Re, resistance; CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. with error bars showing SD

Table 4.6: Description of p-value (t-test) for the effects on IFN-γ of the acute exercise phase in MDI and pump users, comparing before and after, with respect to the resistance and cardiovascular (aerobic).

Acute exercise for IFN-γ			
	Before vs. after exercise	Before vs. after Resistance exercise	After Resistance vs. after cardio exercise
ND	0.89	0.34	0.49
MDI	0.87	0.44	0.35
CSII	0.38	0.35	0.60

4.4.2. Chronic exercise (12sessions)

As mentioned in section 4.3.4 during this part of the study 7 blood samples were taken from each subject (see figure 4.4). The group of pro-inflammatory cytokines including IL-6, TNF-α, IFN-γ, and IL-1β have been associated with β cell destruction in vitro (Suk 2001). To determine the effect of exercise on these pro-inflammatory cytokines, plasma blood samples were collected from CSII, MDI and ND groups in different time points. These samples were collected before exercise and after 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} sessions.

Figure 4.9 demonstrates that throughout the study the level of IL-6 is lower in CSII group compared to MDI and ND groups and this difference was statistically significant (i.e. all P values less than 0.05). The level of IL-6 was high pre exercise in MDI group then decreased over the sessions. EURODIAB study has reported significantly higher levels of IL-6 in T1D sufferers on MDI therapy (Schram, Chaturvedi et al. 2005). Moreover, Jain, Kannan et al. (2003) also showed that in T1D hyper ketonemia elevates the circulating levels of plasma IL-6 (Jain, Kannan et al. 2003). Throughout this study IL-6 level in ND group was increased from its baseline levels (i.e. before any exercise) (P=0.047). In CSII, there was no significant change in IL-6 over the time. MDI and ND groups have almost the same level of IL-6 after sessions 4, 6 and 8 and in MDI blood samples taken at sessions 10 & 12 showed a drop in IL-6 level and were below the level observed in ND.

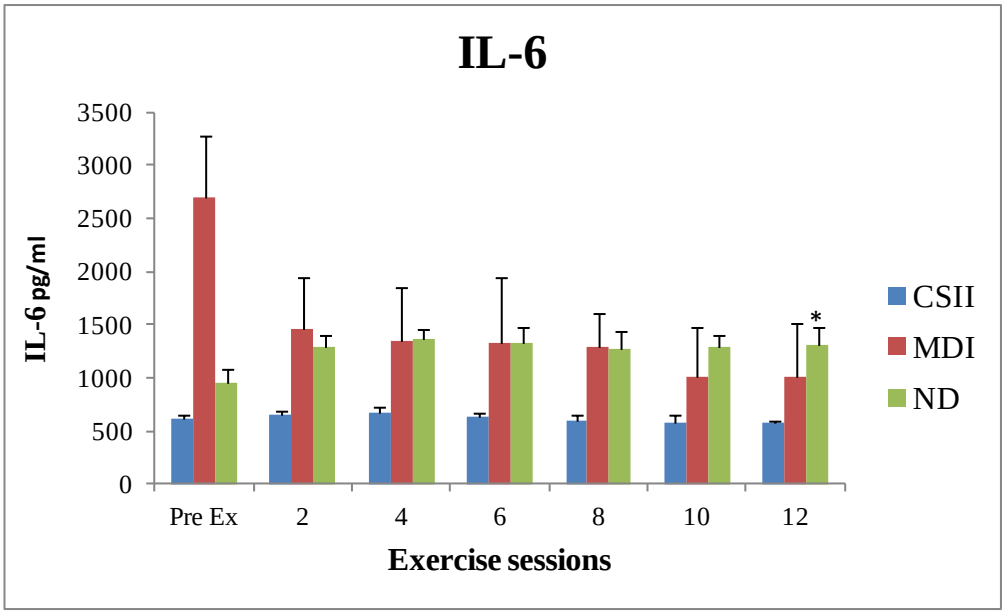


Figure 4.9: Chronic plasma levels of inflammatory cytokines IL-6. Ex, exercise; CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. Several T-tests were conducted across periods of exercise and all p-value were below 0.05. Error bars show SD.

Throughout the study TNF-α among the group was different and this difference was found to be statistically with all P-values less than 0.05. There was a small though statistically significant increase in TNF-α level, as compared with the baseline level, in CSII group after sessions 6 (P=0.055), 8 (P=0.048)10 (P=0.056) and 12 (P=0.056). A significant increase was only seen in the last session in ND group (P=0.039) and no significant change in MDI group (figure 4.10).

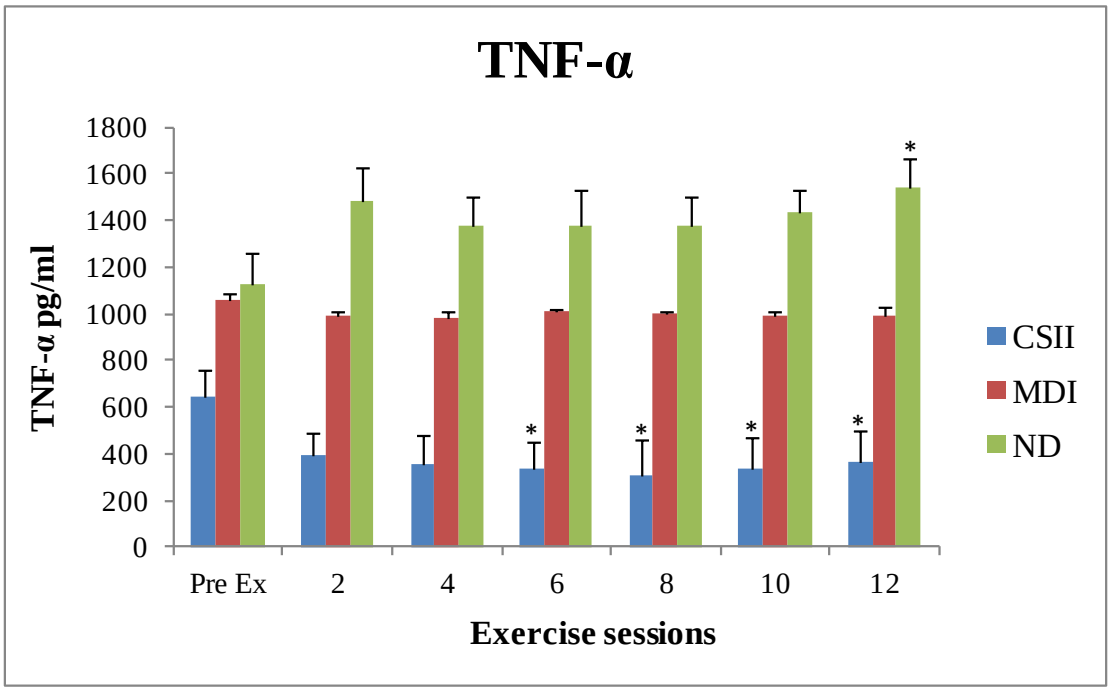


Figure 4.10: Chronic plasma levels of inflammatory cytokines TNF-α. Ex, exercise; CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. P-value was for before exercise and exercise sessions. T-test P-value was for pre exercise and exercise sessions, error bars show SD. P- value *≤0.05.

At all stages of the study statistically significant different level of IL-1β was observed among the groups, (i.e. all P-values were less than 0.05). The level of IL-1β was reduced over the sessions in CSII group. In ND group blood sample taken after session two showed an increase in IL-1β level as compared to baseline level but samples taken

after later sessions showed a small drop in IL-1β level where the difference between session 2 and 12 was statistically significant (P=0.033). In the MDI group, IL-1β was decreased after second session then sustained an increase value after session 4 (figure 4.11).

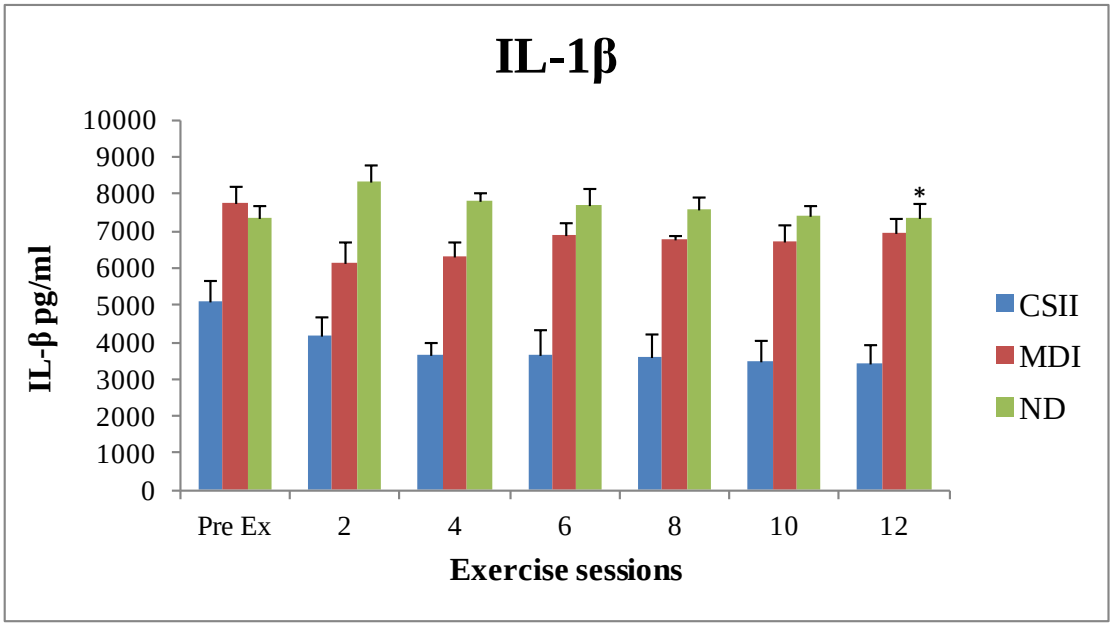


Figure 4.11: Chronic plasma levels of inflammatory cytokines IL-1β. Ex, exercise; CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. P-value was for before exercise and exercise sessions. T-test P-value was for pre exercise and exercise sessions, Error bars show SD. P- value *≤0.05.

The blood samples taken before exercise showed that IFN-γ level in CSII group is lower than the level found in other two groups i.e. MDI and ND. However, sample taken after session 4 and 12 showed that the level of IFN-γ in CSII rose above the levels observed in other two groups. In MDI group the level of IFN-γ declined through the sessions becoming significantly lower in the last two sessions with P=0.018and P=0.031. While in ND group although the level of IFN-γ dropped from its baseline level but small fluctuations in level of IFN-γ was observed during the study but blood sample taken after session 12 showed a significant drop from its baseline reading (figure 4.12).

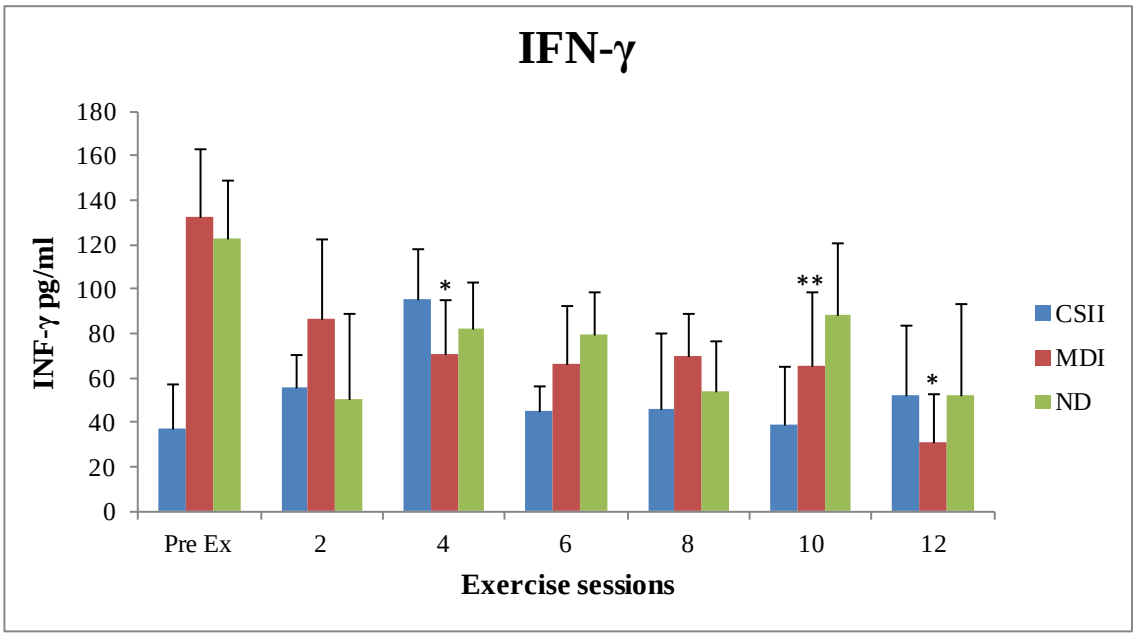


Figure 4.12: Chronic plasma levels of inflammatory cytokines IFN-γ . Ex, exercise; CSII, Continuous Subcutaneous Insulin Infusion; MDI, multiple daily injections; ND, non-diabetic. P-value was for before exercise and exercise sessions. T-test P-value was for pre exercise and exercise sessions, Error bars show SD. P-value *≤0.05, **≤0.01.

4.5. Discussion

The subjects of this study were drawn from Non-Diabetic (ND), T1D sufferers using Multi Daily Injection (MDI) and T1D sufferers using insulin pump as continuous subcutaneous insulin infusion (CSII) groups. A venous blood sample was taken on arrival, during exercise (after the resistance and before cardio) and after the full programme of exercise (including cardio). Clear differences could be seen for the CSII group for many of the parameters when compared to normal and MDI groups.

Before discussing these, it is important to show that the cardiovascular exercise was predominantly aerobic in nature, both for this study and for its use as a pilot for the future work associated with this study. For example, if the cycling amounted to very intense exercise, this might have increased an anaerobic component, particularly in the

early stages. One criticism might be levelled, for example, in the setting of the target heart rate. There is much in the literature about the methodology for doing this. The importance is that if there is an overestimate of resting heart rate, the calculated target heart rate would be too high for the intended moderate intensity, using the Karvonen formula and this might produce an unintentionally high anaerobic component into what should be a mainly aerobic exercise (i.e. the cardiovascular). This equation has been used since the 1950s but has been criticised for its failure to relate directly to VO_{max} and to be based in the original, on too few subjects (Goldberg, Elliot et al. 1988; Tabet, Meurin et al. 2006; Skidmore, Patterson et al. 2008; Shnayderman and Katz-Leurer 2013). Nevertheless, this or similar equations are used and importantly, the much repeated advice is to take the resting heart rate in the morning before rising.

This normal advice on timing of the resting heart rate measurement was not followed for this study because of the overnight variations likely to occur in blood glucose in the diabetic subjects as had been reported and discussed at the beginning of this study. Thus, in diabetes, it is a common thing to have a hyperglycemic value on waking ("dawn phenomenon") and this may not affect heart rate. However, there is also a strong possibility of spontaneous hypoglycemic events that trigger the counterregulatory processes involving glucagon (Mukherjee, Carroll et al. 2011). These are mediated by the sympathetic nervous system and thus epinephrine (Somegyi rebound) (Mukherjee, Carroll et al. 2011). The symptoms of these events, apart from the direct effects of low blood glucose, are palpitations in the early hours via the sympathetic stimulation (Mukherjee, Carroll et al. 2011). Normalisation of this cardiovascular disturbance may take time and require intervention using either pump adjustment to reduce insulin or

increased glucose directly or by injected glucagon. Some T1D people have this several times per month as overt symptoms and others may have it but not sense it each time (Mukherjee, Carroll et al. 2011). Consequently, the heart rate is typically higher in the early morning for insulin users than late at night and actually, the heart rate for healthy people is little different at night than in the early morning, provided they are at rest, as demonstrated numerically in the classic paper by Burger on circadian heart rhythms in normal and diabetic people (Burger, Charlamb et al. 1999). For these reasons, our methodology included instruction to take the heart rate reading in bed at night and volunteers were given heart rate monitors for this purpose. Occasionally they reported that they forgot at night and had done a morning reading, but we accepted the reading (average of three) anyway. We also took a reading of heart rate at the beginning of the exercise event, but this was unrelated to the calculation and was for records, comparisons during the procedures and for safety. This measurement at night for the calculation ensured that the target heart rate for the cycling component should have been in the aerobic region except possibly in the very initial minute or so.

The results of analysis of these samples could be summed up as:

In the acute exercise phase:

- In figure 4.5, the lowest plasma concentrations of IL-6 were found in CSII group. IL-6 was increased significantly in CSII and ND groups. IL-6 level was significantly higher in MDI group than CSII and ND groups. No significant differences in IL-6 level were found among MDI volunteers at the end of this session.

- Figure 4.6 shows, plasma level of TNF-α were decreased in all exercise groups.

- Figure 4.7 is showing the plasma level of IL-1βwas significantly increased in CSII group. In ND group IL-1β level was decreased but not significantly. In MDI group, IL-1β was decreased after resistance exercise while aerobic exercise increased the levelIL-1β.

- Figure 4.8 demonstrate the plasma level of IFN-γ . It was observed that IFN-γ levels in ND and CSII followed the same pattern. IFN-γ was decreased by resistance exercise in both CSII and ND groups while increased in MDI. The lowest level of IFN-γ was found in CSII group. However, aerobic exercise increased the level of IFN-γ in CSII and ND groups. ND and CSII IFN-γ levels were high before exercise but dropped significantly after resistance but after the cardio exercise showed a tendency to rise again.

After this initial analysis subjects were invited to take part in a 6 weeks long (12 sessions) chronic study. This study the results demonstrate that:

- Figure 4.9 reported the plasma levels of IL-6. The CSII group has the lowest level of IL-6 all over the sessions (lower than normal level as in ND group). MDI has the highest level of IL-6 before exercise (high than normal level as in ND group). IL-6 level was decreased in MDI (dropped to normal) and increased in ND group after second session.
- In figure 4.10 the level of TNF-α were below normal before exercise in MDI and CSII groups and then TNF-α level was significantly low in both CSII and MDI groups compare to ND group during all the exercise sessions. There was a significant increase on TNF-α level in CSII group after 3 sessions (6th, 8th, 10th and 12th). While ND group increased TNF-α level after second session but

significantly increased only in the last session. No significant change in MDI group.

- Figure 4.11 shows the level of IL-1β. The lowest level of IL-1β was found in CSII group all over the study. Plasma levels of IL-1β were decreased in all study groups compare to before exercise levels except the second session in ND group where an increase in IL-1β levels were observed. IL-1β was decreased after second session then increased in MDI group.
- Figure 4.12 shows the lowest level of IFN-γ was reported in CSII groups. MDI and ND groups have almost equal of high level of IFN-γ before exercise but this level then decreased over the exercise sessions. The significant decreased on the IFN-γ level was only found in MDI group in the last two sessions. The peak of IFN-γ level for CSII groups was found after 4 sessions.

The cytokine reaction to exercise is different from that provoked by severe infections (Febbraio and Pedersen 2002; Suzuki, Nakaji et al. 2002). For example, the fact that the classic pro-inflammatory cytokine IL-1β, does not increase during exercise, suggests that the cytokine cascade that is provoked by exercise differs significantly from the cytokine cascade prompted by infections (Petersen and Pedersen 2005). Figure 4.13 demonstrates the process of the production of pro-inflammatory cytokines in immune cells. This diagram shows several TLRs and endosome on membrane are activated which eventually lead to translocation of NF-κB and interferon regulatory factors (IRFs) in a MyD88 (myeloid differentiation factor 88)/TRAF TNFr-dependent manner. These translocations activate transcription of multiple pro-inflammatory cytokines such as TNF, IL-6, type-I interferon (IFN) and pro-IL-1β. TNF, IL-6 and IFN are secreted into extracellular space and elicit systemic inflammatory responses. The inflammasome

assembly digests procaspase1 and generates caspase-1, an active form of cysteine protease, which converts pro-IL-1β into the mature active forms IL-1β. Secreted IL-1β and induce systemic inflammatory response (Nakayama and Otsu 2013). In our study we report that the plasma level of IL-1β in the acute exercise was significantly increased in CSII group while a decreased in IL-1β level in ND group. In MDI group IL-1β was decreased after resistance only. In the chronic exercise sessions the lowest level of IL-1β was found in CSII group. Plasma levels of IL-1β were mainly decreased in all study groups compare to IL-1β level before exercise levels.

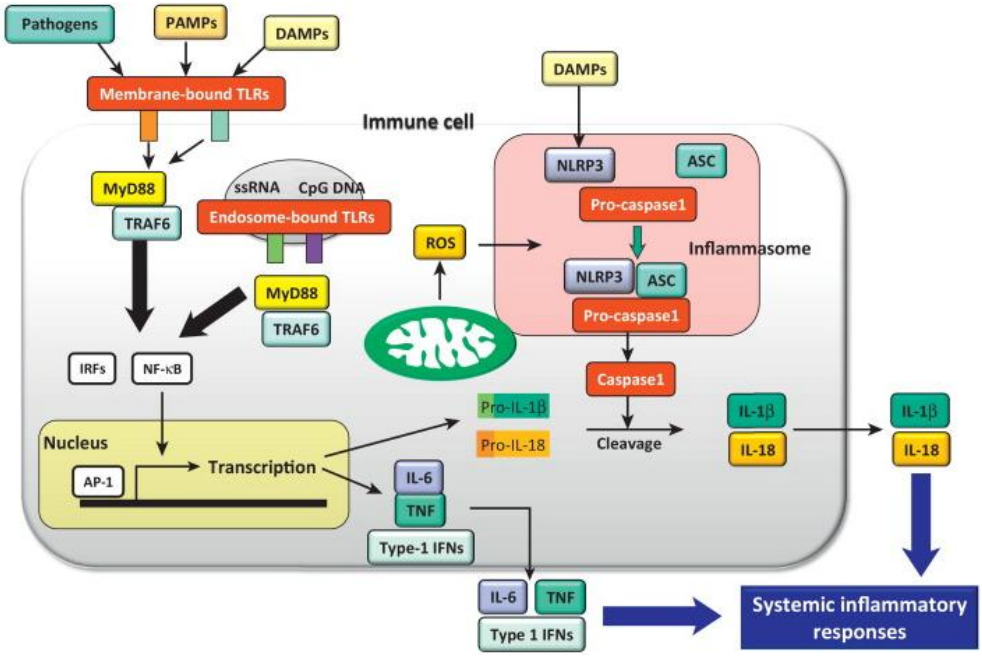


Figure 4.13: Production of pro-inflammatory cytokines in immune cells (see text).

The immune reactive profile for exercise also differs from that of autoimmune diseases like T1D i.e. the immune system acts destructively and usefully for infections, destructively and non-usefully for autoimmune disease and constructively and usefully for exercise. The high level of IL-6 in MDI was reported in the EURODIAB study as significantly high in T1D (Schram, Chaturvedi et al. 2005). Moreover, Jain, Kannan et

al. (2003) showed that hyperketonemic in T1D patents increased level of plasma IL-6 (Jain, Kannan et al. 2003). However the level of IL-6 in CSII was the lower than MDI and this may explain the lack of DKA in CSII group. In this study the level of IL-6 was significantly increased in ND group. The increase in IL-6 after exercise is a remarkably consistent finding (Pedersen & Hoffman-Goetz, 2000; Pedersen et al. 2001; Febbraio & Pedersen, 2002). Pedersen et al. in (2001) and (2003), Febbraio & Pedersen, (2002) reported the increase in plasma IL-6 is clearly demonstrated by muscle contractions without any muscle damage (Pedersen et al. 2001, 2003; Febbraio & Pedersen, 2002). However, the exercise duration and intensity has an effect on the increase of IL-6. Moreover, it has been suggested that the increase in IL-6 after exercise is related to the sympatho-adrenal (realised from muscles) response to exercise (Nehlsen-Canarella et al. 1997). In CSII, there was no significant change in IL-6 over the time. Our result in CSII may also result in the number of carbohydrates ingested by CSII users which was also reported in the Nehlsen-Canarella et al. (1997) study, when they linked the increase in IL-6 level during exercise with the carbohydrate ingestion before exercise session (Nehlsen-Canarella et al. 1997). Febbraio et al. (2002b) as well demonstrated that the release of IL-6 from working muscle is related to carbohydrate ingestion during moderate exercise (Febbraio and Pedersen 2002).

As discussed previously, T1D is an autoimmune disease where autoreactive T cells damage the insulin-producing β-cells in the pancreas (Diana, Gahzarian et al. 2011). Recent pathological studies of T1D have associated both innate and adaptive immune systems' cells with the disease (Diana, Gahzarian et al. 2011).

A NOD mouse study shows that during the growth of T1D, numerous interactions take place between innate immune cells (e.g. macrophages, DC, NK, NKT etc.) and adaptive

lymphocytes, including anti-islet T cells, involving CD4 and CD8 markers as have been noted clinically (Diana, Gahzarian et al. 2011). They observed that IFN-γ is important in this inflammatory mechanism, while Kristiansen and Mandrup-Poulsen (2005) suggest that inflammatory mediators such as TNF-α, IL-1β and IL-6 play a key role in the inflammatory development of both T1D and T2D.

This role of T-Cells in the development of T1D is still a debateable issue because there is another T-cell population which reduces pathological responses of autoimmune system. For example, forkhead box P3 (Foxp3) (see figure 4.14), molecules generated by regulatory CD4 T cells, prevent the advancement of diabetes (Tang and Bluestone 2008). Diana, Gahzarian et al. (2011) in the NOD mouse study discussed the protective function of such a T-cell population (Diana, Gahzarian et al. 2011).

Figure 4.14 is adapter from Herold, Vignali et al. (2013) and explains how regulatory T cells that have lost FOXP3 expression may contribute to autoimmune disease. The molecule forkhead box P3 (Foxp3) is expressed by regulation of CD4 T cells which lead to, inhibition of diabetes development (Tang and Bluestone 2008).In healthy individuals, developing thymocytes that do not express highly self-reactive T cell receptors (TCRs) mature and leave the thymus (left-hand panel). The forkhead box P3 (FOXP3) gene is methylated in these cells. By contrast, highly autoreactive T cells are deleted during development as a part of negative selection. Regulatory T (T_{Reg}) cells also develop in the thymus and, compared with conventional mature T cells, express TCRs that show increased affinity for self-antigens. Owing to the demethylation of the FOXP3 locus and expression of FOXP3 protein, TReg cells have anti-inflammatory functions. However, in patients with type 1 diabetes (T1D) and other autoimmune diseases, TReg cells may not maintain complete demethylation of FOXP3 owing to

defects in interleukin-2 signalling or other mechanisms (right-hand panel). These 'ex-TReg' cells remain autoreactive and, in the absence of FOXP3 expression, can produce potentially pathogenic pro-inflammatory cytokines. It has been suggested that such cells will participate in pathological immune responses to self antigens. In addition, failure to eliminate highly autoreactive T cells during thymic development may lead to the escape of potentially pathologic T cells into the periphery.

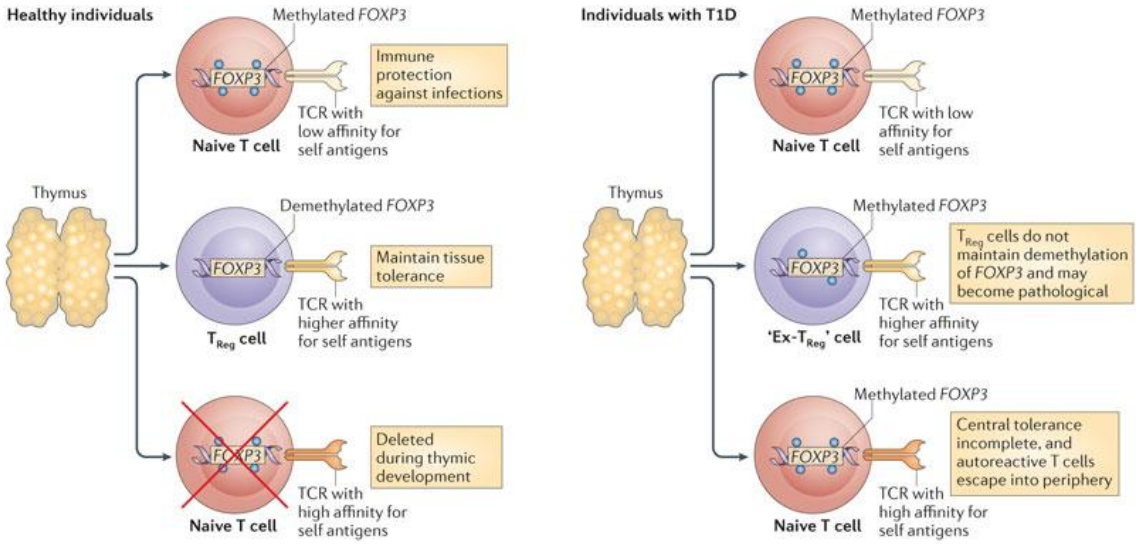


Figure 4.14: How Regulatory T cells, that have lost forkhead box P3 (FOXP3) expression, may contribute to autoimmune disease. Adapted from (Herold, Vignali et al. 2013).

Studies have shown that IL-6 mediate local inflammation and tissue destruction, and curbs the resistance of T cells against apoptosis, encourages activation of T helper cells and regulates the balance between regulatory T cells and Th17 cells (Neurath and Finotto 2011). IL-6 strategies are considered to be an innovative and effective line of action against the inflammatory diseases such as diabetes. Evidence gathered from several studies based on experimental models suggests that autoimmune and chronic inflammatory disease could be treated by blocking the IL-6 signalling (Neurath and Finotto 2011). As IL-6 has an ability to inhibit low-grade TNF-α production, IL-6 may

inhibit TNF-α induced insulin resistance (Nehlsen-Canarella et al. 1997). This statement supports our result in figure 4.5 (IL-6) and figure 4.6 (TNF-α), where our results show the increase in IL-6 and at same time the decrease in TNF-α level in all study groups to almost the same level. The increase in IL-6 level may have an important role in mediating the beneficial health effects of exercise in inactivity and obesity-related disorders such as diabetes (Nehlsen-Canarella et al. 1997) (Nieman, Nehlsen-Cannarella et al. 2008).

In one study neutralising antibodies were administered regularly to blockade IL-6 and as a result suppression of T1D was noticed. Rabinovitch (1998) discussed the role played by IL-6 in experimental T1D development. It was suggested that IL-6, like other cytokines e.g. TNF and Th1 is an important player in the development of the disease.

The present study suggests that in T1D who are using CSII, the IL-6 levels increased in acute (figure 4.5) and chronic exercise (figure 4.9), which demonstrates a positive effect of exercise. As Wallenius et al. (2002) have confirmed the advantage of high level on IL-6 in reduction of body weight in mice when treated with IL-6 (Wallenius, Wallenius et al. 2002). Moreover, Pedersen, Steensberg et al. (2004) concluded the biological roles of IL-6 as: (i) induction of lipolysis; (ii) suppress TNF-α production; (iii) stimulation of cortisol production (Pedersen, Steensberg et al. 2004).These results are supported by Langberg, Olesen et al. (2002) who argued that during exercise connective tissues cause a surge in the level plasma IL-6. While Fischer (2006) noticed that during exercise skeletal muscles produce IL-6 and IL-6 flows from muscle to plasma (Fischer 2006) Furthermore, at the end of the cycling session an elevated level of IL-6 was observed which falls back during the recovery period following the exertion (Steensberg, van Hall et al. 2000; Starkie, Rolland et al. 2001; Steensberg, Febbraio et al. 2001).

However, in the present study, MDI users demonstrated a decline in IL-6 during chronic sessions, which may be caused by lack of insulin in the system. This conclusion is supported by Noneless, Galassetti, Iwanaga et al. (2006) who witnessed effects of exercise and prior hyperglycemia on IL-6 levels in serum and suggested that the level of IL-6 in serum primarily depends on prior glucose levels and physical activity. On the other hand in CSII group below normal levels of IL-6 was observed before exercise but after resistance and cardio exercise sessions a significant increase in the IL-6 level was observed (see Figure 4.5).

As mentioned above in the present study it was observed that levels of IL-6 elevated after exercise while the levels of TNF-α declined. Keller, Keller et al. (2004) observed in knockout mice that decline in TNF-α level may not be associated with IL-6 and caused by some IL-6 independent mechanisms. It has also been reported that IL-6 triggers the production of anti-inflammatory cytokines (Petersen and Pedersen 2005) and inhibits TNF-α production in humans (Plomgaard, Bouzakri et al. 2005) and it is probable that muscle-derived IL-6 provides protection against TNF-induced insulin resistance (Petersen and Pedersen 2005).

In healthy humans as well as occasionally in T1D, high levels of TNF-α modifies insulin signal transduction and causes insulin resistance in the skeletal muscle (Plomgaard, Bouzakri et al. 2005). Therefore, reduced level of TNF-α, as seen after exercise in this study, reduces the insulin resistance in the skeletal muscle. It has been revealed that TNF-α did not have an effect on muscle fatty acid oxidation but amplified fatty acid incorporation into diacylglycerol, which may be involved in the creation of TNF-induced insulin resistance in skeletal muscle (Bruce and Dyck 2004). A rise in TNF-α lead to a rise in insulin resistance in other words actions of insulin are

susceptible to TNF-α arbitrated inhibition when insulin concentrations are low (which was revealed in the current study) and are the least susceptible when insulin concentrations are high (Zhang, Wheatley et al. 2003). This study confirms the fact that diabetes sufferers have an increased level of TNF-α in their skeletal muscle and in their plasma as compared with non-diabetics (see figure 4.10).

It is clear from the above discussion that exercise causes a surge in IL-6 and a decline in levels of TNF-α in T1D sufferer. Taking into consideration the diverse biological profiles of TNF-α and IL-6 and the fact TNF-α can trigger IL-6 release, one theory posits that metabolic syndrome is caused by adipose tissue-derived TNF-α, and that elevated systemic levels of IL-6 depends on levels of TNF-α (Petersen and Pedersen 2005). Figure 4.15 demonstrates that contracting muscle fibres produce and release IL-6, which induces several metabolic effects. IL-6 induces lipolysis and fat oxidation and is involved in glucose homeostasis during exercise. In addition, IL-6 has strong anti-inflammatory effects and may inhibit TNF-induced insulin resistance. sTNF-R, soluble TNF receptor (Petersen and Pedersen 2005).

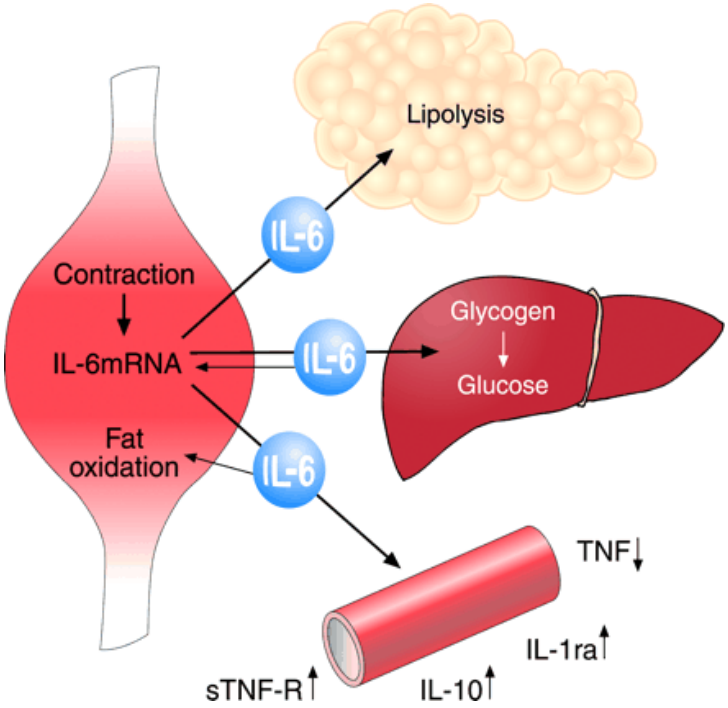


Figure 4.15: Contracting muscle fibres and production of IL-6 (Petersen and Pedersen 2005).

In a study, it has been shown that, in rodents, macrophages generate pro-inflammatory cytokines IL-1β and TNF-α and may play a pathogenic role on β-cells (Arnush, Scarim et al. 1998; Dahlen, Dawe et al. 1998). When compared with other control strains, for example non-obese resistant (NOR) mice, macrophages from NOD mice generate higher levels of the inflammatory IL-1β, and TNF-α cytokines (Uno, Imagawa et al. 2007).

Analysis of blood samples after acute exercise session a rise in IL-1β was observed in CSII group in the present study, which is in line with the findings of Gleeson (2007) who observed an increase in IL-1β during and after acute exercise in the CSII. But in MDI this increase was only witnessed after a cardio i.e. cycling session. This increase in MDi may be caused by high level of insulin in the body.

However, in case of chronic exercise it was observed that in all three groups (i.e. ND, MDI and CSII) the levels of IL-1β dropped (see Figure 4.11). Furthermore, the drop in

IL1-β levels was more significant in CSII as compared to MDI. This further supported the hypothesis that exercise has a positive impact on diabetes sufferers and CSII provides a better mean to manage the blood glucose levels. As reported previously IL-1β is inhibit β cell function as an pro-inflammatory cytokine acting in T1D during the autoimmune process (Maedler, Sergeev et al. 2002). In this study the low level in IL-1β reported in CSII group (figure 4.7 and 4.11) may result in the benefit of CSII therapy, as the low level of IL-1β and TNF-α reported for CSII group (figure 4.6 and 4.10) can indicate the progressing of autoimmune against β cells destruction (Dogan, Akarsu et al. 2006). This means the low level of IL-1β lead to or may be used as an indicator of continuing autoimmune aggression against β cells before the development of extensive β-cell. Baumann, Salem et al. (2012) supported this statements, the secretion high level of both pro-inflammatory cytokines such as; IL-1β (as in figure 4.7 and 4.11), TNFα (as in figure 4.6 and 4.10), and INFγ (as in figure 4.8 and 4.12) in this study, is a fatal destruction process of β-cells (Baumann, Salem et al. 2012).

TNF-α and IL-1β cytokines are usually depicted as pro-inflammatory cytokines and these cytokines arouse the generation of IL-6, and it is clear from the above discussion that higher levels of IL-6 are helpful to manage T1D.

When there are low concentrations of IL-1β the cytokine enhances insulin secretion, increases β-cell replication and lowers apoptosis. The precise opposite of this occurs after prolonged exposure to higher levels of IL-1β in T1D (Donath, Böni-Schnetzler et al. 2010). It is therefore justifiable to propose that under physiologic circumstances, IL-1β that is excreted locally by islet cells, comprising β-cells, plays an key role in the daily maintenance of β-cell mass and function, whilst the long-term and pathologically

heightened levels of islet IL-1β related to inflammation of the islet, causes a decrease in β-cell function and mass in diabetes (Donath, Böni-Schnetzler et al. 2010).

Type 1 T cells mainly generate IFN-γ and TNF-α, and their actions trigger macrophages and killer mechanisms, such as T-cytotoxic cells, therefore pushing the immune system towards cell-mediated immune reactions, which mainly offer protection against intracellular pathogens like viruses (Lancaster, Halson et al. 2004). In autoimmune diabetes NOD mice they found that IFN-γ and TNF-α as an effectors molecules leading to β cell death (Suk, Kim et al. 2001)

In this study IFN-γ levels showed conflicting patterns. But overall it was observable that the IL-6 and IFN-γ levels increased after 4 exercise sessions in CSII groups. In chapter 5, the limitations of this pilot study will be discussed in terms of the selection of the participants. However, these matters have some bearing here too. Inevitably in a study of this kind, it is impossible to match people in many of the respects that may be deemed desirable simply to recruit enough that satisfy some other major criteria. In this chapter, recruits may differ in the oxidative stress, degree of inflammatory change and the lifestyle influences that may or may not have been declared.

During and after the exercise IFN-γ production by T lymphocytes is restricted (Lancaster, Halson et al. 2004). Even following relatively short periods (1–3 wk) of intensified training, marked reductions in the circulating number of T cells producing IFN-γ have been observed (Lancaster, Halson et al. 2004). In anti-IFN-γ antibody-treated mice, IFN-γ blood levels were dramatically decreased TNFα and IL-6 levels remained unaffected by anti-IFN-γ treatment (Matthys, Mitera et al. 1995). Furthermore, IFN-γ can activate macrophages and motivate enhanced pro-inflammatory cytokine production, such as IL-1β and TNF-α (Lehuen, Diana et al. 2010). Moreover in

our study the benefit of the suppression of TNF-α by IL-6 is reported as the low level of TNF-α can play a role in T1D, as the blocking of TNF-α early during pathogenesis may prevented diabetes completely (Christen, Wolfe et al. 2001).

Preventing diabetes in NOD mice provoked by complete Freund adjuvant injection is reliant on the presence of NK cells that generate IFN-γ (Lee, Kwon et al. 2012).

In summary this study examined the effects of exercise on cytokines like IL-6, TNF-α, IL-1β and IFN-γ in ND, MDI and CSII groups. As discussed above it was noticed that exercise leads to an increase in IL-6 and IFN-γ, while causing a reduction in TNF-α and IL-1β levels. The above mentioned literature supported the findings of this study and the hypothesis of the study that exercise (acute and chronic) in diabetics helps to manage the disease and CSII has a positive impact on the T1D.

4.6. Conclusion

- This study has shown that there is a difference between normal healthy volunteer cytokines and those in diabetes. Diabetic levels are known to be higher than ND and it is reasonable to assume that the MDI levels are suppressed compared with the untreated level over the exercise programme. However, the effect of a more continuous infusion appears to be a greater suppression than normal.

- The greater suppression of cytokines in CSII does not appear to be linked with increased risk and in fact seems associated with healthy outcomes (chapter 5).

- These levels can be seen to respond to acute and more chronic forms of exercise in this study. This supports the suggestion that muscle-derived IL-6 plays a key role in exercise-induced leukocyte trafficking. This is important because it has

been demonstrated by different studies that the physiological concentrations of IL-6 provoke an anti-inflammatory, not inflammatory reaction in humans and that IL-6 is independent of TNF-α. The low production of pro-inflammatory cytokine TNF-α concentration may be explained by the inhibiting role of high levels of IL-6 via independent mechanisms during exercise.

- The data produced in the study reveals that a regular exercise regime can lead to a rise in IL-6 and a decline in TNF-α. Furthermore, the reduced levels of TNF-α is associated with the reduced level of IL-1β, while both possess pro-inflammation properties. On the other hand IFN-γ and IL-6 performs anti-inflammatory role in the immune system.

- Diabetes being an inflammation-driven disease can benefit from anti-inflammatory properties of high levels of IL-6 and IFN-γ and reduced levels of TNF-α and IL-1β. The study demonstrate anti-inflammatory effects of taking regular exercise which could provide protection against chronic systemic low-grade inflammation involved in the maintenance, the vulnerability to infection and the progression of the disease.

CHAPTER 5: Effect of exercise on Lipids Profiles and glycemic control

Effect of exercise on lipids profiles and glycemic control for type1 diabetic (MDI and CSII) and non-diabetic (ND)

5.1. Introduction

Diabetes Mellitus is linked with heightened cardiovascular disease (CVD) mortality that is present within all age groups. However, in young people with Type 1 Diabetes (T1D) CVD is considered to be a major risk and is linked with high mortality (McVeigh, Gibson et al. 2013). Patients who have T1D also usually have lipid disorders (Verges 2009; Vergès 2011) and thus it is essential to investigate lipid abnormalities in people with T1D to reduce CVD with this group of people. Hyperglycemia and dyslipidaemia are very prevalent metabolic abnormalities in adults who have T1D and this increases their risk of developing CVD (Maahs, Ogden et al. 2010). T1D patients who have reduced or suboptimal glycemic control will eventually present with heightened LDL levels in comparison to non–diabetic people and T1D patients with optimum glycemic control (Guy, Ogden et al. 2009). The hemoglobin A1C (A1c) is significantly linked with alterations in fasting lipids in adults who have T1D but not taking dyslipidaemia medications (Maahs, Ogden et al. 2010). The important lipid measurements include; low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol (TC) and triglyceride (TG).

Heightened lipid availability decreases insulin-stimulated glucose disposal in the skeletal muscle, which is usually characterised by fatty acid–mediated inhibition of insulin signalling (Szendroedi, Frossard et al. 2012). Reduced insulin sensitivity is linked with amore atherogenic lipid profile in people who have T1D either as adults or

in their youth (Maahs, Nadeau et al. 2011). TG levels are correlated with vascular disease in T1D (Mäkinen, Soininen et al. 2013).

The Diabetes Control and Complications Trial (DCCT) showed that intensive (rather than conventional) insulin therapy is linked with a considerable reduction in LDL, total TC and TG in adults who have T1D (Maahs, Ogden et al. 2010). The improvement of glycemia is suggested as an initial treatment for dyslipidaemia when caring for patients with T1D. Nevertheless, lipid-lowering medicine can also be administered if lipid goals are not obtained (Buse, Ginsberg et al. 2007; Brunzell, Davidson et al. 2008).

5.2. Effect of Exercise on Lipids and glycemia control

When overweight healthy people took part in 16 weeks of aerobic and resistance training, they presented with elevated lipid profiles (Tibana, Navalta et al. 2013). It is a well-established fact that the long-term benefits of frequent aerobic physical activity laid out for the general population can be applied to T1D patients (De Feo, Di Loreto et al. 2006). Yet T1D patients struggle to manage and control blood glucose levels during exercise and this hinders their pursuit for regular exercise (Kilbride, Charlton et al. 2011). Exercise is linked with an heightened risk of hypoglycemic or hyperglycaemic events (Kapitza, Hövelmann et al. 2010). Small-scale studies have revealed that in patients with T1D, resistance training (weight lifting) decreases A1c (Yardley, Kenny et al. 2013). Furthermore, Shriver (2011) after his study reported a statistically significant impact of exercise (aerobic, resistance and combined training) on the management of glucose (A1c). While in T1D sufferers, in contrast to MDI therapy, constant subcutaneous insulin infusion (CSII) is found to be helpful to reduce the occurrence of hypoglycemia during frequent moderate-to-heavy intensity aerobic training and was not

linked with a heightened risk of post-exercise late-onset hypoglycaemia (Yardley, Iscoe et al. 2013).

It has been suggested that the roles of intensive insulin as a treatment of T1D is not only to improve A1c and the prevention of premature cardiovascular events (Nathan, Cleary et al. 2005) but are also linked with lipid profiles (Feitosa, Feitosa-Filho et al. 2013). The improvements of glycemic control without weight gain has been associated with lipids control and this include triglyceride, total cholesterol and LDL (Purnell, Hokanson et al. 1998). CSII is a way of attaining stringent glycaemic control and yet minimising the associated problem of hypoglycemia and possibly of weight gain (Hindmarsh, Peters et al. 2013). T1D patients may have lipids disorders such as high LDL and low HDL (Guy, Ogden et al. 2009) and may influence lipid transfers (Bagdade, Ritter et al. 1991; Bagdade and Dunn 1992). However, in T1D (as distinct from the better known resistance in T2D) the high triglycerides level is linked with insulin resistance (Coen, Dubé et al. 2010).The increased in inflammatory markers such as IL-6 in T1D (as shown in chapter 4) is associated with changes in the lipid profile (Snell-Bergeon, West et al. 2010).However, the relation between intensive insulin treatment and the status of lipid metabolism in T1D need more investigations (Feitosa, Feitosa-Filho et al. 2013) and the benefits of exercise in T1D is not clear yet (Chimen, Kennedy et al. 2012). Moreover, the evidence for T1D in relation to the advantage of exercise on glycaemic control are less clear (Chimen, Kennedy et al. 2012).

5.3. Insulin pump compared to MDI

In comparison to conventional insulin therapies, CSII (or insulin pump) can enhance glycaemic management and promote dietary flexibility in patients with T1D as shown

in chapter 3. There is strong evidence to show that intensive insulin treatments that generate stringent glycaemic control decrease or delay the inception of the long term difficulties associated with diabetes. In adult diabetes the lipids concentration are linked with risk of CVD (Weis, Turner et al. 2001). T1D adult associated with high risk of atherosclerotic disease compared to healthy (Krolewski, Kosinski et al. 1987).Guy, Ogden et al. (2009) found that the lipids level are affected by glycemic control in youth T1D with duration of ~4 years (Guy, Ogden et al. 2009). The same study reported the optimal A1c in T1D youth result in optimal lipid profiles (TC and LDL) compare to non-diabetic youth while the other group of T1D youth with suboptimal A1c have higher standard lipid levels and prevalence of lipid abnormalities (total cholesterol, LDL cholesterol, and non-HDL cholesterol) than non-diabetic youth. Similarly, Petitti, Imperatore et al. (2007) reported that the TC, LDL and triglyceride are increased with increasing A1c in T1D (Petitti, Imperatore et al. 2007).

In T1D adult with poor glycemic control the lipid levels are increased (Guy, Ogden et al. 2009). It has been suggested that the enzyme induction in adipose tissue by insulin, in intensive therapy is typically associated with a marked fall of triglyceride-rich particles (James and Pometta 1990). This statement may explain the low TG and LDL and high HDL in well-controlled T1D. However, the relation between T1D and lipids may be affected by ethnic group (Dias, Brown et al. 2013).

A major study comparing CSII and MDI in T1D with lipid parameters was in 2009. This confirmed that CSII therapy for T1D improved the lipid profile compared to MDI (Derosa, Maffioli et al. 2009). The same study reported that the LDL, HDL, TG and TC were improved after 6 month of CSII therapy compared to MDI. Moreover, even short term (2 weeks) of CSII therapy in T2D improved the lipids such as TG, LDL, HDL, TC

and non-esterified fatty acid (NEFA) (Li, Xu et al. 2004). However, most past studies analysed the different T1Dinsulin route therapies (CSII and MDI)were focused on glycemia control rather than on lipids profiles (Derosa, Maffioli et al. 2009). Moreover, recently Peters et al. (2013) claimed that there is not much evidence to suggest if insulin pump users utilised increased dietary flexibility, and if dietary quality is impacted upon or affects outcomes (Peters, Mount et al. 2013).

The target goals of T1D therapy either CSII or MDI are to achieve normal or near normal glycemia, prevent late vascular complications, reduce hypoglycemia events, improve quality of life, and limited weight gain. As discussed in chapter 3 insulin pump therapy may or can dramatically aid in achieving all of these goals.

5.4. Study Objectives

The purpose of this study was to examine the effects of six weeks of combination exercise (resistance and aerobic), twice a week, on lipid profiles and glycaemic control in T1D using MDI, T1D using CSII and non-diabetic (ND).The ethical approval for this study was gained from De Montfort University (DMU) Ethics Committee, School of Health and Life Science, Leicester, UK.

5.5. Subjects and Methods

5.5.1. Volunteers

As mentioned above three groups of volunteers were involved in this study i.e. T1D using CSII, T1D using MDI and non-diabetic ND. The volunteers were 18-55 years old and not physically active or engaged in any regular exercise or training programmes.

5.5.2. Study Recruitment

Study recruitment was publicised through internal and external adverts as described in section 2.4.1.Initially 49 individuals (ND= 24, MDI= 14, CSII= 11) expressed an interest to participate in this study. However, 14 were excluded because they failed to meet the inclusion criteria, while, 16 participants were unable to attend because of their personal commitments and difficulty in making necessary arrangements to participate. The eventual sample was 19 participants (ND= 7, MDI= 7 and CSII=5) who completed the exercise programme.

5.5.3. Six Weeks Exercise

The participants were required to participate in moderate exercise programme involved two visits per week for six weeks (48 hours between, as improved insulin sensitivity is said to be lost after 48 hours (Shriver 2011). Each visit lasted for more than 90 minutes, again based on trials by others (CDC 2011; Shriver 2011) and as described in figure 5.1, to include stretching upper and lower muscles, a warm up on the bike for 5 min, the resistance exercise, the aerobic exercise, then warm down and rest (full description in sections 2.5.7-2.5.10).

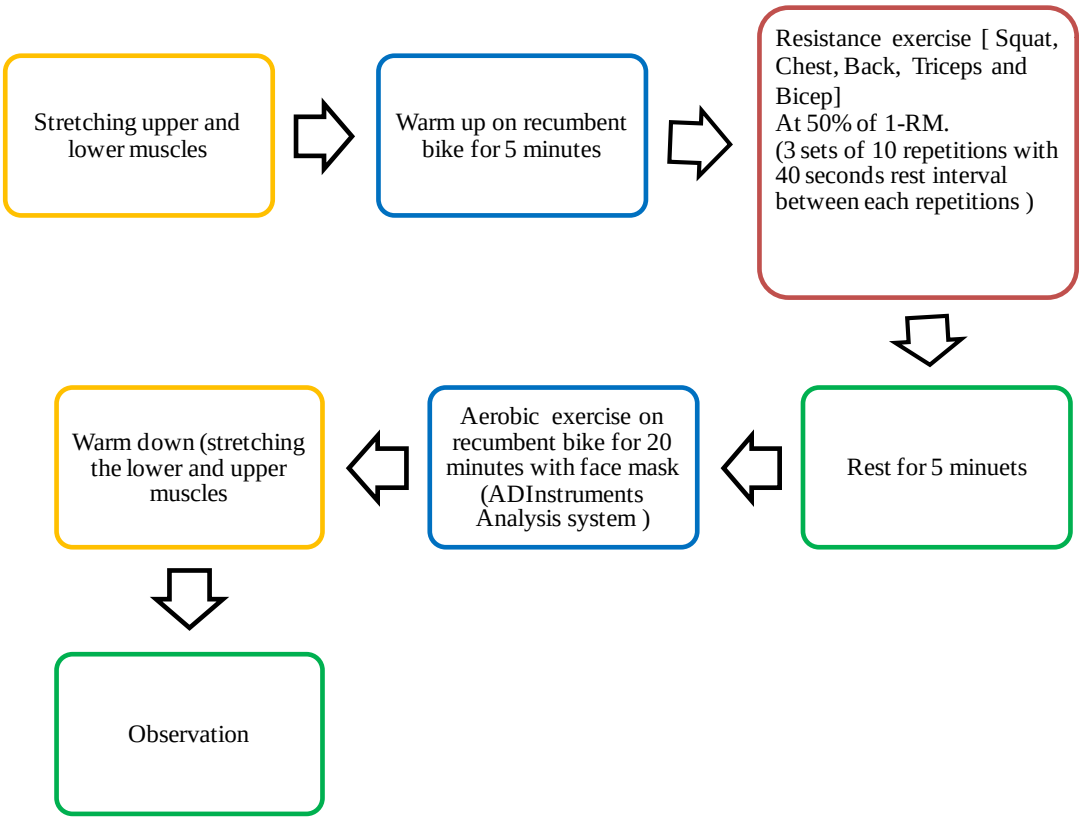


Figure 5.1: Division of the exercise sessions throughout the study

5.5.4. Parameters measured

Lipids profile including; TG, HDL, LDL and TC were measured; before starting the exercise programme and used as the individual base line, after 6 sessions and after 12 sessions of exercise according to UK guidelines and NICE, 2010 (issue date: May 2008 and reissued March 2010) (Cooper, Nherera et al. 2008) (see table 5.1). Body fat % and BMI was calculated before and after study. Rated Perceived Exertion (RPE) was recorded three times in each session; after 1st, 2nd and 3rd sets of resistance exercise and during cardio exercise the RPE was recorded twice; at 10 and 20 minutes. The respiratory exchange ratio (RER) was measured 3 times during cardio exercise; at zero point (after the rest of 5 min after RE), at 10 and 20 min of cardio exercise. T1D volunteers either CSII or MDI groups calculated their carbohydrates daily. T1D people

respond variously to insulin therefore the carbohydrate to insulin ratio was calculated before and after the study for T1D groups.

A1c was measured before first session and after 12 sessions. BG was measured three times during the sessions; before exercise, after resistance and after cardio exercise. Daily insulin (Units/day) used by volunteers during the study were recorded.

Table 5.1: Lipids level according to UK guidelines and NICE, 2010.

Lipid (mmol/l)	Normal	Abnormal	Very abnormal
Total Cholesterol (Tc)	Less than 4.0	4.1-6.0	Greater than 6
LDL-Cholesterol	Less than2.0	2.6-3.9	4.0-4.8
HDL-Cholesterol	>1.4	1.0-1.5	Less than 1.0
Triglyceride (TG)	less than 1.69	1.7-2.25	2.26-5.65

5.5.5. Statistical Analysis

Descriptive statistics like mean, variance and standard deviation were used to express the data. Test of statistical significance based on two tailed t-test and p-value were conducted using 0.05 level of significance (i.e. α=0.05).All data were analysed using the Statistical Package for Social Sciences (IBM SPSS, v.19, Chicago, IL). The carbohydrate to insulin ratio example was calculated as 1 in 10 for example:

When T1D intake 10g of carbohydrate they need 1 unit of fast acting insulin to cover this amount of carbohydrate.

5.6. Results

5.6.1. Characteristics

A total of 19 males who met the selection criteria participated. The samples consisted of 7 diabetic type 1 (MDI), 7diabetic type 1 insulin pump users (CSII) and 7 non-diabetic

(ND). Study characteristics including age, BMI, DM duration and pump duration, BG tests per a day and types of insulin are described in table 5.2.

Table 5.2: Practical study subjects' characteristics; DM, Diabetes mellitus; BG, blood glucose; BMI, body mass index and body fat%. Data are means ± SD

Variables	MDI	CSII	ND
Age, y	36 ±12.5	30.8±8.7	31.4±5.3
DM duration, years	16.4±13.6	14.6±9.8 **Pump duration, years** 4.1±1.7	----------
Types of insulin	Novorapid / Glargine (45%) Novorapid / Lantus (28%) Novorapid / Levemir (13.5%) Novomix (13.5%)	Apidra (15%) Novorapid (60%) Humalog (15%)	----------
BG test frequency (daily)	4.6±0.8	7.8±1.9	----------
BMI Before study After study	 29.3±6.9 29.1±6.9	 25.1±2.5 24.8±2.2	 26.2±3.3 26.2±3.3
Body fat % Before study After study	 31.3±10.9 29.2±11.4	 26.9±3.4 25.08±3.3	 23±7.4 20.7±7

5.6.2. Exercise intensity

The target intensity of resistance exercise (RE) was moderate (11-14) according to the Rated Perceived Exertion (RPE) scale (6-20) (which describes how hard the exercise feels to the participant) (see table 2.3) and estimated maximum heart rate (HR_{max}) was used for cardio (bike) exercise, as in the full explanation provided in section 2.5.5. Table 5.3 shows the RPE during resistance exercise and table 5.4 is the RPE during cardio exercise. The RPE was measured following the completion of each set of RE exercise. The session RPE is a reliable method to measure various intensities of RE exercise (Scherr, Wolfarth et al. 2013).

In order to indicate which fuel (carbohydrate or fat) is being metabolised to supply the body with energy during the 20 minutes of cardio, the respiratory exchange ratio (RER) was used. Williamson, Fuld et al. (2012) confirmed the validity of RER in aerobic

exercise (Williamson, Fuld et al. 2012).This is the ratio between the amount of consumed O_2 and produced CO_2 in one breath and it was measured 3 times; at zero (after the rest of 5 min after RE), at 10 min of cardio and at the end of cardio (see table 5.5). Without any activities and with a light diet RER is about 0.8. However, during intense exercise the RER value can exceed 1, as a result of greater production of CO_2 by the working muscles and more of the inhaled O2 gets used rather than being expelled. As Rubini, Paoli et al. (2012) reported a positive significant correlation between O_2 uptake and CO_2output during exercise (Rubini, Paoli et al. 2012).

The interaction between exercise and meal intake can be determined by RER (Kang, Raines et al. 2013). In this study when RER=0.70 it indicates that fat is the largest fuel source, RER=0.85 suggests a mix of fat and carbohydrates, and a value of RER=1.00 or above is indicative of carbohydrate being the main fuel source. However, in this study the diets of individuals were not controlled. It was difficult to do so with T1D for either MDI or CSII as they must calculate their carbohydrates in order to adjust their insulin basal and bolus rate.

Table 5.3: Rated Perceived Exertion (RPE) for all study groups during resistance exercise (RE). Data are means ± SD

RPE for RE	MDI group sessions											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1st set	13±0.6	12±1	12.7±1.1	12.7±1.1	12.1±1.1	12.7±0.5	12.9±0.4	13±1	12.3±0.5	12.4±1.1	12±0.6	11.7±0.8
2nd set	13.6±0.8	13±0.8	13.7±1.0	13±0.0	13±0.6	13.4±1.0	13.4±1.0	13.1±0.7	13.3±0.8	13.1±1.2	12.4±1.0	12.1±1.1
3rd set	14.6±2.1	14±1.6	14±1.2	13.9±0.7	13.7±1.3	14±1.4	14.1±1.5	13.6±0.8	14.3±1	13.6±1.3	13.1±1.6	12.6±1.5
CSII group sessions												
1st set	12.8±1.3	12.2±0.4	12±0.7	12.4±1.5	12±0.7	11.8±0.4	11.2±0.8	11.2±0.8	12±0	11.5±0.6	11.8±0.4	11.2±0.4
2nd set	14.2±1.3	12.6±0.9	13.2±0.8	13.2±1.8	12.4±0.9	12.6±0.9	12±1.2	11.6±1.1	12.2±0.5	12±0.8	12±0.7	11.6±0.9
3rd set	15.6±1.3	14±1.0	14.2±0.8	14±1.2	13.4±1.3	13.4±1.1	13±1.4	12.8±1.5	13±1.4	12.8±1.5	12.2±1.1	11.8±1.3
ND group sessions												
1st set	11.7±1	11.7±0.8	12±0.8	12±0.8	11.6±0.5	11.5±0.5	11.2±1.3	11.6±1	12±1.5	11.8±2	11.4±1	10.9±1.3
2nd set	13.3±1	13.4±1.1	13.3±0.8	13.4±1	12.6±1	12.7±1.3	12.1±0.7	12.7±1.1	12.4±1.1	12.7±1.7	12.1±0.7	11.9±1.2
3rd set	14.4±2.2	14.1±2.6	13.8±2.4	13.7±2.3	12.8±2.1	12.1±2.5	12.3±2.4	12.3±2.7	12.5±2.5	12.4±2.3	11.7±1.8	11.7±2.0

Table 5.4: Rated Perceived Exertion (RPE) for all study groups during cardio exercise. Data are means ± SD

Cardio exercise RPE	MDI group sessions											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
After 10 min	13.6±1.8	14.0±0.6	12.7±1.1	12.9±1.1	13.0±0.8	13.7±1.3	12.3±1.0	12.1±0.7	12.7±1.1	12.7±1.0	11.9±0.7	11.7±1.0
After 20 min	13.0±1.5	13.1±0.9	12.7±1.0	12.9±0.9	13.1±0.7	13.3±0.8	12.7±1.1	12.3±0.8	13.3±1.4	12.9±1.1	12.0±1.0	11.7±1.0
CSII group sessions												
After 10 min	12.2±0.8	12.6±0.5	12.4±1.5	13.0±1.6	12.2±1.1	12.2±0.8	11.6±0.5	11.8±1.0	12.3±1.3	12.3±1.3	12.0±1.9	11.8±1.1
After 20 min	12.8±1.3	13.0±1.0	12.8±1.8	13.0±1.6	12.6±1.5	12.4±1.1	12.0±1.2	12.0±1.2	12.3±1.3	12.3±1.3	12.0±1.9	11.8±1.1
ND group sessions												
After 10 min	12.3±2.2	13.3±1.7	12.8±0.9	12.4±1.3	12.3±1.8	12.7±2.1	11.9±1.0	12.1±1.1	11.9±1.6	12.0±1.9	11.4±1.0	11.0±1.3
After 20 min	13.7±2.0	14.1±1.9	14.0±1.4	13.3±2.3	12.7±1.8	13.0±2.4	12.3±1.0	12.3±1.1	12.1±2.1	12.4±1.8	11.7±1.5	11.3±2.2

Table 5.5: Respiratory exchange ratio (RER) during cardio exercise. Mean test duration was 1200 seconds Data are means ± SD

RER	MDI group sessions											
	1	2	3	4	5	6	7	8	9	10	11	12
Zero point	1±0.2	1±0.2	1±0.1	1±0.2	1±0.1	1±0.2	1±0.1	0.9±0.1	1±0.2	1±0.2	1.1±0.1	1±0.2
At 10 min	1.1±0.2	1.1±0.2	1.1±0.2	1±0.2	1.1±0.2	1.1±0.2	1.2±0.3	1.1±0.2	1.2±0.2	1.1±0.2	1.1±0.1	1±0.2
At 20 min	1.1±0.2	1.1±0.2	1±0.2	1.1±0.2	1.1±0.2	1.1±0.2	1.1±0.1	1.1±0.2	1.2±0.2	1.1±0.2	1.2±0.1	1±0.2
CSII group sessions												
Zero point	1±0.3	1±0.3	0.9±0.3	0.9±0.3	1±0.2	1±0.1	1±0.2	1±0.2	1±0.1	1±0.1	1±0.2	1±0.1
At 10 min	1.1±0.2	1±0.2	1±0.2	1±0.2	1.1±0.2	1.1±0.2	1±0.2	1±0.2	1±0.2	1±0.2	1±0.2	1±0.2
At 20 min	1.1±0.2	1±0.2	1±0.2	1±0.2	1.1±0.2	1.1±0.2	1.1±0.2	1.1±0.1	1.1±0.2	1±0.2	1±0.2	1.1±0.2
ND group sessions												
Zero point	1±0.2	0.8±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.2	0.9±0.2	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1
At 10 min	1±0.2	0.9±0.1	0.9±0.1	0.9±0.1	1±0.1	0.9±0.1	0.9±0.2	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0
At 20 min	1±0.2	0.9±0.1	0.9±0.1	0.9±0.1	1±.02	0.9±0.1	0.9±0.2	0.9±0.2	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0

5.6.3. Effect of exercise on Lipids

Lipids profiles were measured three times during this study and table 5.6is described the result for all lipids (LDL, HDL, TC, TG) and table 5.7 shows the lipid profiles P value for all study groups.

Table 5.6: Lipids profiles as mean ± SE

Lipids (mmol/L)	**MDI**	**CSII**	**ND**
LDL level			
Before exercise	1.73±0.8	2.00±0.6	1.95±0.6
After 6 sessions	1.87±0.9	1.97±0.7	1.95±0.6
After 12 sessions	1.59±0.7	1.50±0.5	1.85±0.7
HDL level			
Before exercise	1.37±0.7	1.52±0.3	1.30±0.6
After 6 sessions	1.50±0.7	1.55±0.3	1.59±0.7
After 12 sessions	1.64±0.6	1.68±0.2	1.62±0.5
TC level			
Before exercise	4.02±0.7	4.03±0.8	3.92±0.6
After 6 sessions	3.84±0.7	3.93±0.7	3.65±1.0
After 12 sessions	3.72±0.6	3.60±0.6	3.50±0.7
TG level			
Before exercise	1.47±0.9	1.12±0.4	1.34±0.6
After 6 sessions	1.53±0.9	0.90±0.3	1.31±0.2
After 12 sessions	1.37±0.6	0.79±0.2	1.17±0.2
TC/HDL			
Before exercise	2.93	2.65	3.02
After 6 sessions	2.56	2.54	2.30
After 12 sessions	2.27	2.14	2.16
HDL/LDL			
Before exercise	0.79	0.76	0.67
After 6 sessions	0.80	0.79	0.82
After 12 sessions	1.03	1.12	0.88
TG/HDL			
Before exercise	1.07	0.74	1.03
After 6 sessions	1.02	0.58	0.82
After 12 sessions	0.84	0.47	0.72

Table 5.7: Lipids profiles between study groups (P-values)

		CSII vs MDI	CSII vs ND	MDI vs ND
LDL	Before Exercise	0.322	0.946	0.213
	After 6 sessions	0.796	0.881	0.704
	After 12 sessions	0.835	0.389	0.637
HDL	Before Exercise	0.944	0.806	0.670
	After 6 sessions	0.565	0.495	0.738
	After 12 sessions	0.971	0.479	0.445
TC	Before Exercise	0.699	0.751	0.876
	After 6 sessions	0.957	0.928	0.855
	After 12 sessions	0.373	0.867	0.164
TG	Before Exercise	0.495	0.534	0.850
	After 6 sessions	0.095	**0.044**	0.934
	After 12 sessions	0.074	**0.016**	0.335
TC/HDL	Before Exercise	0.696	0.720	0.812
	After 6 sessions	0.942	0.794	0.761
	After 12 sessions	0.599	0.728	0.114
HDL/LDL	Before Exercise	0.440	0.954	0.383
	After 6 sessions	0.507	0.810	0.331
	After 12 sessions	0.613	0.940	0.441
TG/HDL	Before Exercise	0.644	0.386	0.961
	After 6 sessions	0.433	0.658	0.835
	After 12 sessions	0.100	0.264	0.252

5.6.3.1. Low-density lipoprotein cholesterol (LDL)

LDL was decreased from 2mmol/L before exercise to 1.5mmol/L after 12 sessions of exercise in CSII group (Figure 5.2). However, although the trend was probably a decrease, the LDL in MDI and ND groups showed some fluctuation and a smaller overall decline.

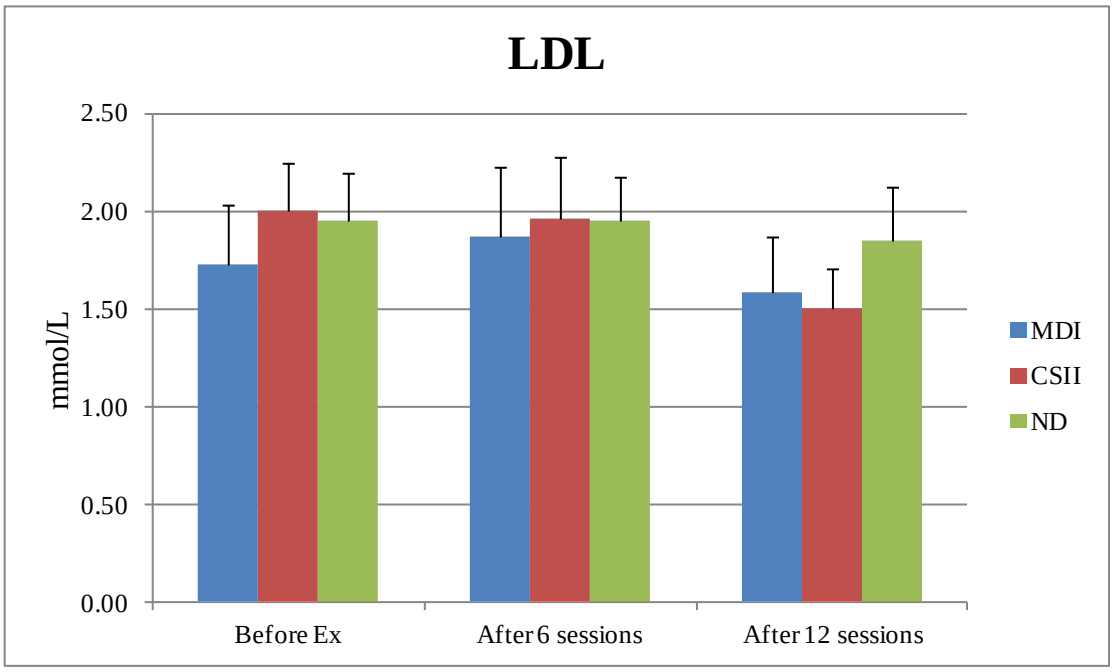


Figure 5.2: LDL, Low-density lipoprotein cholesterol for the subjects before, during (after 6 sessions) and after 12 sessions of exercise. Data are means ± SEM and discussed in the text. The changes were insignificant statistically.

5.6.3.2. High-density lipoprotein cholesterol (HDL)

All study groups showed increase in HDL. Sample taken after six sessions showed a nominal rise in CSII group. However, after 12 sessions all three groups showed a significant rise in HDL (Figure 5.3).

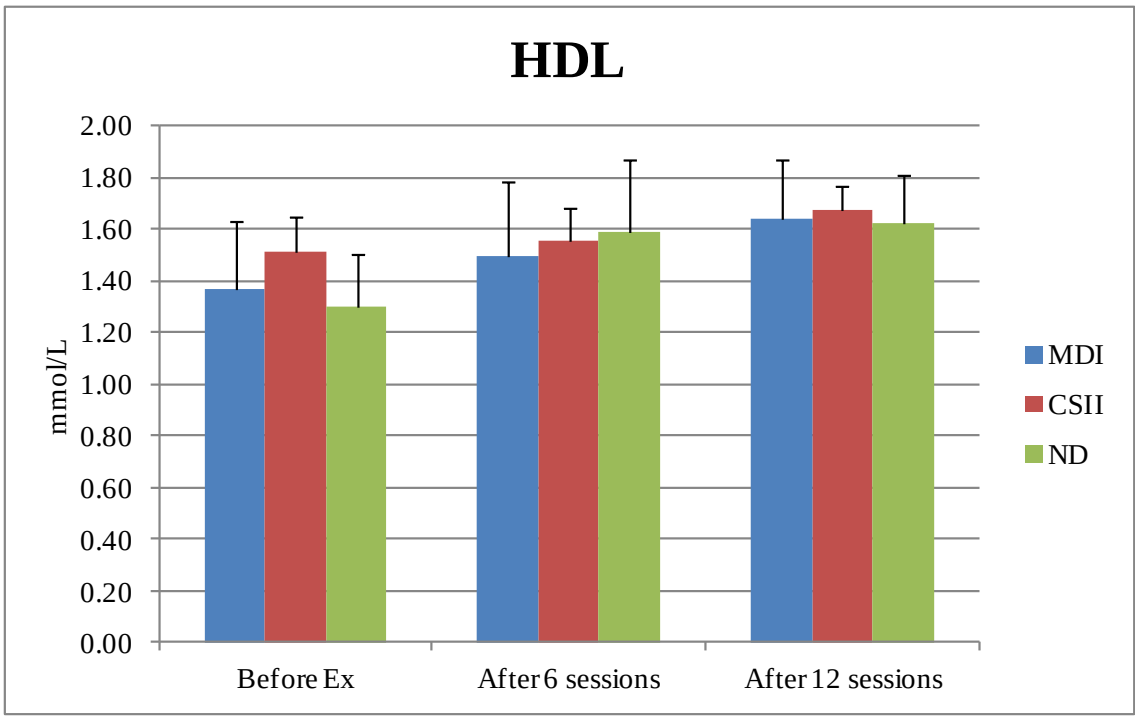


Figure 5.3: HDL, High-density lipoprotein cholesterol for the subjects before, during (after 6 sessions) and after 12 sessions of exercise. Data are means ± SEM and discussed in the text. The changes were insignificant statistically.

5.6.3.3. Total Cholesterol (TC)

The total cholesterol (TC) for ND and CSII groups was significantly decreased after 12 sessions of exercise compared to before exercise ($P<0.01$ and $P=0.026$ respectively). MDI showed a decrease in the level of TC but this was not significant after 12 sessions (Figure 5.4).

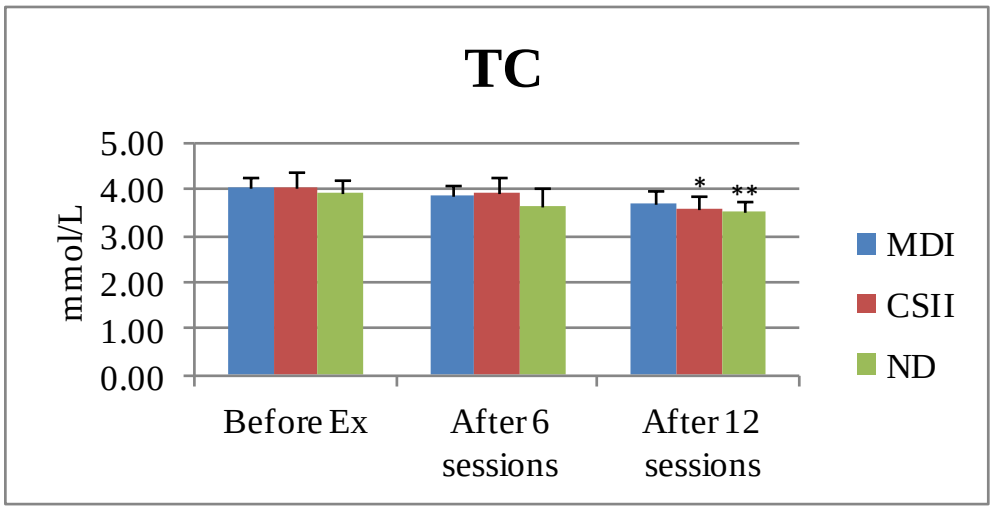


Figure 5.4: Total Cholesterol for the subjects before, during (after 6 sessions) and after 12 sessions of training. Data are means ± SEM; P-value was for before exercise and after 12 sessions of exercise. *≤0.05, **≤0.01. and is discussed in the text.

5.6.3.4. Triglyceride (TG)

In CSII group a statistically significant decline in triglyceride was observed (P=0.03) with values falling from 1.10mmol/L to 0.78mmol/L (Figure 5.5).

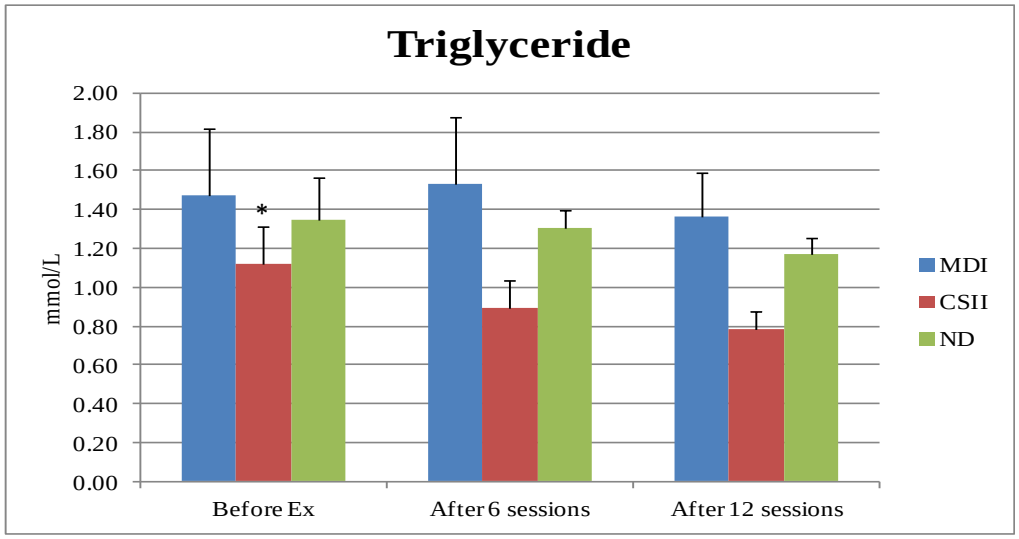


Figure 5.5: Triglyceride for the subjects before, during (after 6 sessions) and after 12 sessions of training. Data are means ± SEM; P-value was for before and after exercise**. *** p-value (≤0.05) i.e. statistically significant was for before exercise and after 12 sessions of exercise and discussed in the text. The other changes were insignificant statistically.

5.6.4. Cholesterol ratio

Further analyses were conducted to determine the cholesterol ratio including (TC/HDL, HDL/LDL and TG/HDL) and table 5.8 showing the normality of these ratios.

Table 5.8: Cholesterol ratio levels

	Preferable	**Ideally**
TC/HDL	Under 5.0	Under 3.5
HDL/LDL	Over 0.3	Over 0.4
TG/HDL	Under 4	Under 2

5.6.4.1. TC/HDL

The total cholesterol/HDL ratio is a useful indicator of cardiovascular disease. All the study groups here had ideal TC/HDL ratios. Moreover the TC/HDL was decreased after 12 sessions of moderate exercise of cardio and resistance for each group. The lowest TC/HDL ratio was found in CSII group, followed by the ND group. The MDI group had the highest TC/HDL ratio; however it is below 3 mmol/L (figure 5.6).

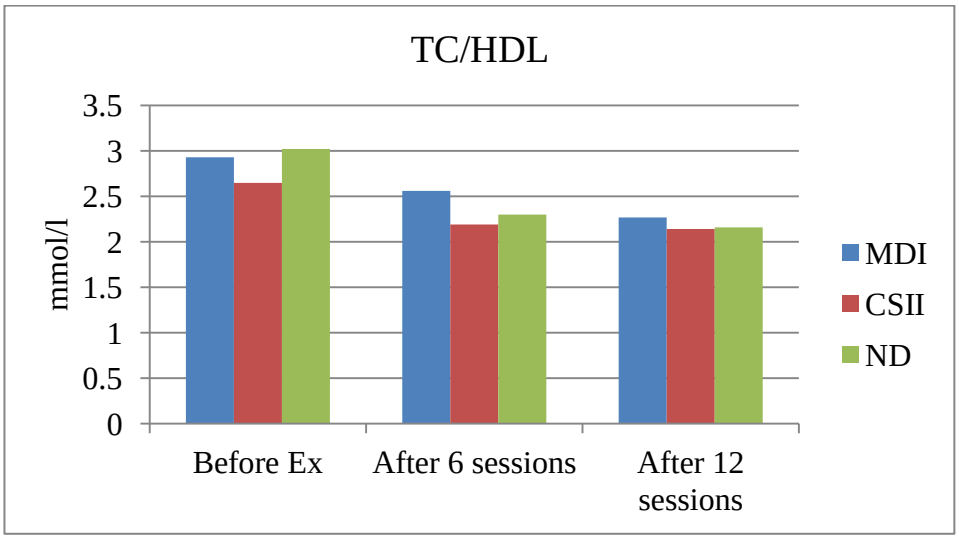


Figure 5.6: Average total cholesterol to HDL ratio (TC/HDL).

5.6.4.2. HDL/LDL

The HDL-cholesterol to LDL-cholesterol ratio (HDL/LDL) was well within the ideal range in all study groups, However, the HDL/LDL ratio was improved over the study (see figure 5.7) for each group. The Pump group and the ND group showed a slightly better response to exercise in this small study.

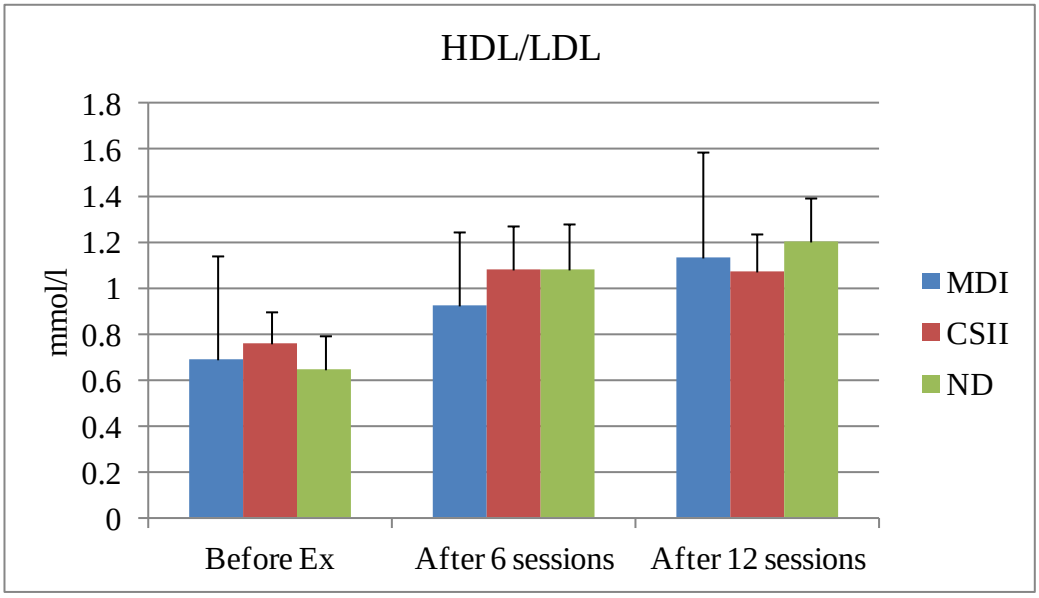


Figure 5.7: Average HDL-cholesterol to LDL-cholesterol ratio (HDL/LDL).

5.6.4.3. TG/HDL

In the CSII group the triglyceride to HDL ratio (TG/HDL) was well within the medically accepted range. The MDI group had a higher ratio compared to ND and CSII groups and the CSII group had a lower profile than the ND throughout the timescale. However, after 12 sessions of moderate exercise all study groups (i.e. CSII, MDI, ND) showed improvement in their TG/HDL ratio, as depicted in figure 5.8

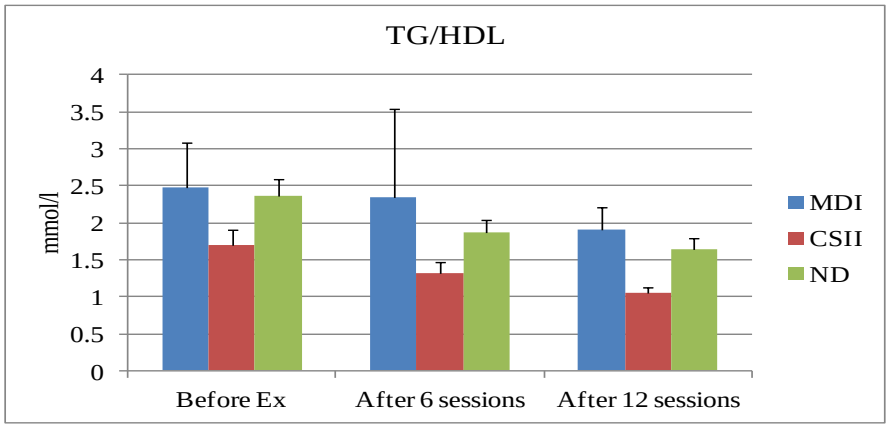


Figure 5.8: Average triglyceride to HDL- cholesterol ratio (TG/HDL). Please see the text for description and discussion.

5.6.5. Effect of exercise on insulin doses

The total amounts of insulin (basal and bolus) used as a mean for each diabetic group during the study are described in table 5.9. The bolus (unit/day) was approximately similar in both CSII and MDI in an exercise day and non-exercise day. The total amount of insulin units used was decreased in both groups for an exercise day. There is no significant difference between CSII and MDI it terms of carbohydrate to insulin ratio (table 5.9). However, the MDI cohort had a higher carbohydrate to insulin ratio compared to CSII. There were no events of hypoglycemia, hyperglycemia or DKA recorded during the exercise sessions for all study groups.

Table 5.9: Daily insulin used by volunteers during the study Mean ±SD

	Exercise day Unit/day		Non exercise day Unit/day		Total insulin		Carbohydrate to insulin ratio e.g. (10g of carbs/insulin units)	
					Exercise day Unit/day	Non exercise day Unit/day		
	Basal	Bolus	Basal	Bolus			Before study	After study
CSII	20.4 ± 9.5	39.8 ±18.2	25.0 ±11.5	45.3 ±12.3	60.2 ±26.9	70.7 ±20.1	1.40±0.5	1.40±0.5
MDI	37.00 ±12.12	42.50 ±24.75	40.67 ±9.02	48.0 ±25.46	79.50 ±35.13	88.67 ±36.69	1.50±0.70	1.50±0.70

5.6.6. Blood glucose level

The blood glucose (BG) was decreased at the end of each session for all groups as described for each group were described separately; figure 5.9 for ND, figure 5.10 for CSII and figure 5.11 for MDI).

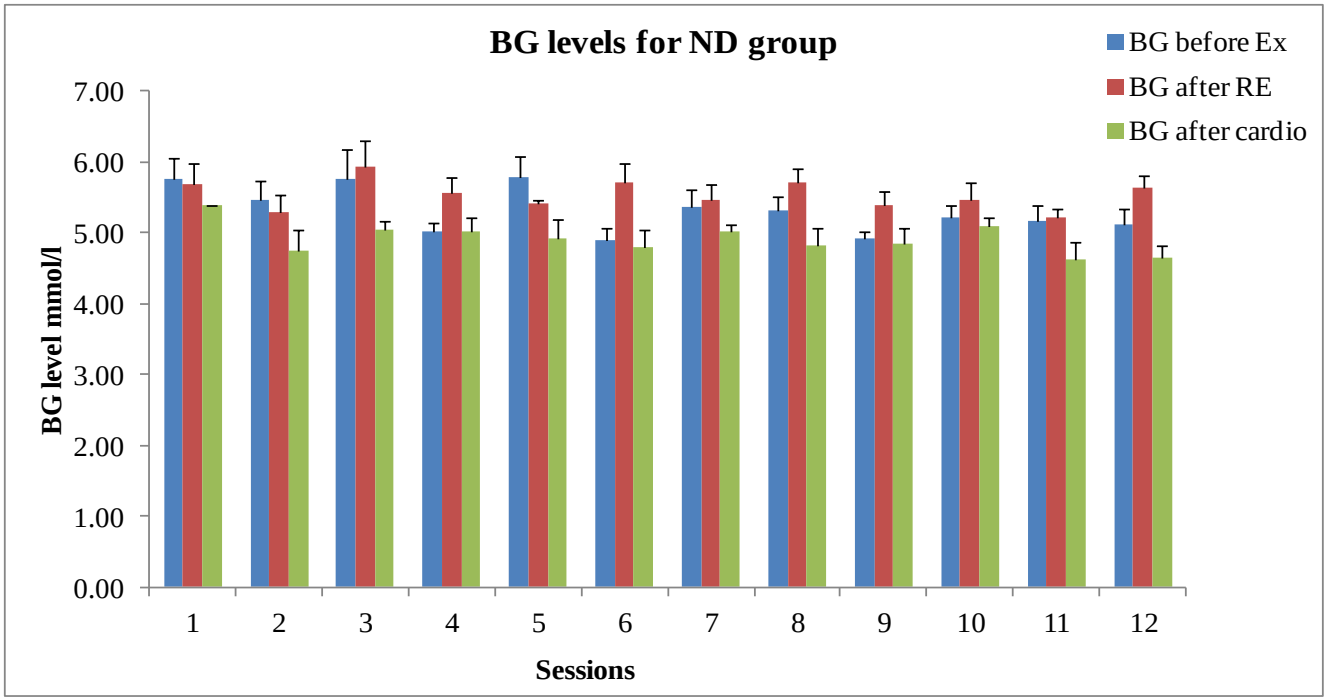


Figure 5.9: BG level for ND group before, after resistance and after cardio exercise. Data are means ± SEM; P-value was for before exercise vs after exercise and after resistance vs after exercise

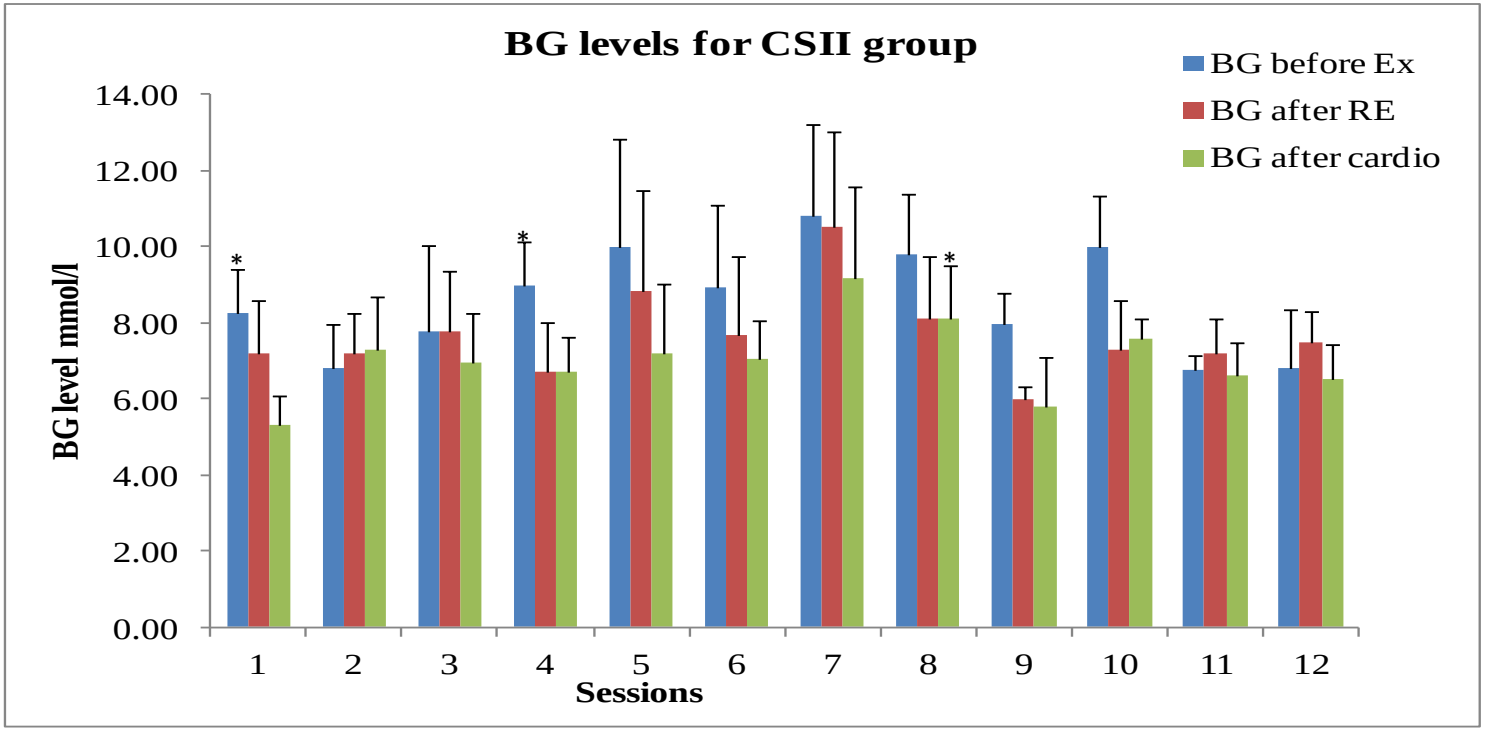


Figure 5.10: BG level for CSII group before, after resistance and after cardio exercise. Data are means ± SEM; P-value was for before exercise vs after exercise and after resistance vs after exercise.

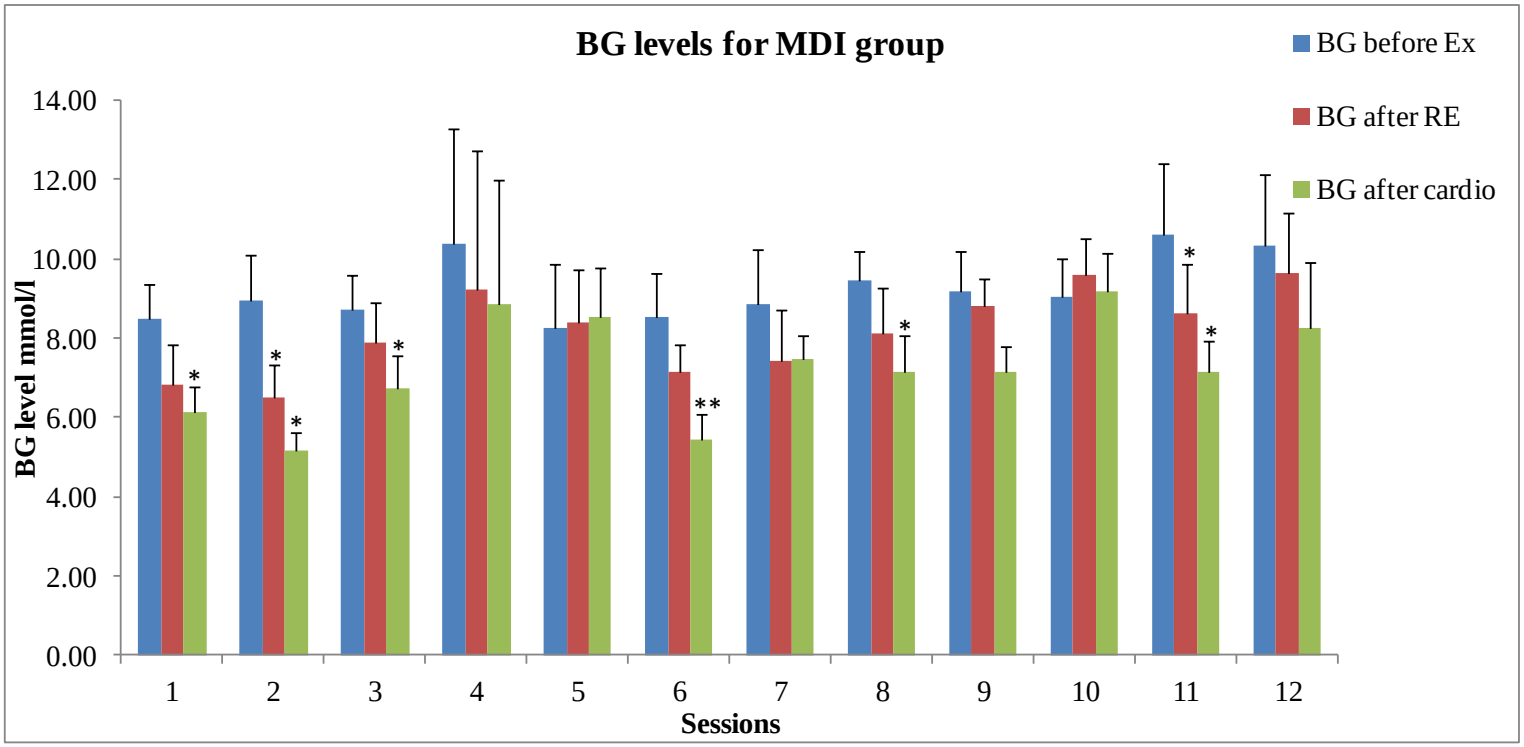


Figure 5.11: BG level for MDI group before, after resistance and after cardio exercise. Data are means ± SEM; Data are means ± SEM; P-value was for before exercise vs after exercise and after resistance vs after exercise.

The A1c level was significantly decreased when compared before and after the completed exercise programme (types of exercise and the intensity) in both CSII and MDI groups (P=0.005 and P<0.001 respectively) (see figure 5.12).

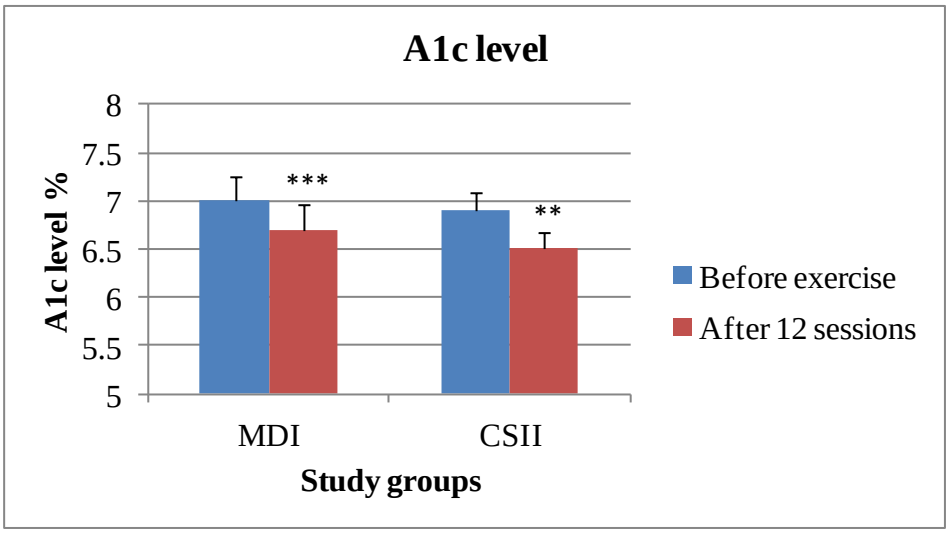


Figure 5.12: A1c for MDI and CSII group before and after 12 sessions of training. Data are means ± SEM; P-value was for before and after exercise. **≤0.01, ***≤0.001.

5.7. Discussion

This study examined the effects of 12 sessions (6 weeks) of moderate intensity of aerobic (bike) and resistance exercise. This was a pilot study, in which the methodology and aims were devised in order to investigate not only the role and effects of exercise, but the responses of participants with alternative forms of insulin delivery. In much in vitro work, matching of conditions is relatively simple and even in vivo work with animal subjects the variables can be minimised in well recognised ways. T1D people, however, will differ in age, body weight, fat distribution as well as duration of disease, experience since diagnosis and the possibility of silent complications. There will be differences in their ability to control their blood glucose profile for a variety of reasons,

including idiopathic, calorie utility-related, dietary and compliance. Whether pump users or not, their insulin dosage profile will be a variable in a study such as the one here, as will their eating habits (even if not their calorie totals) because serious interference with food intake and insulin habit would be clearly likely to have breached health and safety and ethics. Nevertheless, as demonstrated by good A1c readings, all the subjects who were diabetic in this study were well motivated in the maintenance of good BG control and of compliance with keeping to a similar diet the day before and during each study event.

The percentage of male T1D volunteers within the age limit and criteria set in this study will be much less than 0.5% of the male population with pump users forming less than a tenth of these and it turns out that pump users are not particularly forthcoming. Therefore finding even 5 matched pump-user volunteers within a small geographical location was a challenge. Help with recruitment might have been better if we had secured NHS ethical approval and been able to enlist from hospital patient lists, but experience of the research group had shown that this was a long laborious process that was not acceptable due to the timescale of the project. In fact, all participants in the group had to be entirely voluntary anyway (for example, a hospital patient could never be unwillingly seconded) and so inevitably the study groups, diabetic and healthy counterparts, were self-selecting, having answered adverts. Healthy people will not have seen the advertisements in the same places as diabetic subjects and pump users will have seen adverts in web forums, for example, not used by anyone but a pump user.

The overall effect was that standardising the group had to be accepted as a possible limitation on the validity of the study. Future studies will address this to some extent by looking to obtain NHS approval, for example, in order to increase the numbers. Nevertheless, our inclusion and exclusion criteria found subjects who were not very dissimilar to one another except for one overweight (not obese) T1D MDI user. It is obvious from the literature that similar studies have also found recruitment hard and many have similarly small numbers e.g. Jenn, S. (2010), Peake (2006), Hirose (2004) and Pietro Glassett (2006).

The metabolic effects were intended originally to have included an aerobic (cardiovascular) component and a resistance part that would be mainly anaerobic. For the reasons explained in chapter 1, the 50-60% intensity is likely to have constituted anaerobic exercise in the untrained, especially diabetic volunteers. However, the degree to which this was the case is an issue when comparing the untrained diabetic with the untrained healthy volunteers and so the resistance exercise has been termed as that and not specifically as anaerobic in this study.

Given these limitations, the main findings of this investigation are as follow:

- In table 5.2, BMI was not changed in MDI and ND groups whereas in CSII this was slightly decreased after 12 sessions. Nevertheless, the body fat percentages were slightly decreased as in MDI from 31.3 to 29.2, CSII from 26.9 to 25.08 and ND 23 to 20.7.
- The body fat percentage changed slightly in all study groups (table 5.2). MDI group had the highest body fat percentage (31.3%) then CSII (27%) and ND (23%).

- The exercise intensity (RPE) during resistance exercise (RE) for all groups was moderate to high (13-15.6) for the first two sessions which may explain the lack of weight lifting experiences and confidence. However, after couple of sessions the RE intensity back to the target (10.2-12.6); in MDI group the highest RPE number was given is 12.6 while CSII 11.8 and ND 11.7 (see table 5.3). Moreover, the RPE during cardio exercise was changed in all the groups; MDI from 13 to 11, CSII from 12.8 to 11.8 and ND from 13.7 to 11.3 (see table 5.4).
- The ratio between the amount of consumed O_2 and produced CO_2 (Respiratory exchange ratio-RER) was measured to indicate which fuel (carbohydrate or fat) was being metabolised to supply the body with energy during the 20 minutes of cardio. As in table 5.4 the RER was used between 1.0 and 1.1. at the beginning of the study and this high level could explain that the volunteers were uncomfortable with the face mask. Williamson, Fuld et al. (2012) confirmed the validity of RER in aerobic exercise (Williamson, Fuld et al. 2012). Without any activities and with a light diet RER is about 0.8. However, during intense exercise RER value can exceed 1, as a result of greater production of CO_2 by the working muscles and inhaled more of the O_2 gets used rather than being expelled as shown in table 5.4. As Rubini, Paoli et al. (2012) reported a positive significant correlation between O_2 uptake and carbon CO_2 during exercise (Rubini, Paoli et al. 2012).
- The lipid LDL was $\leq$2 mmol/L in CSII group before exercise sessions then reduced after 12 sessions to 1.5 mmol/L. However, MDI group has the lowest LDL level with 1.73 mmol/L then decreased to 1.59 mmol/L after 12 sessions.

ND group started with LDL level of 1.59 mmol/L then slightly decreased to 1.58 mmol/L after 12 sessions (see figure 5.2).

- The CSII group started this study with desirable cholesterol HDL 1.52 mmol/L then slightly increased 1.68 mmol/L. The MDI group started with HDL level 1.37 mmol/L then improved (1.64 mmol/L) after 12 sessions. However, the ND group started this study with borderline high of HDL 1.30 mmol/L then after 12 sessions of exercise become desirable (1.62 mmol/L) (see figure 5.3).
- All study groups started with desirable TC level (<4.03 mmol/L) then after 12 sessions decreased (<3.72 mmol/L). However, CSII group managed to reduce TC significantly after 12 sessions (from 4.03 mmol/L to 3.60 mmol/L) (see figure 5.4).
- All study groups started this study with desirable TG level <1.47 mmol/L. The CSII group has the lowest level of TG (1.12 mmol/L) before exercise, then significantly reduced after 12 sessions (0.80 mmol/L) (see figure 5.5).
- Cholesterol ratio for TC/HDL and HDL/LDL in all study groups was ideal (under 3.5 mmol/L and over 0.4 mmol/L respectively). The TG/HDL ratio for CSII and ND groups was well within the ideal range.
- The MDI group used more insulin unites a day (basal and bolus) compared to CSII group. The total amount of insulin unites used daily was decreased in exercise day in MDI (from 88 units to 80 units) and CSII (from 70 units to 60 units) groups (table 5.9).
- Carbohydrate to insulin ratio were not changed before study and after for both CSII and MDI (table 5.9).

- In this study no hypoglycemia or significant hyperglycemia were recorded during any exercise sessions for any of the study groups.
- The BG level was decreased after each session for all study groups. The BG level was mainly increased after resistance exercise each session for ND group and then decreased after cardio exercise (figure 5.9). In CSII (figure 5.10) and MDI (figure 5.11) groups the BG level was decreased after resistance and cardio exercises as they started with high BG level (8 mmol/L).
- The A1c level was significantly decreased after 12 sessions in MDI and CSII groups.

This study showed that CSII, MDI and ND groups have several benefits of this exercise programme.

The exercise intensity was investigated in this study by the reliability of the session rating of perceived exertion (RPE) scale (6-12). Moderate intensity was associated to be from 11-14 of RPE scale and consisted of 3 sets of 10 repetitions at 55-65% 1RM. This result is supported by the ACSM guidelines and the reliable method to measure various intensities of RE exercise (Scherr, Wolfarth et al. 2013).

The BMI was not significantly improved in all study groups. This result in line with Harris, Kumraoto et al (2009) in a meta-analysis concluded that the BMI was not improved with 6 months of physical activity in schools (healthy children) (Harris, Kuramoto et al. 2009). Other study determining the effect of aerobic (40 min walk or run) versus resistance training on T1D for 12 weeks 3 times, BMI were not changed with either aerobic alone or resistance alone exercise (Ramalho, de Lourdes Lima et al. 2006). However, in healthy people a moderate to high intensity aerobic exercise (60

min/day, 2 to 3 times/week) for 16 weeks resulted in a significantly decreased BMI (Yoshida, Ishikawa et al. 2010).

A combination exercise programme of aerobic and resistance at moderate intensity for 30 min, 5days/week for 12 weeks for overweight and obese adults was reported to produce a significant improvement of BMI, compared to aerobic alone which had no effects on BMI (Ho, Dhaliwal et al. 2012). However, a study by Church et al. (2010) for 9 months of combination exercise observed a significant decrease in BMI compared to RE alone (Church, Blair et al. 2010). The use of insulin is the only therapy for T1D and this often results in weight gain (Hindmarsh, Peters et al. 2013). BMI is an indirect measure of body fat percentage body and lipid concentrations (Lamb, Ogden et al. 2011). The mechanisms involved for the accumulation of fat with exogenous insulin are unclear and further investigations are warranted (Ho, Dhaliwal et al. 2012).

Similarly, combination exercise for 12 weeks decreased but not significant the percentage of body fat as shown in Ho, Dhailwal et al (2012) study and many other studies like Healthy Lifestyle in Europe by Nutrition in Adolescences (HELENA) (Martinez-Gomez, Ruiz et al. 2010).The body fat percentage seems to reflect serum lipid concentrations in obese healthy (Choi, Pai et al. 2002). Lipids profile such as triglycerides is the main component of body fat in health people (Arrese and Soulages 2010). Prabhakaran, Dowling et al. (1999) reporeted the effect of resistance exercise alon for 14 weeks on body fat percentege as well as lipid profile in healthy (Prabhakaran, Dowling et al. 1999). However, Laaksonen, Atalay et al. (2000) reported the improvement of lipids profile after 12- to 16-week (30-60 min, 3-5 times a week) aerobic exercise in young T1D but not the body fat percentage (Laaksonen, Atalay et al. 2000).

The lipids profiles LDL and HDL are improved in this study in all study groups. Ho, Dhaliwal et al (2012) had also shown same result in their study after 12 weeks of training. The decrease of LDL and the increase of HDL in our study is associated with loss of BMI as reported in Guo study (Guo, Kawano et al. 2011).

Improved glycemia control in T1D patients also improves plasma lipid profile (Feitosa, Feitosa-Filho et al. 2013). It has been suggested that the high dosage of insulin in T1D and poor glycemic control may lead to lower LDL; this may explain the low level of LDL in MDI group in this study (Feitosa, Feitosa-Filho et al. 2013). A1c in adult T1D has been significantly associated in change in lipids including LDL, HDL,TG and TC when dyslipidaemia disappeared medications (Maahs, Ogden et al. 2010).This study was comparing T1D with dyslipidaemia medication and without; the high A1c is associated with poor lipids control (TC, LDL, TG and non-HDL). In the present study, we also found the TC and TG were significantly low after 12 sessions (six weeks) of combined exercise in CSII groups. Triglycerides, TC, LDL and HDL were improved significantly in T1D after using CSII therapy (Weng, Li et al. ; Derosa, Maffioli et al. 2009).

Pro-inflammatory cytokines may play a role in lipid profiles. It has been shown that IFN-γ was positively associated with TC and LDL cholesterol in patients (Hocaoglu, Kural et al. 2012). IL-6 is involve in hepatic triglyceride secretion as well as lipoprotein lipase activity (Fernández-Real, Broch et al. 2000). TC and HDL was increased when TNF-α was blocked (Pollono, Lopez-Olivo et al. 2010).

To predict heart disease risk TC/HDL and HDL/LDL ratios are used (Lemieux, Lamarche et al. 2001). In this study the TC/HDL ratio was already ideal in all the

participant groups. Results of this study demonstrate that the TC/HDL ratio was improved in all study groups over the 12 exercise sessions as in figure 5.6. This included the ND group and the reason is probably that the latter were not taking medication, whereas the diabetic participants often were. Consequently there were no very dramatic changes, but the so-called healthy group clearly also benefited in terms of slight but beneficial lipid ratio changes.

Lowering the TG/HDL ratio in our study (figure 5.8) is supported by Nicholls (2011) study which explained the beneficial impact on progression of coronary atherosclerosis in diabetic patients (Nicholls, Tuzcu et al. 2011). Moreover the TG/HDL ratio predicts all-cause mortality in women with suspected myocardial ischemia (Bittner, Johnson et al. 2009). Plasma concentration of TG/HDL ratio might be a useful surrogate estimate of insulin action (Salazar, Carbajal et al. 2012). Moreover, circulating cholesterol and triglyceride levels are associated with vascular injury in T1D (Mäkinen, Soininen et al. 2013). Lipoproteins are responsible for transporting lipids, and alterations in their subclass distributions may partly explain the increased mortality in individuals with T1D (Mäkinen, Soininen et al. 2013).

Cholesterol metabolism seems to play a role in vascular health beyond serum lipids in T1D (Koponen, Hallikainen et al. 2011). This could related to the insulin role as a central player in the regulation of lipid metabolism (Vergès 2009).

Abnormalities in lipid levels are observed in T1D patients with poorly controlled glycemia, (i.e. increased triglycerides and LDL cholesterol), or in micro- or macro albuminuria (i.e. with a background of increased triglycerides and LDL cholesterol, but decreased HDL cholesterol). However, T1D with good glycemia control show normal

or slightly decreased triglyceride and LDL-cholesterol levels and, sometimes, increased HDL-cholesterol levels. However, even with good glycaemic control in T1D, qualitative abnormalities of lipoproteins can sometimes be observed and abnormalities are not fully explained by hyperglycemic tendency. Peripheral hyperinsulinemia, associated with subcutaneous insulin administration (to deal with high blood glucose) has been implicated. The precise consequences and pathways of such qualitative lipid changes on the development of cardiovascular disease in T1D patients are, as yet, still unknown (Vergès 2009).

In this study, T1D patients (MDI or CSII) tended to decrease their total insulin doses in an exercise day, so this differed from our survey. De Mol, De Vries (2011) reported the decrease in insulin doses by 14.2% when T1D patients did aerobic exercise (de Mol, de Vries et al. 2011). In the meta-analysis by Kennedy, Nirantharakumar et al. (2013) the reduction of insulin doses around the time of exercise time was associated with decreased hypoglycemia after exercise (Kennedy, Nirantharakumar et al. 2013). Moreover in Hall, McDonald et at (2013) assessed the role of combination exercise (resistance and aerobic) for 3-6 weeks on insulin sensitivity in T1D,and their results suggested that this mixture of exercise may improve glucose tolerance, with each exercise type leading to differential improvements (ie decreased requirement) for exogenous insulin (Hall, McDonald et al. 2013).

In one study, the reduction of basal insulin during exercise created a trend towards hyperglycemia in T1D with CSII therapy (Younk, Mikeladze et al. 2011). Yardley, Kenny et al. (2012) reported that the combination of resistance and aerobic exercise with T1D patient reduced the severity of post-exercise hypoglycemia and improved the glycemic stability throughout exercise for individuals with T1D when they performed

resistance exercise before aerobic exercise (Yardley, Kenny et al. 2012). Moreover, Yardley, Iscoe et al. (2013) concluded that the regular moderate-to-heavy exercise in T1D using CSII was more likely to limit post exercise hyperglycemia compared to MDI users and did not increase the risk of post-exercise late-onset hypoglycemia (Yardley, Iscoe et al. 2013). Hyperglycemia in T1D is most likely during high intensity exercise because of counterregulatory effects (Yardley, Kenny et al. 2012) whereas hypoglycemia can happen at rest after moderate intensity (McCrimmon 2011). Moreover, recently Davey, Howe et al. (2013) reported the moderate intensity increased the risk of hypoglycemia during exercise and several hours after (Davey, Howe et al. 2013). Several factors have been associated with exercise-related hypoglycemia such as start exercise session with BG level below 5 mmol/L, insufficient carbohydrate intake increased duration of moderate-intensity exercise (McCrimmon 2011).

In this study the BG level was commonly reduced after cardio exercise sessions but not at hypoglycemia level which is similar to Yardley, Iscoe et al. (2013) finding with MDI and CSII groups when they had similar reductions in glucose levels during moderate intensity exercise (Yardley, Iscoe et al. 2013). Moreover, Guelfi, Jones et al. (2005) concluded that the decline in BG levels is more with moderate-intensity exercise compared with intermittent high-intensity exercise in T1D (Guelfi, Jones et al. 2005). As shown in this study the aerobic exercise is a more likely cause of a decrease in BG level than resistance exercise and Yardley, Kenny et al. (2012) found similarly (Yardley, Kenny et al. 2012). Moreover, they recommend performing aerobic exercise after resistance exercise. This regimen will cause less of a decline in blood glucose during exercise in individuals with T1D than when exercise is performed in the opposite order.

This study concludes that A1c was significantly decreased in MDI and CSII groups after 12 sessions of resistance and aerobic exercise (figure 5.12). However, not all others have observed the same. In one study of T1D, exercise was largely unsuccessful in showing a benefit on A1c (Chimen, Kennedy et al. 2012) and we postulate that this could result from hyperglycemia immediately after exercise using inappropriate protocols such as the predominance of resistance exercise.

Salem, AboElAsrar et al. (2010) reported that exercise improves glycemic control among T1D (Salem, AboElAsrar et al. 2010) in children with T1D. Regular physical activity has been recommended to control glycemia without increasing the risk for severe hypoglycemia (Herbst, Bachran et al. 2006). Physical activity has provided clear evidence of improved glycaemic control in T2D patients (Chimen, Kennedy et al. 2012). In T1D the improvement of A1c leads to improve lipids profile (Maahs, Ogden et al. 2010) as shown in LDL for CSII group (figure 5.2), HDL in CSII and MDI groups (figure 5.3), TC for CSII and ND groups (figure 5.4) and triglyceride in CSII group (figure 5.5) and cholesterol ratios (figure 5.6-5.8)

5.8. Conclusion

- Physical activity at moderate intensity that includes a combination of aerobic and resistance training has provided clear evidence of improving glycaemic control. Others have found that reductions in A1c demonstrated the advantages of resistance exercise but not aerobic exercise in T1D (Yardley, Iscoe et al. 2013).

- We found that performing resistance exercise before aerobic exercise is more beneficial because this may lead to less hypoglycemia during exercise in individuals with T1D.
- For one individual who responded with hyperglycaemic levels post exercise, this improved over the six weeks.
- The reduction in basal insulin rate before exercise was safe and effective in reducing hypoglycemia because doing so may attenuate the decline in glucose levels during subsequent aerobic exercise.
- Aerobic exercise after resistance exercise seems to lead to lower reliance on glucose supplementation during exercise. In the ND group, this order of exercise was shown to increase the use of lipids as a fuel source during exercise.
- The lipid profiles were improved for volunteers, including LDL, HDL, TC and TG plus the various ratios, but not the BMI or body fat.
- Regular moderate physical activity should be recommended in patients with T1D. Resistance exercise should be programmed in before cardiovascular exercise.

Chapter 6: Epilogue

6.1. General Discussion

The overall objective of this thesis was to further the understanding of the effect of exercise on T1D patients who received insulin either via MDI or via CSII. This study was conducted by employing three groups of volunteers i.e. T1D using CSII, T1D using MDI and ND.

Key observations from these studies are discussed in the following sections.

I. The most important parameter evaluated in T1D patients using CSII was the A1c. It was observed that after using CSII the A1c level dropped as compared to before. Moreover, pump users concluded that they calculated their carbohydrates and following a diet, consumed more frequent meals, exercised frequently. Exercise was mainly aerobic (walking) at moderate to high intensity for 3 times weekly. Insulin pump users were asked if they regularly counted carbohydrates intakes and 99.4% participants answered that they did. CSII users had desirable lipid levels; ie total cholesterol, LDL cholesterol and HDL cholesterol (75%, 68% and 65% respectively). They reported that the exercise decreased the BG level compared to before exercise. However, they very rarely had hypoglycemia events during exercise sessions and stated that hypoglycemia was not of particular concern to them as a barrier to exercise. The last point evaluated was part of their opinion of quality of life and treatments with a pump. Pump users reported that their daily life was much better than those on MDI therapies as CSII have more freedom from worry about daily injecting before meals and better control over their BG readings. Currently CSII is the closest choice

available to the physiologic method of insulin delivery, offering the possibility of more flexibility and more precise insulin delivery than MDI.

II. One of the most important goals of this thesis was to determine the effects of combined exercise training; resistance exercise (resistance training ie weight lifting) and aerobic training (cycling exercises) on the immunological changes (IL-6, IL-1β, TNFα and INFγ) in patients with T1D using MDI or CSII and non-diabetic.

The effect of exercise on (IL-6, IL-1β, TNFα and INFγ) was divided into acute and chronic effects as described in chapter 4. The results of the immunological changes indicate the importance of the immune response and its occurrence of IL-6, IL-1β, TNFα and INFγ in T1D. Both studies show the strong association of these cytokines (IL-6, IL-1β, TNFα and INFγ) with T1D as autoimmune diabetes. This link between these cytokines as secreted by T-cells and macrophages, with the pathogenesis of T1D has been a focus in some recent studies. However, the relationship of combined exercise (resistance and aerobic) and plasma cytokines (IL-6, IL-1β, TNFα and INFγ) with T1D either MDI or CSII and non-diabetic has been explored for the first time in the current study.

III. The other important goals of this study were to determine the effects of this exercise training on; BMI, body fat %, RPE, RER, lipid ratios, total amount of insulin (basal and bolus), BG and A1c changes in patients with T1D using MDI or CSII and non-diabetic.

The BMI and body fat were not significantly affected by the 12 sessions (6weeks) of moderate intensity of aerobic and resistance exercise in both study groups. The Rated Perceived Exertion (RPE) decreased during cardio and

resistance through the exercise sessions in all groups. However, Respiratory exchange ratio (RER) was unchanged during cardio exercise in all groups. The LDL and HDL were not affected by this exercise programme while TC was significantly affected in the ND and CSII groups (increased and decreased respectively). The TG was decreased significantly only in the CSII group. The total amount of insulin was decreased on exercise days but not significantly in MDI and CSII. The BG level was mainly increased after resistance only in ND group and then back to normal or lower after cardio exercise. Cardio exercise was decreased the BG level in both MDI and CSII. The A1c was advantageously and significantly affected by the 12 sessions of resistance and cardio exercises.

6.2. Recommendations

The studies in this thesis highlight several directions for future research:

1. The need to develop use of autoimmune markers (especially TNFα) for proper diagnosis in the screening of diabetes mellitus and to describe patients who will benefit from early insulin therapy and who may eventually benefit from specific immunotherapy.
2. The need to determine if cytokine markers can be used as indicators for T1D progression and can be used to monitor the efficacy of therapy and in TID patients and in those who are at risk of developing T1D.
3. The biology of cytokines needs to be investigated to improve our understanding in regulating autoimmune diabetes in humans and possibility of potential therapies involving cytokines, such as specific blockers.

4. The need to advise T1D patients to change to insulin pump therapy, as our results indicates that this is a major improvement over MDI, using lipid, glucose and immune markers as the criteria for assessment.
5. Exercise should be one of the major tools of diabetes therapy since its effectiveness, as shown here, can be proved by objective metrics.
6. This research using moderate intensity exercise (resistance and aerobic) has shown beneficial results following a single session in term of immunological and following six weeks of both exercise types in people with T1D.
7. Where a combination is used, there is greater advantage if the resistance exercise comes first.
8. Recommendations are needed to improve the education program for those treating T1D, especially by extending the use of insulin pumps, whether for children or adult. The restriction in the UK to their use mainly in children and in difficult to treat adults, as imposed by NICE is short-sighted. This research result can be used to guide this process because it is clear that exercise works synergistically with pump use to great advantage in T1D.

6.4. Limitations

There are some limitations with respect to this work that need to be considered:

In this study there were limitations that were noted.

- The main limitation was sample size. The target number of volunteer was to recruit 60 subjects: 20 T1D on MDI therapy, 20 non-diabetic and 20 T1D on insulin pump therapy. However, fewer were recruited in all groups and because pumpers were rarer, fewer still of these were found. The subjects who were recruited underwent tests to make sure that they were matched, safe and suitable

on the basis, for example, of background health and body measurements. Several were rejected in this process. Greater volunteer numbers and even sized groups would have improved the confidence in the comparison of the study groups (ND, MDI and CSII). On reflection, NHS ethical approval would have increased the pool of possible recruits and although NHS ethical approval can take more than a year, it would have been worth doing.

- The tissue storage regulations limited this study in practical terms to frozen (-80C) plasma and serum only and not for the blood cells. For this reason that we were not able to do the cellular assays like Peripheral Blood Mononuclear Cells (PBMCs). This, in addition to cytokine measurements, would have provided a more informative profiling of the cellular immune system such as monocytes and lymphocytes (T cells, B cells, and natural killer (NK) cells) to describe the diabetic status.
- This study is the first to apply the immunological and metabolic parameter measurements to assess the response of pump users in comparison to MDI, to this combination of exercise. Thus there is no precedent available to compare and contrast the findings of this study and this did limit the discussion.
- As mentioned previously a survey was conducted using a questionnaire with structured questions. It is a general practice to conduct a pilot study before the launch of the original survey but in this study this was not done here. During the development of the questionnaire advice was taken from health professionals and researchers especially statisticians with specialist knowledge of questionnaire design to make the questions simple and understandable. It is a common practice to use triangulation i.e. multitude of methods are used to verify the results of the

study. Unfortunately, in this study the only scope for the triangulation was to compare the survey with the experiences of the exercise volunteers and with other studies in the literature. Every care was taken during the data analysis to use variety of statistical techniques to analyse the data and check the validity, consistency and significance of data. It is probably important to note that this study itself was a pilot for others planned, but the questionnaire results might well have been improved if a very small trial group had been recruited. An example is that the shortcomings of the lipid readings section might have been improved.

6.5. Future work

This study has raised number of additional questions and areas for future research.

The observations on the effect of acute and 12 sessions of aerobic and resistance exercise on ND and T1D using CSII or MDI as insulin therapy indicate that the advantages of these exercise for T1D patients. It would therefore be interesting to investigate:

- insulin sensitivity in both groups.
- use of Peripheral Blood Mononuclear Cells (PBMCs), the immune system technical for blood cells which a critical component, such as monocytes and lymphocytes, with the lymphocyte population consisting of T cells, B cells, and natural killer (NK) cells.
- the comparison of T2D using insulin with T1D for the same exercise programme and cytokines. Since pumps are being developed for T2D users, a further comparison might be incorporated.

- a focus on subjects from a single ethnic group of sufficiently large sample size such as Saudi Arabia or south Asia, since the diabetes problem may be different in ways this study could determine.

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APPENDIX

A. Insulin types in the market

B. Insulin pump in the market

C. De Montfort University Insulin Pump Users Survey 2009 publication

D. De Montfort University Insulin Pump Users and Exercise Survey 2011

E. Ethics approval

F. Standard Operating Procedure (SOP)

G. Volunteer Information Sheet (VIS)

H. Consent Form

I. Volunteers Health Screen

J. Volunteer Record Sheet

K. Visit Measurements

L. Assessment of caloric intake

Appendix A

List of insulins in the market

The table highlights the various insulin types, their manufacturers, the insulin source and how it's available. It also highlights insulin ranges that have been discontinued.

Insulin type	**Name of insulin**	**Manufacturer**	**Source**	**Vial, cartridge or prefilled pen**
Rapid-acting analogue	Humalog	Lilly	analogue	vial & cartridge
	Humalog	Lilly	analogue	prefilled pen
	Novorapid	Novo Nordisk	analogue	vial
	Novorapid Penfill	Novo Nordisk	analogue	cartridge
	Novorapid Novolet	Novo Nordisk	analogue	prefilled pen
Long-acting analogue	Lantus	Aventis	analogue	vial, cartridge & prefilled pen
	Levemir	Novo Nordisk	analogue	cartridge
Short-acting	Human Actrapid	Novo Nordisk	human	vial
	Actrapid Pen*	Novo Nordisk	human	prefilled pen
	Actrapid Penfill*	Novo Nordisk	human	cartridge
	Human Velosulin*	Novo Nordisk	human	vial
	Pork Actrapid*	Novo Nordisk	pork	vial
	Humaject S*	Lilly	human	prefilled pen
	Humulin S	Lilly	human	vial & cartridge
	Hypurin Bovine Neutral	CP Pharmaceuticals	beef	vial & cartridge
	Hypurin Porcine Neutral	CP Pharmaceuticals	pork	vial & cartridge
	Insuman Rapid	Aventis Pharma	human	vial & cartridge
	Insuman Rapid Opti Set	Aventis Pharma	human	prefilled pen

Medium & long-acting	Humulin I	Lilly	human	prefilled pen
	Humulin I	Lilly	human	vial & cartridge
	Humulin Lente	Lilly	human	vial
	Humulin ZN	Lilly	human	vial
	Human Insulatard	Novo Nordisk	human	vial
	Insulatard Penfill	Novo Nordisk	human	cartridge
	Human Insulatard Pen*	Novo Nordisk	human	prefilled pen
	Human Monotard*	Novo Nordisk	human	vial
	Pork Insulatard*	Novo Nordisk	pork	vial
	Human Ultratard*	Novo Nordisk	human	vial
	Hypurin Bovine Isophane	CP Pharmaceuticals	beef	vial & cartridge
	Hypurin Bovine Lente	CP Pharmaceuticals	beef	vial
	Hypurin Bovine Protamine Zinc	CP Pharmaceuticals	beef	vial
	Hypurin Porcine Isophane	CP Pharmaceuticals	pork	vial & cartridge
	Insuman Basal	Aventis Pharma	human	vial & cartridge
	Insuman Basal OptiSet	Aventis Pharma	human	prefilled pen
Analogue mixtures	Humalog Mix25	Lilly	analogue	cartridge
	Humalog Mix25	Lilly	analogue	prefilled pen
	Humalog Mix50	Lilly	analogue	prefilled pen
	NovoMix 30	Novo Nordisk	analogue	prefilled pen & cartridge
Mixtures	Humaject M3	Lilly	human	prefilled pen
	Humulin M2	Lilly	human	cartridge
	Humulin M3	Lilly	human	vial & cartridge
	Humulin M5	Lilly	human	vial
	Human Mixtard 30**	Novo Nordisk	human	vial
	Human Mixtard 50	Novo Nordisk	human	vial

	Human Mixtard 10 Pen	Novo Nordisk	human	prefilled pen
	Human Mixtard 20 Pen	Novo Nordisk	human	prefilled pen
	Human Mixtard 30 Pen**	Novo Nordisk	human	prefilled pen
	Human Mixtard 40 Pen	Novo Nordisk	human	prefilled pen
	Human Mixtard 50 Pen	Novo Nordisk	human	prefilled pen
	Mixtard 10 Penfill	Novo Nordisk	human	cartridge
	Mixtard 20 Penfill	Novo Nordisk	human	cartridge
	Mixtard 30 Penfill**	Novo Nordisk	human	cartridge
	Mixtard 40 Penfill	Novo Nordisk	human	cartridge
	Mixtard 50 Penfill	Novo Nordisk	human	cartridge
	Pork Mixtard 30*	Novo Nordisk	pork	vial
	Hypurin Porcine Isophane 30/70 mix	CP Pharmaceuticals	pork	vial & cartridge
	Insuman Comb 15	Aventis Pharma	human	vial & cartridge
	Insuman Comb 15 OptiSet	Aventis Pharma	human	prefilled pen
	Insuman Comb 25	Aventis Pharma	human	vial & cartridge
	Insuman Comb 25 OptiSet	Aventis Pharma	human	prefilled pen
	Insuman Comb 50	Aventis Pharma	human	vial & cartridge
	Insuman Comb 50 OptiSet	Aventis Pharma	human	prefilled pen

* Denotes that the insulin range is discontinued. ** Mixtard 30 withdrawn on December 31, 2010. (Adapted from diabetes.co.uk)

Appendix B

Comparisons of Current Insulin Pumps

Links **800#'s**	Animas (877) 937-7867	Accu-Chek (800) 280-7801	Medtronic (800) 933-3322	Insulet (781) 457-5000	Sooil US (866) 747-6645	Tandem Diabetes (877) 801-6901
Model	Ping 	Spirit® 	Paradigm Revel 	OmniPod 	DiabecareIIS 	t:slim 
Dim. [mm]	51 x 77 x 18	80 x 47 x 24	523: 50.8x76.2x20.3 723: 50.8x91.4x20.3	pod: 41x61x18 pda: 66x110x26	46 x 77 x 19	79.5 x 50.8 x 15.2
Volume	5.525 ci 90.54 cc			Pod: 2.7 ci PDA: 11.5 ci	4.3 ci 67 cc	3.746 ci or 61.38cc
Weight	3.9	2.8, 4.8	523: 3.53 oz	OP: 1.2 oz	1.9 oz	3.95oz. with full

[oz]		with battfull cartridge inf set	723: 3.81 oz	(full res.) PDM: 4.0 oz (w/ batteries)		reservoir
Reservoir Size	200u plastic	315u	523: 176 u 723: 300 u	200u	300u plastic	300u
Connection	Luer lock	Luer lock	Proprietary	Built-in	Proprietary	Luer Lock
Screen Size						60.198
Colors	blue, silver, black, pink, green	Blue, with 30 pump skins in colors and styles	blue, clear, pink, purple, smoke or customize	white	Black, Gray, Pink, Green, White	Standard Black. Case available in 5 colors: aztec black, midway silver, alpine white, pacific purple, roselle pink Black screen with blue, orange, red, grey text

Basal Increment	0.025u	0.1u from 0.1 to 25.0 u/hr	0.025 u	0.05 u u/hr, up to 30 u/hr	0.1 u/hr or 0.01 u/hr	0.001 at programmed rates ≥ 0.1 units/hr
Total Basals	12/day	24/day	48/day	48/day	24/day	16/day
Basal Profiles	4	5	3	7	1	6
Basal Interval	30 min	60 min	30 min	3 min	60 min	1 minute increments
Basal Delivery	varies, 0.2 u/hr every 3 mins	every 3 mins	varies, 0.6 u/hr = every 10 mins		every 4 mins	Every 5 mins
Temp Basal	-90% to +200% in increments of 10% for 0.5 to 24 hours (30 min increments)	in 10% increments from 0% to 200%, and 15 min to 24 hr	+/- 0.1 u increment as single basal rate for 0.5 to 24 hrs or as % of current basal	% or u/hr (1-12 hrs, in 30 min increments)	10% increments from 0% to 200% and up to 12 hours	6 temporary basal rates. Duration 15 minutes to 72 hours. Increments of 1 minute. From 0% to 250% of current basal (in

						increments of 1%).
Bolus Increments	0.05 visual or audio, 0.1, 1.0, 5.0 audio	0.1, 0.2, 0.5, 1.0, 2.0	.025, 0.1 visual, 0.5 or 1.0 visual or audio, remote extra	0.05, 0.1, 0.5, 1.0u	0.1, .05, 1.0u	0.05 - 25 units
Carb and Correction Factors	yes, carb and BG values can be entered into the pump or meter-remote	yes, manual carb, BG from Accu-Chek BG monitor	yes, manual carb, BG direct from BD meter or manual entry	yes	Yes, manual carb	Yes, manual carb
Bolus Type	units or carbs: standard, extended, combination	quick, scroll, extended, multiwave	units or carbs: standard, extended, combination	Meal, correction, meal & correction; normal, extended, combination	Normal, extended, combination	units or carbs, normal, quick, extended
1 u Bolus Duration	1 or 3 sec	5 sec	30 sec	40 sec	12 sec	~ 30 sec
Battery	AA lithium or alkaline x 1	AA x 1 Alkaline or Rechargeable	AAA for pump, A23 for remote	AAA x 2 (PDA)	1/2 AA 3.6v lithium	Integrated rechargeble lithium polymer battery

Battery Life	4-6 with lithium, 2-4 with alkaline	4 week	3 weeks	4 weeks	8-10 weeks	7 days with a full charge
Motor	DC	DC	DC	stepper	DC	Micro-Delivery Technology
Memory	non-volatile: 500 boluses, 270 basals, 120 daily totals, 60 alarms, 60 primes, 900 BG levels	non-volatile: 90 days (4,500 events); history recall of last 30 boluses, alerts, daily insulin totals, and temporary basal rate increases	4000 events, volatile (basal & history loss can occur): 24 boluses, 7 day totals	90 days of data (up to 5400 records)	Last 500 boluses, primes and daily totals. Last 100 alarms (all time and date stamped)	Up to 90 days (11,000 events)
Software Download	ezManager Max, downloads in 3 minutes with dongle and software that is available at Animas	Pocket Compass with Bolus Calculator , insulin pump configuration software, IR Communication Port	Medtronic CareLink® Therapy Management System and ParadigmPAL™ 3.0 Software at Medtronic	Omnipod Extension for the CoPilot Health Management System	none	t:connect Diabetes Management Application; Mac and PC compatible, Micro USB download of t:slim Pump and supported BG meters

Water?	12 ft for 24 hrs	IPX 8, 60 minutes at 2.5 meters	splash resistant	watertight	Watertight	IPX7 Rating. Watertight - tested at 3 feet for 30 minutes.
Extra Features	Meter-remote offers wireless bolus calculation and delivery within 10 ft. ezCarb software stores up to 500 food items from Calorie King database on the meter-remote. Calculator features for carbohydrates, blood glucose corrections and insulin; reminders for time of day and when to perform blood glucose checks. Pump and meter-remote are available in Spanish. PC and Mac downloads are available with ezManager Max software	choice of standard, advanced or custom selectable user menus, icon- and menu-driven programming, backlit display, reversible display screen, 12 languages, audible or vibrating bolus confirmation and alerts.	Enhanced CGM Feature, Predictive Alerts, Low and High Alerts, Rate of Change Alerts, REAL-Time Trend Graphs	backlight, reminders & alerts, child lock, integrated Freestyle meter, 1000 common foods in PDA, Tubeless	Carb Counting Program, Auto Dose capability, Bolus frequency restrictions, preset meal and default bolus. PIN# programming and access to functions including daily maximums and mode settings for healthcare professions / caregivers. Backlight, icon menu. Auto display of remaining insulin and remaining battery life. Lock-out feature with PIN unlock. Twelve languages	Only touch screen insulin pump in the US
Guarantee	4 years	4 years	4 years	4 years	4 years	4 years

Adapted from: diabetesnet.com

It is a pleasure to accept your revised manuscript entitled "Insulin pump users would not rule out using an implantable artificial pancreas: experiences of diabetes self-management by insulin pump users and their attitudes toward a non-electronic implantable closed loop insulin pump (INSmart)" in its current form for publication in Practical Diabetes.

Thank you for your contribution.
Sincerely,
Dr. Rustam Rea
Co-Editor, Practical Diabetes
rustam.rea@nhs.net

Insulin pump users would not rule out using an implantable artificial pancreas: experiences of diabetes self-management by insulin pump users and their attitudes toward a non-electronic implantable closed loop insulin pump (INSmart)

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ABSTRACT

Aim: To establish the limitations of open loop continuous subcutaneous insulin infusion (CSII) as perceived by current users of the technology, and to ascertain their interest in and requirements for a non-electronic implantable closed loop insulin pump, INSmart, currently under development for the treatment of type 1 diabetes. INSmart has been surgically implanted in the peritoneum in animal models and continuously restored normoglycemia.

Methods: A bottom-up survey design was used to determine both positive and negative experiences of patients currently using CSII to define the performance characteristics they would require from a non-electronic, implantable closed loop insulin pump.

Results: 360 insulin pump users completed the survey. All respondents had type 1 diabetes, were predominately from English-speaking countries and had been diagnosed before age 34 years. Most had well controlled blood glucose (BG) according to their self-reported HbA1c results. They reported a reduction in this value after transferring to CSII from multi-dose injections. However, 70% of pump users had more than three hypoglycaemic episodes per week. 80% reported self-measured BG values above 10 mmol/L three or more times per month. 94% respondents considered a (non-electronic implantable) closed loop insulin pump would make their BG management easier and improve their quality of life.

Conclusions: The majority of respondents felt there were still many disadvantages to current external insulin pumps such as their constant visible presence, rotation of insertion sites and skin inflammation. These shortfalls could be overcome by a device, such as INSmart that provides a relatively instant feedback mechanism for controlling insulin release due to its proposed location in the peritoneal cavity. Running Head: Attitudes Toward a Non-electronic Implantable Closed Loop Insulin Pump (INSmart)

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Introduction

Successful glycaemia management in diabetes requires mean blood glucose (BG) concentrations that result in glycated haemoglobin (HbA1c) values close to the normal range, while avoiding hypoglycaemia. Although of proven efficacy, it is difficult to achieve this chronically using multi-dose insulin injections or open-loop continuous subcutaneous insulin infusion (CSII), as evaluated in the Diabetes Control and Complications Trial (DCCT) [1,2] for patients with type 1 diabetes.

The attraction of a closed loop insulin delivery system which can maintain normoglycaemia is obvious to both patients and health care services that have to deal with the costs of poor diabetes control around the world. [3]. In order to produce an effective closed loop system, insulin needs to be released and metabolised over an appropriate time scale to minimise fluctuations in BG levels. Several methods for accomplishing closed loop control have been developed in both human and animal models [4-6] but the "perfect" artificial pancreas remains elusive [7,8], because of limitations in one or more of the contributory components of a closed loop system, namely delivery devices and sensors.

External insulin pumps or CSII are driven by mechanical force and provide a continuous infusion of a short-acting insulin delivered from a soft cannula under the skin. The major drawbacks to this therapy however, are primarily the slow absorption of insulin into the plasma, the need to re-site subcutaneous cannulas every 48 hours in order to minimise the risk of tube blockages and skin infection at the insertion sites. These contribute to delays and failures in response and have contributed to the difficulty in converting a CSII system to a closed loop system. Similarly, subcutaneous glucose sensors which have become part of some integrated CSII systems rely on the difference between subcutaneous glucose and BG being proportional to the rate of change taking place in BG [9], this time lag limits the sensitivity of continuous glucose monitors to detect hypoglycaemia; algorithms can be produced to mitigate this where there are sufficient data from sequential readings to give the BG/time gradient.

Intraperitoneal insulin infusion offers a more physiological route for insulin delivery devices, producing greater porto-systemic and hepatic insulin gradients, and controls hepatic glucose metabolism more efficiently. Recent research [10, 11] in our laboratory has focused on producing an implantable insulin delivery device (INSmart) which would deliver insulin to the peritoneum in an automated fashion linked to changing glucose levels (Fig. 1a & b). INSmart delivers insulin via a glucose-sensitive gel which acts as both a sensor and controller of the amount of insulin released (Fig. 1c). The glucose-sensitive gel comprises polymerised derivatives of dextran and a glucose-sensitive lectin, concanavalin A. The highly viscous gel that forms due to the equilibrium binding between the dextran and the lectin binding sites impedes insulin release. These changes in the presence of glucose as the binding sites are disrupted resulting in a reduction in the viscosity of the gel that facilitates insulin release. This process is both reversible and repeatable, being sensitive to the changes in glucose levels that occur in the peritoneal cavity. The gel layer is therefore both the sensor and the delivery port in this design and contains no electronics or moving parts. The benefits of an INSmart device for the treatment of diabetes are that it could provide automated control preventing hypoglycaemia and also the long term harm from hyperglycaemia. However the associated risks from an implantable device could arise from surgery, leakage of the insulin reservoir and infection.

A prototype design was used to demonstrate the feasibility of this novel approach by restoring normoglycaemia in diabetic rats [12] and pigs [13] for up to 5 weeks but would require some redesign to provide it with biocompatibility, reliability and security to be optimal for clinical use. In designing a clinically-testable prototype it is important to take account of the views of prospective users of such a device. To gain insight from the potential users' perspective about the characteristics that they would expect of such a device it was decided to conduct a survey of current users of CSII.

2. Method

We surveyed CSII users to determine their current approach to glucose management, their appreciation of its importance and to understand the practical difficulties of achieving desired control with their current pump therapy. Questions were multiple choice or open ended and addressed BG management, basal and bolus delivery, diet, hypo- and hyperglycaemia as well as short and long term insulin pump management. We also wanted to know what they thought about the concept of a surgically implantable pump such as INSmart, if it could bring the advantage of closed loop functionality. A closed loop INSmart device or 'artificial pancreas' could present an alternative to pancreatic or islet transplants, and to electronic-sensor controlled pumps, assuming biocompatibility, predictability and security can be assured.

Survey Design, distribution and response collection

An international survey of patients with diabetes currently using CSII was carried out. This was aimed at gauging their opinions of whether a closed loop implantable insulin pump was an attractive proposition, the premise being that since this group of patients already managed their diabetes in a partly automated way, they might offer unique insights about the concept.

The questionnaires were produced in English and distributed to insulin pump users through various channels. Advertisements were placed in various local and national media (such as newspapers) within the UK, and in publications from various diabetes charities such as Diabetes UK. An interactive web-based version of the survey (Survey Monkey®) was also available via a dedicated website for participants who wanted to submit responses via the internet. The UK Diabetes Network and 'Pumpers' also distributed copies to members on their databases. Finally we used social networking sites such Twitter® and Facebook® to publicise the survey.

Participants answered 56 questions which were either multiple choice or open ended, relating to their background, the insulin pump brand being used, the type of insulin used in the pump, basal and bolus doses, infusion set, insertion sites, the current quality of glycaemic control as evidenced by self-reported Hb1Ac concentration and the frequency and severity of hypo- and hyperglycaemic events, and self-reported diabetes complications. Specifically they were asked about the practical difficulties they experienced with CSII in achieving their glucose targets. Finally they were asked to respond to a description of the implantable closed loop insulin pump, INSmart.

Analysis of responses

The responses from Survey Monkey® were downloaded in Microsoft Excel® and then coded before inputting into SPSS®. All postal responses were entered manually using the same coding directly into SPSS®.

3. Results

Pump users current approach to glucose management

360 completed surveys were received and analysed. 30.4% of responses were from the United Kingdom, which is predominantly where the survey was widely distributed and advertised. Many responses were also collected from the US (39.9%), Canada (2.8%) and 0.35% of the total responses each from Australia, France, India, Israel and Switzerland, however 25% of respondents did not disclose which country they lived in. About 88% of the survey respondents were below the age of 34 at diagnosis of diabetes. 36.4% were between the age of 0-11 years, 27.5% between the ages 12-21, and 24.4% between 22-34 years of age and are thus likely to have type 1 diabetes [14]. All responses collected from respondents under the age of 17 were completed by their parents.

Fig. 2a shows the most commonly used pump was the Medtronic Paradigm® device (57.6%) and Novorapid® (insulin Aspart) and Humalog® (insulin lispro) were the insulins most commonly infused (Fig. 2b). Respondents were asked whether the pump they used was chosen by them, or had been given to them by their medical advisors. As 44% had been given the pump by their medical advisors this suggests that the choice was made by diabetes physicians and/or Diabetes Specialist Nurses rather than patient choice.

When insulin pump users were asked about the amount of insulin they infused over a 24 hour period 50.6% used 20-40 Units, 24.4% used 40-60 Units and 12.7% have used more than 60 Units. Most (57.3%) reported infusing a basal rate of 0.5-1 Units/hr, with 20.3%

using 1-2 Units/hr and 18.4% using 0.5 Units/hr. Only 3.2% of respondents infused a basal rate of more than 2 Units/hr.

Most respondents (52.2%) used the standard or 'spike' bolus to cover meals.

The majority of respondents (65.7%) had a HbA1c value between 42-64 mmol/mol (6.0-8.0%), a broadly acceptable range [1,15]. 13.9% had HbA1c values between 32–42 mmol/mol (5.1%-6.0%), indicative of overly tight control, associated with a significant risk of hypoglycaemia. 2.8% had HbA1c values above 76mmol/mol (9.1%) and 0.3% had values above 86mmol/mol (10%) which indicates very poor glucose control. 77.8% people could recall their HbA1c result before starting CSII; 57.3% reported that it had improved subsequently.

About 70% of the respondents reported having a hypoglycaemic episode at least once a week. In most cases (39.9%) respondents were able to sense that they were hypoglycaemic and 51.6% of these respondents confirmed that this occurred at BG below 4 mmol/L. 79.4% respondents reported BG values above 10 mmol/L more than three times in the month preceding the survey, and most (68.7%) claimed that they would respond by taking a correction bolus straightaway. However, 9.8% reacted to elevated BG by waiting 60-90 minutes before re-testing their BG and 10.1% by drinking water. Some respondents would change their infusion set, in case it had become blocked.

Pump users' reactions to a description of an implantable closed loop pump (INSmart)

Fig. 3 summarises the responses from an open question asking the respondents what they thought about closed loop insulin delivery from a device like INSmart, which removes the necessity to adjust insulin in response to changes in BG but possibly not to eliminate entirely the need for routine BG tests. Over 90% of respondents were in favour of closed loop insulin delivery and gave reasons for these views. 31.5% of respondents thought that having a closed loop system would provide them with better BG control than their current insulin pump treatment, in particular 10% of the respondents thought that a closed loop system would offer the best possible chance of achieving glycaemic control in the non-diabetic range. The majority of respondents felt there were still many disadvantages to current external insulin pumps such as their constant visible presence, rotation of insertion sites, cannula site irritation/infection and skin inflammation. The concept of a so-called artificial pancreas is widely acknowledged by interested parties as the “holy grail” in insulin delivery and BG management and although only 10% of respondents actually selected this answer, many of the other responses encompassed elements of the concept. Other common responses included: ‘it would fit into their lifestyle more easily’ suggesting that they would be able to forget about the constant vigilance required from BG testing and insulin administration. ‘It would be accurate, safe and sensitive’ which highlights that most people with diabetes still have issues relating to BG control as well as safety.

Only 4% of respondents did not think that closed loop delivery would be an attractive proposition. The main concern from these responses related to a possible failure of the device indicating that they would not feel safe or comfortable allowing a device to deliver their insulin automatically. Other responses included concerns that the device would not allow the user to make their own adjustments and that they would constantly worry that the device would fail. A more obvious reason for not finding this type of device attractive for respondents was they would find the insertion surgery invasive and undesirable. These responses suggest insulin pump users tend to be well adapted to the demands of running a pump safely and effectively and it is not surprising that they would identify not only the advantages, but also the potential disadvantages and hazards of an implantable closed loop system.

Table 1 shows the positive responses to a question where respondents were asked if what their opinions would be regarding a closed loop insulin pump that needed to be implanted under the skin. It can be seen that the main concerns to an implantable closed loop delivery device relate to the surgery and the refilling of the insulin in such a device. The main negative responses to an implantable insulin pump related to concerns about the surgery itself and possible resulting infection, as well as device safety, the concept of an implanted device and the impact on others including children.

Fig. 4 shows the general preference for refilling an implantable insulin pump, such as INSmart, was weekly (40.5%). This was a deliberately open ended question and the reason most respondents opted for this preference was that they felt this would allow them to remember to refill the reservoir on a set time every week.

4. Discussion

The bottom-up survey was designed to gain an understanding of insulin pump therapy together with users' experiences of their condition and treating it with infused insulin. This was aimed at gauging their opinions of whether a closed loop implantable insulin pump was an attractive proposition, the premise being that since they already manage their diabetes in a partly automated way, they might be particularly perceptive about the prospect in ways not obvious to others.

Many of the background responses implied that pump users were all type 1 and that they had been diagnosed early in life. The majority of the respondents were from the UK and North America. The lack of responses from France may have been as a result of the survey being written in English as Sulmot et al. [16] have reported that insulin pump use in the country, especially for children and adolescents with type 1 diabetes, increased 10 fold between 2001 and 2007. A higher proportion of patients with type 1 diabetes in the US use pumps compared with UK residents and these are funded by the medical insurance companies. In the UK, the criteria for pump use are somewhat different and depend more on the local Commissioners implementing NICE guidelines [17] for pump use.

Clear choices emerged for the pump brand and the insulin type. Bartalo et al. [18] has shown that there are no pharmacokinetic or pharmacodynamic differences in the absorption profiles of insulin lispro and aspart and conclude that the use of short acting insulin in CSII therapy provides a small but statistically significant improvement in glycaemic control compared with regular insulin. Glycemic control was also dependent on the infusion line and has been shown to deteriorate after 48 hours of use leading to an incremental loss of glycemic control [19].

In this survey, quantities of insulin used per day and the dose rate used were variable but within expected ranges. In general terms, pump users are reported to need about 80% of the dose given to type 1 people by injection, and this relates to the efficiency of converting long acting insulins to diffusible insulin that can reach the plasma. Basal insulin needs were found to be below 1Units/hr for most of the respondents. Insulin requirements are believed to increase during the night and early morning (dawn phenomenon) due to a decrease in insulin sensitivity caused by cortisol and growth hormone secretion. Basal insulin requirement begins peaking in juveniles (<20 years) before midnight and maintains a relative high throughout the night [20], drops in the morning and increases again from noon to midnight. Basal needs for adults (> 20 years) show a more abrupt peak in the morning followed by a drop off until noon and gradually increasing in the evening. Basal rates thus require fine tuning by those on insulin pump therapy.

The bolus pattern, although subject to variation depending on the circumstance, tended towards the standard spike bolus for the respondents in this survey. A spike bolus delivers the incremented dose of insulin in a short time similar to a subcutaneous injection and as most insulin pump users were well versed in judging their insulin input in response to their meals, this method gave adequate blood glucose control. An extended square wave bolus used by 5.1% of respondents, delivers a larger dose of insulin spread over a longer period of time such as an hour or two and useful when eating foods high in protein. The delay in the delivery of carbohydrates from the digestive system when eating and digesting protein can approach the insulin duration-of-action, so in these cases the blood glucose level is better controlled by a slow extended release of insulin that matches the profile of carbohydrates entering the bloodstream. 24.4% of respondents used a combination bolus (standard + extended), as often one method of bolusing does not fit the elevated blood glucose levels from the different types of carbohydrates present in their meal. This provides a large initial dose of insulin, and extends the tail of the insulin action. It is appropriate for high carbohydrate and high fat meals such as pizza and chocolate cake.

A super bolus (1.6% respondents) considers the basal rate delivery of insulin following the bolus as part of the bolus and can be borrowed ahead and given together with the bolus. This type of bolus is often

used to prevent hypoglycaemia. Cukierman-Yaffe et al. [21] has reported that there is a significant relationship between glycaemia indices and the use of a bolus calculator (a feature is several insulin pumps). Diabetes patients that used the bolus calculator in 50% of their boluses had a lower HbA1c and mean BG value suggesting better glucose control.

Most responders had very well controlled glucose as described by their HbA1c and reported an improvement after transferring from MDI. However, 70% had more than three hypos per week. Frequent troublesome hypoglycaemia with MDI is an indication for CSII and we did not ask whether this frequency had reduced since starting CSII. However, 90% of pump users said they could detect an oncoming hypo and that, for them, it became a problem only if the BG dropped below 4 mmol/L.

Continuous glucose monitoring (CGM) using a Guardian sensor® has been shown to improve HbA1c values over a 12 week period and lower the incidence of hypoglycaemia compared with self-monitoring of BG in CSII users [22, 23]. There was, however, a high incidence of drop outs for CGM due to patient discomfort. These findings are similar to those reported by a Juvenile Diabetes Research Foundation trial [24] which also found a significant improvement in HbA1c of young diabetes patients who used a sensor, although they did not find an alteration in the incidence of hypoglycaemic events.

By contrast, 80% of respondents had BGs above 10 mmol/L three or more times per month and their remedy was either to give an additional bolus (70%) or watch and wait (10%) or drink water (10%). While 10 mmol/L is the upper limit of normal BG levels, this may in practice indicate that levels are much higher. Together this information about glucose control reveals that although convenient, pump therapy might be less effective than reported, although not necessarily less effective than MDI therapy. It may be that an anonymised survey elicits information that differs from other sources for a variety of reasons that relate to surveys in general as well as to diabetes. It also implies that despite being on a reliably constant basal dose of insulin and with boosts conveniently selected for delivery to a tailored pattern coupled with features such as electronic memory and safety lockout features, respondents were commonly above the target BG range. An increase in BG with CSII may result from an occlusion of the infusion line or cannula, although more commonly problems arise from human error, for example inaccurate carbohydrate estimation, inaccurate insulin carbohydrate ratios, insulin sensitivity factors as well as lifestyle factors such as exercise and stress. Whether the post prandial BG peak would be detected would depend on the user testing at the relevant times.

The positive attitude towards an artificial pancreas such as INSmart focused on the control of BG and user independence as well as improved quality of life. Negative responses were perceptions about relying on an automated system that could possibly fail or not be reliable. The concept of an implantable device rather than an external (and therefore easily-removable) pump was clearly worrying to some. There were comments about the need for comfort, the safety of implantation and maintenance including refill which would all need to be demonstrated for an INSmart type device to secure approval from the Medical Devices Directive in the UK [25] (FDA in the US).

The behaviour, attitude and use of existing external pump users from the open ended questions from this survey provided some useful feedback toward a redesign of the existing device which has now successfully been implanted into diabetic pigs. It is apparent that current external pumps have shortcomings which an implantable INSmart device could overcome:

•Automated delivery of insulin to real time changing glucose levels by the fast uptake of glucose in the peritoneum

•No changing of infusion lines, rotation of sites and not visible

•No moving parts or electronic power requirements

•No need to regularly check BG levels

•No need to bolus for mealtimes

However, an implantable INSmart device would still need to overcome risks such as leakage of insulin or smart gel, infection and surgery.

5. Conclusion

The general consensus from the survey was that most respondents felt that an implantable artificial pancreas would be a close match to a functioning healthy pancreas and therefore appealing. The vast majority of respondents felt that there are many disadvantages to using current external insulin pumps such as its constant visible presence, rotation of insertion sites and skin inflammation, the need for frequent BG testing and adjustment of insulin flow in the absence of feedback from a sub-cutaneous implanted sensor. These shortfalls could be overcome by a device, such as INSmart that provides a relatively instant feedback mechanism for controlling insulin release due to its location in the peritoneal cavity. Its performance would be a much closer match to a fully functioning healthy pancreas and therefore very appealing to the pump users surveyed. The key requirements of an INSmart like device identified by the survey are that it needs to be comfortable to 'wear', safe and reliable and easily refilled on a weekly basis.

Competing interests

Nothing to declare

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List of Figures and Tables

Figure 1a shows a depiction of an implantable INSmart device (b) the placement of an INSmart device in the human body (c) shows the mechanism of insulin release in different glucose conditions from the smart gel present in the INSmart device as a thin layer.

Fig. 2a: Type of insulin pump used by the survey respondents (numbers above bars denote the percentage of respondents)

Fig. 2b: Type of insulin infused by pump (numbers above bars denote the percentage of respondents)

Fig. 3 shows what insulin pump users thought about closed loop insulin delivery (numbers above bars denote the percentage of respondents)

Fig. 4: How often would you prefer the pump to be filled (numbers above bars denote the percentage of respondents)

Table 1: Insulin pump users thoughts about the concept of a closed loop insulin pump implanted under the skin (numbers in column denote the percentage of respondents)

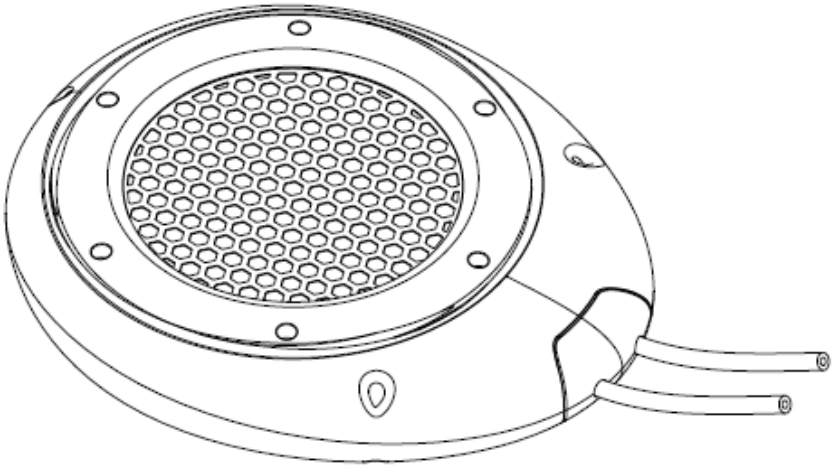

Figure 1a A design version (CAD) of the artificial pancreas for human implantation

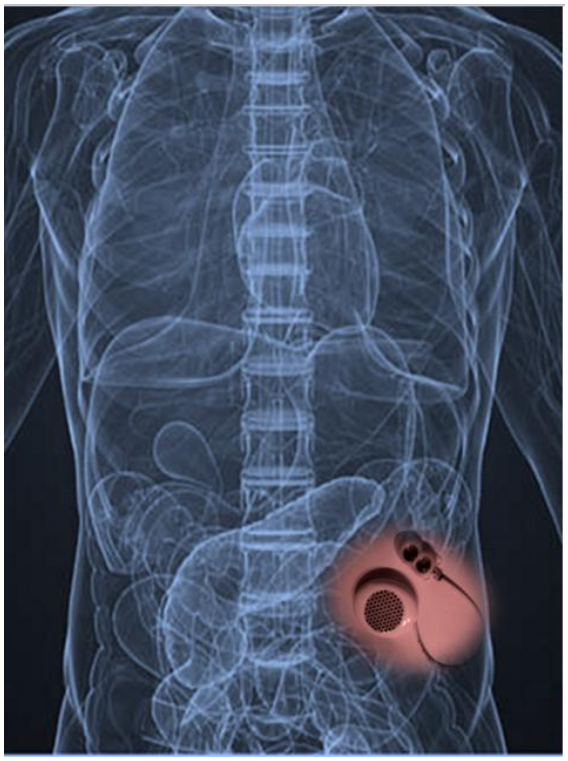

Figure 1b Intended siting of the artificial pancreas

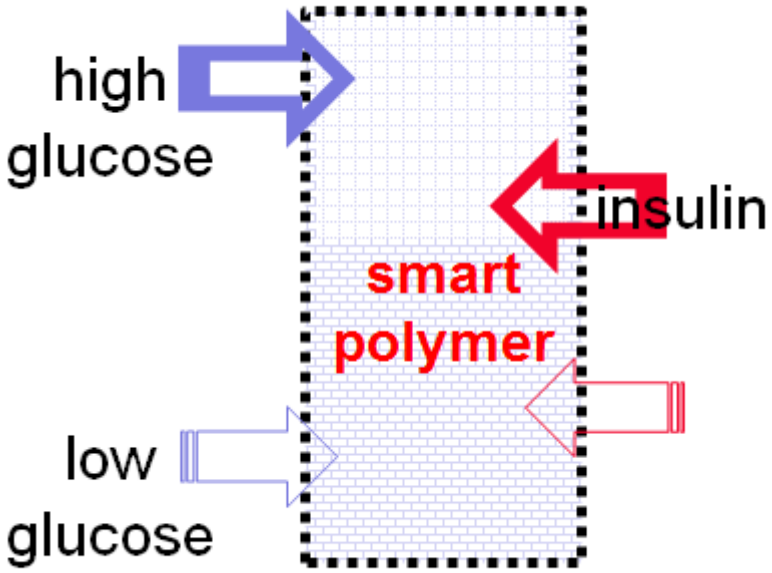

Figure 1c The mechanism in outline of the chemical artificial pancreas

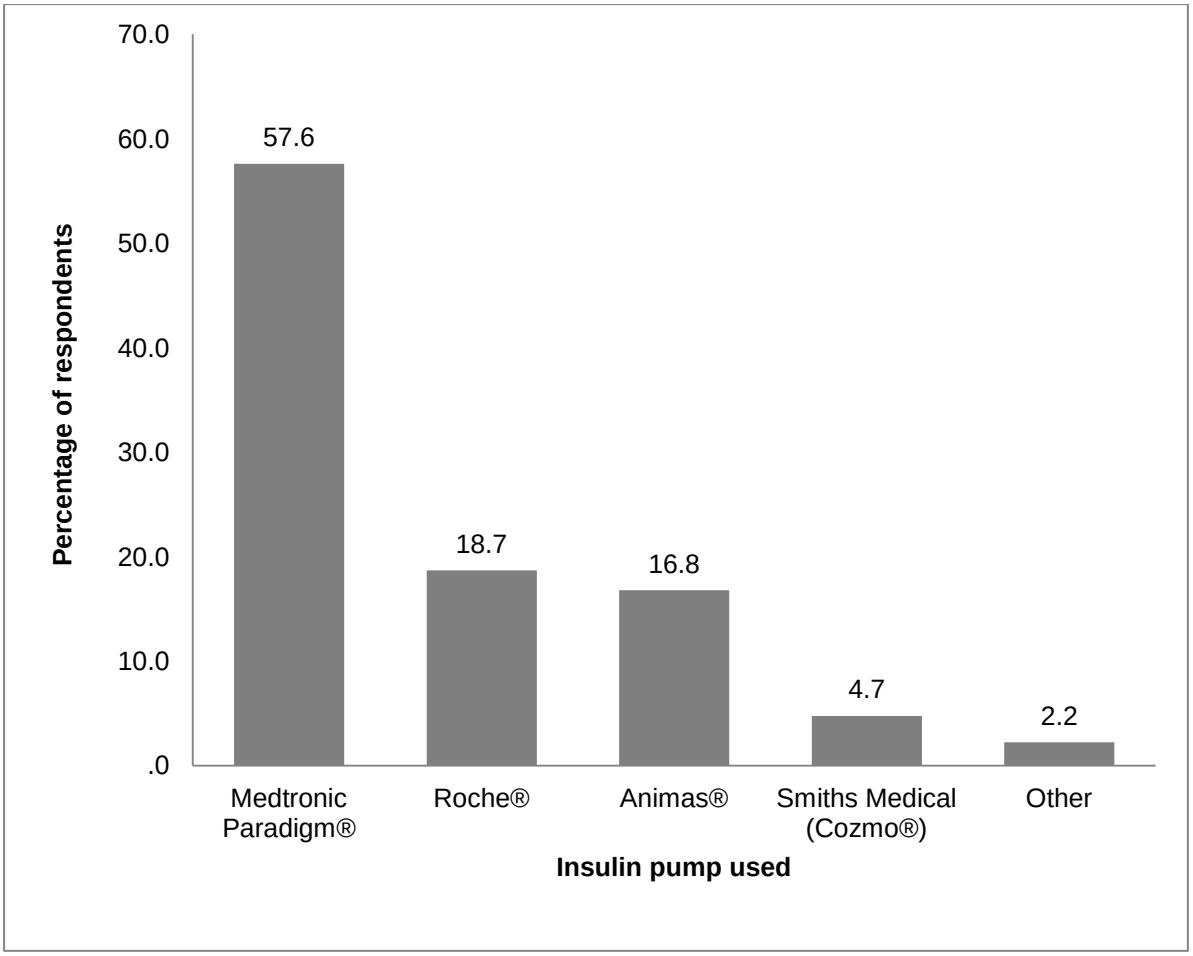

Figure 2a Pumps reported used by the survey respondents

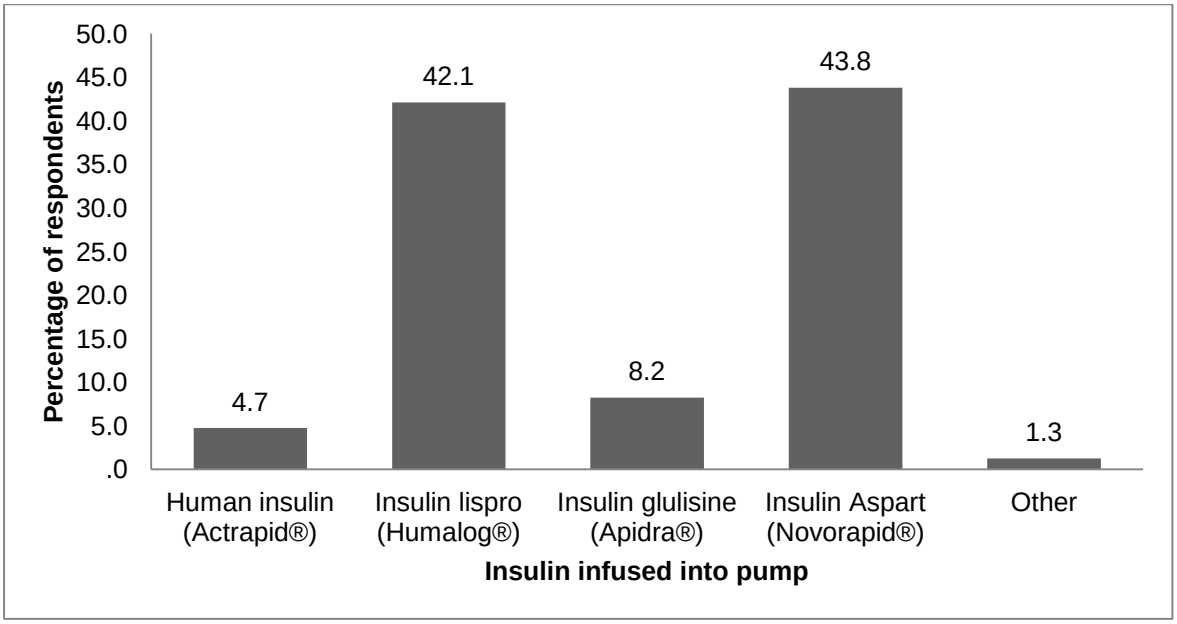

Figure 2b Insulins reported used by respondents

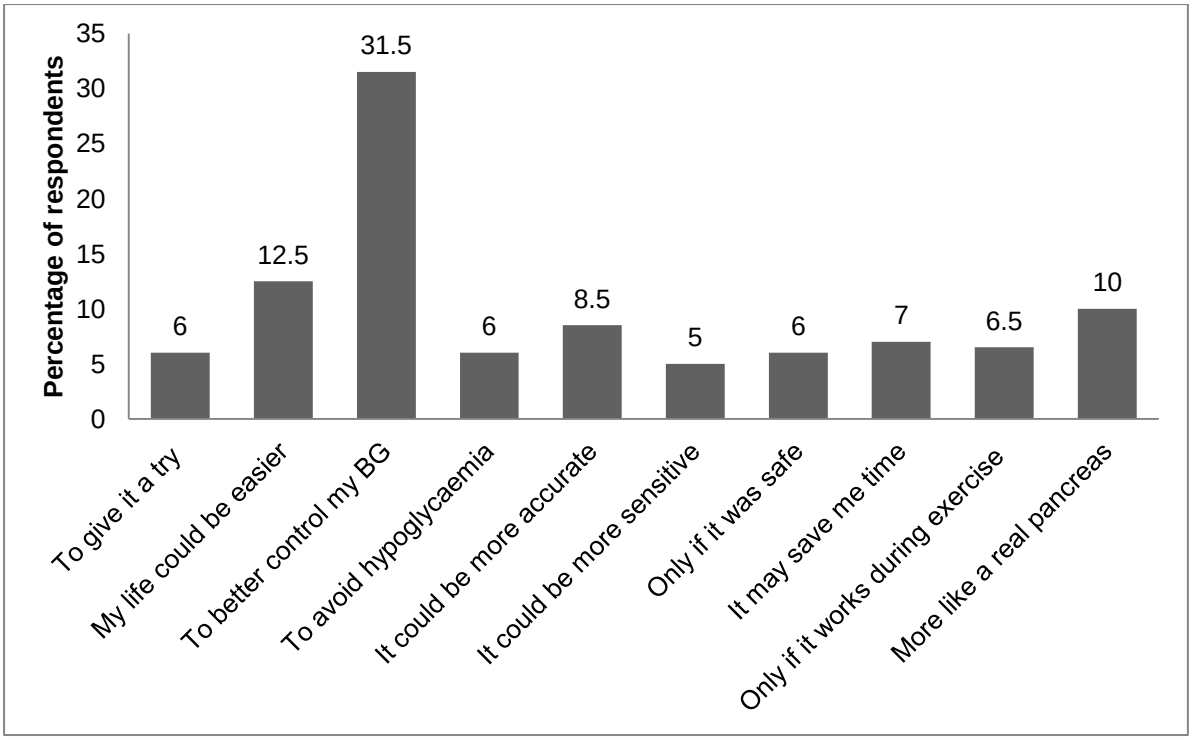

Figure 3Perceptions of the acceptability of the implantable artificial pancreas

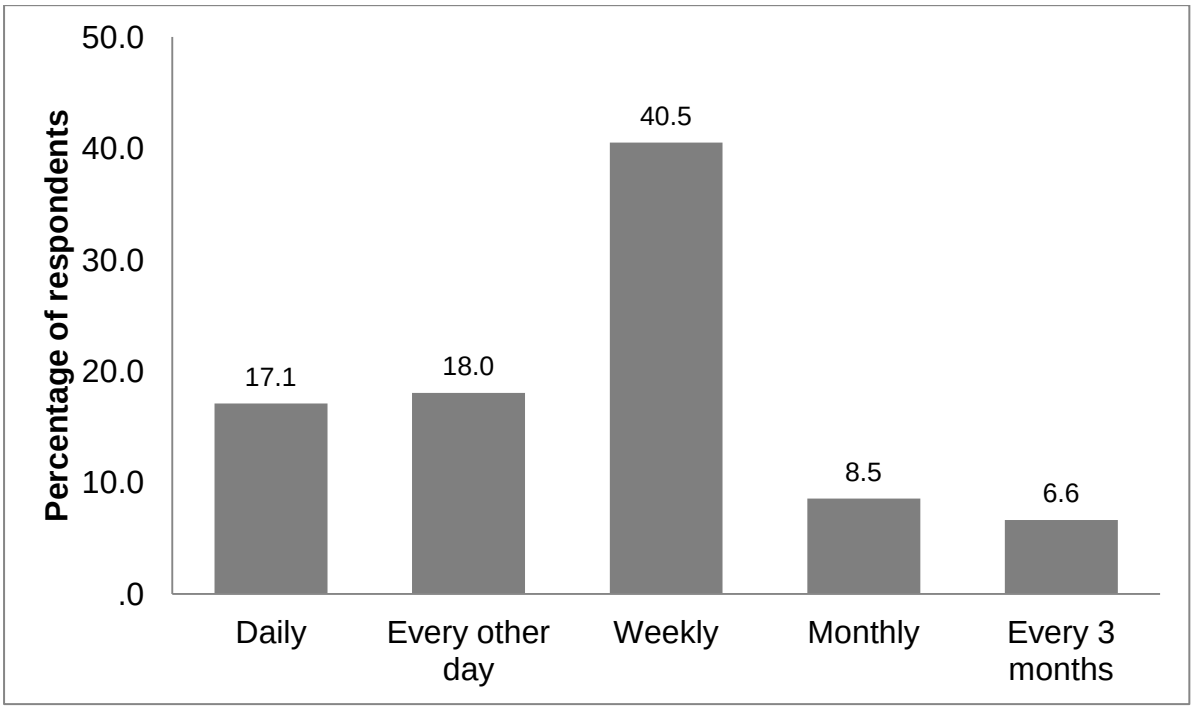

Figure 4Respondents suggestions of a convenient refill period

Insulin pump users thoughts about the concept of a closed loop insulin pump implanted under the skin	%
Positive responses	
Would make feel like a happy, relaxed normal person	1.8
To control my blood sugar better	14.5
Children would prefer this	0.9
Only if it was comfortable, effective and made me feel better	13.1
It has some advantages over current pump	3.2
Having it implanted would not bother me as long as it was provided to others	3.2
If it sensed BG better and was available free	1.8
It would be better because I wouldn't need to be remove for exercising and swimming	3.2
Only if it is not dangerous (infection, re-fill, size, painful, rejected)	21.3
It may improve my quality of life (less stress, noticeable)	12.2
Negatives responses	
Only if it was small in size	4.5
It would depends surgery to implant it	4.5
Only if there is no catheter and no scar tissue	4.1
It scares me	2.3
Only if it was easier to manage	2.3
I would be willing to try	1.4
I couldn't trust it	2.2
I need more information	5.4

Table 1: Insulin pump users thoughts about the concept of a closed loop insulin pump implanted under the skin (numbers in column denote the percentage of respondents)

Appendix D

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DE MONTFORT UNIVERSITY
LEICESTER

Leicester School of Pharmacy
Faculty of Health & Life Sciences

De Montfort University Insulin Pump Users and Exercise Survey 2011

Return to:

FREEPOST RSAT-ZSRT-RRGG

INsmart Diabetes Survey

Faculty of Health & Life Sciences

De Montfort University

The Gateway Leicester

Your answers will be treated as strictly confidential

Please tick (✓) appropriate box.

Section A: About you

Q1. Are you?

1- □ Female
2- □ Male

Q2. How would you best describe your occupation?

1- □ Professional
2- □ Skilled
3- □ Semi- skilled
4- □Manual labour
5- □ Student
6- □ Retired
7- □ Others

Q3. How old are you?

1- □ Between 1-10 years
2- □ Between 11-20 years
3- □ Between 21-30 years
4- □ Between 31-40 years
5- □ Between 41-50 years
6- □ Between 51-60 years
7- □ Between 61-80 years
8- □ Over 80 years

Q4. How old were you when your diabetes was first diagnosed?

1- □ Between 1-10 years
2- □ Between 11-20 years
3- □ Between 21-30 years
4- □ Between 31-40 years
5- □ Between 41-50 years
6- □ Between 51-60 years
7- □ Between 61-80 years
8- □ Over 80 years

Q5. What is your highest level of education?

1- □ PhD
2- □ Master degree
3- □Bachelor
4- □ You are still in full time education under bachelor
5- □ You have not had any formal education
6- □ Other, please specify ……..

Q6. What is your ethnic group? (Please tick one box from section A to E)

a. WHITE

1. □ British

2. □ Irish
3. □ Any other White background (Please write in……)

b. MIXED

4.□ White and Black Caribbean
5. □ White and Black African
6. □ White and Asian
7. □ Any other mixed background (Please write in……)

c. ASIAN OR ASIAN BRITISH

8. □ Indian
9. □ Pakistani
10. □ Bangladeshi
11. □ Any other Asian background (Please write here ……..)

d. BLACK OR BLACK BRITISH

12. □ Caribbean
13. □ African
14. □ Any other Black background (Please write in…….)

e. CHINESE OR OTHER ETHNIC GROUP

15. □ Chinese
16. □ Any other ethnic group (Please write in…….)

Q7. Which country do you live in?

1.□The UK	2.□US	3.□Australia	4.□Canada	5.□Israel
6.□India	7.□Switzerland	8.□France	9.□Saudi	10.□Other

Q8.If you are from the UK, what is your approximate income before tax (UK£)?

1- □Unemployed
2- □0-10,000
3- □10,000-20,000
4- □20,000 – 30,000
5- □ 30.000- 40.000
6- □40,000- 50.000
7- □ 50.000-100.000
8- □Over 100,000

Section B: <u>about your diabetes</u>

Q9. How was your diabetes diagnosed?

1- □ By your GP
2- □ By hospital clinic
3- □ By friend/ family
4- □ By ambulance/ A&E (Accident and Emergency)
5- □ By your self
6- □ By medical cheek-up (work, insurance)
7- □ Others please state……

Q10. What type of diabetes do you have?

1- □ Type 1
2- □ Type 2
3- □ I don't know
4- □ Other Please state

Q11. Is there a history of diabetes in your family?

1. □No
2. □ If Yes Please state who**……..**

Q12. Please state your weight, height

1. □Height……
2. □Weight……..

Q13. What was your HbA1c when you were diagnosed with diabetes? (if known)

1- □Below 5 % (31)
2- □Between 5.1 and 6 % (31 and 42)
3- □Between 6.1 and 7 % (43 and 53)
4- □Between 7.1 and 8 % (54 and 64)
5- □Between 8.1 and 9 % (65 and 75)
6- □Between 9.1 and 10 % (76 and 86)
7- □Over 10.1 %(86) please state…….

Q14. What was your average HbA1c before using insulin pump therapy?

1- □Below 5 % (31)
2- □Between 5.1 and 6 % (31 and 42)
3- □Between 6.1 and 7 % (43 and 53)
4- □Between 7.1 and 8 % (54 and 64)
5- □Between 8.1 and 9 % (65 and 75)
6- □Between 9.1 and 10 % (76 and 86)
7- □Over 10.1 %(86) please state…….
8- □Don't know

Q15. What is your average HbA1c now that you are using insulin pump therapy?

1- □Below 5 % (31)
2- □Between 5.1 and 6 % (31 and 42)
3- □Between 6.1 and 7 % (43 and 53)
4- □Between 7.1 and 8 % (54 and 64)
5- □Between 8.1 and 9 % (65 and 75)
6- □Between 9.1 and 10 % (76 and 86)
7- □Over 10.1 %(86) please state…….
8- □Don't know

Q16. What do you think your HbA1c should be?

1- □Below 5 % (31)
2- □Between 5.1 and 6 % (31 and 42)
3- □Between 6.1 and 7 % (43 and 53)
4- □Between 7.1 and 8 % (54 and 64)
5- □Between 8.1 and 9 % (65 and 75)
6- □Between 9.1 and 10 % (76 and 86)
7- □Over 10.1 %(86) please state…….
8- □Don't know

Q17.In the last 12 months, have you had any of the following tests?

	Yes	**No**	**Don't know**
1. Your blood pressure taken by a doctor or nurse.			
2. A cholesterol test by a doctor or nurse.			
3. An eye test where a photograph of the back of your eyes was taken.			

4. Your bare feet were examined.			
5. You have had your weight checked by a doctor or nurse.			

Q18. For each question please tick yes or no.

	Yes	No
1. Has your eyesight suffered as a consequence of your diabetes?		
2. Do you have diabetic kidney disease?		
3. Do you require dialysis?		
4. Have you had a kidney transplant?		
5. Is your usual blood pressure normal?		
6. Do you take any medication to control your blood pressure?		
7. Are you on lipid lowering medication (for high cholesterol or triglycerides)?		
8. Have you ever had a heart attack?		
9. Do you ever have chest pain due to angina?		
10. Have you ever had heart bypass surgery (coronary artery bypass)?		
11. Have you ever had a balloon angioplasty or a coronary stent placed?		
12. Have you ever had, or suspected that you had a stroke?		

Q19. Please tell us what you cholesterol levels are, if know?

1. Total cholesterol level …….
2. High-density lipoprotein (HDL)……..
3. Low-density lipoprotein (LDL)……

Q20. Do you take medication to lower your cholesterol such as statins?

1- □Yes
2- □No

Your insulin Pump

Q21. What kind of insulin pump do you use?

1- □Medtronic Paradigm
2- □Roche
3- □Animas
4- □Smiths Medical – Cozmo
5- □Accu-Chek Spirit
6- □Other, please state

Q22. How many years have you been using a pump?

1- □Less than 1 year
2- □Between 1 and 3 years
3- □Between 3 and 5 years
4- □More than 5 years

Q23. What kind of insulin do you infuse into your pump?

1- □Human insulin - Actrapid®
2- □Insulin lispro - Humalog®
3- □Insulin glulisine - Apidra®
4- □Insulin Aspart - Novorapid® or Novolog
5- □ Humulin S®
6- □ Humulin I®
7- □ Insulatard® (Isophane)
8- □ Mixtard®
9- □ Humulin M3®
10- □ Levemir® (detemir)
11- □ Lantus® (Glargine)

12- □Other, please state……...

Q24. Typically, what are your basal rates throughout a non-exercise day?

1- □ Less than 0.5Unit/hr
2- □ Between 0.5Unit/hr and 1Unit/hr
3- □ Between 1Unit/hr and 2Unit/hr
4- □ More than 2Unit/hr

Q25. How do you bolus for meals on a non-exercise day?

1- □ Standard bolus (Spike)
2- □ Extended bolus (Square wave)
3- □ Combination bolus (Spike and Square wave)
4- □ Super bolus (Increased spike)
5- □ Other, please specify……

Q26. What was the total amount of insulin you typically use on a non-exercise day?

1- □Less than 20Units
2- □Between 21Units and 30Units
3- □Between 31Units and 40 Units
4- □Between 41Units and 50 Units
5- □Between 51Units and 60 Units
6- □More than 61 Units, please state……

Q27. How many times you test your blood glucose on a non exercise day?

1- □1-3 times
2- □4-6 times
3- □More than 6 times

Q28. Typically, what are your basal rates throughout an exercise day?

1- □ Less than 0.5Unit/hr
2- □ Between 0.5Unit/hr and 1Unit/hr
3- □ Between 1Unit/hr and 2Unit/hr
4- □ More than 2Unit/hr

Q29. How do you bolus for meals on an exercise day?

1- □ Standard bolus (Spike)
2- □ Extended bolus (Square wave)
3- □ Combination bolus (Spike and Square wave)
4- □ Super bolus (Increased spike)
5- □ Other, please specify……..

Q30. What was the total amount of insulin you typically use on an exercise day?

1- □Less than 20Units
2- □Between 21Units and 30Units
3- □Between 31Units and 40 Units
4- □Between 41Units and 50 Units
5- □Between 51Units and 60 Units
6- □More than 61 Units, please state……

Q31. Do you use pre-exercise bolus?

1. □No
2. □Yes, please state how much……

Q32. What is acceptable blood glucose (BG) before exercise?

1- □ Below 4 mmol/l (72 mg/dl)
2- □ Between 4-7 mmol/l (72-126 mg/dl)
3- □ Above 7 mmol/l (126 mg/dl)

Q33. How does exercise change your blood glucose?

1- □ Increase your blood glucose
2- □ Decrease your blood glucose
3- □ No change

Q34 .How many times do you test your blood glucose on an exercise day?

1- □1-3 times
2- □4-6 times
3- □More than 6 times

Q35. If your blood glucose was less than 4mmol/L (72 mg/dl) pre-exercises, what would you do?

1. □Miss out your pre-exercise bolus
2. □Reduce your pre-exercise bolus
3. □Leave the exercises today
4. □Postpone exercise for another day
5. □Take some carbohydrate then exercise
6. □Nothing
7. □Other, please state**……**

Q36. If you experienced a hypoglycemia (low blood glucose) after exercise what action would you take?

1-□ Reduce the insulin rate
2-□Increase the insulin rate
3-□ Wait to re-check the blood glucose
4-□Drink water or any drink with low calories
5-□Seek medical help
6-□Other (please specify)……..

Section C: <u>Your attitude to exercise</u>

Q37.How important is participating in sport and exercise to you?

1-□Important
2-□ No view
3-□ Not important at all

Q38 .Have you always led an active exercise lifestyle?

1- □Yes
2- □No
3- □Please explain how ……..

Q39. Typically what is your blood sugar value pre-exercise?

1- □Below 5 mmol/l (90 mg/dl)
2- □Between 5.1 and 6 mmol/l(91-108 mg/dl)
3- □Between 6.1 and 7 mmol/l (109-126 mg/dl)
4- □Between 7.1 and 8 mmol/l (127-144 mg/dl)
5- □Between 8.1 and 9 mmol/l(144-162 mg/dl)
6- □Between 9.1 and 10 mmol/l (163-180 mg/dl)
7- □Over 10.1 mmol/l, (181 mg/dl) please state…….

Q40. Please tick (✓) appropriate box

	Yes, please state	**No,** please state
1. Is your pump switched on during exercise?		
2. Do you have any pump problems during exercise?		

Q41. If you don't switch your pump on during exercise, do any of the following apply?

1- □You are not sure about the pump sensitivity during exercise
2- □You are worried about discomfort
3- □You worry that your blood glucose goes up to abnormal level (hyperglycemia)
4- □You worry that your blood glucose goes down (hypoglycemia)
5- □Interferes with exercise program
6- □Advised by medical professional to switch pump off during exercise
7- □Other…….

Q42.How much exercise did you participate in before you started your pump therapy?

1- □None
2- □Less than 1 hour a week
3- □between 1-3 hours a week
4- □More than 5 hours a week
5- □ Other…….

Q43.Has this changed since you have been on your pump?

1- □No
2- □Yes, please state……..

Q44.What would you say the level of intensity is when you exercise?

1. □**Light**(Easy, does not induce sweating unless it's a hot, humid day, no noticeable change in breathing patterns.)
2. □**Moderate**(Somewhat hard, sweat after about 10 minutes of exercise. Breathing becomes deeper and more frequent.)
3. □**Heavy**(Hard, sweat after 3-5 minutes. Breathing is deep and rapid.)

Q45.Typically, what type of exercise do you do?

1- □Aerobic exercise (e.g. walking, cycling, jogging and swimming)
2- □Anaerobic exercise (e.g. resistance training and weight lifting)
3- □A mixture of aerobic and anaerobic
4- □Other……..

Q46. Please select all that apply.

1. Walking	
2. Cycling	
3. Free Weights	
4. Swimming	
5. Sit ups/ Push ups	
6. Yoga	
7. Team Sports Basketball/Football	
8. Running	
9. Boxing/ Kick Boxing	
10. Combination, e.g: combined walking and weight lifting	
11. Cross-training (combining of exercises to work various parts of the body)	
12. Other. Please state**……**	

Q47. Are there any barriers preventing you from taking part in more sport?

1	Health reasons	2
3	Lack of motivation	4
5	Embarrassment about how I look .eg overweight or fitness lack	6
7	Lack of time	8
9	It does not interest me	10
11	It is too expensive	12
13	Lack of transport	14
15	Fear of Injury	16
17	Don't know	18
19	Other, Please state…….	

Q48. Typically, how many days in the week do you undertake physical activity?

1. □Every day
2. □1-2 days
3. □ 3-5 days
4. □ None

Q49. In a typical exercise session how long do you spend participating in sport or exercise?

1. □Less than 30 minutes.
2. □ From 30 to 1 hour.
3. □From 1 to 2 hours.
4. □From 2 to 3 hours.
5. □From 3 to 4 hours.
6. □More than 4 hours.
7. □None

Q50. How many times do you exercise per day?

1. □Once
2. □Twice
3. □3 times.
4. □More than 3 times.

Q51. Being physically active is good for the following, for each factor please place a tick in the box that you most agree with most?

	Agree	No view	Disagree
1. General Health			
2. HBA1c			
3. Blood glucose			
4. Hypoglycemia			
5. Hyperglycemia			
6. Diet			
7. Any additional comments**……**			

Q52. Where do you typically exercise?

1. □ At school/ college/ work/ university
2. □ In a sports team (e.g. football, netball)
3. □ In a class or club (e.g. aerobics, dance, etc)
4. □ Gym

5. □ On your way home or school.
6. □ Don't know
7. □ Elsewhere, please state……

Q53. Are you a member of any sport centre or physical activity group?

1. □Yes
2. □No

Q54. How often do you climb the stairs at home? (**Average over the last 3 months)**: please tick one

1- □None
2- □1 to 5 times a day
3- □6 to 10 times a day
4- □11 to 15 times a day
5- □16 to 20 times a day
6- □More than 20 times a day

Q55. Typically when do you do your exercises? Tick all that apply

1. □Morning
2. □Afternoon
3. □Evening
4. □After meal
5. □Before meal

Q56. How often do you suffer with hypoglycaemia during a week?

1- □None
2- □Once
3- □2 or 3 times
4- □More than3 times

Q57. Has exercise increased or decreased the number of hypoglycaemia you have?

1- □Increase
2- □Decrease
3- □ No effect

Q58. Do you experience hypoglycaemia while exercising?

1- □Always
2- □Frequently
3- □Rarely
4- □Never

Q59. How many times per week is your measured fasting blood glucose 10 mmol/L (180 mg/dl) or above?

1. □ Once
2. □Twice
3. □ More than twice times
4. □ Never

Q60. What do you do if your blood sugar is over 10mmol/L (180mg/dl)?

1. □Reduce the insulin rate
2. □Increase the insulin rate
3. □Wait to reduce the blood glucose
4. □Drink water or any drink with low calories
5. □Seek medical help

Q61. Have you ever experienced diabetic ketoacidosis as a consequence of exercise?

1- □Yes
2- □ No

Section D: Your diet

Q62. Q. How many calories do you think you eat and drink in a typical day?

1. □1500 or less
2. □ Over 1500 up to 2000
3. □ Over 2000 up to 2500
4. □ Over 2500 up to 3000
5. □ Over 3000 up to 3500
6. □3500 or more
7. □I don't know

Q63. Do you count carbohydrates regularly in order to help you to control your diabetes?

1. □Yes
2. □No

Q64. Do you eat special diabetic food/ drink? (e.g. Low calorie, soft drink, diabetic chocolate etc…)

1. □Yes
2. □No

Q65. Which of these are important to you?

1- □ Reducing fat
1. □ Staying motivated
2. □ Eating better
3. □ Finding someone to exercise with
4. □ Discovering a physical activity that you like
5. □ To reduce your HbA1c value
6. □Others**,** please state…….

Q66. Do you see a dietician?

1- □Frequently
2- □Regularly
3- □ Never

Q67.Which of the following factors influenced your decision to participate in sport? Please tick all that apply

□ Because friends/ family do it	□To improve your health
□ Because doctor advised you	□To relieve stress and relax
□ To keep well with your diabetes	□You are overweight
□ To improve your physical appearance, self-motivation	□ Because of HbA1c is high
□ Because you are unfit	□ Don't Know
Dietician	□DSN (Diabetic Specialist Nurse)
□ Other (please specify)……	

Q68. Do you follow a medically approved dietary programme?

1.□ No

2.□ Yes (please specify)……

Q69. How would you describe your diet approach to you?

1.□Your eating habits are healthy

2.□You are too busy to find healthy foods or meals

3.□ You don't know enough about good nutrition or how to eat healthily

4.□ Sometimes, you try to eat healthy food

5.□ You can’t resist junk food

6.□ Your eating habits are poor

Q70.What other factors affect your eating habits?

1.□ Your parents and/or family don’t eat healthily.

2.□ Your school / work don’t offer healthy choices at lunch or mealtimes

3.□ You and your friends are always going out to eat and socializing over food and you end up eating lots of junk food.

4.□You really don’t feel like it’s worth it or have the motivation to try to change your habits.

5.□ You eat healthy diet

6.□ Others

Q71. Do you drink alcohol?

1- □Yes

2- □No

1-□ Yes, but not on the exercise day

Q72. Do you smoke on an exercise day?

1-□Do not smoke

2-□ Yes, please state how many…….

Q73. How often are you ever too unwell or stressed to exercise?

1. □Frequently
2. □Sometimes
3. □Rarely
4. □Other (please specify)

Q74 .Have you experienced any of the following symptoms after exercise?

1. □Bleeding
2. □Chafing
3. □Flushing Hives
4. □Hypothermia
5. □Muscle cramps
6. □Red face
7. □Shortness of breath
8. □Urinary (pain,colour or blood)
9. □None

Q75. Please write any comments you would like to add

Appendix E

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DE MONTFORT UNIVERSITY
LEICESTER

HLS FREC Ref: 855

17th November 2012

Joan Taylor
Pharmacy
HLS

Dear Joan,

Re: Ethics application – Use of exercise-based physiological monitoring to compare calorie turnover in the two main types of diabetes and where different therapies are used (ref: 855

I am writing regarding your application for ethical approval for a research project titled to the above project. This project has been reviewed in accordance with the Operational Procedures for De Montfort University Faculty of Health and Life Sciences Research Ethics Committee. These procedures are available from the Faculty Research and Commercial Office upon your request.

I am pleased to inform you that ethical approval has been granted by Chair's Action for your application. This will be reported at the next Faculty Research Committee, which is being held in January 2012.

Should there be any amendments to the research methods or persons involved with this project you must notify the Chair of the Faculty Research Ethics Committee immediately in writing. Serious or adverse events related to the conduct of the study need to be reported immediately to your Supervisor and the Chair of this Committee.

The Faculty Research Ethics Committee should be notified by e-mail to HLSFRO@dmu.ac.uk when your research project has been completed.

Yours sincerely,

Professor Paul Whiting
Chair
Faculty of Health and Life Sciences
Research Ethics Committee

Appendix F

dmu.ac.uk DE MONTFORT UNIVERSITY LEICESTER **Pharmacy** **Faculty of Health & Life Sciences**

Standard Operation Procedure (SOP)

Title of Project: The effects of aerobic and resistance exercise on diabetes

Name of Principal Investigators: Prof MJ Taylor, Mr.Ahmed Alsabih and Mr.Mohamd Alblihed

Preliminary procedures

Before enrolling in the study the volunteer will be asked to attend a screening visit where we will:

- discuss and complete confidential questionnaires regarding his health, family history and physical activity level.
- measure his blood pressure.
- measure his height and weight
- provide an opportunity for him to ask questions
- familiarise him with equipment to be used in the study and teach him how to use the recumbent ergometer bike and how to lift the weights safely in the multigym machine.
- This session will also be used to determine the intensity of exercise (moderate intensity) on the recumbent ergometer bike and how much weight he should lift in the resistance exercise session later.

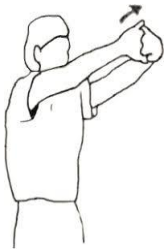
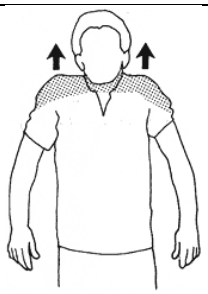
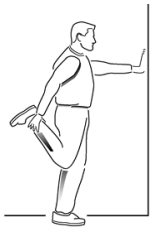
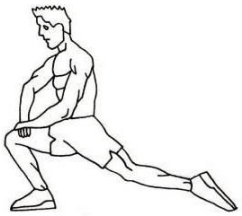
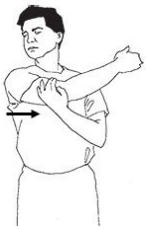
These preliminary procedures will enable us to determine whether the volunteer suitable to safely participate in the study or not.

Main experimental trials

The main experimental trials will involve 2 x 2 hour exercise sessions a week for a 6- week period,(exercise session includes rest + final observation of volunteer for hypos etc…). Blood glucose levels will be monitored before, during and after each session, using a standard finger prick test. Cholesterol, High density lipoprotein and Triglyceride will be monitored before, during and after exercise programme and after the second session in each week of 6 weeks of exercise programme using a finger prick test. There may be occasions when the volunteer may not be allowed to participate if that or another health issue is deemed to threaten wellbeing.

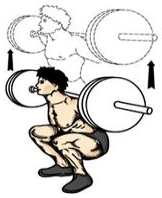
Each exercise session will consist of a combined exercise protocol of 30 min of resistance exercise (3 sets of 8 -10 repetitions at 50 – 60% of one-repetition maximum strength 1-RM) using upper and lower muscle groups followed by 30 min moderate cycling at 50 – 60% of predetermined maximum heart rate (HR_{max}) or ratings of perceived exertion (RPE).
The exercise session will include the following 6 phases:

1. Warm-up: start with cycling for 5 - 10min on the recumbent ergometer bike at low to moderate intensity (30 to 40% of HR_{max}). Warm-up is an essential part of the exercise session and designed to prepare the body for exercise, increase body temperature and to reduce the potential post-exercise injury or pain (muscle stiffness).
2. Stretching: stretch lower and upper muscle groups for 5 min

 loosens upper arm and chest muscles	 Stretching shoulder, middle back, arms, hands, fingers, wrist	 Stretching the back and shoulder muscles	 Stretching the chest, top of shoulder and lower arm muscles
 Stretching the shoulders and neck	 Stretches triceps, top of shoulders, waist	 Stretching the quadriceps	 Hip flexor stretches
 Chest stretch for pectoral muscle	 Stretches inner thigh, groin	 Stretches side of shoulder and back of upper arm	

3. Resistance exercise: this phase will include 30 min of five different exercises working upper and lower muscle groups (3 sets of 8 -10 repetitions at 50 – 60% of one-repetition maximum strength 1-RM) and using the Multigym machine

Working muscle groups as follows: (3 sets of 8 -10 repetitions each exercise)

 a. Squat	 b. Incline bench press	 c. lat curl pull down	 d. Triceps	 e. Biceps

4. Cycling

The volunteer will do moderate cycling at 50 – 60% of predetermined maximum heart rate (HR_{max}) or ratings of perceived exertion (RPE) for 30 min. The volunteer and the bike will be attached to monitoring equipment that can produce instant graphical representation of performance linked to work done and produce different metabolic parameters such as Oxygen consumption (VO_2), Carbon Dioxide Production (VCO_2), Respiratory Exchange Ratio (RER), Heart rate (HR) etc. The volunteer attachment will be via a breathing mask and by ECG stick-on electrodes.

5. Cool-down: cycling for 5 - 10min on the recumbent ergometer bike at low to moderate intensity (30 to 40% of HR_{max}). The purpose of the cool-down phase is reducing the risk of injury and brings the body physiological responses back to normal such as heart rate and blood pressure.

6. Stretching: stretch lower and upper muscle groups for 5 min.

I ……(NAME AND ADDRESS OF PARTICIPANT) would like to participate in a focus group/interview in the DMU research study 'Investigating views on acceptable methods for controlling blood sugar'.

_____ _____

Signed by Participant date

Name ……..Address……..

Postcode…….. Telephone number …….

Appendix G

Leicester School of Pharmacy **Faculty of Health & Life Sciences**

Volunteer Information Sheet (VIS) General practitioner

Title of Project: The effects of aerobic and resistance exercise on diabetes

Name of Principal Investigators: Prof MJ Taylor, Mr.Ahmed Alsabih and Mr.Mohamd Alblihed

Date 26/10/2011

Dear general practitioner

We would like to give you some information and details about the research study. This information is just to understand why the research is being done and what it would involve for the volunteer. Please take time to read the following information carefully. Talk to the volunteer about the study if you wish.
Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

As part of the growing research work into diabetes, School of Pharmacy, De Montfort University (DMU) are working to investigate the effects of combined exercise programme (aerobic and resistance) on blood glucose, metabolic and immunological parameters that could help people with the management of their diabetes.
This research involves a combination of two types of exercises and this is where we need the volunteer help. We would like to invite the volunteer to take part in this study which will help us to understand the role of exercise and how it can help people with diabetes to maintain a healthy body weight and to possibly better manage their blood glucose level.
We will keep volunteer information strictly confidential and nobody other than the research team will have access to the volunteer personal information.
Before any research goes ahead it has to be checked by De Montfort University Research Ethics Committee. They make sure that the research is fair.

Why the volunteer have been invited?

The volunteer have been invited to take part in this study because he is male and have either Type 1 or Type 2 diabetes, and his age between 18 - 55.

Does he have to take part?

It is up to him to decide. We will answer any questions he has about the study and go through this information sheet. We will then ask him to sign a consent form to show he has agreed to take part. The volunteer is free to withdraw at any time, without giving a reason. This would not affect the standard of care he is receiving from his doctor or hospital.

What will happen to the volunteer if he takes part?

Preliminary procedures

Before enrolling in the study the volunteer will be asked to attend a screening visit where we will:

- discuss and complete confidential questionnaires regarding his health, family history and physical activity level.
- measure his blood pressure.
- measure his height and weight
- provide an opportunity for him to ask questions
- familiarise the volunteer with equipment to be used in the study and teach him how to use the recumbent ergometer bike and how to lift the weights safely in the multigym machine.
- This session will also be used to determine the intensity of exercise on the recumbent ergometer bike and how much weight the volunteer should lift in the resistance exercise session later

These preliminary procedures will enable us to determine whether the volunteer is suitable to safely participate in the study.

Main experimental trials

The main experimental trials will involve 2 x 2 hour exercise sessions a week for a 6- week period. (Exercise session includes rest + final observation of volunteer for hypos etc…).

Blood glucose levels will be monitored before, during and after each session, using a standard finger prick test. Cholesterol, high density lipoprotein and triglyceride will be monitored before, during and after exercise programme and after the second session in each week of 6 weeks of exercise programme using a finger prick test. There may be occasions

when the volunteer may not be allowed to participate if that or another health issue is deemed to threaten wellbeing.

Each exercise session will consist of a combined exercise protocol of 30 min of resistance exercise (3 sets of 8 -10 repetitions at 50 – 60% of one-repetition maximum strength 1-RM) using upper and lower muscle groups followed by 30 min moderate cycling at 50 – 60% of predetermined maximum heart rate (HR_{max}) or ratings of perceived exertion (RPE).

The exercise session will include the following 6 phases:

1. Warm-up: start with cycling for 5 - 10min on the recumbent ergometer bike at low to moderate intensity (30 to 40% of HR_{max}). Warm up is an essential part of the exercise session and designed to prepare the body for exercise, increase body temperature and to reduce the potential post-exercise injury or pain (muscle stiffness).
2. Stretching: stretch lower and upper muscle groups for 5 min as shown in standard operating procedure.
3. Resistance exercise: this phase will include 30 min of five different exercises working upper and lower muscle groups (3 sets of 8 -10 repetitions at 50 – 60% of one-repetition maximum strength 1-RM) and using the multigym machine
4. Working muscle groups as shown in standard operating procedure.
5. Cycling

   The volunteer will do moderate cycling at 50 – 60% of predetermined maximum heart rate (HR_{max}) or ratings of perceived exertion (RPE) for 30 min. The volunteer and the bike will be attached to monitoring equipment that can produce instant graphical representation of performance linked to work done and produce different metabolic parameters such as Oxygen consumption (VO_2), Carbon Dioxide Production (VCO_2), Respiratory Exchange Ratio (RER), Heart rate (HR) etc…. The volunteer attachment will be via a breathing mask and by ECG stick-on electrodes.
6. Cool-down: cycling for 5 - 10min on the recumbent ergometer bike at low to moderate intensity (30 to 40% of HRmax). The purpose of the cool-down phase is reducing the risk of injury and brings the body physiological responses back to normal such as heart rate and blood pressure.
7. Stretching: stretch lower and upper muscle groups for 5 min.

Expenses and payments

We would like to pay expenses to the volunteer and to offer an incentive of £50 each on completion of 50% and £120 on completion of 95% of the dates agreed to.

What are the possible benefits of taking part?

There may be no benefits to the volunteer but as a result of being involved in this study he will receive health and fitness information about himself including fitness tests and body measurement. The findings of this study will be published in scientific Journals so that understanding about how exercise can help people with diabetes to improve their health and control their weight and blood glucose levels. This information may contribute towards improved exercise guidelines for the diabetic patients.

We will provide the volunteer with feedback about the main study findings and also about his own results and would be delighted to explain our findings and discuss possible implications with him.

What if there is a problem?

The chance of something going wrong is small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that the volunteer will only participate if it is safe for him to do so. However, if he has any concerns at any time about any aspect of the way he has been approached or treated during the course of this study, he should ask to speak to the researchers who will do their best to answer his questions (contact details below), and the normal De Montfort University complaints mechanisms will be available to him.

Will the volunteer taking part in the study be kept confidential?

All information that is collected about the volunteer during the course of the research will be kept strictly confidential. Any information about him, which leaves the University, will have his name and address removed so that he cannot be recognised from it.

What will happen if the volunteer don't want to carry on with the study?

While we do not expect the programme to cause the volunteer to become upset if this does happen then he will have the option to pause or stop his participation immediately, he may continue only if he wanted to. If he withdraw from the study, we will destroy all his identifiable data, but may use the data collected up to his withdrawal.

Who has reviewed the study?

This study has been reviewed and approved by the Faculty of Health and Life Sciences Ethics Committee at De Montfort University. Approval does not guarantee that he will not come to any harm if he takes part. However, approval means that the committee is satisfied that the

volunteer rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that he has been given sufficient information on which to make an informed decision.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting Ahmed Alsabih on p09053155@myemail.dmu.ac.uk or Mohamd Alblihed on p06004947@myemail.dmu.ac.uk or Prof.Taylor on 01162506317 or mjt@dmu.ac.uk .

Appendix H

Leicester School of Pharmacy

Faculty of Health & Life Sciences

Centre Number:
Study Number:
Patient Identification Number for this trial:

Consent Form

Title of Project: The effects of aerobic and resistance exercise on diabetes

Name of Principal Investigator: Prof MJ Taylor, Mr.Ahmed Alsabih and Mr.Mohamd Alblihed

Please initial at the end of each point

1. I confirm that I have read and understand the Participant Information Sheet dated. (05/10/2011) (Version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. I understand that agreeing to take part means that I am willing to undertake some exercise and giving samples in the above study.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected.
3. I understand that any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party. No identifiable personal data will be published. The identifiable data will not be shared with any other organisation.
4. I understand that confidentiality can be guaranteed for information which I might disclose in any session or visit I attend. I understand that this information will be used only for the purpose(s) set out in this statement and my consent is conditional on the University complying with its duties and obligations under the Data Protection Act 1998.
5. I agree to take part in the above study.

Participant Name…….. Signature…….Date…….

Person Taking consent……...Signature……Date……..

When completed, copy for patient; copy for researcher site file.

Appendix I

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y aculty of Health & Life Sciences

Volunteers Health Screen for Study Volunteers

Name: …… Volunteer Identification Number…….

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

At present, do you have any health problem for which you are:

(a) on diabetes medication, prescribed or otherwise yes [ ] no [ ]

(b) other medication yes [ ] no [ ]

(b) attending your general practitioner yes [ ] no [ ]

(c) on a hospital waiting list yes [ ] no [ ]

In the past two years, have you had any illness which required you to:

(a) consult your GP yes [ ] no [ ]

(b) was this related to diabetes yes [ ] no [ ]

(b) attend a hospital outpatient department yes [ ] no [ ]

(c) be admitted to hospital yes [ ] no [ ]

Have you ever had any of the following:

(a) Convulsions/epilepsy yes [ ] no [ ]

(b) Asthma yes [ ] no [ ]

(c) Eczema yes [ ] no [ ]

(d) Heart problems yes [ ] no [ ]

(e) A blood disorder yes [ ] no [ ]

(f) Head injury yes [ ] no [ ]

(g) Digestive problems yes [ ] no [ ]

(h) Hearing problems yes [ ] no [ ]

(i) Problems with bones or joints	yes [ ]	no [ ]
(j) Disturbance of balance/co-ordination	yes [ ]	no [ ]
(k) Numbness in hands or feet	yes [ ]	no [ ]
(l) Disturbance of vision	yes [ ]	no [ ]
(m) Thyroid problems	yes [ ]	no [ ]
(n) Kidney or liver problems	yes [ ]	no [ ]
(o) Chest pain	yes [ ]	no [ ]
(p) Any other health problems	yes [ ]	no [ ]

Do you currently smoke yes [ ] no [ ]

Have you ever smoked yes [ ] no [ ]

If so, for how long did you smoke and when did you stop? ……

How many units of alcohol do you typically drink in a week? …….

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

……

……

……

……

Name and address of your GP

……

Letter received from GP ☐

Appendix J

Private and confidential

De Montfort University Diabetes and Exercise Group 2012
Volunteer Record Sheet

Volunteer Identification Number:

TITLE…….… FIRST NAME……..… SURNAME……..…
DATE OF BIRTH……..…. AGE……MALE / FEMALE …
CORRESPONDENCE ADDRESS……....
…… POSTCODE: ……..…….
HOME TELEPHONE: …….. MOBILE: ……..……..
WORK TELEPHONE ……
EMAIL ADDRESS: …….
Please tick ☐ I am happy for De Montfort University Diabetes and Exercise Group to contact me by email
(We may will be sending key information via emails, so please include an up to date email address that you will check on a regular basis)
PERSON TO CONTACT IN CASE OF EMERGNCY……..……
RELATION TO YOU……..……..…….
HOME TELEPHONE: …….. MOBILE: ……..….
EMAIL ADDRESS: ……..

Type of diabetes? Healthy ☐ Type 1 ☐ Type 2 ☐ Other ☐
Number of years with DM duration……..…
Do you have any other medical conditions? Yes/ No
If yes, please state: ……..……
Do you have any specific dietary requirements? Yes/ No
If yes, please state: …….
Name of your GP……. Tel……
Address ……Post Code…….
Do you have any existing medical conditions Yes / No
If yes, please state……..……..…
Any infectious diseases Yes / No
If yes, please state ……..……..
Have you been in hospitalised the past year Yes / No
If yes, why ……
History of acute diabetic complications

Number of Hypoglycemia (low blood sugar) event in last month……..
Number of Hyperglycemia (high blood sugar) event in last month ……..…….
Number of Diabetic ketoacidosis event in last month……
Are you taking any medications, pills or drugs……..... If yes, please list and state dose

……..

Do you have any of the following?

	Yes	No		Yes	No
Heart Disease			Liver Disease		
High Blood Pressure			Kidney Disease		
Rheumatic arthritis			Hepatitis		
Heart Murmur			Asthma		
HIV Positive /Aids			Tuberculosis (TB)		
Any allergy			Stroke		
Tumour (Cancer) History			Epilepsy		
Terminally or mentally ill			Any recent surgery		
Restriction in physical activity because of disease			Had an active infection		
Participation in exercise (aerobic or resistance) 2 times or more weekly for 20 minutes or longer per session during the previous 6 months					

Diabetes mellitus DM

Type of insulin……..…….
Units / day…….…….
Injections / day……..…….
Blood glucose testes / day …….

PLEASE RETURN THIS FORM TO: De Montfort University Diabetes and Exercise Group 2012

Participant Name…….. Signature…….Date…….

Appendix K

Visit Measurements sheet

	TRIAL 1						TRIAL 2					
Sessions number	**1**	**3**	**5**	**7**	**9**	**11**	**2**	**4**	**6**	**8**	**10**	**12**
1. Subject name/Ref	**Name**						**Ref:**					
2. Date	/ /						/ /					
3. Food inventory sheet												
4. Temperature of the lab												
5. ADI calibration												
6. Zero all input												
7. Chest polar Attached												
8. RER at rest 5 min on (ADI)												
9. BP & HR at rest	/ mm Hg				bpm		/ mm Hg				bpm	
10. BG before exercise	mmol/L						mmol/L					
11. Stretching for 5 min												
12. Warm up on the bike for 5 min (at 50-55 % HRR)												
Resistance Sets	**1st**		**2nd**		**3rd**		**1st**		**2nd**		**3rd**	
13. Exercise 1 Squat												
14. Exercise 2 Chest												
15. Exercise 3 Back												
16. Exercise 4 Triceps												
17. Exercise 5 Bicep												
	RPE		**HR**				**RPE**		**HR**			
18. After 1st set of Resistance												
19. After 2nd set of Resistance												
20. After 3rd set of Resistance												
21. BG after Resistance	mmol/L						mmol/L					
22. BP & HR after Resistance	/ mm Hg				bpm		/ mm Hg				bpm	
23. 5 minute break (attach the 3 leads ECG, pulse rate and mouth piece)												
24. Start cardio exercise cycling												
	RPE		**HR**		**BG**		**RPE**		**HR**		**BG**	
25. At 10 min of Cycling												
26. At 20 min of Cycling												
27. Unattached the ECG leads, HR chest polar and mouth piece)												
28. Stretching for 5 min												
29. BP & HR after Cycling	/ mm Hg				bpm		/ mm Hg				bpm	
30. BG after Cycling	mmol/L						mmol/L					

	Visits		
	1st	**6th**	**12th**
31. Height			
32. Weight			
33. Body water %			
34. Body Fat %			
35. BMI			
36. Blood Gases			
A. Blood pH			
B. pCO2 mmHg			
C. pO2 mmHg			
37. Electrolytes			
A. K+ mmol/L			
B. Na+ mmol/L			
C. Ca+ mmol/L			
D. Cl- mmol/L			
E. Lactate mmol/L			
38. Lipids profile			
A. LDL mmol/L			
B. HDL mmol/L			
C. Total cholesterol mmol/L			
D. Triglyceride mmol/L			
39. Creatinine µmol/L			

40. HbA1c	**1st**			**12th**				
41. Waist (cm):	**1st**			**12th**				
42. Hip (cm):	**1st**			**12th**				
43. Waist/HIP Ratio:	**1st**			**12th**				
44. Blood samples for Immuno assay	**Acute exercise**			**Chronic exercise**				
	Before Exercise	**After Resistance**	**After Cycling**	**2nd**	**4th**	**6th**	**8th**	**12th**

Appendix L

Assessment of caloric intake

FOOD INVENTORY INSTRUCTIONS

It is important that you weigh and record everything that you eat and drink except water for theday prior to each main experimental trial. Please do not take any alcohol on these days.

Please (i) start a separate page for each day.
(ii) start a separate line for each item.
(iii) for the day before exercise, try to eat same food as similar as you can.

Column 1
Record meal and time and place of eating.

Column 2
Describe each item as accurately as possible, stating where relevant:

(i) Type and brand
(ii) Whether food is fresh, dried, canned, frozen, salted, smoked, etc.
(iii) Whether food is cooked, if so give method of cooking e.g. fried, baked, etc.

Column 3
Record the weight of each item after cooking:

(i) Place scales on a level surface
(ii) place plate or container on top of scales
(iii) press 'ON/Reset' button to turn on scales
(iv) once zero appears, add first item of food
(v) record weight displayed
(vi) press reset button before weighing next item

Wherever possible, record weights in grams. If this is not possible, record weights in household measures (e.g. sugar or jam in teaspoons, stating whether level, rounded, or heaped).

Column 4
Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

Columns 5 and 6

Please leave blank.

If food consists of several items, please list each on a separate line i.e. instead of writing 'one cheese sandwich', record separately the weights of bread margarine, cheese, etc.
Please remember to record all drinks, as well as food, giving weights where possible, or volumes if these are known. Record separately the weight of added milk and sugar.

An example is shown overleaf.

Food Inventory - Example

Name:.....Date:.....

1	2	3	4		
Time/Place	**Description of food/drink**	**Weight of food/drink (g)**	**Weight of container/ leftovers (g)**	**Leave Blank**	
				5	**6**
Breakfast	Cornflakes (Kelloggs)	28			
8:30am	Milk (Sainsbury's virtually fat-	48			
Home	Bread (Mothers Pride, large	76			
	sliced, toasted)				
	Flora margarine	7			
	Robinsons lemon marmalade	12			
	Coffee (instant)	2			
	Milk (whole pasteurised)	10			
Lunch	Cheese (Cheddar)	55			
1:00pm	Bread (white, crusty)	76			
Pub	Butter	4			
	Chutney (2 teaspoons)				
Snack	Coffee (instant)	2			
3:30pm	Coffee-mate	6			
Office	Mars Bar	35			
	Apple	76	8 (core)		
Dinner	Turkey Fillet (frozen, grilled)	102			
6:30pm	Potatoes, old, boiled	320	74		
Home			(leftover)		
	Peas (Birds Eye, frozen, boiled)	50			
	Heinz tomato ketchup	14			
	Yoghurt (Ski strawberry thick and	162	10		
	creamy)		(carton)		
	Coffee, filter	148			
	Milk (Sainsbury's virtually fat-	8			