



The effects of a combined exercise programme aerobic and resistance on blood glucose, incretin, metabolic and inflammatory mediators that could control the diabetes and improve insulin sensitivity in type 2 diabetes patients

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Abbreviations:

Abbreviations	Definitions
NADH	Nicotinamide adenine dinucleotide
FADH ₂	Flavin adenine dinucleotide
BG	Blood glucose
FBG	Fasting blood glucose
ND	Non diabetes
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TRG	Triglyceride
TC	Total cholesterol
HDL	High density lipoprotein
LDL	low density lipoprotein
VLDL	very low-density lipoproteins
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
HR	Heart rate
RPE	Rate of perceived exertion
1RM	One repetition maximum
IL-6	Interleukin 6
IL-4	Interleukin
TNF	Tumor necrosis factor
CRP	C- reactive protein
LEPT	Leptin
RETN	Resistin
OS	Oxidative stress
ROS	Reactive oxygen species

RNS	Reactive nitrogen species
IRS	Insulin-resistance syndrome
GLP-1	Glucagon like peptide
GIP	Glucose-dependent-insulinotropic polypeptide
DPP4-I	Dipeptidyl peptidase 4 Inhibitor
SU	Sulphonyl urea
GLUT-4	Glucose transporters type 4
SGT2-I	Sodium glucose cotransporter 2 inhibitor
ADA	American Diabetes Association
WHO	World Health Organisation
IDF	International Diabetes Federation
CVD	Cardiovascular disease
AGE	Advanced Glycation End-products
RAGE	Receptor of Advanced Glycation End-products
Hb-AGE	Heamoglobin Advanced Glycation End-products
TAG	Triacylglycerols
BMI	Body mass index
WC	Waist circumference
MetS	Metabolic syndrome
PAD	Peripheral artery disease
CAD	Coronary artery disease
CHD	Coronary heart disease
MI	Myocardial infarction
CNS	Central nervous system
HTN	Hypertension
CysC	Cystatin C

GFR	Glomerular filtration rate
CKD	Chronic kidney disease
GD	Gestational diabetes
AUC	Area under the curve
SEM	Standard error of mean
WAT	White adipose tissue
SAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue
FFA	Free fatty acid
BAT	Brown adipose tissue
EX	Exercise
RE	Resistance exercise
AE	Aerobic exercise
S1	Session one
S6	Session six
S12	Session twelve
Pre	Before
Post	After
RER	Respiratory exchange ratio
CO ₂	Carbon dioxide
O ₂	Oxygen
VO ₂	Volume of oxygen consumed per minute
VCO ₂	Volume of carbon dioxide produced per minute
CHO	carbohydrate
EE	Energy expenditure
IC	indirect calorimetry
IR	Insulin resistance
H ₂ O ₂	hydrogen peroxide
OGTT	Oral glucose tolerance test

NGT	Normal glucose tolerance
IS	Insulin sensitivity
MTT	Meal glucose tolerance test
HIEC	hyperinsulinemic euglycemic clamp
ITT	Insulin tolerance test
IST	Insulin suppression test
SSPI	Steady state of plasma insulin
SSPG	Steady state of plasma glucose
CIGMA	Continuous infusion of glucose with model assessment
FSIVGTT	Minimal model analysis of frequently sampled intravenous glucose tolerance test
IV	Intravenous
RIST	Rapid Insulin Sensitivity Test
HOMA	Homeostasis Model Assessment
QUICKI	Quantitative insulin sensitivity check index
FPP	Finger prick procedure
SPSS	Statistical Package for Social Science
μL	Microliter
Kcal/L	The energy release from metabolism for each L of VO ₂
NICE	National Institute for Health and Care Excellence
LADA	Latent Autoimmune Diabetes of Adults
ELISA	Enzyme-linked immunosorbent assay
EDTA	Ethylene diaminetetraacetic Acid
ATP	Adenosine-5'-triphosphate
ADP	Adenosine-5'-diphosphate

GTP	Guanosine triphosphate
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
FADH	Flavin adenine dinucleotide
HMG CoA reductase	3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A reductase
ADI	ADIstruments Analysis System

Abstract

An excessive number of calories consumed daily, in addition to a sedentary lifestyle, are the main causes of increasing type 2 diabetes (T2D) prevalence worldwide (LEE, H.K. et al., 2010). Diabetes is usually accompanied by hypertension, lipid disorders and obesity. The aim of this study is to show that combination exercise is effective. It will compare T2D and non-diabetes (ND) volunteers doing combination exercise consisting of resistance and cycling. The interventions are minor and fairly short consisting of 12 episodes of exercise over 6 weeks, yet this was enough to produce measurable change and improvement. This included the re-categorization of two T2D volunteers to being ND, using normal metrics.

Method: in each exercise session the participant should perform a combined exercise program consists of 30 min of resistance exercise followed by 20 min moderate cycling. This is done twice a week for 6 weeks. At the beginning of each session the participants have to stretch-up for 11 steps of stretching, then he/she must cycle for five minutes to warm up. The RE consist of 3 sets, in each set the volunteer performed (squat, chest, back, biceps and triceps) 10 times. HbA1c, lipid profile, weight, BMI, waist and lung capacity also measured at baseline and the 12th exercise session. OGTT were done at the beginning, after 1st session and finally after session 12. Moreover, BG and HR measured before exercise, after 1st, 2nd and 3rd RE then after 10 and 20 minutes of AE. BP also checked before exercise after RE and then after AE. RPE were checked after 1st, 2nd and 3rd RE as well as after 10 and 20 minutes AE. Blood samples collected at base line after these sessions (S1, S2, S4, S6, S8, S10 and S12), centrifuge and refrigerate the plasma to be analysed later by a commercially-available enzyme-linked immunosorbent assay (ELISA) and by The Evidence Investigator™ Biochip Array technology (Randox, UK).

Results: After just six weeks, there was a reduction in the HbA1c level for the T2D volunteers which is significant ($P= 0.000$). Moreover, in ND the reduction was also significant ($P= 0.000$). In T2D, the OGTT result shows that the AUC of BG for the 1st

OGTT was $(30.55 \pm 2.5 \text{ mmol/L/h})$ but then decreased in the 2nd and 3rd OGTT, finally decreasing to $(24.4 \pm 1.9 \text{ mmol/L/h})$. This reduction reflects a clear improvement in the IS in T2D. It shows that the reduction of BG in the OGTT after chronic effect of exercise was better than acute effect (after the first session). Meanwhile in ND, the AUC for the 1st OGTT was $(14.9 \pm 3.2 \text{ mmol/L/h})$ and although became $(13.9 \pm 3.0 \text{ mmol/L/h})$ in the 2nd OGTT returned to the original in the third reading. In the T2D group who are using (Metformin and SGT2-I group), their result shows elevation in GLP-1 in the assessment of both acute and chronic effect of the programme. GLP-1 in this group was (3.9 ± 1.5) and increased to (8.4 ± 1.2) , ($P= 0.345$) after S1 and then increased more to (11.0 ± 0.8) , ($P=0.196$) after 6 weeks of exercise.

Conclusion: In T2D and ND combination exercise has a beneficial effect on HbA1c, the improvement was higher in T2D. The anthropometric variables (weight, waist, BMI and lung capacity) improved significantly as well in T2D and ND. OGTT shows a very significant improvement of BG and insulin level in both groups that was higher in T2D, which shows that there was an improvement in insulin sensitivity. Exercise is also important to improve GLP-1 secretion. Despite the range of studies on incretin undertaken here, still there is a need to compare the effect of exercise and different types of pharmacological therapy on GLP1. This study compared the effect of exercise on T2D plus medication in volunteers. It has been found that within T2D group only Metformin and SGT2-I group was improved. Both SGLT2 inhibitors and metformin have been found to affect body weight and this may explain the improvement of GLP-1 level, suggesting an area for future investigation. Finally, exercise has different effect on the inflammatory markers, it shows elevation of IL-6 in T2D after 6 weeks of exercise. CRP represent significant reduction after 6 weeks as well which illustrates that exercise has good impact in decreasing inflammation by reducing CRP. Moreover, there is a little published data on the effect of exercise and medication type on the inflammatory markers. The present study illustrates a significant reduction in Cystatin C after 6 weeks within T2D group who are using (metformin and DDP4-I). Therefore, there is increasing concern that some medication plus exercise improve inflammatory markers which need further investigations.

Publications:

- 1- Alsubaie NS, Alharbi BM, Sahota TS and Taylor MJ (2019) The Effects of A Combined Exercise Programme Aerobic and Resistance on Blood Glucose, and Incretin Hormone that Could Control the Diabetes in Type 2 Diabetes, Journal of Metabolic Syndrome.
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Chapter 1

1.1 Introduction

Diabetes mellitus (DM) is a chronic disease characterized by chronic persistent hyperglycaemia (Goyal and Jialal, 2018). This hyperglycaemia accompanied with metabolic disorder leads to organ damage and serious complications. Type 2 diabetes (T2D) is a global epidemic which has increased with increasing obesity and sedentary life style (Goyal and Jialal, 2018). Diabetes is usually accompanied by hypertension, lipid disorders and obesity. Recent studies show that a reduction in HbA1c cause 35% relative risk reduction for fatal/nonfatal cardiovascular disease. Also 56% reduction in CVD when this reduction of HbA1c is accompanied with systolic blood pressure (SBP) decrease (Eeg-Olofsson et al., 2016). Moreover, 75% reduction in CVD if reduction of HbA1c and SBP accompanied with decrease in non-HDL level (Eeg-Olofsson et al., 2016). Cardiovascular complications are the leading cause of morbidity and mortality between patients with T2D. Asian had the highest prevalence with kidney complications among T2D subjects (Zheng, Ley and Hu, 2018). T2D is considered as a serious disease, which needs immediate intervention. This intervention depends on the severity of the case. It could be either diet and exercise or pharmacological intervention by using oral anti-diabetic medication to control blood glucose levels within normal levels. It is necessary to check routinely to discover T2D at an early stage because identification and early treatment can prevent or ameliorate further complications in pre-diabetes, diabetes or metabolic disorder. Diet and exercise can potentially prevent the development of T2D of many of those at risk or in early stages (Diabetes UK, 2018).

1.1.1 Research questions

- 1- Are there demonstrable health differences and improvement in HbA1c after the six weeks exercise intervention between groups that can be attributed to the intervention?
- 2- Does exercise improve insulin sensitivity? Do the improvements correlate with medication type?
- 3- Does the improvement affect incretin involvement? Do the improvements correlate with medication type?
- 4- Does exercise change the inflammatory nature of T2D? Do the improvements correlate with medication type?

1.1.2 Aims

The aim of the study is to investigate the effects of a combined exercise programme (i.e. a combination of cardio “aerobic” and resistance) on blood glucose, incretin, metabolic and inflammatory mediators that could control the diabetes and increase insulin sensitivity in T2D. The aim of this study also is to demonstrate that combination exercise is essential for T2D. Moreover, it is going to compare two intervention groups of both T2D and ND to see if the changes in primary and secondary outcome are significant between the two groups. This study is also looking for the changes in incretin level within T2D group to find if there is any effect of exercise on the secretion of this hormone. Moreover, to state the effect of different medication on this hormone.

1.1.3 Objectives

- 1- Starting recruit volunteers.
- 2- Apply for ethical approval.
- 3- Organise for screening visit, take medical histories and decide the inclusion and exclusion criteria.
- 4- Search to understand the insulin and oral agent medication efficacy: becoming a diabetes educator.
- 5- Arrange to have training to learn first aid, phlebotomy, defibrillation techniques, oxygen administration, adrenaline administration.
- 6- Prepare for visits to diabetology group to participate in clinical and academic discussions relating to both types of diabetes.
- 7- Ensure to train for oral glucose tolerance tests (OGTT) and measuring HbA1c, incretin, and inflammatory mediator (using RANDOX and ELISA machine).
- 8- Record metabolic parameters.

The following section presents critically evaluated literature that relates to the field of study. The information is extracted from previous research studies that give useful information and insight on the topic of study. A literature search was conducted by using electronic databases (Science direct, google scholar, Medline, Embase, Sport medicine, PubMed, CINAHL, Cochrane library, and Scopus) from April 2015 until January 2019. This thesis will be organised in various sections under the chapters. The first chapter will contain introduction, the researcher will provide a background information on the prevalence of diabetes mellitus, NHS and global health expenditures, definitions, classification of diabetes diagnosis, symptoms of diabetes mellitus, complications, management of T2D as the main areas of chapter one. In chapter two, the researcher will focus on metabolism, the thesis will focus on carbohydrate, fat and protein metabolism. As well as the physiology of insulin production. Chapter three is the methodology then chapter 4, 5 ,6 and 7 are is the main exercise programme but each one has different findings and different analysis and has different research questions, aim and literature review.

1.2 The prevalence of diabetes mellitus

According to the International Diabetes Federation (IDF), it is estimated that globally more than 415 (8.4%) million people have diabetes (Einarson et al., 2018). The incidence of diabetes is increasing rapidly and that number is expected to increase to more than 642 million by 2040 (Monje, Catena and Borgnakke, 2017; Diabetes UK, 2018). Approximately, 90% of diabetes worldwide have T2D. The percentage of individuals who have been diagnosed with T2D in the UK is approximately more than 2.7 million, but 750,000 individuals have the symptoms but remain undiagnosed (Diabetes UK, 2018). T2D is a global disease, but is more common in developed countries. In the future, its prevalence is expected to increase in Africa, Asia, and the Middle East. It is expected to increase in those countries by an estimated 50% by 2030 (Forbes and Cooper, 2013). Globally, T2D prevalence is 90% (Zheng, Ley and Hu, 2018). Countries in Asia such as China and India are examples where T2D is most prevalent. Common risks associated with the diagnosis of T2D in such countries is genetics factors, unhealthy eating combined by a lack of exercise (Zheng, Ley and Hu, 2018). Stevens et al, found that T2D is accompanied by decrease quality of life and a reduction in life expectancy up to 10 years (Stevens et al., 2015). According to Aguiree et al., (2013) in the countries with high-income, the prevalence of people with T2D is about (87% to 91%), while in type 1 diabetes is (7% to 12%), and finally the prevalence of other types of diabetes is (1% to 3%)(Aguiree et al., 2013).

1.3 NHS and global health spends

In the UK, diabetes cost approximately 10% of UK NHS health expenditure, and it expected to rise to 17% by 2035/2036. The cost of diabetes to the NHS is over (£1.5m) an hour or 10% of the NHS budget for England and Wales, or more than £25,000 every minute on diabetes (Diabetes UK, 2018). Annually, the estimated number that spent on treating diabetes and its complications is £14 billion (Diabetes UK, 2018).

Global health spends on diabetes and its complications were costed as at least £411 billion in 2013. By 2035, the estimated number could exceed USD 627 billion (Aguiree et al., 2013).

1.4 Definitions

1.4.1 Diabetes mellitus

Diabetes mellitus is a metabolic disorder of various aetiologies accompanied with disturbances of carbohydrate, fat and protein metabolism as well as chronic hyperglycaemia (elevated blood glucose). The condition is due to defects in the secretion of insulin, insulin action, or both (Ozougwu et al., 2013; Kharroubi and Darwish, 2015; American Diabetes Association, 2018). Diabetes is a condition in which glucose in the blood rises to higher levels than the normal range (4-8mmol/L).

1.4.2 Type 2 diabetes mellitus

T2D is a progressive metabolic disorder characterised by hyperglycaemia (Goyal and Jialal, 2018). That can be due to insulin resistance (IR) in peripheral tissues, deficiency of insulin secretion in the pancreas (Goyal and Jialal, 2018; Kerner and Brückel, 2014; American Diabetes Association, 2018). This form of diabetes is not usually rapidly fatal because usually some endogenous insulin is still being produced by the pancreas (Goyal and Jialal, 2018; Kerner and Brückel, 2014).

1.4.3 Type 1 diabetes mellitus (T1D)

T1D specifies the progression of beta-cell destruction, which finally causes diabetes mellitus (Bornstein, 2018). In this kind of diabetes, there is absolute insulin deficiency thus exogenous insulin is essential for survival, to prevent the development of ketoacidosis, coma and death. T1D occur due to autoimmune processes that lead to beta-cell destruction. This accompanied with the existence of anti-GAD, islet cell or insulin antibodies (Kerner and Brückel, 2014; American Diabetes Association, 2018).

1.4.4 Haemoglobin A1c (HbA1c)

HbA1c is presenting the plasma glucose concentration over the last two to three months and used mmol/mol or percentage to measure it (Vijayakumar et al., 2017; Goyal and Jialal, 2018).

1.4.5 Metabolic Syndrome

Metabolic syndrome is the term for some risk factors for both coronary heart disease (CHD) and T2D. If the patient has three or more of five metabolic risk factors, they are considered to have metabolic syndrome (Ryl et al., 2015). These risk factors are the following:

- a- Obesity, if the waist measurement ≥ 35 inches for women and ≥ 40 inches for men.
- b- High level of triglyceride (TG).
- c- Low level of HDL cholesterol level.
- d- High blood pressure (hypertension)
- e- High level of fasting blood glucose (diabetes mellitus).

1.5 Classification of diabetes

Diabetes has been classified into the following categories (Monje, Catena and Borgnakke, 2017; Kerner and Brückel, 2014; ADA, 2016; Goyal and Jialal, 2018):

1. T1D.
2. T2D.
3. LADA (latent autoimmune diabetes in adults) is classified as type 1 diabetes.
4. Maturity-onset diabetes of the young (MODY)
5. Gestational diabetes.
6. Diabetes associated with causative clinical states, diseases, drugs and/or chemicals.

1.5.1 Type 1 diabetes mellitus (IDDM)

Millions of people worldwide have been affected by T1D, which is also known as insulin-dependent diabetes mellitus (IDDM) and its prevalence (5-10%) of diabetes subjects (American Diabetes Association, 2018; Goyal and Jialal, 2018). In this condition, T cell-mediated destruction of β cells in the islets of Langerhans causes absolute insulin deficiency, but the reason for this auto-immune destruction is not fully understood (American Diabetes Association, 2018; Mustonen et al., 2018; Wang et al., 2017; Bornstein, 2018). It has been found that there are high levels of T cells in T1D accompanied with a decline in C-peptide levels (Linsley et al., 2018). This leads to a total lack of insulin secretion from the pancreas, causing catastrophic hyperglycaemia, resulting in a disturbance in the metabolism of carbohydrate, fat and protein, that also produces ketones leading to ketoacidosis which is rapidly fatal if untreated (American Diabetes Association, 2018). This β cell destruction occurs rapidly in children and adolescent while it is slow in adult (American Diabetes Association, 2014; Goyal and Jialal, 2018). T1D is common in adolescence and childhood but it can happen as well in adult even in the 80th and 90th decades of life

which called (LADA) (American Diabetes Association, 2018). T1D is due to genes and environmental factors such as viruses nevertheless, recent studies have proved that less hygiene correlate to incidence of autoimmune disease (Bornstein, 2018).

1.5.2 Key features of T1D

According to American Diabetes Association, (2018):

- ✓ Treatment with insulin is essential throughout the patient's lifetime.
- ✓ It entails beta cell destruction, which causes complete insulin deficiency and lack of plasma C-peptide.
- ✓ Patients are predisposed to ketosis.
- ✓ Usually, the onset of the disease is acute.
- ✓ Peak onset time is at pre-school age (4-6 years) and around puberty (10-14 years); however, it can occur at any age.
- ✓ Presence of specific autoantibodies.

1.5.3 Environmental factors

Numerous environmental factors which might be aetiological and/or pathogenic in T1D (Wang et al., 2017; Katsarou et al., 2017). The exposure to some environmental factors for long periods of time causes certain variation in genes, this genetic predisposition is linked to the human leukocyte antigen (HLA) genes which produce protein that play a crucial role in immune system regulation (Adi and Gerard-Gonzalez, 2018). Another factors are thought to contribute to the development of T1D, and may comprise one or more triggers such as multivitamins deficiency especially in neonates who were not breast feed, vitamin D deficiency, and viral or microbial infections (Wang et al., 2017; Katsarou et al., 2017; Adi and Gerard-Gonzalez, 2018). In humans, evidence shows that there is a higher rate of onset of the disease reported in the winter, while the lower rate reported at the end of spring and summer (Scobie and Samaras, 2012). Basically, the presence of β -cell-directed autoantibodies associated with HLA alleles is the best predictor to diagnose T1D (Adi and Gerard-Gonzalez, 2018).

1.5.4 Pathogenesis

It has been found that in T1D, most of the cases had an immunological association to disease pathogenesis (Adi and Gerard-Gonzalez, 2018). Not all patients with T1D have these features (Atkinson, Eisenbarth and Michels, 2014). There are two kinds of T1D; type 1A occurs due to a cell-mediated autoimmune attack on β cells (autoimmune) diabetes. Some (85–95%) of patients with T1D have type 1A which show positive antibodies (Adi and Gerard-Gonzalez, 2018). Type 1B (idiopathic) diabetes with no antibodies which have unknown etiology (Atkinson, Eisenbarth and Michels, 2014; American Diabetes Association, 2018; Adi and Gerard-Gonzalez, 2018).

1.5.5 Type 2 diabetes (T2D)

The prevalence of T2D is very high worldwide (DeFronzo et al., 2015; Goyal and Jialal, 2018). This type is a multifactorial disease characterised by IR, relative insulin deficiency and advanced beta cell deterioration, which affects insulin secretion (Christensen et al., 2014; DeFronzo et al., 2015; Goyal and Jialal, 2018). T2D is a condition that is normally characterised by the body failing to use the available insulin, as produced in the pancreas (Christensen et al., 2014; DeFronzo et al., 2015). These pathophysiological defects cause an imbalance between the rate of glucose production and its disposal. The rise in the former and decline in the latter leads to hyperglycaemia. This is caused by a condition called IR which is due to a reduction of glucose disposal by peripheral tissues, mainly muscles and increased production of hepatic glucose (Meah and Juneja, 2015). During the initial stages of diabetes, the pancreas produces extra insulin in an attempt to utilize glucose. However, over time, the pancreas fails to generate sufficient insulin to keep the blood glucose at the recommended levels (Christensen et al., 2014; DeFronzo et al., 2015). As such, the individual suffers T2D because of a lack of adequate levels of insulin. Alternatively, it may sometimes result from the failure of the body's cells to react to insulin. This persuades the glucose to remain in the blood without being used as a source of fuel for the production of energy (Christensen et al., 2014; DeFronzo et al., 2015; Samour and King, 2012). The Insulin signalling pathway: food is consumed, the food is then digested in the stomach, and finally the small intestine will subsequently release

glucose which is transported through the blood circulation. Out of the five types of specific protein called glucose transporters “GLUT”, the GLUT-4 protein is considered the most insulin-sensitive and it is known as the insulin dependent glucose transporter which uptakes glucose only into the skeletal muscle and adipose tissue in cases of normal insulin secretion (Mann and Bellin, 2016; Codario, 2011). Other transporters such as GLUT1, GLUT2 and GLUT3 are known as insulin independent glucose transporters, which can be found in vital organs such as pancreatic β cells, kidneys, brain, red blood cells (RBC) and placenta. Therefore, GLUT2 in β cells permits glucose to be transported within β cells to stimulate the secretion of insulin (Mann and Bellin, 2016).

This pathway usually starts by a simple mechanism called simple glucose-stimulated insulin secretion (GSIS) where glucose is transported into the β cell and then metabolised to release ATP. The ATP triggers a cascade of signals in the β cell which is important for glucose to stimulate insulin production. GLUT1 and GLUT3 have been found in recent studies to have played an essential role in β cells like GLUT2 which show that any mutation in the gene encoding GLUT2 does not cause a defect in the production of insulin (Mann and Bellin, 2016). After food ingestion, glucose is transported to the β cell and converted via hormone glucokinase to glucose 6 phosphate (G6P). This hormone is called glucose sensor in the β cell. In the mitochondria, ATP are produced via the Krebs cycle as a result of metabolised G6P. The ATP then binds to and closes the ATP-dependent potassium channel which prevents the potassium to quit the β cell and this process is called cell membrane depolarisation. This depolarisation triggers voltage-gated calcium channels which elevate calcium influx and increase intracellular calcium concentration. The high level of calcium in the cytoplasm stimulates insulin and C-peptide production (Mann and Bellin, 2016). This GSIS is also augmented by amino acid (AA), free fatty acid (FFA) and glucagon-like peptide-1 (GLP-1). Insulin secretion can be stimulated also when some AA enters the Krebs cycle and acts as a substrate to convert G6P to then generate ATP. ATP then does the same process of depolarising the β cell membrane to stimulate insulin secretion (Mann and Bellin, 2016). The pathway of how FFA stimulates insulin secretion is still unclear. The basal insulin secretion increased along with the chronic elevation of FFA however this prevents the bolus insulin secretion (GSIS). High levels

of lipolysis in adipose tissue elevates FFA in the circulation and causes IR and T2D (Mann and Bellin, 2016). Furthermore, the pancreas stimulates insulin secretion in the blood stream to enhance glucose clearance from the blood stream (Mann and Bellin, 2016; Saltiel and Kahn, 2001). Insulin has three target cells, skeletal muscle, liver and adipose tissues and each cell has insulin receptors to enable insulin to enter inside the cell and trigger glucose transporters e.g. GLUT4 (in the skeletal muscle and adipose tissue) and GLUT2 (in the liver) (Mann and Bellin, 2016; Jaldin-Fincati et al., 2017). Insulin-induced GLUT4 translocation from intracellular to the cell membrane has been found in the early 1980s (see figure 1) (Thorens and Mueckler, 2009). The GLUT4 proteins are stored in intracellular vesicles of skeletal muscle and adipose tissue and then transferred to the cell surface in the plasma membrane as translocation of GLUT4 to the cell surface (Codario, 2011).

The role of these transporters is to transport glucose on the cell membrane from the outside to the inside of cells to store it or use it as energy for different body functions. In adipose tissue, insulin stimulates glucose storage as TG and inhibits lipid breakdown (lipolysis). Moreover, in the skeletal muscle and liver, insulin stimulates storage of glucose as glycogen, insulin stimulates protein synthesis. All these cascade to normalise blood glucose, however, in T2D there are some defects in this process starting in failure of IR which lead to hyperglycaemia (Mann and Bellin, 2016; Saltiel and Kahn, 2001). In T2D, skeletal muscle cells in IR subjects are characterized by a lower ability to utilise extracellular glucose. The level of GLUT4 becomes low in those subjects, because of the impairment in their translocation to the membrane. This is due to either an insulin signalling mechanism impairment or due to an accumulation of GLUT4 in the membrane (Navale and Paranjape, 2016). This defect affects the pathway between the transporter (GLUT4) and the receptor before enhancing the glucose entering the cell, which causes IR (Mann and Bellin, 2016).

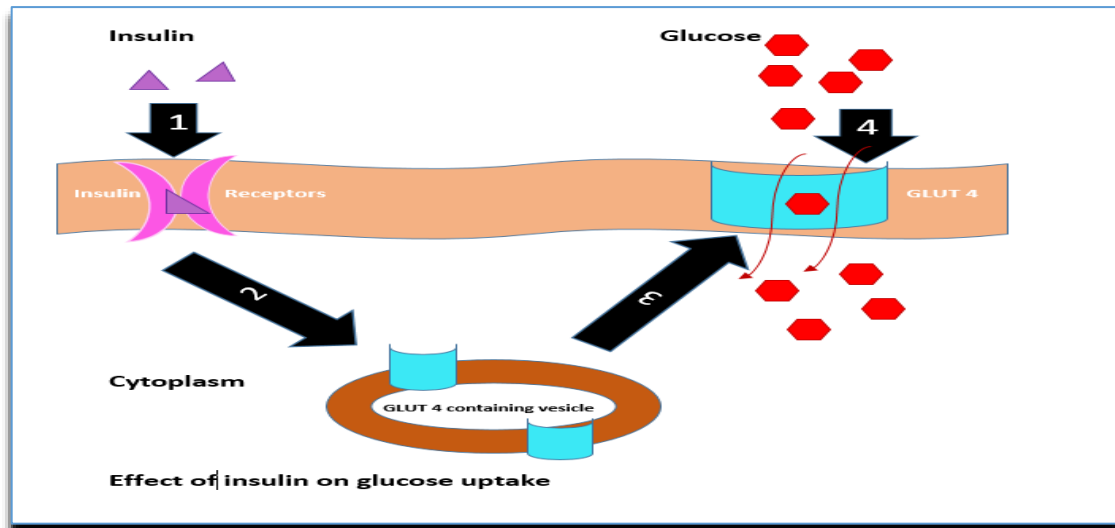


Figure 1: effect of insulin on glucose uptake

There are three counter-regulatory hormones which help the body when it suffers from hypoglycaemia. These hormones are glucagon, adrenaline and cortisol. Glucagon stimulates hepatic glucose production via glycogenolysis or by production of glucose from AA and glycerol via gluconeogenesis (Mann and Bellin, 2016). It also stimulates lipolysis and the carrying of FFA to the liver. Cortisol enhances the catabolism of protein to the AA which acts as a substrate for gluconeogenesis and impaired glucose uptake by insulin. Moreover, adrenaline inhibits insulin secretion directly, enhancing glycogenolysis, adipocytes lipolysis and IR (Mann and Bellin, 2016). In addition, the defects in incretin hormones lead to a reduction in the postprandial insulin release by the beta cells, as well as increased production of glucagon by the alpha cells (Garduno-Garcia et al., 2018; Michaliszyn et al., 2014). Additionally, the renal tubular reabsorption of glucose is increased as a result of upregulation of sodium glucose co-transporters-2 (SGLTs-2) and a β -cell deficiency. All these factors cause fatigue in β -cells because of their huge workload, indicating that insulin therapy may be necessary for longstanding T2D (Meah and Juneja, 2015). Recently, the effect of incretin has attracted a great deal of attention. In humans, the main incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Nauck and Meier, 2016; Fava, Dong and Wu, 2016). Both hormones enhance glucose-dependent insulin secretion and are quickly deactivated by dipeptidyl

peptidase-4 (DPP-4); however, only GLP-1 minimises glucagon secretion (Nauck and Meier, 2016; Fava, Dong and Wu, 2016). The incretins have a direct effect on the satiety centre, which suppresses appetite, delays gastric emptying and causes an increase in beta cell neogenesis as well as inhibit apoptosis. These two hormones are secreted from the intestinal cells in response to food intake. GLP-1 is synthesised from proglucagon in the l-cells of the small intestine and GIP in the k-cells of the proximal intestinal mucosa (Nauck and Meier, 2016; Fava, Dong and Wu, 2016). GLP-1 levels are decreased in T2D patients but still they can react with this hormone (Rhee et al., 2014; Nauck and Meier, 2016).

By administration of exogenous GLP-1, glycaemic control can be achieved, it has an advantage in reducing body weight (Nino et al., 2017; Htike et al., 2017). T2D suffer from extreme production of hepatic glucose, although they have a high level of insulin (Okamoto et al., 2017). In these individuals, insulin resistance is characterised by increased hepatic glucose production and fasting hyperinsulinemia. It is clear that in T2D, the ability of insulin to suppress hepatic glucose production is minimised (Okamoto et al., 2017). Accordingly, inappropriate insulin secretion or the inability of insulin receptors to effectively respond to insulin, leads to impaired glucose homeostasis and T2D (Page and Johnson, 2018). Risk factors for the development of T2D include sedentary lifestyle, family history, obesity, high waist circumference, history of gestational diabetes in women, being older than 40 years and ethnicity, such as being of South Asian, African-Caribbean or black African descent (Stevens et al., 2015).

1.5.6 Causes of type 2 diabetes

The pancreas is a large complex gland that is found behind the stomach, and consists of α , β , δ cells (Mann and Bellin, 2016; Thomas and Philipson, 2015). The role of β cell in the body is to produce insulin, which transfers the glucose that is available in the blood to recipient cells, so that it can be used as energy in the body (Mann and Bellin, 2016; Thomas and Philipson, 2015). While α cells role is to produce glucagon which release glucose in case of hypoglycaemia.

According to Schwartz, et al (2016), all types of diabetes are created from defects in the β cell in the pancreas. This β cell impairment affects insulin production. In this theory, IR only represents a primary deficiency in insulin production as well as approximately third this people are going to have diabetes in the future (Schwartz, et al, 2016).

There are several causes of such failure, which are referred to as risk factors. One of the risk factors for T2D is age. Researchers have shown that people aged 45 or older stand a higher chance of developing diabetes conditions. However, this age drops to 25 in the case of Asians (Jones et al., 2012). Genetic factors also play a critical role in the acquisition of T2D. Studies have proven that the condition is genetically inherited; as such, people who have close relatives with the condition are at greater risk (Jones et al., 2012). Overweight and obesity also magnify the chances of contracting the disease (Martin-Timon et al., 2014; Hsu et al., 2015). It is therefore advisable for Asians to maintain body weight within the recommended range of BMI (18.5 to 24.9 kg/m²) by undertaking regular exercise and observing a healthy diet and lifestyle (Jones et al., 2012). Finally, ethnicity is a risk factor; past studies have shown that individuals whose origin is south Asian, black African, African-Caribbean or Chinese may be more likely to developed T2D (Watt et al., 2018; Diabetes UK, 2018; Ogurtsova et al., 2017).

1.5.7 Key features of T2D

According to (American Diabetes Association, 2016), the key features of T2D are:

- ✓ Insulin resistance (associated with obesity); relative insulin deficiency.
- ✓ Slow development of hyperglycaemia.
- ✓ Risk factors: obesity, age, ethnicity, unhealthy diet, smoking and physical activity.
- ✓ Cardiovascular risk factors: hypertension, dyslipidaemia and glucose intolerance.
- ✓ Gestational DM.
- ✓ Stronger genetic correlation than T1D.

- ✓ Strong association with CVD.
- ✓ Risk of heart failure that is two to three times higher than in people without diabetes.
- ✓ Risk of cerebrovascular disease; patients are three times more likely to suffer a stroke.
- ✓ Risk of diabetic nephropathy, neuropathy, and retinopathy.

1.5.8 Some factors associated with developing T2D

Some factors associated with developing T2D (Kerner and Brückel, 2014; American Diabetes Association, 2018):

1. Genetic syndromes: muscular dystrophy and lipodystrophic disease.
2. Pancreatic diseases: chronic pancreatitis, post pancreatectomy and cystic fibrosis.
3. Endocrinopathies: primary aldosteronism, Cushing syndrome, acromegaly, pheochromocytoma, glucagonoma and polycystic ovaries.
4. Medication: such as thiazide diuretics, beta-blockers, glucocorticoids, phenytoin, nicotinic acid, catecholamines, tacrolimus, oestrogen, progesterone and antidepressants, especially clozapine, olanzapine and risperidone.
5. U.S. regulators have shown that six anti-psychotic medications can increase the risk of impaired glucose tolerance (IGT) and diabetes:
 - a. Zyprexa (olanzapine)
 - b. Risperdal (risperidone)
 - c. Clozaril (clozapine)
 - d. Seroquel (quetiapine)
 5. Geodon (ziprasidone)
 - e. Abilify (aripiprazol)
6. Chemicals: Tetrachlorodibenzoparadioxin.

1.5.10 Gestational diabetes mellitus (GDM)

This type of diabetes is when high blood glucose is discovered at any time during pregnancy and is categorised as GDM (Baz, Riveline and Gautier, 2016; Goyal and Jialal, 2018). Generally, gestational diabetes occurs at 24 - 28 weeks of pregnancy (ADA, 2016). During pregnancy, GDM women have a sevenfold risk of developing T2D for several years compared to with normal glucose tolerance (NGT) women (Baz, Riveline and Gautier, 2016). It has been found that higher than 25% of women with GDM could have T2D in future as they developed insulin resistance before and after pregnancy (Baz, Riveline and Gautier, 2016; Goyal and Jialal, 2018).

1.6 T2D, obesity and inflammatory markers

The progression of T2D usually preceded by large vessel atherosclerosis (Esser et al., 2014). Both disorders are linked to abdominal obesity and the insulin-resistance syndrome (IRS). The IRS involves hyperinsulinemia, glucose intolerance, hypertension and dyslipidaemia (high triglyceride and low high-density lipoprotein [HDL]) which are together known as metabolic syndrome (Feijóo-Bandín et al., 2016; Esser et al., 2014). The pathogenesis of obesity-related IR and T2D involves the activation of the immune system and a chronic inflammation process. Inflammatory markers are considered as risk factors for the progress of T2D and its macrovascular complications (Esser et al., 2014). In case of obesity, liver, muscle, pancreas and adipose tissue are all sites of inflammation. It has been considered that T2D is an inflammatory disease (Esser et al., 2014). Thus, in the management of T2D the anti-inflammatory therapies might play important role in the prevention of the disease (Esser et al., 2014). Chapter 8 shows more information about inflammatory markers.

1.7 Glycosylated proteins (AGE products, and their receptors RAGE)

AGE is defined as Advanced Glycation End-products while RAGE is defined as the Receptor of Advanced Glycation End-products. The hypothesis of AGE formation, suggests that glucose binds to chemically sensitive amino acid protein which accelerate chemical modification of this proteins to form AGE during hyperglycaemia which contributes to the pathogenesis of diabetic complications such as atherosclerosis, nephropathy, neuropathy and retinopathy (Singh et al., 2014; Yamagishi, Fukami and Matsui, 2015). RAGE can be produced in most of the body cells to induce inflammation. The RAGE pathway cooperates with cytokines, lipopolysaccharides, oxidised low-density lipoprotein (LDL), and glucose activated cellular response. RAGE facilitated persistent cell stimulation is crucial in diabetes and its complications, tumour growth, autoimmune and infectious inflammatory diseases (Singh et al., 2014). The creation of advanced glycation end-products (AGEs) is associated with failure in glycaemic control. In renal failure patients, chronic

inflammation will elevate oxidative stress (OS) level which will cause rapid AGE formation and the kidney unable to eliminate it (Singh et al., 2014). Anti-AGE policies may have a key role in the treatment of diabetic patients. Numerous of possible drug candidates such as AGE inhibitors have been stated recently (Saleh, 2015). There is evidence to show in diabetes that glycation of haemoglobin and production of Hb-AGE stimulate cellular OS to release potent oxidants. This causes oxidative damage to endothelial cell and produces inflammatory response. These inflammatory mediators play crucial role in the progression of atherogenesis and the diabetic vascular complications (Saleh, 2015). High production of AGEs has a crucial role and underlies the pathophysiology of chronic diabetic complications (Piperi et al., 2015). AGEs in the mitochondrial cause production of high amount of reactive oxygen species (ROS). The AGEs are responsible for activating the RAGE. The latter has a negative effect of damaging intracellular cells and increasing the level of cytosolic ROS. Also, it activates the nuclear factor kappaB (NF-Kb), increases the level of cytokines and stimulates the endoplasmic reticulum to cause oxidative stress (Piperi et al. 2015; Pinto-Junior et al. 2018). Recent findings have demonstrated a link between regulating AGE/RAGE and microRNAs in complicated micro and macrovascular complications for persons suffering from diabetes. These studies argued the rule of miRNAs as novel therapeutic agents in the complications of diabetes (Piperi et al., 2015). Moreover, AGEs IR by decreasing content of GLUT-4 protein (Pinto-Junior et al., 2018).

1.8 Diagnosis

It is very important to do routine checks for blood sugar, blood pressure, and lipid in those patients who are at high risk of developing diabetes. The earlier patients are diagnosed, the greater the information will help in managing diabetes properly and avoid complications. If impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) is discovered early, this could help in diabetic onset delay or prevention (Christensen B et al., 2014). Impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) are the cases when the blood glucose level is higher than normal but still not reach the level of T2D. Individuals with IFG or IGT (see table 1) are known as pre-diabetic, and they have a high risk to develop T2D. In people with IFG or IGT, it is valuable to have pharmacological and lifestyle interventions to minimize the risk of progression to T2D. Lifestyle interventions is also beneficial in preventing other chronic disease not only T2D (Stevens et al., 2015). During the last decade, the diabetic diagnostic tests have been improved. Table 1 demonstrates the diagnostic criteria according to the American Diabetes Association (ADA) (Vijayakumar et al., 2017):

- Fasting glucose ≥ 7.0 mmol/L (126 mg/dL) is generally diagnostic of diabetes mellitus.
- 2h Post oral glucose tolerance test (OGTT) ≥ 200 mg/dL (11.1 mmol/L).
- HbA1c $\geq 6.5\%$ (48 mmol/mol).

Prediabetes was defined as HbA1c levels between 5.7 and 6.4%, FPG (100 - 125 mg/dL), or 2h post OGTT (140 - 199 mg/dL) according to American Diabetes Association (ADA) criteria (Vijayakumar et al., 2017).

	Normal	Pre diabetes	Diabetes
HbA1c	< 5.7% < 39 mmol/mol	5.7 % -6.4% (39-46 mmol/mol)	$\geq 6.5\%$ ≥ 48 mmol/mol
FBG	3.9- 5.5 mmol/l (70-100mg/dl)	5.6-6.9mmol/l (100- 125mg/dl)	> 7.0 mmol/l (≥ 126 mg/dL)

Table 1: HbA1c and FBG levels.

1.8.1 Impaired fasting glucose

If the fasting glucose level is between 5.6 and 6.9 mmol/L (100– 125 mg/dL), this is considered as prediabetic and needs an OGTT to confirm that (Vijayakumar et al., 2017).

1.8.2 Oral glucose tolerance testing (OGTT)

ADA states that, in those patients who are at risk, glucose tolerance should be checked if the level of glucose cannot be detected by other diagnostic tests. An oral glucose tolerance test is made up of two blood tests. The first blood test is a fasting blood sample, after which 75 g of oral glucose solution is given as a drink, and then a further blood sample is taken after 2 hours to measure glucose level (American Diabetes Association, 2018;Christensen B et al., 2014).

	Normal	Pre diabetes	Diabetes
BG	< 7.8 mmol/L (140 mg/dL)	7.8- 11.0 mmol/L (140-199 mg/dl)	≥ 11.1 mmol/L (200 mg/dL)

Table 2 Glucose level 2h post OGTT for ND, Pre-diabetes and T2D.

This test is very important in high risk patients to discover diabetes at an early stage. In women with impaired fasting glucose, GTT is the test of choice. ADA recommendation is that tests should be repeated on a different day to confirm the result (ADA, 2016).

1.8.3 Glycated haemoglobin levels (HbA1c)

HbA1c measures the quantity of glucose that is being carried by the haemoglobin in the body (Vijayakumar et al., 2017; Goyal and Jialal, 2018). The HbA1c measures the blood glucose levels for the previous two to three months. The latest recommendation ADA, is that the glycated haemoglobin HbA1c in a patient with diabetes is > 6.5 % (48 mmol/mol). A value of less than 6.5% (48 mmol/mol) does not exclude diabetes diagnosed using other glucose tests (see table 1) (Vijayakumar et al., 2017).

1.9 Symptoms of diabetes mellitus

Table 3 summarises the symptoms of diabetes and the cause behind these symptoms according to ADA.

Table 3: Symptoms of DM (American Diabetes Association, 2018)

Symptoms	Due to
Polyuria	Osmotic diuresis associated with clearance of glucose from blood.
Polydipsia	Water loss from polyuria.
Tiredness/lethargy	Reduced ability to utilise glucose
Weight loss	Breakdown of protein & fat as alternative energy source.
Genital itching	Local effect of glucose that excreted in the urine.
Blurred vision.	Change of shape of lens due to hyperglycemia.

1.9 Complications

Acute complications of T1D are hyperglycaemic hyperosmolar nonketotic coma, Ketoacidosis, and hypoglycaemia which is rare in T2D (Tiwari, 2013). Chronic complications are divided into microvascular and macrovascular disorders, which increase morbidity and mortality of the disease (Siersma et al., 2017; Li et al., 2018; Goyal and Jialal, 2018).

In insulin-sensitive tissues such as liver, muscle, and heart, high fatty-acid flux leads to oxidative damage, while in noninsulin-sensitive tissues such as kidney, eye, and nervous system are affected by high level of blood glucose and fatty acid levels which resulted in ROS-induced diabetic complications (Tiwari, 2013; Sharma, 2015; Hecker and Wagner, 2017).

Table 4: Chronic complications

Macrovascular	Microvascular
Atherosclerotic diseases	Nephropathy
Ischemic heart disease	Neuropathy
Stroke	Retinopathy
Peripheral Vascular Disease PVD	

Protein glycation and production of advanced glycation end products (AGEs) play a significant role in the diabetic complications as explained above in section (1.7) as well as some other diseases like osteoporosis, aging and rheumatoid arthritis (Singh et al., 2014). During diabetes and inflammation, RAGE, the development of receptors is enhanced in certain cells. When AGEs and RAGE Interact on macrophages, this causes OS (explained more lately in section 5.3) (Singh et al., 2014). Microvascular and macrovascular are common in both T1D and T2D patients. Some of microvascular

complications are blindness, kidney disease, and gangrenous ischemia changes causing amputations (Gedebjerg et al., 2018; Kuniss et al., 2018). According to the quality report 2016, the prevalence of long-term complications in T2D subjects are: 24.2% neuropathy, 0.7% amputation, 8.2% retinopathy, 0.4% blindness, 12.5% nephropathy and 0.6% dialysis (Kuniss et al., 2018). Diabetes complications presented in one-third of recently diagnosed T2D patients (Gedebjerg et al., 2018).

1.9.1 Microvascular complications

1.9.1.1 Diabetic nephropathy

Diabetic nephropathy is a condition that results to the damage of the kidney due to elevation of BG. As such, the damaged kidney becomes 'leaky' and allows large amounts of protein to be discharged to urine. This condition can advance and become severe and cause kidney failure. Diabetic nephropathy is a major cause of kidney disease in a majority of patients that requires renal replacement, and also linked to cardiovascular mortality (O'Brien, Patrick and Caro, 2003; Valmadrid et al., 2000). The oxidation of protein produces carbonyls of the existence of OS (Tiwari, 2013; Hecker and Wagner, 2017). The higher production of this carbonyls play a crucial role in the pathogenesis of nephropathy among diabetes patients (Tiwari, 2013; Hecker and Wagner, 2017). Diabetic nephropathy consists of two types, microalbuminuria and macroalbuminuria. Uncontrolled diabetes and high blood pressure causes diabetic nephropathy, which needs pharmacological treatment and protein diet restriction (Tiwari, 2013). When the extracellular glucose level is increased to (30 mmol/l), the production of intracellular ROS increases also and recruit podocyte apoptosis and podocyte depletion. It concludes that podocyte apoptosis/depletion is the early leading cause of diabetic nephropathy in murine T2D models (Wada and Makino, 2013). Three major pathways showing abnormality of intracellular metabolism have been identified in the progress of diabetic nephropathy are formation of AGE products, stimulation of polyol and PKC (protein kinase C) pathways, and glomerular hyperfiltration which induce intra-glomerular hypertension (Wada and Makino, 2013). Furthermore, recent studies show that the inflammation pathways play significant

roles in the development of diabetic nephropathy (Yap, Frankel and Tam, 2017). These studies support the development of new therapeutic agents (Yap, Frankel and Tam, 2017; Wada and Makino, 2013). High concentration of protein in urine leads to renal impairment and chronic kidney disease, which could finally cause end stage renal disease. Tight glycaemic and blood pressure control will decreased renal perfusion pressure, which assists in the prevention of renal disease (Pugliese and Porta, 2018). Screening for diabetic nephropathy in T2D should be at diagnosis and then annually (Handelsman et al., 2015).

1.9.1.2 Diabetic retinopathy

Diabetic retinopathy is the term for disorders of the retina which occur as a result of diabetes mellitus. When diabetic retinopathy occurs, the resulting changes are in retinal blood vessels. There are two main types of retinopathy: non-proliferative and proliferative. In non-proliferative retinopathy, which is the more common, capillaries behind the eye swell and form pouches. Non-proliferative retinopathy varies between mild, moderate, and severe, depending on the numbers of blood vessels that have been blocked (ADA, 2016). In some cases, after several years of developing retinopathy, it progresses to a more serious form (proliferative retinopathy). Here, the damage of the blood vessels increases which leads to a complete blockage of the vessel. As a result, new blood vessels start growing in the retina responded to that closure. Diabetic retinopathy is a chronic progressive threatening disease of the retinal microvasculature associated with the persistent high blood glucose levels and other conditions associated with diabetes mellitus e.g. hypertension (Ghanchi et al., 2012). However, retinopathy rates are higher among: people with T1D and in people with longer duration of diabetes (American Diabetes Association, 2018). Uncontrolled blood glucose can also cause cataracts, glaucoma and occlusion of retinal artery and retinal vein (American Diabetes Association, 2018). In T2D who are using an oral hypoglycaemic agent or who control their blood sugar on life style changes alone, about 60 % of them develop retinopathy after 15 years (Singh et al., 2014). AGEs had a critical effect in loss of lens transparency by glycation of eye lens protein which cause cataract. Cataract is a major cause of blindness in developed and developing countries.

In diabetes mellitus patients the progression of cataract is increased. It is recommended to consume low AGEs-content food to delay the development of cataract (Singh et al., 2014). Diabetic retinopathy screening in T2D should be at diagnosis and then annually, if it is negative retinopathy should be checked every 2 years (Handelsman et al., 2015).

1.9.1.3 Diabetic neuropathy

Diabetic peripheral neuropathy is a common diabetes complication which increases the rate of morbidity and mortality (Malone, 2016; Hussain and Adrian, 2017). In hospitalized diabetes patients, 30% have peripheral neuropathy (Malone, 2016). Furthermore, in diabetes patients, 60-70% of them have developed mild to severe peripheral neuropathy (Habibur Rahman et al., 2016). The symptoms of neuropathy are paraesthesia, striking pain, numbness, or weakness in the feet and hands and finally sensory loss (Malone, 2016). Approximately 1 in 10 people with diabetes develop a foot ulcer, which can cause a serious infection (Diabetes UK, 2018). Furthermore, diabetic neuropathy causes ulceration of lower limbs, reduced healing of wounds which result in gangrene, and subsequently the need for amputation (Hussain and Adrian, 2017). Tight glycaemic control, and good foot care may greatly lower the risk of these outcomes (Pemayun et al., 2015; Hussain and Adrian, 2017). There are various causes for lower extremity amputation such as HbA1c \geq 8%, peripheral arterial disease, hypertension, and hypertriglyceridemia. Immediate treatment of comorbidities like hypertriglyceridemia and hypertension are essential to minimize risk of amputation (Pemayun et al., 2015). In diabetes, the mitochondria abnormalities and mitochondria associated OS are susceptible to ROS which cause damage for mitochondrial DNA and nuclear DNA which lead to diabetes neuropathy (Sandireddy et al., 2014). Deregulation of fission and fusion proteins that control the number and the shape of mitochondria cause damage of the cell functions and might result in degeneration (Ramesh et al., 2012). In T2D, OS leads to numerous adverse effects on cells physiology e.g. lipid peroxidation. This lipid peroxidation results in neural disorders (Ramesh et al., 2012). Basically, OS has been shown to have a role in

the pathogenesis of diabetic neuropathy in animal models. Some clinical trials show that the administration of α -lipoic-acid “antioxidant” leads to an improvement in the nerve conduction velocity and minimize neuropathic symptoms (Ramesh et al., 2012). Moreover, AGEs might damage nerve fibres because of their effect on matrix metalloproteinases. The neuropathy screening should be started after 5 years after diagnosis of T1D and then annually, while in T2D it should be at diagnosis and then annually (Handelsman et al., 2015).

1.9.2 Macrovascular complications

Macrovascular complications manifest as cardiovascular disease (CVD) such as peripheral vascular disease (PVD), stroke, and coronary heart disease (CHD) (Chawla et al., 2016). The main causes of CVD are coronary atherosclerosis, and metabolic syndrome in people who have T2D. It has been reported that hyperglycaemia, is associated with a high risk of new cardiovascular events (Chawla et al., 2016). OS due to T2D affects the cell physiology such as lipid peroxidation (Tiwari, 2013; Ramesh et al., 2012). In T2D, atherosclerosis and cardiomyopathy result from IR while FFA causes an increment of mitochondrial ROS; also, ROS inactivates anti-atherosclerosis enzyme but lipids are the main targets (Chawla et al., 2016). The toxic effect of hydroperoxides on the cell is direct or by degradation of cell by the hydroxyl radical which is very toxic (Tiwari, 2013). Hydroperoxides react with copper and iron and produce malondialdehyde (MDA) which destroy cell membranes. The structure of lipid and metabolism shows changes in diabetes patients especially those with vascular complications (Tiwari, 2013). Notably, in atherosclerosis cholesterol specifically, LDL cholesterol is the main contributor to atherosclerosis. Nevertheless, in T2D dyslipidaemia, TRG and VLDL have been found to be elevated with normal or slightly high LDL and low HDL (Rask-Madsen and King, 2013).

In coronary atherosclerosis, plaque builds up inside the arteries, which causes narrowing, decreases the flow of oxygen-rich blood to the heart. The damage of the inner layers of the coronary arteries is due to some factors such as; high blood glucose level in IR or diabetes, high blood cholesterol level, high blood pressure, presence of

albuminuria and smoking (Chawla et al., 2016). Inflammation also worsens metabolic syndrome and increases the risk of CHD and heart attack (American Diabetes Association, 2018; Chawla et al., 2016). A common cause for such complications is ROS which is a result of processes commenced and accelerated through chronic hyperglycemic conditions (Chawla, Chawla and Jaggi, 2016; Sandireddy et al., 2014).

1.10 Management of type 2 diabetes mellitus

The target of treatment in diabetes patients listed in (table 5) which shows the levels of BG, blood pressure (BP) and the lipid profile. Table 6 also states the BG level in pregnant women while fasting and 2h after OGTT.

Table 5: Target of treatments as stated by (Baxter et al., 2015; Inzucchi et al., 2015; American Diabetes Association, 2018) as the following:

	Test	Level
Blood glucose	FPG	< 5.6 mmol/l (70–130 mg/dL)
	Post-prandial (2hrs)	< 7.8 mmol/l (< 180 mg/dL)
	HbA1c	< 6.5% (48 mmol/mol)
Blood pressure		< 130/80
Lipids profile	TC	< 4 mmol/l (<156mg/dl).
	LDL	< 2.59 mmol/l (<100 mg/dL; <70mg/dL an option in those with existing cardiovascular disease.
	HDL	Greater than 40 mg/dL for men, greater than 50 mg/dL for women
	TRG	< 1.7 mmol/l (150 mg/dL).

Table 6: For Pregnant women with diabetes the BG target is (American Diabetes Association, 2018; NICE 2015):

	fasting	1 hour after meals	2 hours after meals
Blood glucose level	< 5.3mmol/l	< 7.8mmol/l	< 6.4mmol/l

Inzucchi et al., (2015) believed that, at early stage of T2D the non-pharmacological treatment preferred as first option for three months and then if still no improvement in blood glucose, the pharmacological treatment should be initiated.

1.10.1 Hypoglycaemic agents

Table 7 shows the hypoglycaemic agent that used in T2D (Garber et al., 2017; Baxter et al., 2015; BNF, 2018).

Table 7: Medication list for T2D

Class of medication	Examples	NICE guideline recommendations	Efficacy	Mechanism of action	Caution & Contraindications	Side effects
1-Biguanides	Metformin	Considered as the 1st line treatment, Metformin “drug of choice in T2D” especially in obese patients BMI>25kg/m ² . The dose should start low, and increased slowly.	1%–2% A1c reduction and had some benefit in TG reduction and weight loss.	Activate AMP kinase and decrease hepatic glucose production.	Caution: In renal impairment the dose should adjusted. It should be stopped if eGFR < 30ml/min/1.73m ² .	Gastrointestinal disorder (diarrhoea, nausea, vomiting, and abdominal cramping), CKD, lactic acidosis.

					<p>Contraindications:</p> <p>Severe renal impairment if SrCr > 150 micmol/L and eGFR < 30ml/min/1.73m2. Ketoacidosis, Heart failure, Lactic acidosis</p>	
<p>2-Suphonylureas (SU)</p>	<p>2ndgeneration Glyburide Gibinclamide Glipizide Gliclazide Glimipride</p>	<p>Consider as 1st line therapy when:</p> <p>A- Metformin is not tolerated or C.I. B- Non-obese patients. C- Rapid response is required.</p>	<p>1%–2% A1c reduction.</p>	<p>Closes K ATP channels on b-cell plasma membranes and stimulate the pancreas to secrete more insulin.</p>	<p>Contraindications:</p> <p>Porphyria, pregnancy, breast feeding, and severe hepatic impairment</p>	<p>Hypoglycaemia, and weight gain.</p>

		Used as combination with Metformin in obese patient with uncontrolled DM.				
3- Meglitinides (glinides)	Repaglinide Nateglinide	Used in combination with Metformin in obese patients when Metformin alone failed. Repaglinide can be used as monotherapy or in combination with Metformin. Nateglinide can only use in combination with Metformin.	0.5%–1.5% A1c reduction. Rapid acting for meal time flexibility	Closes K ATP channels on b-cell plasma membranes and increase insulin secretion.	Contraindications: Ketoacidosis, pregnancy, breast feeding, and severe hepatic impairment	Hypoglycaemia, and weight gain.

4-Thiazolidinediones (TZD)	Pioglitazone Rosiglitazone	2nd/3rd line add to: A- Metformin & SU where insulin C.I or not tolerated. B- SU where metformin is not tolerated. C- Metformin as alternative to SU where risk of hypoglycaemia significant.	0.5%–1.4% A1c reduction. As well as both increase HDL-C, but Pioglitazone has more positive effects on reducing LDLC & TG than Rosiglitazone.	Activate nuclear transcription factor PPAR-γ and increase insulin sensitivity	Contraindications: Pregnancy, breast feeding, and severe hepatic impairment, heart failure, bladder cancer, and patient with high risk of fracture.	Weight gain, edema, heart failure, bone fracture, and altered blood lipid (increase LDL ‘rosiglitazone’).
5- Alfa- Glucosidase inhibitors:	Acarbose Miglitol	Consider as an alternative therapy in people who are unable to use other oral drugs.	0.5%–0.8% reduction in A1c	Inhibit intestinal alfa-glucosidase enzyme and delay intestinal carbohydrate digestion and absorption.	May enhance hypoglycaemic effects of insulin and sulphonylureas.	Gastrointestinal disorder (diarrhea, flatulence).

<p>6-Dipeptidyl Peptidase 4 Inhibitors (DPP4-I)</p>	<p>Sitagliptin Vildagliptin Saxagliptin Linagliptin Alogliptin</p>			<p>Inhibits DPP-4 activity, increasing postprandial active incretin (GLP-1,GIP) concentrations. Increase insulin secretion and decrease glucagon secretion (glucose dependent).</p>	<p>Contraindications: Diabetic Ketoacidosis (DKA), pregnancy, breast feeding and renal impairment</p>	<p>Angioedema/urticarial and other immune-mediated dermatological effects, upper respiratory tract infection (URTI), Hepatotoxicity and gastrointestinal disturbance.</p>
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7-Bile acid sequestrants	Colesevelam			Binds bile acids in intestinal tract, increasing hepatic bile acid production and (decrease hepatic production level and increase incretin level)		Constipation, increase triglycerides, and may decrease absorption of other medications.
8-Dopamine-2 agonists:	Bromocriptine (quick release)			Activates dopaminergic receptors, modulates hypothalamic regulation of metabolism, and increase insulin sensitivity.		Dizziness/syncope, nausea, fatigue, and rhinitis.

<p>9-Sodium–Glucose Cotransporter 2 Inhibitors (SGLT 2 I)</p>	<p>Canagliflozin, Dapagliflozin, Empagliflozin.</p>			<p>Inhibit SGLT2 in the proximal nephron, blocks glucose reabsorption by the kidney, facilitates its excretion in the kidney increasing glucoseuria. It is independent on the action of insulin which minimise hypoglycaemia unlike TZD.</p>		<p>Genitourinary infections, polyuria, volume depletion/hypotension, dizziness, increase LDL, and increase creatinine (transient).</p>
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10-GLP-1 receptor agonists	Exenatide, liraglutide Albiglutide Lixisenatide Dulaglutide.	0.5%–1.1% reduction in A1c		Activates GLP-1 receptors, increase insulin secretion and decrease glucagon secretion (glucose dependent), slow gastric emptying, and increase satiety.	Contraindications: Severe gastrointestinal disease, DKA.	Gastrointestinal disorder (nausea, vomiting, and diarrhea), increase heart rate, C-cell hyperplasia/medullary thyroid tumors in animals, pancreatitis.
11-Amylin mimetics	Pramlintide			Activate amylin receptors, decrease glucagon secretion, slow gastric emptying and increase satiety.		Gastrointestinal disorder (nausea, and vomiting), hypoglycaemia unless insulin dose is simultaneously reduced.

1.10.2 Insulins

Insulin is a peptide hormone that can be purified from bovine or porcine pancreas which is responsible for regulation of CHO and fat metabolism. It was discovered in early 1921 by Banting and Best (Baeshen et al., 2014; Kubota et al., 2017). In 1982, a new field was developed by using genetic engineering in the production of insulin in *E. coli* and yeast which was approved by FDA and the human insulin was the first medication that has been produced by using recombinant DNA technology by Eli Lilly Company. Human insulin is produced by β -cell and consists of 51 amino acids in two chains connected by 2 disulfide bonds (Baeshen et al., 2014).

Insulin has different effects on the body which are listed below (Baxter et al., 2015; Garber et al., 2017; Inzucchi and Majumdar, 2015; Mann and Bellin, 2016):

- ✓ Maintain blood glucose level within 4-6 mmol/l.
- ✓ Convert glucose to glycogen in skeletal muscle (Glycogenesis).
- ✓ Convert excess glucose to fat in adipose tissue (lipogenesis).
- ✓ Stimulate protein synthesis.
- ✓ Reduce new glucose output by inhibiting converting of glycogen to glucose (glycogenolysis) and preventing breakdown of protein to energy (gluconeogenesis).
- ✓ Prevent break down of fat (lipolysis).

The uses of insulin could be in T1D and T2D (Baxter et al., 2015; Garber et al., 2017; Inzucchi and Majumdar, 2015). T2D individuals who are recommended to use insulin e.g. T2D who have inadequate blood glucose control on optimised oral hypoglycemic agents, contraindications to oral hypoglycemic agents or have recurrent symptoms of hyperglycaemia or unexplained weight loss. Moreover, T2D who are pregnant or considering pregnancy or with acute illness or recurrent infection.

1.10.2.1 Types of insulins

Table 8 illustrates different types of insulins (Baxter et al., 2015; Garber et al., 2017; Inzucchi and Majumdar, 2015; BNF, 2018).

Table 8: Insulins List.

Insulin type	Examples	Company	Dosage form	Onset (min)	Peak activity (hr)	Duration (hr)
Rapid acting (Analogue)	Humalog (Insulin lispro)	Lilly	Vial, cartridge and prefilled pen	15 min	1.5 h	2-5 h
	Novorapid (Insulin aspart)	Novonordisk	Vial	10-20 min	1-3 h	3-5 h
Short acting (Soluble)	Actrapid	Novonordisk	Vial, cartridge and prefilled pen	< 30 min	1.5-3.5 h	7-8 h
	Humilin S	Lilly	Vial and cartridge	30-60 min	1-6 h	6-12 h
	Insuman Rapid	Sanofi Aventis	Vial, cartridge and prefilled pen	< 30 min	1-4 h	7-9 h

Intermediate acting	Humulin I	Lilly	Vial, cartridge and prefilled pen	½- 1 h	1-8 h	22 h
	Insulatard	Novonordisk	Vial, cartridge and prefilled pen	<1.5 h	4-12 h	24 h
	Insuman Basal	Sanofi Aventis	Vial, cartridge and prefilled pen	< 1 h	3-4 h	11-20 h
Long acting	Lantus (Glargine)	Sanofi Aventis	Vial, cartridge and prefilled pen	2.5 h	-	24 h
	Levemir (Detemir)	Novonordisk	Cartridge	2.5 h	-	24 h
Mixture	Mixtard 30/70 or 50/50	Novonordisk	Vial and cartridge	< 30 min	2-8 h	24 h
	Novo Mix 30	Novonordisk	Cartridge and prefilled pen	< 10-20 min	1-4 h	24 h
	Humalog mix 25 or 50	Lilly	Vial, cartridge and KwikPen	15 min	2h	22 h
	Insulin comb 25	Sanofi Aventis	Vial, cartridge and prefilled pen	30-60 min	2-4	12-19 h

1.10.2.2 Clinical applications of insulin:

Insulin acts on specific receptors available on the cell membrane, the density of these receptors are different according to type of cell e.g. liver, muscle and adipose tissue have the highest density (Mann and Bellin, 2016). Proinsulin has two chains connected together by C-peptide which is later separated to release insulin and C-peptide. The insulin contains A- and B- chains which are connected together by two sulphide bonds (Mann and Bellin, 2016; McCulloch, 2018). Insulin receptor is a receptor tyrosine kinase (RTK), it is glycoprotein which had 2 extracellular subunits (extracellular) which connected to 2 transmembrane subunits (in cytoplasm) by disulphide bonds (Mann and Bellin, 2016; McCulloch, 2018). Both insulin and C-peptide are co-released from β cell however, C-peptide only useful in clinical situation to quantify the endogenous insulin production in those patients who are using insulin. Activates insulin receptors, which allow insulin to stimulate the glucose to transport across cell membranes which increase glucose disposal through use in body energy or store it as glycogen in the liver and skeletal muscle. Alternatively, it is stored as TG in the adipose tissue. Furthermore, insulin decreases hepatic glucose production by inhibiting glucagon secretion (Mann and Bellin, 2016; McCulloch, 2018). The adverse effect of insulin are hypoglycaemia, and weight gain.

1.10.2.3 Insulin regimens:

Insulin regimens classified as the following: (Diabetes UK, 2016; Scobie and Samaras, 2012).

1.10.2.4 Basal Bolus

A basal-bolus injection regimen is injecting four or more injections over the day. It consists of an injection at each meal and one at night. This regimen is closest to the physiological Insulin profile, i.e. like ND. A basal-bolus regimen can be used in T1D and T2D. Regular self-monitoring blood glucose (SMBG) is required for this regimen. The role of basal insulin is to control BG levels at stable levels during fasting periods. It works over a relatively long period of time and therefore basal insulin will be either

long acting insulin or intermediate insulin. The role of bolus insulin used at meal times is to keep BG levels under control after each meal i.e. to stop the post-prandial rise that would occur despite basal dosing. Short acting insulin or rapid acting insulin used before meal to act quickly. Multi daily injections consist of basal (once daily of intermediate or long acting Insulin) and bolus (3 or more short or rapid acting insulin doses).

1.10.2.5 Insulin dose adjustment:

(Diabetes UK, 2018; Scobie and Samaras, 2012) illustrate that:

Once daily regime: often starts with 10 units at bed time.

Twice daily regime: 2/3 is given the morning and 1/3 in the evening depending on meal sizes.

Basal Bolus regime: ½ is given at bed time with the remaining ½ split between the 3 bolus injections.

1.10.2.6 Injection technique:

- 1- Abdominal area (fast).
- 2- Arms (medium to fast)
- 3- Thigh (low)

1.10.2.7 Insulin pump:

According to Diabetes UK, an insulin pump consists of rapid or short acting insulin which filled into a portable device attached to the body by a catheter located below the skin (Diabetes UK, 2016). It is considered as a superior alternative to insulin injections because there is no need to inject the skin many times. Moreover, it facilitates the blood glucose level control. In the UK, there are approximately one in every 1,000 diabetic patient uses an insulin pump (Diabetes UK, 2018).

1.10.2.8 Artificial pancreas:

Artificial pancreas (AP) idea was first established in the 1970s, it is made up with a big device which had limitations (Saleem et al., 2017). During the last few decades, many researchers have improved the (AP) or automated control system (Saleem et al., 2017). The idea of AP consists of an integrated closed-loop control (CLC), combining continuous glucose monitoring (CGM) and insulin pump (continuous subcutaneous insulin infusion “CSII”), which improve glycaemic control in diabetes (Breton et al., 2012; Saleem et al., 2017). This device intended to optimize blood glucose level within normal range (3.9–10 mmol/L). It is also decrease blood glucose from 7.73 to 6.68 mmol/L without increasing hypoglycemia, and this reduced variability overnight. It has been conclude that, this artificial pancreas shows improvement toward automated CLC (Breton et al., 2012). The missing feedback of glucose sensing has a fundamental drawback for closed loop control (Saleem et al., 2017). Numerous ideas have been developed after receiving feedback in other researches such as proportional integral design (PID) control, fuzzy logic control and adaptive control (Saleem et al., 2017). Moreover, the most widely used control approach is the model predictive control (MPC), because it has ability to handle a wide range of scheme constraints. In essence, to overcome the problems of regulating insulin in AP studies is still challenging (Saleem et al., 2017). Recent study shows that dual-hormone artificial pancreas had the lowest risk of hypoglycemia, however the differences were not significant which need further study to prove that (Haidar et al., 2017).

Chapter 2 (Metabolism)

2.1 introduction

Metabolism is the term used for various biochemical reaction which provides required energy to the body and helps in maintaining biological functions such as, biosynthesis, heat generation and muscle contraction (Evans and Heather, 2016). Energy is derived from carbohydrate, fat and protein, while glucose is the main source of energy in cells due to its solubility and fast movement which make it easy to transport (Evans and Heather, 2016). Anabolism is the process of storing energy from energy-rich substrate, while catabolism is the processes degrading substrates to biologically energy which used in the movement or other anabolic mechanism. Obesity is a result of imbalance of these pathways (Evans and Heather, 2016). Szablewski, (2011) illustrates that glucose metabolism is a very important process in most physiological functions. It is also produced inside the body by process called gluconeogenesis in the liver and kidney from non-carbohydrate source. Glucose is used as a source of energy by catabolising and producing adenosine triphosphate (ATP) and as a source of starting material for biosynthetic reactions. This process is conducted in peripheral tissues including muscle kidney and the brain (Szablewski, 2011; Mann and Bellin, 2016). In the case of low blood glucose level that may cause, seizures, loss of consciousness and finally death. Glucose homeostasis is the process that normally controls blood glucose level around 90 mg/dl (5 mmol/l). Insulin is the dominant glucoregulatory hormone, which regulates the plasma glucose concentration. In T2D, if the glucose concentration is very high after meals, insulin is required to stimulate the utilisation of glucose to prevent hyperglycaemia (Maughan, 2013; Giugliano, Ceriello and Esposito, 2008; Szablewski, 2011; Mann and Bellin, 2016). During hypoglycaemia, glucagon, another pancreatic glucoregulatory hormone, acts on the liver to increase hepatic glucose production within minutes. Carbohydrate consumption provokes increases the concentration of insulin, and decreases the concentration of glucagon (Maughan, 2013; Szablewski, 2011). Any impairment in the regulation of hepatic glycogen metabolism lead to disorder in blood glucose homeostasis and are

significantly accompanied with metabolic disorders e.g. T2D (von Wilamowitz-Moellendorff et al., 2013; Mann and Bellin, 2016).

2.2 Carbohydrate definition

Carbohydrates are molecular compounds consist of three elements: carbon, hydrogen and oxygen (Evans and Heather, 2016). It is the main source of energy in human nutrition (Stylianopoulos, 2013). Carbohydrates which called saccharide are classified into, simple sugars and complex carbohydrates. Simple sugar is the monosaccharide e.g. glucose while complex sugar consists of more than one monosaccharide such as disaccharides (two sugars) e.g. sucrose, polysaccharides (more than two sugars) e.g. starch and cellulose (Dashty, 2013). The enzyme amylase which is produced in the mouth, stomach and small intestine, converts starch and sugar from food into simpler sugars (Dashty, 2013). Sugars commonly found in the diet are disaccharides which include:

1-Sucrose commonly known as table sugar, which is hydrolysed by enzyme invertase (sucrase) into one mole of D-Glucose and one mole of D-Fructose. They bound together by (1- α to 2- β orientaion).

2- Lactose is the milk sugar, which is divided by lactase enzyme into one galactose carbohydrate and one glucose carbohydrate. These are bound together by a (1-4 glycoside bond in β orientation).

3-Maltose which hydrolysed by maltase enzyme into (two molecules of D-Glucose), it is the result of starch breakdown. The link is between the first carbon atom of glucose and the fourth carbon of another glucose molecule (1-4 in α orientation).

2.3 Carbohydrate pathway

To provide the primary energy fuel, carbohydrates are hydrolysed and transferred into the blood as glucose. This process begins with the absorption of carbohydrates from the stomach wall. Disaccharides or polysaccharides are hydrolysed in the small intestine by three pancreatic enzymes: amylase, maltase, invertase and also by lactase, which is secreted by the intestinal wall. In the portal vein, the monosaccharides are absorbed and then transported to the liver. The glucose is then converted to glycogen in the liver (see section 5), and primarily stored in the cells of the liver as well as the skeletal muscle tissue (Maughan, 2008).

2.4 Carbohydrate or Glucose metabolism

Metabolism as explained above consists of catabolism which is the produce of energy and anabolism which is the storage of energy (Evans and Heather, 2016). Metabolism pathway understanding play essential role in the management of several diseases such as diabetes (Evans and Heather, 2016). The energy molecules that used in metabolism are consist of two groups, the first is the phosphate group which is the high energy e.g. adenosine triphosphate (ATP), guanosine triphosphate (GTP) and creatine phosphate. The second group is the hydride ion carrier group e.g. nicotinamide adenine dinucleotide (NADH), Flavin adenine dinucleotide (FADH₂) and nicotinamide adenine dinucleotide phosphate (NADPH) (Evans and Heather, 2016; Salway, 2016). Glucose molecule uptake into the cell membrane by glucose transporters (GLUT), which is immediately phosphorylated to glucose-6-phosphate (G6P) by the enzyme hexokinase (liver and pancreas: glucokinase) (Evans and Heather, 2016; Mann and Bellin, 2016). G6P then either stored as glycogen in the muscle or enters glycolysis process (see figure 4) (Consitt et al., 2016). G6P plays an essential role in the metabolism of carbohydrate, which can be used for glycolysis process and glycogen synthesis (glycogenesis), while in case of glycogen breakdown (glycogenolysis) and non-carbohydrate precursors (gluconeogenesis) the G6P can be produced (Evans and Heather, 2016; Mann and Bellin, 2016). These will discussed more lately in this section.

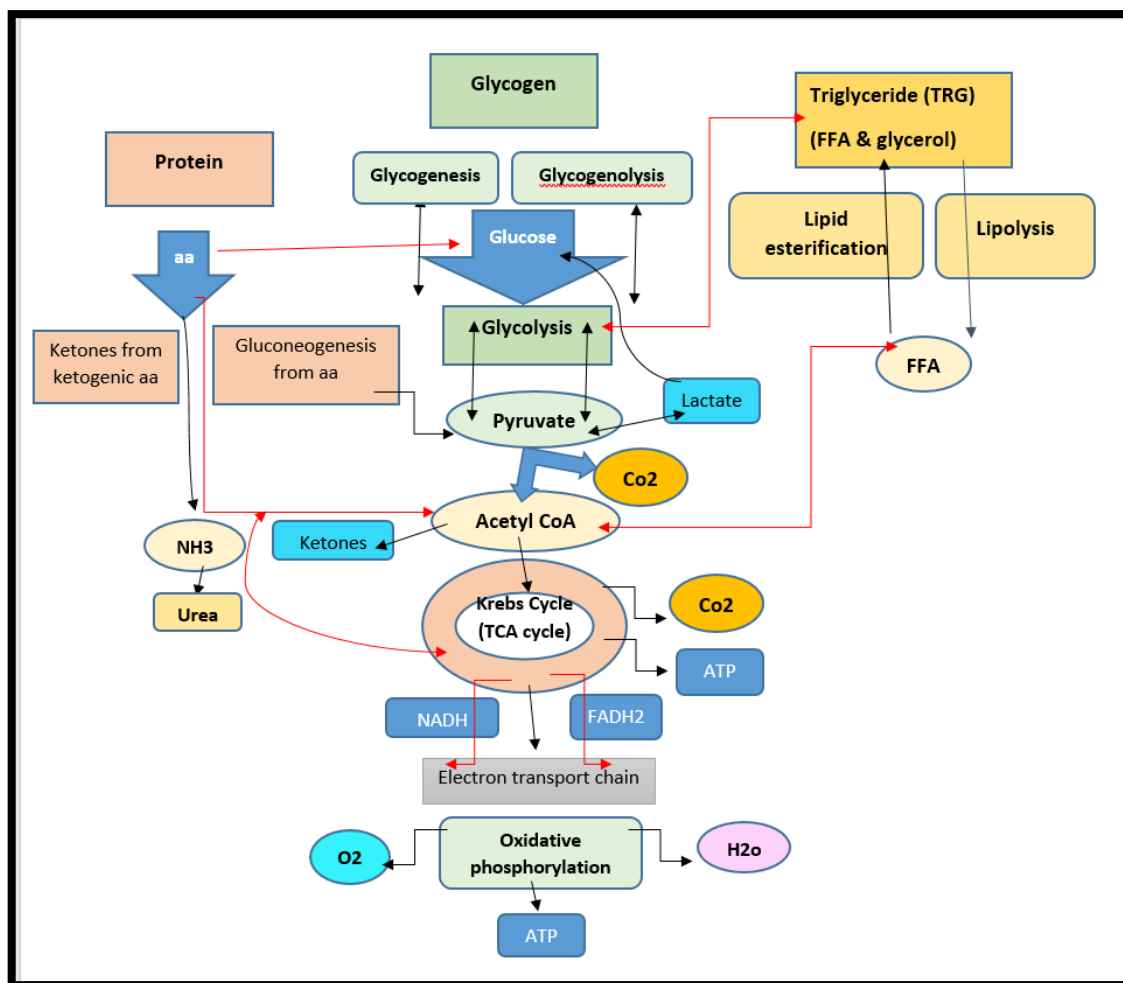


Figure 2: Metabolism of carbohydrate, Protein and fat.

2.5.1 Glycolysis (Anaerobic)

Glycolysis is the process of glucose metabolism to obtain energy through different chemical reaction (Locasale, 2018). This is the oldest biochemical pathway, it has been discovered at 1950s which is a pathway that represents the basic principles of achieving biological from metabolic biochemistry (Locasale, 2018). Figure 2 shows that each molecule of glucose converted to 2 molecule of pyruvate, 2 ATP, and 2 NADH (Evans and Heather, 2016). This part of the process is called anaerobic, and it occurs in the cytoplasm, in the presence or absence of oxygen. After the digestion of carbohydrates, insulin stimulates the entering of glucose from the blood into cells for storage (Dashty, 2013). Glycolysis is controlled by hormonal and metabolic signals which stimulate insulin secretion in the pancreas and inhibit glucagon release (Evans and Heather, 2016). During exercise, glucose is transferred to muscle as source of

energy (Dashty, 2013). In glycolysis, Six-carbon glucose molecules are degraded to three-carbon pyruvate. After that, pyruvate transport to mitochondria and decarboxylated to acetyl-CoA and then further metabolized to lactate (Evans and Heather, 2016). By glycolysis, glucose breakdown in the muscle provides energy, while in liver, extra glucose is broken down to pyruvate, and then to acetyl-CoA, to be used in lipogenesis (lipid synthesis) (Evans and Heather, 2016). Glycolysis is the main pathway providing ATP from glucose or glycogen in tissues to be used for internal body function or in different body movements (Evans and Heather, 2016).

2.5.2 Krebs cycle, Aerobic (Prep Step - CoA) or tricarboxylic acid cycle

This is a sequence of chemical reactions that are a common characteristic of aerobic organisms that results to release of energy from acetyl-coA oxidation obtained from eating carbohydrates, fats and proteins in diet. Dashty, (2013) states that, pyruvate and lactate can be additionally oxidized to Carbon dioxide (CO_2) and water (H_2O) by a process called the Krebs' (tricarboxylic acid) cycle in the mitochondria. Degradation of glucose for releasing its energy for the anabolic pathways starts from glycolysis and continues to the Krebs or tricarboxylic acid (TCA) cycle in the mitochondria. It called aerobic because it needs oxygen to be completed. Krebs cycle occurs in the inner mitochondrial membrane (see figure 3). Cells break down food such as CHO through multiple pathways e.g. in the glycolysis process the breakdown of glucose molecules releases energy, which is captured by energy carrier molecule adenosine triphosphate ATPs and NADH. In this process glucose is converted to 2 pyruvate molecules in the mitochondria where each pyruvate is oxidised to acetyl CoA. Acetyl CoA (2 carbon atom) reacts with oxaloacetic acid (4 carbon) and produce citrate (6 carbon) to then enter citric acid cycle. Acetyl CoA can also be generated by break down of fats and amino acids. In this circular reaction part of the citric acid cycle, carbon atom is oxidised and release waste product carbon dioxide (CO_2). In this way energy released stepwise and capture by energy carriers including 3 NADH, 2 ATP, and 1 FADH_2 . Moreover, this citrate is again oxidised to oxaloacetic acid and combined with acetyl CoA to release citrate and start citric acid cycle and finally generate energy. As it is explained in the beginning each glucose converted to 2 pyruvate molecules in the

preparatory process of Krebs cycle. As explained each pyruvate start the process of Krebs cycle to release energy, from glycolysis 2 ATP, 2 NADH and extra 2 NADH from preparatory stage. Furthermore, in citric acid cycle there is 1 ATP, 3 NADH, and 1 FADH₂ hence if multiple each one by 2 the result is 2 ATPs, 6 NADH, 2 FADH₂. The net result is 4 ATP, 10 NADH, and 2 FADH₂. Every NADH contain 3 ATPs, and every FADH₂ have 2ATPs, so 10 NADH contain 30 ATP, 2 FADH₂ contain 4 ATP and the 4 ATP that already in Krebs cycle. Finally, totally 38 ATPs are released via Krebs cycle to produce energy inside the cells. Acetyl CoA is the general catabolic intermedaiator which enter Krebs cycle and generate ATPs regardless of whether the starting molecule is glucose, fat or amino acid (Dashty, 2013;Evans and Heather, 2016).

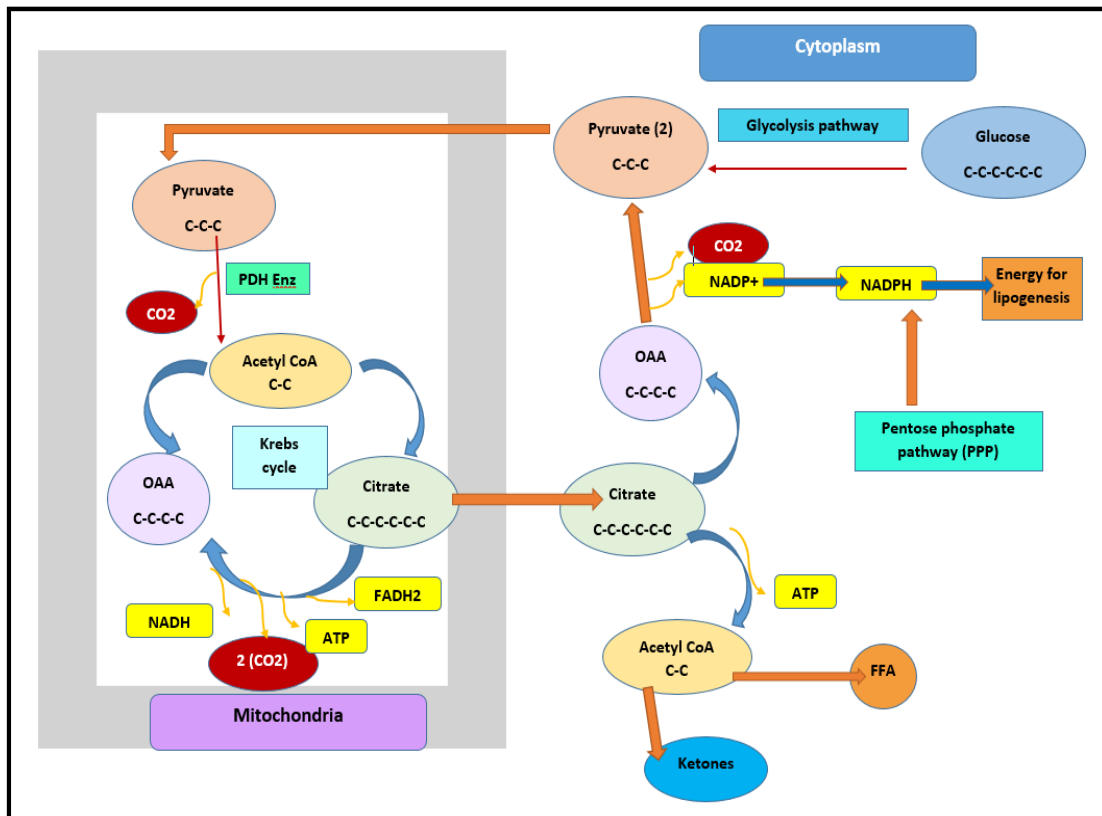


Figure 3: Krebs cycle

2.5.3 Glycogenesis

Glycogenesis is the process of glycogen synthesis from glucose (see Figure 4), which happens mainly in the liver and skeletal muscle and stored there. It is synthesised when the body is in a state of rest, and is activated by insulin when the glucose level becomes high, commonly after the consumption of carbohydrates (Dashty, 2013; Evans and Heather, 2016). This process will explain more in details later in (section 2.12).

2.5.4 Glycogenolysis

Glycogenolysis is the breakdown of glycogen to produce glucose (see Figure 4), it mainly occurs in the liver, skeletal muscle and kidney. This occurs to supply glucose for glucose dependent tissue when the blood glucose level becomes low (Dashty, 2013; Evans and Heather, 2016). Generally, glycogenolysis plays crucial role when the body responds to the production of adrenaline and the regulation of glucose levels in the blood (Goff and Chen, 2016; Ward, 2014). Any impairment in the regulation of hepatic glycogen metabolism lead to disorder blood glucose homeostasis and significantly accompanied with metabolic disorders e.g. T2D (von Wilamowitz-Moellendorff et al., 2013). This part will also discuss more lately in (section 2.12).

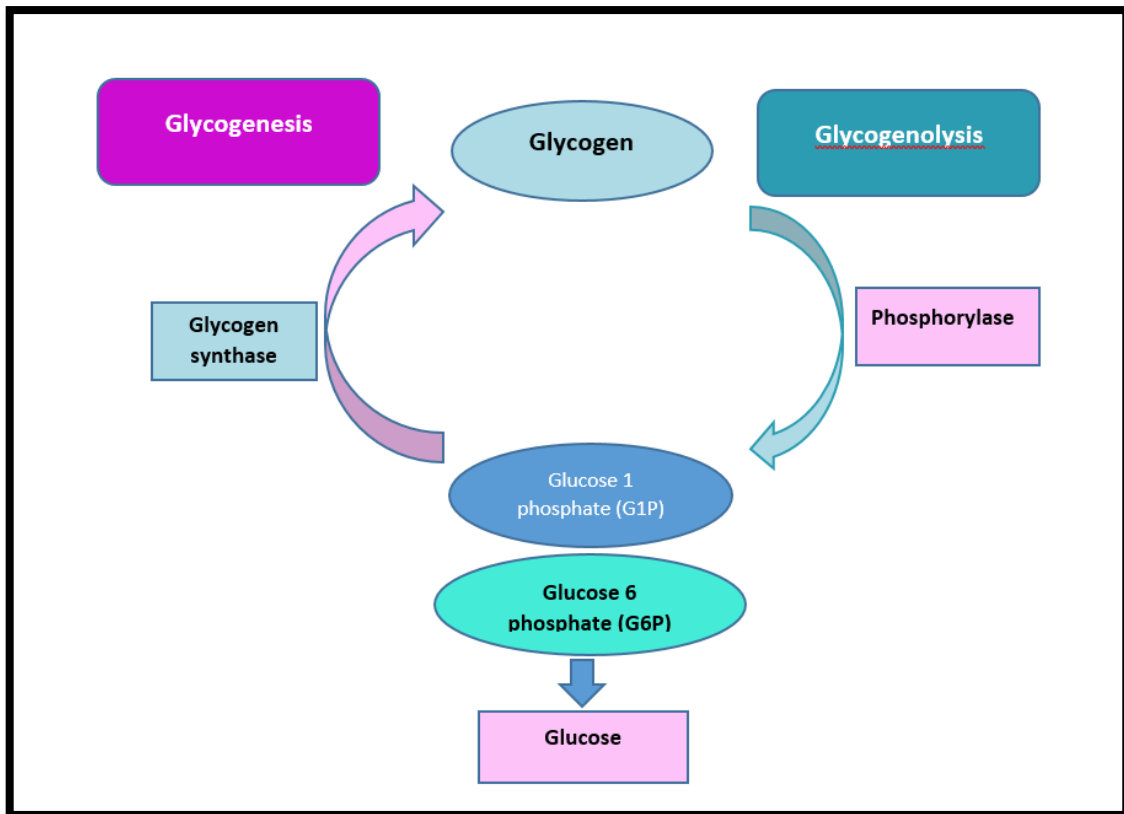


Figure 4: Glycogenesis and glycogenolysis

2.5.5 Glycogen Storage differences between liver and muscles, and Hepatic and Fat and Muscle cell responses to glucose

Glycogen is a branched glucose polymer in the form of glucose, which serves as energy storage during periods of nutritional sufficiency for utilisation as needed (Evans and Heather, 2016). Claude Bernard discovered liver glycogen in 1857 (Peter et al., 2012). Glycogen is the essential storage system because it allows the body to generate glucose quickly during both sedentary and athletic activities and maintains blood glucose (Jensen et al., 2011). Glycogen is the freely stored form of glucose, present mainly in the muscles and the liver (Evans and Heather, 2016). The muscles contain three to four times more glycogen than the liver due to the large muscle mass within the body. About 350 gram of glycogen stored in skeletal muscle that is enough for contraction of the muscle for 70 minutes, while there are about 100 gram of glycogen stored in the liver that is enough for body supply for about 12 hours (Evans and

Heather, 2016). Glycogen metabolism is regulated by hormones such as insulin, glucagon and adrenaline “epinephrine” except in diabetes the insulin is exogenously added (Peter et al., 2012; Evans and Heather, 2016). When glucagon is released if hypoglycaemia is threatened, it stimulates the liver to discharge some of the stored glycogen to maintain blood glucose levels (Peter et al., 2012).

2.5.6 Glycogen metabolism in the liver and muscles

Glycogen metabolism as explained above is regulated by three hormones, insulin, glucagon and epinephrine (Goff and Chen, 2016). In the post prandial the glycogen stored mainly in the liver, while after exercise primarily in the skeletal muscle (Adeva-Andany et al., 2016). This metabolic system consists of glycogen synthase that stores energy and glycogen phosphorylase which produces energy in the form of glucose released directly into plasma (glycolysis). Glycogen synthase is making glycogen to store it which activated by the anabolic hormone (insulin) (Goff and Chen, 2016; Agius, 2015). In this process glucose enter cells through transporters, then it will phosphorylate to G6P, then G6P going through isomeration to G1P and create uridine 5'-diphosphate-glucose which contribute glycogen synthesis (Adeva-Andany et al., 2016). Glycogen phosphorylase catalyses the breakdown of glycogen to glucose to use it for energy, this activated by catabolic hormones glucagon and epinephrine (Goff and Chen, 2016). In this process glycogen is converted to G1P and then to G6P and finally produce glucose (Adeva-Andany et al., 2016). Glycogen phosphorylase activity is regulated by allosteric mechanism and by enzyme phosphorylation and dephosphorylation which is reversible (Adeva-Andany et al., 2016). Liver glycogen phosphorylase isoenzymes have different response to regulatory process than muscle. The liver is responsible for glucose level regulation in the plasma (Adeva-Andany et al., 2016; Peter et al., 2012). The enzyme glycogen phosphorylase in the muscle is highly activated by AMP and by elevated glycogen level in the muscle (Adeva-Andany et al., 2016). The main candidates to regulate muscle glycogen synthesis are GLUT4, hexokinase and glycogen synthetase (Adeva-Andany et al., 2016; Peter et al., 2012). The liver stores glucose as glycogen, it can then break down to glucose but the liver is not using this for energy, it will release it to blood circulation when BG level become

below normal level. In the liver the enzyme glycogen phosphorylase is the isoenzyme of muscle phosphorylase and has different action than the one in the muscle; because in the liver this enzyme is sensitive to glucose molecules. After consuming a meal that leads to high glucose levels, the glucose acts as an allosteric inhibitor and binds to the special regulator known as the “allosteric”. This makes a change in the structure of “phosphorylase a” from an active state to an inactive state which will stop glycogen from breaking down to glucose (Agius, 2015). Hence, the liver will stop producing its own glucose after consuming a meal. However, in case of low BG, the liver cell is converted from an inactive state to an active state leading to the breakdown of glycogen to glucose which is then released into the blood stream (Adeva-Andany et al., 2016). Along the glycolytic pathway, glucose 6-phosphate is metabolized to pyruvate and lactate (Lavin, 2012; Evans and Heather, 2016). It has been found that during exercise, fatigue, impaired muscle performance and muscle glycogen depletion all have strong association. Patients with Glycogen Storage Disease, had a complete elimination of muscle glycogen synthase which causes weakness, pain and cramps in the muscle as well as cramps and poor exercise performance (Xirouchaki et al., 2016). There are several mechanisms for fatigue during exercise yet the main reason for that is still unclear (Xirouchaki et al., 2016). The hypothesis behind fatigue is the glycogen depletion. Different researches propose that during exercise, glycogenolysis is essential for energy supply and muscle contraction generally because muscle glycogen is important for glucose to enter glycolysis pathway (Xirouchaki et al., 2016).

2.5.7 Gluconeogenesis

The good balanced between glucose utilization and endogenous glucose production or dietary glucose delivery is necessary for controlling normal blood glucose level (Goff and Chen, 2016). Glucose is produced from three different procedures: absorbed from the intestine after carbohydrate consumption, glycogenolysis, and gluconeogenesis (Giménez-Cassina et al., 2014). Carbohydrate metabolism continues even in the lack of dietary intake and induces the body to produce new glucose, using non-carbohydrate sources and regulated by glucagon hormone (Giménez-Cassina et al., 2014; Evans and Heather, 2016; Dashty, 2013). In the case of glucose, the precursors are lactate, pyruvate, glycerol, and glucogenic amino acids. This is conducted mainly in the liver, and to a lesser extent in the cortex of the kidneys (Evans and Heather, 2016). Generally, the non-carbohydrate precursor are specific amino acids (such as alanine), glycerol (resulting from fat breakdown) and lactate (derived from muscles) (Schutz, 2011). Examples of catabolism is the breakdown of glucose “glycolysis” and breakdown of glycogen “glycogenolysis”. Anabolism process such as synthesis of glucose “gluconeogenesis” and the synthesis of glycogen such as “glycogenesis”. Gluconeogenesis is activated in the shortage of endogenous and exogenous glucose supply, where the body uses fats and amino acids instead of carbohydrates to produce glucose. This process is vital in case of metabolic stress, such as starvation (Evans and Heather, 2016; Schutz, 2011). Glycerol maintains glucose supply via this process for the body tissue during fasting, by activation of stored body fats (see figure 2). This response will only occur if adequate ATP is available to the cell. The liver has a crucial role in controlling blood glucose homeostasis by balancing glucose uptake and storage through glycogenesis and the production of glucose through glycogenolysis and gluconeogenesis if BG threatens to become low (Szablewski, 2011; von Wilamowitz-Moellendorff et al., 2013). Thus, in the postprandial state, the liver converts glucose to glycogen and TG, while in the post-absorptive state, it produces glucose by glycogenolysis and gluconeogenesis (von Wilamowitz-Moellendorff et al., 2013).

2.6 Fructose metabolism

The main source of energy in cells is glucose, as stated previously but cells can also utilise fructose in cases where a high amount of sucrose (glucose and fructose) have been ingested. The muscle, adipose tissue and kidney contain hexokinase (HK), which is necessary to phosphorylate fructose to produce fructose-6-phosphate (F6P) (Dashty, 2013). F6P is consumed directly through the glycolysis pathway. The liver however, contains only glucokinase (GK), so fructose needs to be converted first to glucose in the liver to complete the glycolysis pathway (Dashty, 2013).

2.7 Pentose phosphate pathway:

Pentose phosphate pathway (PPP) is an alternative pathway to glycolysis and TCA cycle for oxidation of glucose. It should be noted that no ATP are consumed or produced in this pathway (Evans and Heather, 2016; Arese and Gallo, 2014). Primarily, glucose is oxidized by enzyme hexokinase and a molecule of ATP is utilized to produce G6P in the 1st process of glycolysis pathway. PPP is the biochemical reaction that oxidized the G6P (6 carbon) by glucose 6 phosphate dehydrogenase (G6PDH) to 6-phosphogluconate and generate NADPH. The 6-phosphogluconate is converted to ribulose-5-phosphate (5 carbon) and CO₂ and a second molecule of NADH (Evans and Heather, 2016; Arese and Gallo, 2014). This pathway is an important source of NADPH in most tissues (Kuehne et al., 2015). It is also an important source of ribulose-5-phosphate which is used in nucleotides (DNA and RNA) and in aromatic amino acids synthesis. While NADPH provides energy for some anabolic reaction e.g. lipogenesis and also to maintain the storage of antioxidant inside the body (Evans and Heather, 2016; Arese and Gallo, 2014).

2.8 Fats

Dietary lipids are an essential source of energy. TG from ingested dietary lipids is absorbed by gut, emulsified by bile acid, and then hydrolysed in the intestinal lumen with different pancreatic lipases to release free fatty acid (FFA) and glycerol (Hussain, 2014). Lipids are either endogenous lipids, which are synthesised within cells, or exogenous lipids, which are derived from dietary fat. They are categorised by their insolubility in water, and have a different range of biological functions. They are essential in cell membranes as phospholipids, and as a major source of stored energy in adipose tissue as triacylglycerols (TAGs), which is known as a TG (Griffin, 2013; Hussain, 2014). Serum lipids contain TG, phospholipids, cholesterol and their component fatty acids (FA). These serum lipids are transferred from intestine to peripheral liver and tissues, to be utilised and stored in macromolecular complexes of lipid and protein called lipoproteins (Griffin, 2013). TG is not water soluble so it needs to transport into the blood circulation as lipoproteins, which known as (chylomicrons). Lipids then release from the transporter by lipoprotein lipase (LPL) and catalysed by pancreatic lipase to FFA and water soluble glycerol and enter circulation (Griffin, 2013). Obesity, T2D, and CVD diseases associated with defect of enteric lipid homeostasis (Song, Veenstra and Perrimon, 2014).

2.8.1 Lipid digestion and absorption

The average daily intake of fat in a Western diet ranges from between 50 and 100 g, and provides between 35–40% of total energy (Griffin, 2013). This diet contains mostly TG, phospholipids and cholesterol. The fat is emulsified in the stomach by the breakdown by lipases and is solubilised with bile salts in the duodenum. This can then be absorbed through the walls of the upper small intestine (Griffin, 2013).

2.8.2 Lipid metabolism

Griffin (2013) illustrates that, fats play a key role in the process of energy storage. Once fats have been ingested, they are formed as TG. This process starts with the fat catabolism process that comprises of splitting FA from glycerol with the help of enzyme lipase. Insulin controls the action of lipase. In the case of insulin deficiency, there is great accumulation of FA, which are converted in the liver to the ketone bodies which results in ketoacidosis (Griffin, 2013). Any disturbances in lipid metabolism in adipose tissue, skeletal muscle, liver, gut and pancreas play an essential role in the progress of IR, impaired glucose metabolism and T2D (Stinkens et al., 2015). Low fat diet help in preventing and/or reversing these disturbances in FA metabolism (Stinkens et al., 2015).

2.8.3 Lipogenesis

Lipogenesis is the process for the formation TG or FA which starts in the mitochondria (Griffin, 2013; Hussain, 2014). FA synthesis is obtained from three sources, diet, adipose cells and liver. The exogenous source produce TG which enters the circulation as chylomicrons, while in the liver glucose is converted to FFA and then to TG which is released into the circulation as very low-density lipoprotein (VLDL). The last source is the adipose cells which produce TG or FFA in the circulation. In the blood circulation, VLDLs interact with lipoprotein lipase (LPL) in the skeletal muscle, cardiac and adipose tissue, where TG used for storage or release energy (Griffin, 2013; Hussain, 2014). Lipogenesis is an anabolic process which obtains energy from different pathways, first through Krebs cycle and TCA which produces Acetyl CoA and releases ATP and the second pathway is PPP which releases NADPH (see figure 3) (Evans and Heather, 2016).

2.8.4 Lipolysis

Lipolysis is the process when TG, stored in adipose tissue, starts to decompose and release glycerol and free fatty acids, free fatty acids then enter the blood circulation, which is essential when the body needs more energy (Griffin, 2013). TG consists of glycerol connected to 3 fatty acids by 3 ester bonds. TG can be hydrolysed through several steps to diglycerides and then monoglyceride and finally glycerol and FFA. During lipolysis, 3 water molecules are hydrolysed 3 ester bonds within TG molecules in the presence of 3 different enzymes. TG is hydrolysed first by enzyme adipose triglyceride lipase (ATL) to give diglycerides (2 ester bond and 2 FA) and one FFA. Moreover, this diglycerides hydrolysed by hormone sensitive lipase enzyme (HSL) to produce monoglyceride (1 ester bond and 1 FA) and 1 FFA. Finally, monoglyceride is hydrolysed by enzyme monoglyceride lipase to release glycerol and FFA (Griffin, 2013). In the blood stream, the free fatty acids bind to serum albumin to transfer to tissues (Griffin, 2013). Free fatty acids oxidized to generate ATP which take place in the mitochondria. FFA then degrades to acetyl-CoA that totally oxidized via Krebs cycle (Griffin, 2013). Glycerol also enters the blood stream and is absorbed in the liver and kidney to participate in gluconeogenesis (see figure 3) and glycolysis pathway. Different hormones induce lipolysis such as glucagon, adrenaline, nor-adrenaline and growth hormone (Mann and Bellin, 2016).

2.8.5 Lipoproteins

Lipids are insoluble in water and plasma; therefore, they need to combine with a protein to form a lipoprotein. Lipoprotein facilitates the transportation of lipids in the body (Griffin, 2013). It has spherical surface which is bounded by hydrophilic cover of phospholipid monolayer and has free cholesterol and is covered by a large protein, apolipoprotein which helps to stabilized all the structure (Hussain, 2014). The role of lipoproteins is to carry endogenous lipids that are synthesized in the liver; and exogenous lipids, which re-synthesized in the gut from dietary fats to peripheral tissue (Griffin, 2013).

The first lipoprotein is chylomicrons which result from TRG absorption in the intestine. It has a large spherical structure lipoprotein and low molecular weight and it carries exogenous TG to tissues. The second is very-low-density lipoprotein (VLDL), which is produced in the liver and released into the bloodstream to transport and supply body tissues with endogenous TG. The third is intermediate-density lipoprotein (IDL) which result from catabolism of VLDL. The fourth is Low-density lipoprotein (LDL) the final product of catabolism of VLDL and transfers cholesterol to the peripheral tissues. (Griffin, 2013). The accumulation of the cholesterol in the artery walls occurs if the concentration of LDL is high, which can lead to atherosclerosis. The fifth lipoprotein is high-density lipoprotein (HDL) which produced from liver and intestine acts to transfer cholesterol from the cells to the liver for elimination. It is very important to protect artery walls from atherosclerosis that can cause cardiovascular problems (Griffin, 2013).

2.8.6 Insulin resistance and intestinal lipoprotein production

The role of insulin in adipose tissue skeletal muscle cells is to induce the migration of the GLUT4 transporter to the cell membrane to enable uptake of glucose. Insulin stimulates lipoprotein lipase (LPL) to remove FFA from chylomicron and VLDL to be uptake by adipocytes (Mann and Bellin, 2016). In case of lipogenesis, intracellular glucose is converted to glycerol-3-phosphate that combine and esterified with FFA to produce TG in adipocytes (Morgantini et al., 2014). In the liver, excess glucose from a diet also enters Krebs cycle in the mitochondria and produces pyruvate, then Acetyl CoA, then citrate which transports to cytoplasm and is converted again to Acetyl CoA and this process finally produces cholesterol by enzyme HMG CoA reductase (see figure 3) (Mann and Bellin, 2016). Acetyl CoA can also converted to FA which combined to glycerol (obtained from glucose before enter Krebs cycle) to produce TG (Morgantini et al., 2014). This lipid (cholesterol and TG) combine in the liver to apoproteins phospholipids to form two kinds of lipoprotein (HDL and VLDL). VLDL is responsible for transporting FA and TG to body tissues for energy or for storage in

adipose tissue (Arca, 2015). VLDL also converted to LDL which is responsible for transportation of cholesterol to body tissue or LDL which is moved to the liver again to recycle and form more lipoprotein or excreted in the bile duct (Arca, 2015). On the other hand, lipolysis occurs in adipocytes in case of fasting or exercise to release energy (Norikazu Maeda, 2017). Any defect of lipid metabolism leads to a higher level of fat in the body “dyslipidaemia”, obesity, insulin resistance, T2D and CVD (Hussain, 2014). In the IR individual there are increase of TG-rich lipoproteins level and decrease in HDL cholesterol level (Arca, 2015; Hussain, 2014). The release of chylomicron is elevated in obesity, insulin resistance and T2D (Hussain, 2014). Different studies show the link between IR and dyslipidaemia (Hussain, 2014). Overproduction of VLDL is mainly evident in T2D (Arca, 2015). This proves that diabetes is also associated with high fat level not only high glucose level this will discuss more lately in (chapter 5).

2.9 protein

2.9.1 Structure and function of proteins

CHO and fat are the main source of energy. Protein also provides some energy at 4 kcal/g; though proteins predominant action is to act as a source of amino acids (AAs) and to provide essential substrate to the body (Hayamizu, 2017). In case of starvation when the intake of lipid and CHO is inadequate, AAs can be used as source of energy (Hayamizu, 2017; Bender, 2012). Essentially, there is no storage form of AAs like CHO “glycogen” or fat “TG”. Thus, muscle protein will breakdown if necessary, to provide energy (Hayamizu, 2017). Protein is the second largest component of tissue, after water. It makes up about 17% of the weight of an average adult (Emery, 2015). After ingested a protein rich meal, gastric juices containing hydrochloric acid released in the stomach to facilitate proteins breakdown. Stomach secretes pepsin to break the peptide bonds between amino acids and produce smaller protein units (Emery, 2015). This will transfer to small intestine where the pancreatic enzymes chymotrypsin and trypsin assist the breakdown of this protein as well as extra enzyme released by small intestine help in this process. Small intestine muscle contractions (which need ATP) help in mix the protein and deliver it to the site of absorption (Emery, 2015). It will then transfer released amino acids (AAs) to blood circulation then to liver. In the liver, some AAs used to resynthesize new protein while minimum number of AAs can breakdown to nitrogen (contain ammonia). Finally, the nitrogen is the end-product of protein metabolism, which excreted in the urine as urea (see figure 2) (Emery, 2015). In the liver, most AAs are deaminated and the carbon skeleton are used in two process ketogenesis or gluconeogenesis. Alternatively, they are used as an energy fuel for liver (Bender, 2012). Some AAs convert in the liver to glucose or FAs. This glucose is either stored as glycogen or used as source of energy and FAs are also either stored as TG in the adipose tissue or used for energy (Hayamizu, 2017; Bender, 2012). AAs can be used to produce purine and pyrimidine bases which are used for nucleotide synthesis (RNA and DNA) (Newsholme et al., 2011). That glucose from AAs can be then converted to pyruvate and OAA by catabolism via Krebs cycle - these AAs that form pyruvate are called “glucogenic AAs”. Pyruvate and OAA are substrates for

gluconeogenesis and can produce glucose again in the liver and kidney (Hayamizu, 2017; Bender, 2012). FA converted to Acetyl CoA or acetoacetate “this called ketogenic AAs”. Lysine and leucine are examples of AAs that produce ketogenic intermediate Acetyl CoA or acetoacetate. Alanine and arginine are producing glucogenic intermediate. Moreover, isoleucine, phenylalanine and tyrosine produce both glucogenic and ketogenic precursors (Bender, 2012). Acetyl CoA or acetoacetate (2C) is not used as a precursor for gluconeogenesis due to the lost 2 carbon inform of Co₂ when forming citrate (6C) in Krebs cycle. Therefore, they are not able to increase OAA which can be used in gluconeogenesis. Acetyl CoA and acetoacetate are good precursors for ketone synthesis or energy production in a fasting state, while in a feeding state can produce FA (Bender, 2012). Insulin plays major role in regulating metabolism of carbohydrate, lipids, and protein in different tissues (Burgos et al., 2016). Therefore, any resistance to insulin lead to T2D. In insulin resistance individuals, there is a defect of protein metabolism which prove that there are link between T2D and whole body protein metabolism impairment (Burgos et al., 2016).

2.10 Lactate

Lactate and lactic acid are appear in the blood in case of anaerobic metabolism in absence of oxygen in the tissue to support normal metabolic demands (Consitt et al., 2016). In the skeletal muscles lactate produces by anaerobic glycolysis in the absence of oxygen. Lactate then transports to the liver and converts to glucose. Glucose then returns to the skeletal muscles and is metabolized back to lactate CO₂ and H₂O (Consitt et al., 2016). The normal plasma lactate concentration is 0.3–1.3 mmol/litre (Phypers and Pierce, 2006). For many years it has been believed that lactate was only a waste product as a result of anaerobic exercise (Gladden, 2004). Lactate research started from the 19th century, when Pasteur proposed that lactate was produced by lack of oxygen during muscle contraction. In 1920, Otto Meyerhof suggested that glycogen was a precursor of lactate. In 1923, Hill and Lupton proposed that when muscle contracted the lactic acid immediately released as energy donor (Gladden, 2004). Lactic acid was consider as the main cause of fatigue (Hermansen, 1981). Moreover, in the 20th century George Brooks started to discover the actual lactate's role in exercise and metabolism. In high intensity exercise, the body unable to use oxygen immediately to create energy and this called anaerobic mode when body energy begun to break down to pyruvate and then to lactate (Gladden, 2004). The lactate production conducted in the cytoplasm via glycolysis process after production the intermediate metabolite pyruvate from glucose. Pyruvate is the end product of glycolysis which enters mitochondria through mitochondrial pyruvate carriers (MPC) (Consitt et al., 2016). In the aerobic state, pyruvate is then converted to acetyl CoA by pyruvate dehydrogenase (PDH) which enter the Krebs cycle. Lactic acid level is usually low under these conditions. While in anaerobic conditions, pyruvate is converted by lactate dehydrogenase (LDH) to lactic acid the only reaction that can regenerate NAD⁺ allowing further glycolysis (Consitt et al., 2016). High plasma lactate level associated with insulin resistance, obesity and T2D (Consitt et al., 2016). Lactate has the essential role in preventing glycolysis and insulin signalling which also inhibits insulin-stimulated glucose uptake (Consitt et al., 2016). Research propose that the accumulation of lactate is due to over production of pyruvate higher than oxidation of pyruvate which

involving PDH role (Consitt et al., 2016). Moreover, this accumulation increased in hyperinsulinemia but the mechanism is not clear. PHD has been found higher in lean healthy subjects while in insulin resistance subjects and elderly found impaired (Consitt et al., 2016). Exercise indicated to improve PHD activity especially in younger individuals (Consitt et al., 2016). This study suggested that three months of endurance exercise improved PHD phosphorylation, which minimize lactate accumulation in older participants (Consitt et al., 2016).

2.11 Physiology of insulin production

Insulin has a crucial role in controlling metabolism of carbohydrate, lipids, and protein in the body tissues (Burgos et al., 2016). Generally, β - cells stimulate insulin secretion when the glucose is released in circulation (Figure 5). Thus, to remove this glucose from circulation and use it either in the body energy as ATP or store it as glycogen in the muscle or as TG in the adipose tissues (Rutter et al., 2015).

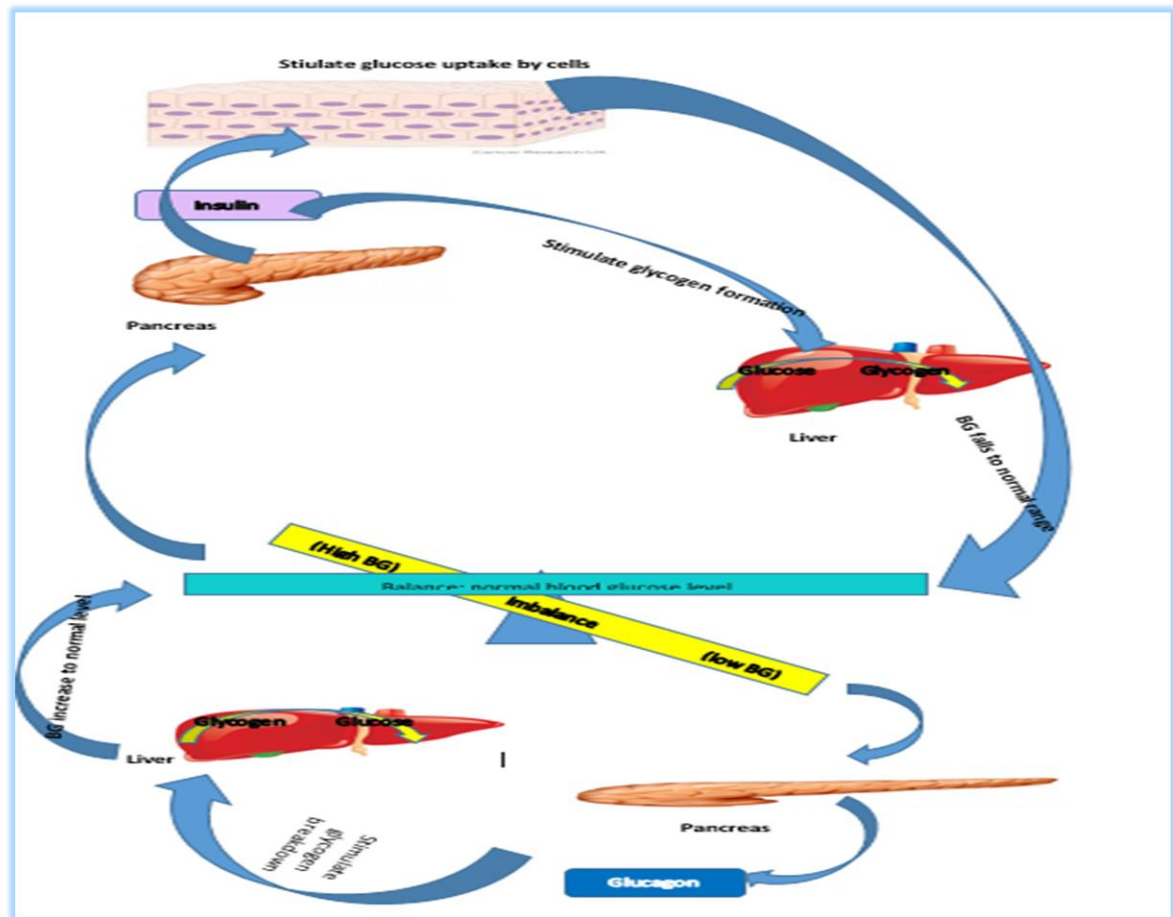


Figure 5: the organs that control plasma glucose level.

2.11.1 Insulin secretion in the nondiabetic individual

In non-diabetes, the liver, while fasting, releases glucose at a rate of approximately (2.0 mg/kg/min) and it is responsible for the balance of glucose homeostasis by both glycogenolysis and gluconeogenesis (Wilding, 2014; Meah and Juneja, 2015). The kidneys reabsorb most of this glucose to release it into circulation by gluconeogenesis

(15–55 g per day), (approximately 180 mg/dL), so that less than 0.5 g is excreted per day. This retained amount (known as basal glucose) is metabolised by a balanced secretion of basal insulin to maintain euglycaemia. During the 4.5-hour postprandial period, an elevation in renal glucose release (>2-fold), which cause a further bolus secretion of insulin (known as prandial) to control the glucose metabolism (Wilding, 2014; Meah and Juneja, 2015). Additional hepatic actions of insulin on adipose tissue and muscle to prevent the gluconeogenic substrates production (such as lactate, alanine, and glycerol) as well gluconeogenic energy substrates (e.g., FFAs), which lead to successive inhibition of hepatic glucose production HGP (Wilding, 2014)

2.11.2 Insulin secretion in individuals with type 2 diabetes

In T2D patients, both renal and hepatic glucose production are increased due to elevation in gluconeogenesis (Wilding, 2014; Meah and Juneja, 2015). The production of hepatic glucose from glycogenolysis and gluconeogenesis is about 5 times higher (2.5 mg/kg/min), due to multiple defects. In addition, the renal threshold is inappropriately raised up to 240 mg/dL as a result of upregulation of SGLT-2 transporters, which prompts the kidneys to reabsorb extreme amounts of glucose and leads to the existence of hyperglycaemia (Meah and Juneja, 2015). During the 4.5-hour postprandial period, glucose production has been increased in T2D approximately by 30% (100 g vs 70 g) higher than healthy individuals (Wilding, 2014; Meah and Juneja, 2015). In case of diabetes, gluconeogenesis has important role. Therefore, it has been found elevated in diabetes subjects (Wilding, 2014; Mann and Bellin, 2016). Insulin and catecholamines (eg, adrenaline) play important role in regulating renal gluconeogenesis which take place within proximal tubule cells in the renal cortex (Wilding, 2014; Mann and Bellin, 2016). Insulin reduces renal gluconeogenesis directly, and minimizes the production of gluconeogenic substrates, such as lactate and glycerol which decreases glucose production into the circulation. On the other hand, adrenaline enhances renal gluconeogenesis, increases renal glucose production, increases the supply of gluconeogenic substrates, inhibits insulin secretion, and decreases renal glucose uptake (Wilding, 2014; Mann and Bellin, 2016).

2.12 Metabolism in Non-diabetes and Diabetic Individuals

As indicated in (section 2.5.3), the principal metabolic effects of insulin are glycogen synthesis stimulation in skeletal muscle, and liver. Moreover, it increases glycolysis and minimizes gluconeogenesis in the liver (Szablewski, 2011; Griffin, 2013; Mann and Bellin, 2016). Insulin also stimulates the active transport of plasma amino acids into muscle as well as stimulates muscle protein synthesis directly (anabolic action) while depressing protein breakdown. Insulin stimulates the fatty acid synthesis (lipogenesis) and storage in adipose and hepatic tissues as TG. In adipose cells, insulin inhibits hormone-sensitive lipase activity, preventing the breakdown of the lipid (lipolysis). By preventing lipolysis, the free fatty acid decreased in the circulation (Szablewski, 2011; Griffin, 2013; Mann and Bellin, 2016). Generally, glycogen converts to glucose to give energy which is required during physical activity. On the other hand, in a sedentary life style, after consuming CHO if the stored glycogen in the body is already high so then another process called de novo lipogenesis occurs which produces fat from carbohydrates (Jensen et al., 2011; Ameer et al., 2014). This process occurs in the cytoplasm by converting glucose through glycolysis to pyruvate as explained above in (section 2.5.2) which is then in mitochondria converts to Acetyl CoA and finally to FFA in the circulation or stores in the form of TG (3 FFA glycerol molecules) in adipocytes and leads to obesity (see figure 2) (Evans and Heather, 2016; Mann and Bellin, 2016). However, in case of doing activity this stored glycogen become less in the muscle so this TG stores as glycogen and then could be convert to glucose which called gluconeogenesis (Evans and Heather, 2016; Mann and Bellin, 2016). TG hydrolysis to (glycerol and FFA), glycerol converts to pyruvate and then glucose and finally glycogen while FFA converts to Acetyl CoA. While in case of protein metabolism as explained above (section 2.9), most AAs are deaminated and the carbon skeleton are used in ketogenesis or gluconeogenesis process or can be used as source of energy for liver functions (Bender, 2012). In the liver, via Krebs cycle 50% of AAs converts to pyruvate and then glucose while the other 50% converts to Acetyl CoA and then FFA or in the TCA cycle (Bender, 2012). The role of insulin in fat and muscle cells is to ensure that the glucose transporter (GLUT4), is stimulated and delivered to the surface of the cell

from a location that is intracellular. In this case, insulin ensure that carbohydrate is stored in the body as glycogen and after consumption of a diet rich in carbohydrate, the blood glucose is rapidly removed from the circulation (Jensen et al., 2011; Govers, 2014). The processes are less effective in diabetic individuals than in non-diabetic people. However, in insulin resistant and T2D subjects, stimulating glucose disposal by insulin is reduced (Jensen et al., 2011). In T2D, if glycogen stores are full then the body started to synthesis De novo lipid from the glucose (Jensen et al., 2011; Ameer et al., 2014).

Chapter 3 (Methodology)

3.1 Training

First aid, defibrillation and phlebotomy training (Collecting venous blood samples should be taken by using aseptic technique via the vacutainer system).

3.2 Exercise study design

The diabetes and exercise physiology lab (DEPL) had been established by the research team which had the exercise equipment with good ventilation and room temperature control. The lab and equipment checked regularly for safety.

3.3 Recruitment of volunteers

Ethical approval had been obtained by DMU Ethics committee. The recruitment was by different way, posters distributed in the diabetes awareness day, in some schools, on twitter, diabetic UK, and DMU official website. Confidentiality is very important so all data saved securely by code and number, all volunteer sheets stored securely.

3.4 Volunteer information sheet

This sheet explains the whole trial to the volunteers including aim and objectives. This will also provide the information about what they are going to do from week one to week six, what kind of test, and the offer that they will get by completing six weeks.

3.5 Volunteer health screen

This form contains patient name, email address, contact number, and home address. This is also made to take the patient past medical history, medication history, physical activity, and family history. Moreover, if they have any medical problem or acute illness to decide that they can participate or not depending on the study exclusion and inclusion criteria. This form should be kept in secure place only the research team can access to it.

3.6 Volunteer consent form

This form to confirm that the volunteers had read the volunteer information sheet and agree to participate in the trial as well as they can withdraw any time if they cannot continue in the study for some reasons.

3.7 Confidentiality

To make sure that the volunteer information is confidential, each volunteer information recorded with their initial and type of diabetes (code). Only research team know what that code means.

3.8 Exercise equipment

3.8.1 Multi-use gym machine

This machine (Body Craft Jones Maxrack 3D Machine) is used to perform resistance exercise which consists of squat, chest, back, biceps, and triceps. Each step should be done for ten times and then repeat all steps for three sets.

3.8.2 Ergometer bike

The ergometer bike (Lode, Corival Recumbent, US) is used before starting any type of exercise to warm up and for aerobic exercise as well (see Figure 6).



Figure 6: Ergometer bike

3.8.3 AD Instruments machine (ADI) Power lab system

ADI Analysis System (PL3516/P ADInstruments, 16 channels, Australia) (see Figure 7). This instrument comprises a face mask, gas mixing chamber, gas analyser (oxygen and carbon dioxide), long tube to connect the face mask with gas mixing chamber. It is important to calibrate ADI each time before the volunteer starts cycling to give accurate reading. Calibration can be conducted by using bag contains (16% oxygen and 4% carbon dioxide). All equipment e.g. tube, face mask, nose clip, head cover should be cleaned after each volunteer to avoid any infection.



Figure 7: AD Instruments machine (ADI) Power lab system

3.8.4 HbA1c analyser machine (Quo-Test A1c reagent kit)

Que-Test A1c reagent kit is used to measure Glycated haemoglobin (HbA1c). By using finger pricking procedure (FPP) and squeeze a large drop of blood on the cartridge then place it into the analyser. In approximately 5 minutes the result will appear on the screen as well as a print of the result (see Figure 8).



Figure 8: HbA1c analyser machine (Quo-Test A1c reagent kit)

3.8.5 Lipid profile measurement machine Cholestech LDX analyser

Cholestech LDX analyser (Cholestech Corporation 3347 Investment Boulevard, Hayward, CA 94545, USA) is used to measure lipid profile (TC, HDL, LDL, and TG). It consists of the analyser, test cassette, optical check, tube to collect blood, and 35 μ l fixed volume pipette. By using Finger Print Procedure (FPP), with draw a few amount of blood by pipette and place it on the test strip (cartridge) and then insert the strip in the machine by using buttons "RUN", "DATA" and "STOP". In approximately five minutes the result will printout (see Figure 9).

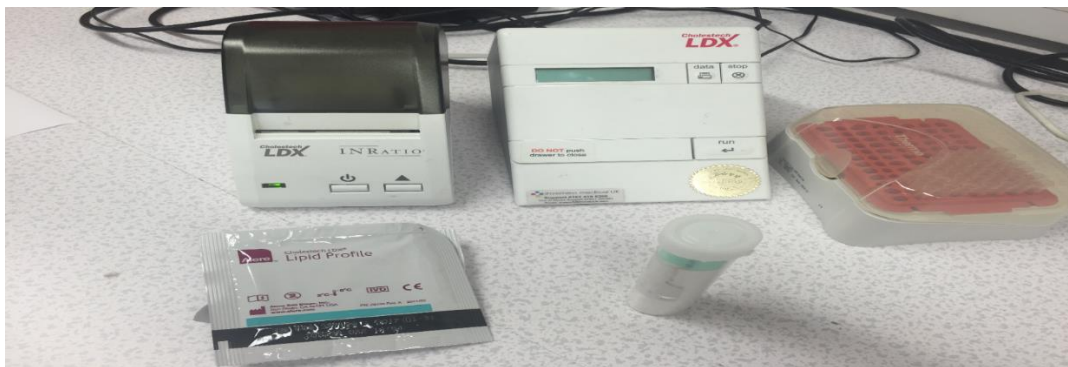


Figure 9: Lipid profile measurement machine Cholestech LDX analyser

3.8.6 Blood glucose monitor by Finger pricking procedure (FPP)

This process used to measure blood glucose (BG) during the exercise sessions and the OGTT. First, sterile the finger with the alcohol swab, leave it until dry. Use the lancet device to prick the finger, squeeze a big drop enough to measure BG. Insert a strip in the blood glucose kit (Contour, BAYER, Ontario, Canada,) place the drop of blood on the strip, then the result will display on the screen immediately (see Figure 10).



Figure 10: Blood glucose monitor by finger pricking procedure (FPP)

3.8.7 Lactate monitoring by (FPP):

Lactate was measured in two time points, the first one before exercise once the volunteer arrive and the second one immediately after the cycling has completed. This is conducted by using FPP as explained above in section (8.8.6). Insert the lactate Pro 2 strips in the blood lactate test meter (Lactate Pro 2, Konan-cho, Amstelveen, the Netherlands) (see Figure 11).



Figure 11: Lactate monitor

3.8.8 Heart rate (HR) monitor:

The patient wears the HR monitor (Polar) on the chest which acts as a sensor. The patient then checks the watch (Polar) at the following intervals: 1) at resting, 2) after each set of resistance exercise (RE), 3) during 20 minutes of aerobic exercise (AE) (see Figure 12).



Figure 12: Heart rate (HR) monitor

3.8.9 Rate of perceived exertion (RPE)

By using Borg scale (1976) during RE and AE. This scale started from 6 till 20, that to show that the perceived level of exercise to be between very very light to maximum exertion (see Figure 13).

Rating of Perceived Exertion Borg RPE Scale		
6	Very, very light	How you feel when lying in bed or sitting in a chair relaxed. Little or no effort.
7		
8		
9		
10		
11	Fairly light	Target range: How you should feel with exercise or activity.
12	Somewhat hard	
13		
14		
15	Hard	
16		
17	Very hard	How you felt with the hardest work you have ever done.
18	Very, very hard	
19		
20	Maximum exertion	

Figure 13: Rate of perceived exertion (RPE) Borg scale of perceived exertion 1976

3.8.10 Blood pressure monitor (BP)

Arm blood pressure monitor (Omron M10-IT, Omron Healthcare Co., Ltd, Japan) is used to measure blood pressure of the volunteers during the exercise session. The volunteer should sit on the chair and relax for 10 minutes at least to make sure the reading is correct. The BP set is cuffed around the arm at the same level of the heart. The monitor then applies pressure to the arm in order to measure the BP (see Figure 14).



Figure 14: Blood pressure monitor (BP)

3.8.11 Collection and storage of venous blood samples

Venous blood samples should be taken by using aseptic technique via the vacutainer system (see Figure 15). Venous blood samples collected from each volunteer (in combination exercise) total 21 samples. In the first session for OGTT, a total of four samples (first one before drinking the oral glucose while fasting, the second sample was taken after 30 minutes, the 3rd one after one hour, then finally the 4th one after 2 hours of drinking the glucose. In the second visit which is the first exercise session a total of three blood samples were taken, the first one before starting exercise, the 2nd after resistance exercise, and the 3rd one after aerobic exercise. The following day of the 1st exercise session, another OGTT is conducted and another four samples are taken as the 1st OGTT. In the 2nd exercise session only one sample at the beginning then after that blood samples have been taken on 4th, 6th, 8th, 10th, 12th, and 4 samples are taken in the same manner as the previous two.

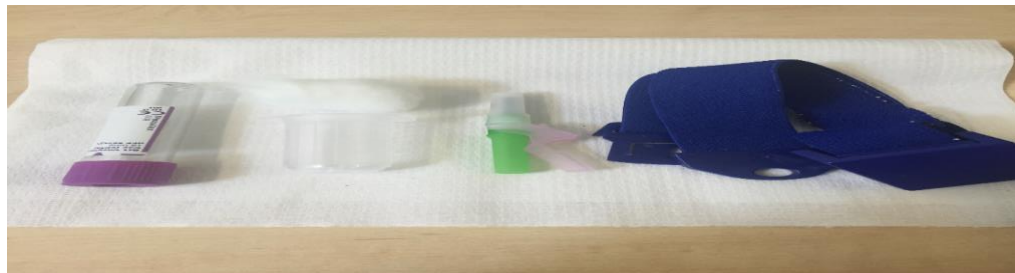


Figure 15: Collection and storage of venous blood samples

3.9 ELISA machine

This machine is equipped to scan and quantify colour reactions produced when chromophores bond to antibodies with which the antigen of interest interacts. ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and measuring peptides, proteins, hormones and antibodies. It is used to analyse insulin sensitivity and incretin level (see Figure 17). The general principle of ELISA involves immobilizing an antigen to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a quantifiable product (Merckodia, 2009). A highly specific antibody-antigen interaction is the most important component of the detection procedure. One of the advantage of this method is the step where non-bound material can be easily separated from bound ones during the assay. This ability to wash away non-specifically bound ingredients makes the ELISA a great tool for quantifying specific analytcs. In ELISA, various antigen-antibody combinations are used, always including an enzyme-labelled antigen or antibody, and enzyme activity is measured calorimetrically. The enzyme activity is measured using a substrate that changes colour when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values. Depending on the antigen-antibody combination, the assay is called a direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA (Merckodia, 2009).

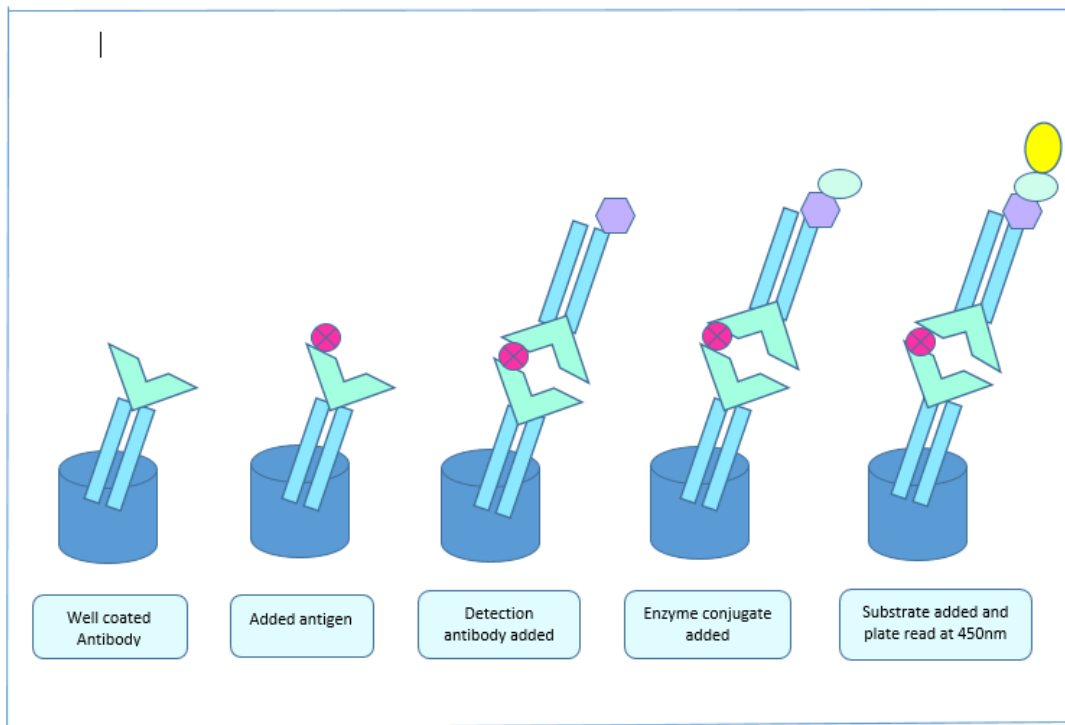


Figure 16: Procedure of ELISA

In the present study, insulin and incretin will be analysed using a commercially-available enzyme-linked immunosorbent assay (ELISA) with <0.01 cross reactivity with pro-insulin (Merckodia AB, Uppsala, Sweden). The Microplate Reader with a plate shaker (IKA® MTS 2/4 digital, Germany) and Manta Software, (Labtech LT-4000, Ireland) are used to read and analyse the tests. Merckodia is a solid phase two-site enzyme immunoassay. It uses a direct Sandwich technique where two monoclonal antibodies are directed against separate antigenic elements on the insulin or incretin. When samples are incubated, the insulin present in the sample reacts with both the anti-insulin antibodies bound to the microplate wells and peroxidase-conjugated anti-insulin antibodies. And the unbound enzymes labelled antibodies are removed with a simple washing step, followed by the addition of a detection agent, 3,3',5,5'-tetramethylbenzidine (TMB). After incubation, an acidic solution is added to bring the reaction to a stop and a colorimetric measurement is conducted (Merckodia, 2009).



Figure 17: ELISA machine and Mercodia Kit

3.9.1 The procedure of insulin as the following:

- 1- Mix two enzyme conjugate solution to prepare enzyme conjugate 1X and also need to prepare wash buffer solution.
- 2- Add 25 μ l of calibrators and the samples into wells in order.
- 3- Pipette 100 μ l of enzyme conjugate 1X to wells.
- 4- Incubate wells on the plate shaker for 1 hour at room temperature (18-25° C) and (700-900 rpm).
- 5- Wash wells quick wash for 6 times.
- 6- Pipette 200 μ l of substrate TMB in the wells.
- 7- Incubate on the bench at room temperature (18-25° C) for 15 minute.
- 8- Add 50 μ l of stop solution to the wells and shake it on the plate shaker to mix it for 5 seconds.
- 9- Read on the ELISA machine within half an hour to see the results.

3.9.2 The procedure for GLP-1 as the following:

- 1- Prepare wash buffer solution and prepare enzyme conjugate 1X by mixing two different vials of enzyme conjugate.
- 2- Mix 5 ml substrate reagent A with 5 ml substrate reagent B to prepare substrate working solution.
- 3- Add 25 μ l of calibrators and the samples into wells in order.
- 4- Add 50 μ l of enzyme conjugate 1X to wells.
- 5- Incubate wells on the plate shaker for 2 hours at room (18-25° C) and (700-900 rpm).
- 6- Wash wells quick wash for 6 times.
- 7- Pipette 100 μ l of substrate working solution into each well.
- 8- Incubate on the bench in the dark for 15 minutes at room temperature (18-25° C).
- 9- Use a microplate reader for chemiluminescence. Read all visible light (glow) with an integration time of 1 second.

3.10 RANDOX Machine

The Evidence Investigator™ (Randox, UK) is a Multiplexing ELISA technology (see Figure 18). This machine is used to measure the level of C-peptide, CRP, cystatin, Interleukin -6 (IL-6), Tumour Necrosis Factor α (TNF- α), leptin and resistin.

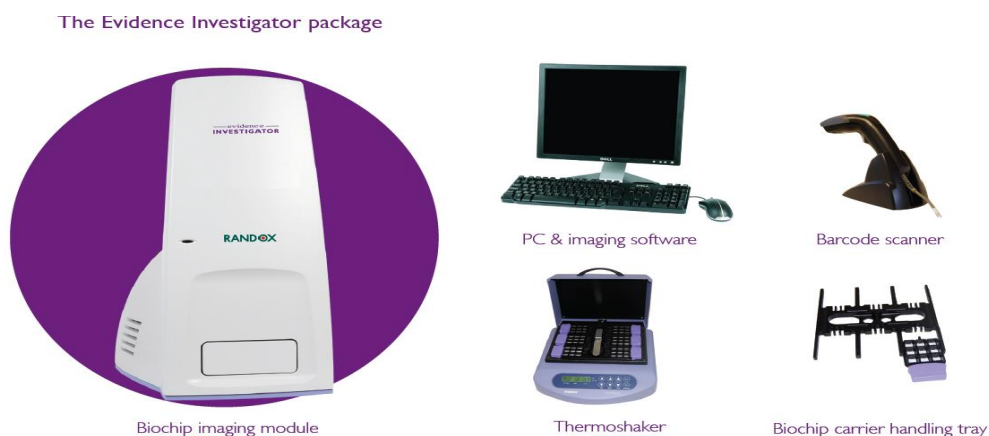


Figure 18: RANDOX Machine

The Evidence Investigator™ consists of Metabolic Syndrome Array I and Array II.

3.10.1 The Evidence Investigator™ Metabolic Syndrome Array I (METS I)

The Evidence Investigator™ Biochip Array technology is used to analyse blood sample for different test such as for insulin, IL-6, leptin, resistin and TNF α .

3.10.2 Reagent composition

1. METS I DIL ASY (1 × 14ml), is the assay diluent 20 mM Tris Buffered saline pH 7.5 containing protein, surfactant, blocking agents and preservatives.
2. METS I CONJ (1 × 20ml), is the conjugate 20 mM Tris buffered saline pH 7.5 containing protein, surfactant, preservatives and assay specific antibodies labelled with horseradish peroxidase (HRP).
3. METS I BIOCHIP (54 BIOCHIPS), solid-phase substrate containing discrete test regions of immobilised antibody.
4. METS I CAL (9 × 1ml), 9 vials of lyophilised base material containing analytes for the full array.

5. REAG SGNL-EV840 (1 × 10ml) and LUM-EV840 PX (1 × 10ml), mixed together in a ratio of 1:1 and give the working Signal reagent-EV40.
6. BUF WASH (conc) (1 × 32ml), 20 mM Tris buffered saline, pH 4.4 containing surfactant and preservatives.
7. Calibrator Concentration Disc and Barcodes I.

Quality control kit: this contains a number of small vials with red cover to be used as quality detector of the procedure. For one METS I kit we need 3 vials of control.

3.10.3 Procedure

- 1- Prepare samples to make sure that it dissolved before you start.
- 2- All materials should be equilibrated to room temperature before use.
- 3- Add 1 ml of double deionised water to the 9 vials of calibrator and 3 vials of control and then roll for 30 minutes.
- 4- The 6 Biochip carriers should be attached to the handling tray.
- 5- Pipette 200 µl of assay diluent in each well (total of 54 well).
- 6- Pipette 100 µl of calibrator, control, and then the samples in order.
- 7- Place the handling tray to the base plate of the thermoshaker and incubate it for 1 hour at +37° C and 370 rpm.
- 8- Mix half the quantity of washing buffer with 500 ml distilled water to use it in washing the well.
- 9- Remove the handling tray with the 6 carriers from the thermoshaker. Discard the reagents and then wash with the mixture that we prepare, carry out 2 quick wash, tap the side of handling tray and then carry out 4 long wash each one stays for 2 minutes.
- 10- After the wash pipette 300 µl conjugate into each well.
- 11- Place the handling tray to the base plate of thermoshaker and incubate it for 1 hour at +37° C and 370 rpm.
- 12- While waiting for that to finish prepare the mixture (working Signal reagent-EV40) and protect from light.

- 13- Remove the handling tray with the 6 carriers from the thermoshaker. Discard the reagents and then wash with the mixture that we prepare, carry out another 2 quick wash, and 4 long wash.
- 14- After washing take off the first carrier and pipette 250 μ l of the mixture (working Signal reagent-EV40), protect the biochip from light and wait for 2 minutes then place it into the Evidence Investigator™.
- 15- Repeat same step to rest of 5 biochips.
- 16- The image will be captured automatically by software.
- 17- Finally, you can check all your results in the computer.

3.10.4 The Evidence Investigator™ Metabolic Syndrome Array II (METS II)

This array has the same composition and same process except two steps only:

- 1- The dilution of samples with 300 μ l of the diluent is required, this diluent is available in separate box.
- 2- Washing process here is different, 6 long washes instead of 4 long washes.

3.11 Main exercise trial

The study will involve 2 x 2 hours exercise sessions a week for a 6 weeks period.

3.11.1 Introduction and orientation visit

During the first visit of the volunteer, the volunteer is normally asked to complete some medical and personal information forms in order to obtain their initial data. During this visit, the student may use the opportunity to introduce the process to follow and also provide an initial orientation. This would include showing the volunteer the equipment and letting them try resistance exercise, cycling, and the testing equipment.

3.11.2 One repetition maximum (1RM)

Before the volunteer start he must do a one repetition maximum (1RM) assessment to see what weight he can lift in resistance exercise by starting from low weight and increase it gradually to the maximum weight. 50-60% of this weight is used for the moderate exercise. Also, the target heart rate is calculated from a formula by using (Resting heart rate and age). But the 1RM is from a measurement. This target heart rate should always be used when volunteer is cycling in the lab. Blood glucose levels will be monitored before, during and after each session, using a standard finger prick test. In addition, HbA1c, cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG) will be measured at the baseline in the first session, and after last exercise session of six weeks programme using a finger prick test. Weight, height, BMI, waist, and lung capacity should be taken at baseline in the first session and then after six weeks.

3.11.3 Study design

Each exercise session will consist of a combined exercise protocol of 30 min of resistance exercise followed by 20 min moderate cycling (see Figure 19). The oral glucose tolerance test (OGTT) will be done to assess insulin sensitivity at the beginning and end of the programme and blood samples will be screened for other immunological parameters. For exercise sessions once the volunteer arrive, he must attach a polar chest for heart rate monitor; then he/she asked to sit to check his BG by FPP. After that blood pressure reading is taken and he should undergo the complete 11 steps stretching routine, then the volunteer must cycle for five minutes to warm up which is necessary before performing any kind of exercise to minimize post exercise pain or muscle stiffness. After warmup he will start the first set of resistance exercise by doing all steps for 10 times (squat, chest, back, biceps, and triceps) and then the second and third set similar to the first one.



Figure 19: Combination exercise programme.

3.11.4 Study groups

The intervention group are T2D and ND who are doing combination exercise.

Study group are classified in five groups

- 1- T2D volunteers with no medication.
- 2- T2D volunteers on Metformin only.
- 3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4- I).
- 4- T2D volunteers on Metformin and SGT2-I.
- 5- Non diabetes (ND).

3.12 Analysing process

3.12.1 Analysing venous blood sample the following

Inflammatory markers such as IL-6, TNF, leptin, resistin, cytoxin and CRP were analysed by using RANDOX and sampling time in the following sessions, 1st, 2nd, 4th, 6th, 8th, 10th, and 12th. While insulin was analysed by using ELISA, the sampling time was 4 times point during the three OGTT. Finally, GLP-1 was also analysed by using ELISA during 1st, 2nd, 4th, 6th, 8th, 10th, and 12th sessions.

3.12.2 Analysing by FPP we analyse the following

- BG Measure frequently before exercise after 1st, 2nd and 3rd set of RE then 10 and 20 minutes after AE.
- HbA1c (measure at the start and after the six weeks).
- OGGT (measure the start after S1 and after the six weeks).
- Lipid profile (measure at the start and after the six weeks).
- Lactate testing (measure before exercise and after AE).

3.12.3 Measuring the changes during the six weeks' exercise programme in the following

- HbA1c, BG, blood pressure, heart rate, and RPE.
- Insulin sensitivity.
- GLP-1.
- Inflammatory markers.

3.12.4 Analysing by the ADI the following

- Aerobic to anaerobic change using gas exchange:

Oxygen consumption (V_{O_2}), respiratory exchange ratio (RER), carbon dioxide production (V_{CO_2}), heart rate (HR), and bike revolution per minutes (RPM).

3.12.5 Anthropometric measurement

Measure body composition twice at baseline before start and then finally at the end after 6 weeks. This include, weight (Kg), height (cm), body mass index (BMI), lung capacity and waist (cm).

3.13 Study population

- 1- Healthy subjects (ND).
- 2- T2D.

3.13.1 Sample size

The target of the sample size for this study is 30 Volunteers.

3.13.2 Intervention group

(17 T2D) doing combination exercise and (8 ND) doing combination exercise.

3.13.3 Selection criteria for volunteers

In this study our target volunteers are T2D, and ND with age group from 18-60 years old who met the inclusion criteria.

3.13.4 Exclusion criteria

People who had any of the following were not allowed to participate

- Heart disease.
- Liver disease.
- Kidney disease.
- Uncontrolled and very high blood pressure > 160/95 mmHg.
- Injury.
- Bleeding.
- Epilepsy.
- Acute exacerbated bronchial asthma.
- Osteoporosis.
- Arthritis.
- Mentally ill.
- Had recent surgery.
- Using medication increase blood sugar e.g. steroids.
- Using medication mask hypoglycaemic symptom e.g. beta blockers.

3.14 Statistical analysis

Data was analysed by using Excel 2010 and SPSS (version 22), Values were expressed as mean with standard error of mean (SEM). Pre and post exercise samples were compared using a paired-samples t-test and the level of statistical significance was act at ($P \leq 0.05$).

Chapter 4 The effects of a combined exercise programme on the body blood glucose, HbA1c, blood pressure, heart rate, lipid and other anthropometrics variables in T2D and ND.

4.1 Research question

Are there demonstrable health differences and improvement in HbA1c, blood glucose (BG), blood pressure (BP), heart rate (HR), lipid and other anthropometrics variable after the six weeks exercise intervention in T2D and ND that can be attributed to the intervention?

4.2 Aim:

The aim of the study is to investigate the effects of a combined exercise programme (i.e. a combination of cardio “aerobic” and resistance) on the body in T2D and ND participants. This by assessing the improvement in HbA1c, BG, BP, HR and lipid. Moreover, to see if there are any reduction in weight, BMI and waist. The aim of this study also to see the effect of these exercise on the body fitness.

4.3 Definitions

4.3.1 Physical activity

This is the contraction of skeletal muscle that occurs due to body movement. This movement needs energy spending additional to resting energy expenditure (Adeva-Andany et al., 2016).

4.3.2 Exercise

A type of physical activity: designed, organized body movement repeated in constant manner to improve or sustain physical fitness (Lucotti et al., 2011).

4.3.3 Resistance exercise

Resistance exercise (RE) is mainly anaerobic exercise which means that the body is not using oxygen in the total support of movement. This type of exercise uses strength of the muscle to move a weight or do an effort against a resistive load (Lucotti et al., 2011). RE such as weight lifting and using weight machines (Lucotti et al., 2011).

4.4 Aerobic exercise

Aerobic exercise (AE) is the type of exercise where oxygen is used for energy production from glucose. Typically it increases heart rate (HR) and cardiorespiratory fitness from movement and exercise such as cycling running, swimming, bicycling, jogging and walking (Lucotti et al., 2011). AE involves regular, repeated, and constant movements of similar large groups of muscle for at least 10 min interval (Lucotti et al., 2011). For diabetes, the American Diabetes Association (ADA, 2015) recommends performing at least 150 minutes per week of moderate-intensity physical activity (50 - 70% of maximum heart rate) or 90 minutes at least weekly of vigorous aerobic exercise (more than 70% of maximum heart rate) (Garber et al., 2017). Performing regular exercise at least 3 days a week, but not more than 2 consecutive days without doing any kind of physical activity. ADA, (2015) also recommends three times weekly of resistance exercise with three sets of 8 - 10 repetitions of weight lifting.

4.5 Effect of exercise in the body

The cell energy is known as ATP which is energy currency. In case of muscle contraction, this ATP is broken-down by the myosin ATPase into adenosine diphosphate (ADP) and inorganic phosphate (P₁) offers the energy for muscle contraction (Maughan, 2013). During rest and to cover resting energy requirements of the cell, each muscle cell has approximately one billion ATP molecules. The body will use these molecules and after that will regenerate it in 2 minutes (Maughan, 2013). On the other hand, in intensive exercise, the requirements of ATP of some muscle cells is estimated as 100 times bigger than during rest (Maughan, 2013). This part will discuss different studies on the effect of exercise on the body in term of HbA1c, blood pressure, lipid profile and anthropometrics variables.

Sigal et al., (2007) found that aerobic or resistance exercise training alone improved glycemic control, but the effects were more evident with both combined. Their study established that previous works had evaluated the effects that aerobic training and resistance training had on the glycemic control in patients suffering from T2D. The effects were assessed using the values of HbA1c that were obtained from the trials. As such (Sigal et al., 2007) set out a randomized controlled trial with the objective of determining the effects that training alone had on T2D patients. The project also included studying the effects of resistance training, and a combination of aerobic and resistance training on the values of HbA1c among patients suffering from T2D. It also involved 251 T2D adult whose age ranged between 39 and 70 years and control group (Sigal et al., 2007). The change obtained was a significant reduction in the HbA1c in the aerobic training compared with control group (-0.05% and $P = 0.007$), while in the resistance group compared with control was significant (-0.38% and $P = 0.038$). The result in the combined exercise group was additional reduction in the HbA1c by -0.46% when compare with aerobic training group ($P = 0.014$) and -0.59% in comparison with resistance training group ($P = 0.001$) (Sigal et al., 2007). In conclusion, a combination of both aerobic and resistance training produces a significant improvement compared to aerobic training alone or resistance training alone. Changes in blood pressure and lipid profile were insignificant in all groups. Another study has been observed that in T2D, only the combination, but not aerobic and resistance training alone compared with the non-exercise group, improved HbA1c level (Church et al., 2010). The objective of the Church study was to examine the ways in which T2D patients could benefit from aerobic training only, resistance training alone and a combination of both. The effect was determined using HbA1c values. Just like the previous studies, Church used a randomised design in which 262 sedentary participants. T2D participants with HbA1c level between 6.5% and 11% (Church et al., 2010). The control group had 41 individuals, 73 practised resistance training for three days a week, 72 did aerobic training and 76 did combined both aerobic and resistance training. The result of the reduction of HbA1c in the combination training group vs the control group was (-0.34% and $P = 0.03$). While in aerobic training the reduction in HbA1c was (-0.24% and $P = 0.14$). In the resistance training the reduction in HbA1c was (-0.16% and $P = 0.32$). It concluded that this combination achieves HbA1c levels that cannot be

achieved if either aerobic or resistance training is done alone (Church et al., 2010). According to Yardley the order in which different type of exercise is carried out may be important, performing resistance exercise before aerobic exercise improved glycemic control during exercise; it also reduced the severity and duration of post-exercise hypoglycemia diabetes patients. The research study carried out by (Yardley et al., 2013) hypothesised that resistance exercise had the capacity to reduce HbA1c. The researchers set out the aim of examining the acute impacts that resistance training had on glycaemia. There were significant changes in the glucose levels in plasma attenuated to the involvement of exercise. The level of glucose in plasma was noted to have reduced from 8.4 ± 2.7 to 6.8 ± 2.3 mmol/L. It has been found that performing RE then AE improve HbA1c and decrease post exercise hypoglycaemia (Yardley et al., 2013).

Aguiar et al., (2014) conducted six months of exercise RCT among men who are at high risk of developing T2D in Australia. They hypothesised that a parallel-group, assessor-blinded, randomised controlled trial could be used. The approach used was characterised by the fact that it had to last six months (Aguiar et al., 2014). The primary outcome of this particular exercise was that the significant change of weight at the end of the six months (Aguiar et al., 2014). There were also several secondary outcomes of the study which include; physical activity, fasting plasma glucose, body composition, diet quality, waist circumference, muscular fitness and HbA1C levels (Aguiar et al., 2014). Other changes that were included blood pressure and body fitness. (Aguiar et al., 2014) concluded that such exercises and programs significantly improved the risk factors of T2D patients.

This research aimed at comparing the impacts of resistance training and aerobic training, as well as the combination of both on the glycaemic control, body composition and cardiovascular risk factors among patients suffering from diabetes (Yavari et al., 2012). They used a sample size of 80 that consisted of 37 men and 43 women and divided them randomly. There were four groups, each with twenty people. The groups were divided so that the members could undergo different exercises such as resistance, aerobic, control and combined training (Yavari et al., 2012). The exercise involved training that was done three times a week for the entire

year. The results showed that the HbA1c levels had dropped significantly in three of the four groups. The control group did not have any obvious change. The most significant changes that the individuals experienced were improvements in blood pressure, postprandial glucose, VO₂max and muscular percentage (Yavari et al., 2012). A combination of any two exercises led to a significant improvement in such parameters as HbA1c and alone (Yavari et al., 2012). This study is important to this research because it shows that better positive changes are obtained when the two methods are combined.

It has been observed that premenopausal women with T2D report greater effort during exercise than the control group without diabetes, as measured by the Rating of Perceived Exertion (RPE) scale (Huebschmann et al., 2015). Overweight, sedentary women aged 50–75 years with (n=26) or without T2D (n=28) have been enrolled in the Huebschmann study. Participants completed submaximal cycle ergometer exercise at 30 W and 35% of individually-measured peak oxygen consumption (35% VO₂peak). Exercise effort was assessed by RPE and plasma lactate concentration. According to the Huebschmann results showed that in T2D, VO₂ peak was low comparing to controls (p=0.003). The RPE shows no significantly improved in T2D versus controls (30 W: Control, 10.4±3.2, T2D, 11.7 ±2.3, p=0.08; 35% VO₂peak: Control, 11.1±0.5, T2DM, 12.1±0.5, p=0.21). Nevertheless, lactate level was higher in T2D than in controls (p=0.004 at 30 W; p<0.05 at 35% VO₂peak). Higher RPE was associated with higher lactate, higher heart rate, and a hypertension diagnosis (p<0.05 at 30 W and 35% VO₂peak). The conclusion shows that in older women the exercise effort was greater with T2D than controls because skeletal muscle unable to use glucose as energy fuel (Huebschmann et al., 2015).

4.6 Method:

4.6.1 Study design

In each exercise session the participant should perform a combined exercise program consists of 30 min of resistance exercise followed by 20 min moderate cycling. This is done twice a week for 6 weeks. At the beginning of each session the participants have to stretch-up for 11 steps of stretching, then he/she must cycle for five minutes to warm up. The RE consist of 3 sets, in each set the volunteer performed (squat, chest, back, biceps and triceps) 10 times. HbA1c, lipid profile, weight, BMI, waist and lung capacity also measured at baseline and the 12th exercise session. OGTT were done at the beginning, after 1st session and finally after session 12. Moreover, BG and HR measured before exercise, after 1st, 2nd and 3rd RE then after 10 and 20 minutes of AE. BP also checked before exercise after RE and then after AE. RPE were checked after 1st, 2nd and 3rd RE as well as after 10 and 20 minutes AE. All the blood test measured by using FPP as discussed before in (section 3.8.6).

4.6.2 Intervention group

The intervention group are T2D (n=17) 4 female and 13 male. ND (n=8) 5 female and 3 male who are doing combination exercise.

Medication	Number of Volunteers
Pravastatin 40mg OD	1
Atorvastatin 20mg OD	3
Metformin 500 mg BD	3
Metformin 850 TDS	1
Metformin 1000 mg BD	2
Sitagliptin 50 mg OD (DPP4-I)	1

Sitagliptin 100mg OD (DPP4-I)	2
Empagliflozin 10 mg OD	2

4.7 Results and discussion

4.7.1 Anthropometrics variables

Table 9: T2D anthropometric variables:

T2D	Pre 1st Se Ex	Post 12th Se Ex	P value
Age (years)	48.6 ± 2.6	-	-
Height (cm)	170.35 ± 4.8	-	-
Weight (kg)	92.0 ± 4.3	90.0 ± 4.5	0.001
Waist (cm)	105.2 ± 3.6	102.3 ± 3.6	0.000
BMI (kg/m ²)	30.8 ± 1.0	30.2 ± 0.9	0.001
Lung Capacity	381.8 ± 39.7	423.6 ± 33.6	0.001

ND anthropometric variables:

Table 10: Anthropometric variables for ND on combination exercise. Data expressed as Means ± SE, and P value.

ND	Pre 1st Se Ex	Post 12th Se Ex	P value
Age (years)	31.6 ± 4	-	-
Height (cm)	167.4 cm ± 4.4	-	-

Weight (kg)	58.86 ± 1.2	58.22 ± 1.2	0.490
Waist (cm)	73.7 ± 1.0	71.4 ± 0.5	0.052
BMI (kg/m²)	21.2 ± 1.2	20.9 ± 0.9	0.382
Lung Capacity	380 ± 26.7	580 ± 39.8	0.008

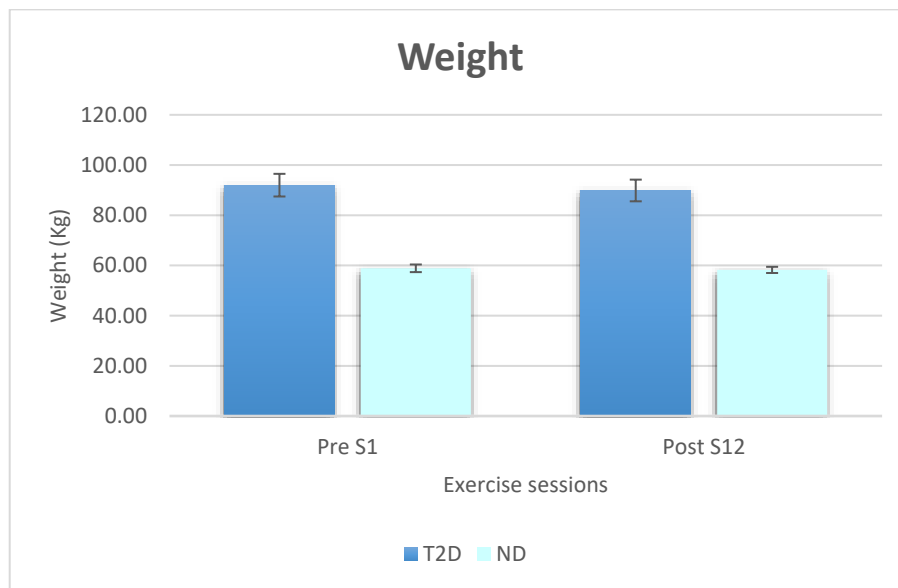


Figure 20: Weight changes across whole exercise sessions in both groups

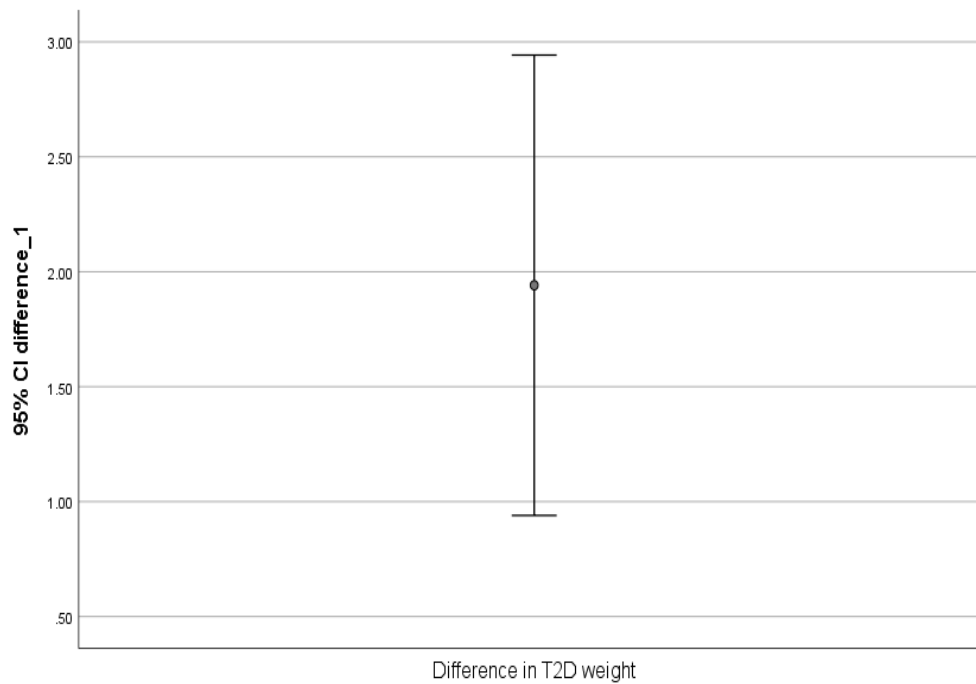


Figure 19a: T2D weight shows CI 95% higher than zero which mean this difference is significant

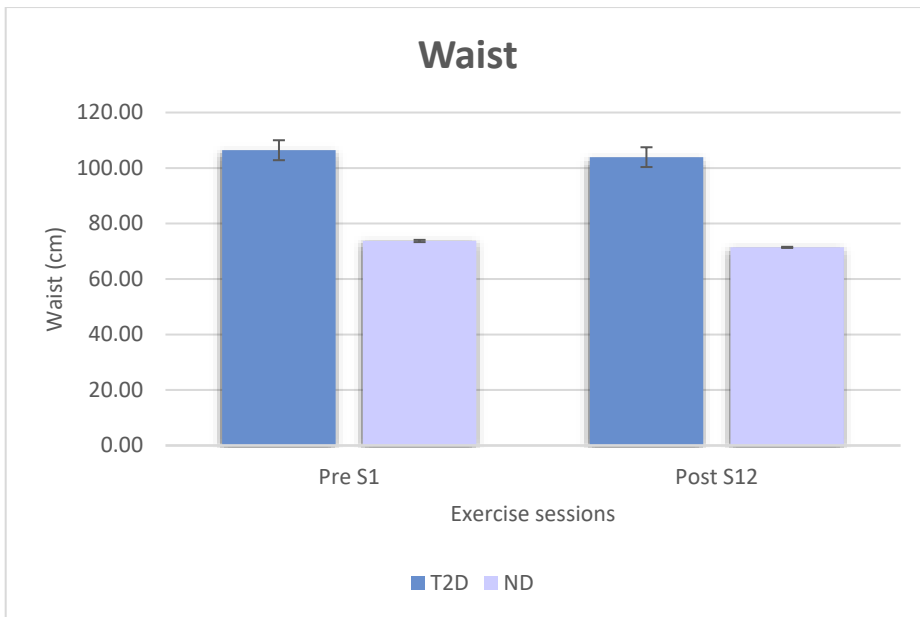


Figure 21: Waist changes across whole exercise sessions in both groups

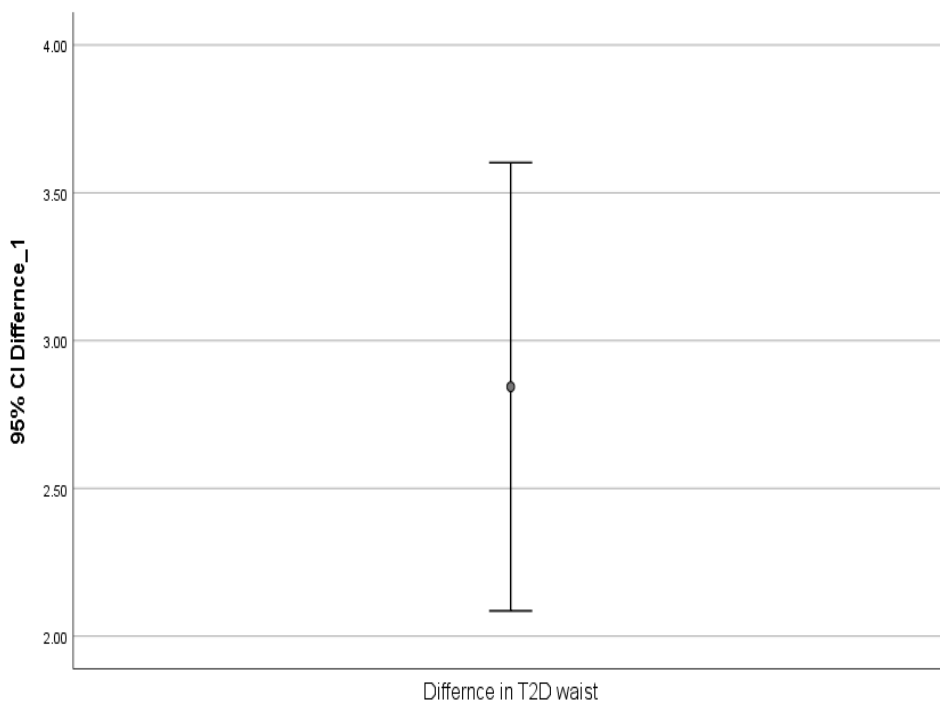


Figure 20a: T2D waist shows CI 95% higher than zero which mean this difference is significant.

Error difference (waist Pre- waist Post) > 0

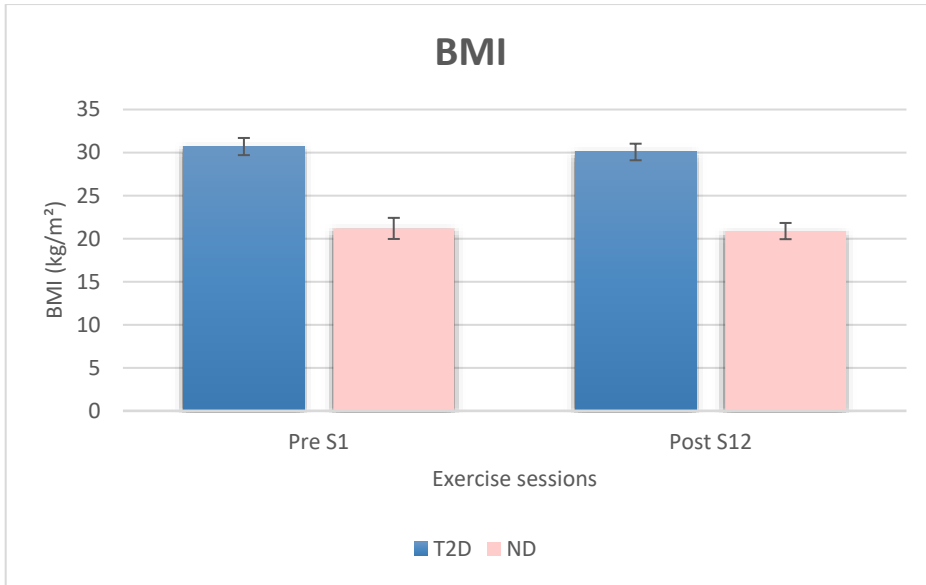


Figure 22: BMI changes across whole exercise sessions in both groups



Figure 21a: T2D BMI shows CI 95% higher than zero which mean this difference is significant

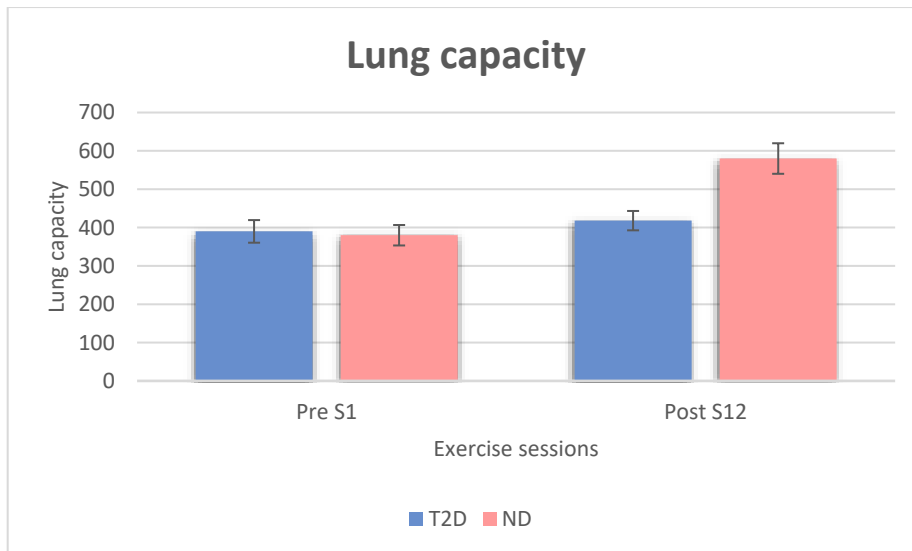


Figure 23: Lung capacity changes across whole exercise sessions in both groups

Data for all study groups are presented in section (9.1.1), table (10) for T2D and section (9.2.1), table (25) for ND. They show the results Pre S1 of Ex and Post S12 of combination exercise across the six weeks and demonstrate the chronic effect of exercise on those variables. In T2D the weight reduction was significant which decreased from (92.0 ± 4.3) to (90.0 ± 4.5) and $(P < 0.001)$, the waist was (105.2 ± 3.6) and decreased to (102.3 ± 3.6) which is also significant $(P < 0.000)$. Moreover, the BMI reduction was significant, it was (30.8 ± 1.0) and decreased to (30.2 ± 0.9) and $(P < 0.001)$. This sort of finding reflects previous study conducted in obese older adults that also demonstrated improvement in insulin sensitivity (IS) and other cardiometabolic risk factors such as CRP (Bouchonville et al., 2014). It has been also found similar significant reduction in weight, waist and BMI in previous studies conducted among T2D (Aguiar et al., 2014; Sigal et al., 2007). There was a significant improvement in lung capacity for T2D as well which improved from (381.8 ± 39.7) to (423.6 ± 33.6) and $(P < 0.001)$. In ND, the result of weight reduction improved from (58.86 ± 1.2) to (58.22 ± 1.2) . It has no significant changes when comparing pre S1 and post S12; in pre S1 (58.86 ± 1.2) and post S12 (58.22 ± 1.2) , $(P \text{ value} = 0.490)$. BMI shows no significant reduction in pre S1 (29 ± 0.4) and decreased to (28.1 ± 0.2) , $(P \text{ value} = 0.382)$. The result of waist reduction was close to be significant, pre S1 (21.2 ± 1.2) post S12 (20.9 ± 0.9) ,

(P value =0.052), and previous studies found it significant (Aguiar et al., 2014; Sigal et al., 2007). Finally, lung capacity improved as well with significant reduction, pre S1 (380 ± 26.7), post S12 (580 ± 39.8), and (P value =0.008).

4.7.2 HbA1c:

Table 11: HbA1c changes in T2D and ND exercise group. Data expressed as Means \pm SE, % of differences, and P value.

	Pre S1	Post S12	P. value
HbA1c mmol/mol (T2D)	55.7 \pm 2.7	48.9 \pm 2.1	0.000 which is < 0.001
HbA1c mmol/mol (ND)	34.6 \pm 0.6	31.2 \pm 0.2	0.003

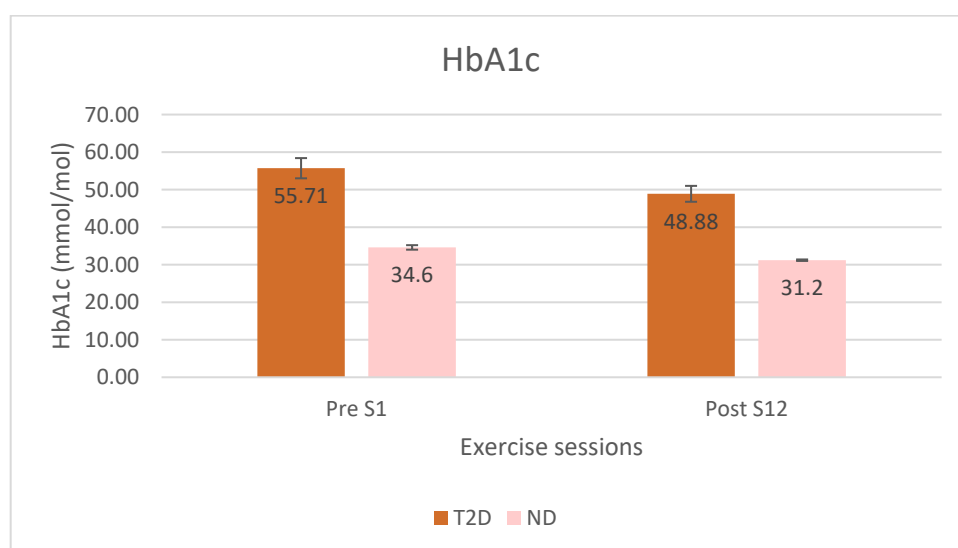


Figure 24: HbA1c changes across the exercise sessions in both group

In T2D and ND as in figure 23, there was significant reduction in the HbA1c level. These results demonstrate improvement in both study group. However, the improvement in HbA1c was higher in T2D and represent higher reduction compared to previous studies (Sigal et al., 2007; Church et al., 2010). In T2D the reduction was (-6.8 mmol/mol “0.7 %”) and in ND the reduction was (3.4 mmol/mol “0.4 %”). In the present study, HbA1c results in both T2D and ND has been significantly decreased after 12th sessions of combination exercise which would give benefit in decreasing their risk of having CVD or microvascular complications. As explained before in chapter 1, the reduction in HbA1c level usually associated with a 15% -20% reduction in major CVD events and a 37% decrease in microvascular complications (Selvin et al., 2004; Stratton et al., 2000). Another previous studies has found that a reduction in

HbA1c in the group who performed combination exercise of AE and RE shows reduction in HbA1c more than RE group and AE group. These finding proved that AE and RE alone reduce HbA1c, however a combined of AE and RE showed more improvement than those of either programme alone (Aguiar et al., 2014; Sigal et al., 2007; Church et al., 2010). A combination of RE and AE cause a significant improvement in HbA1c compared with when either aerobic or resistance training was used alone (Yavari et al., 2012).

4.7.3 Blood Glucose level

T2D and ND BG changes during 12 sessions of combination exercise:

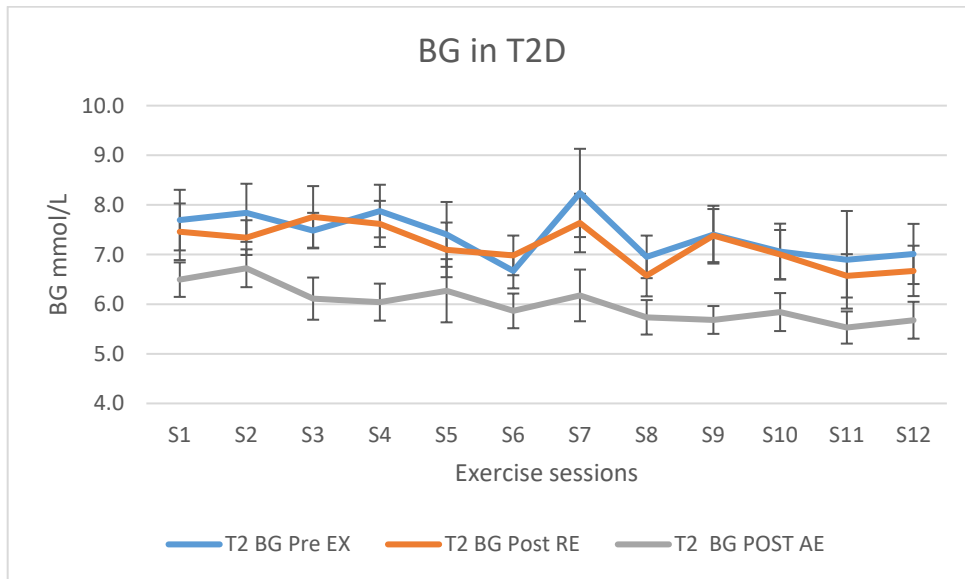


Figure 25: BG changes across the whole exercise sessions in T2D group.

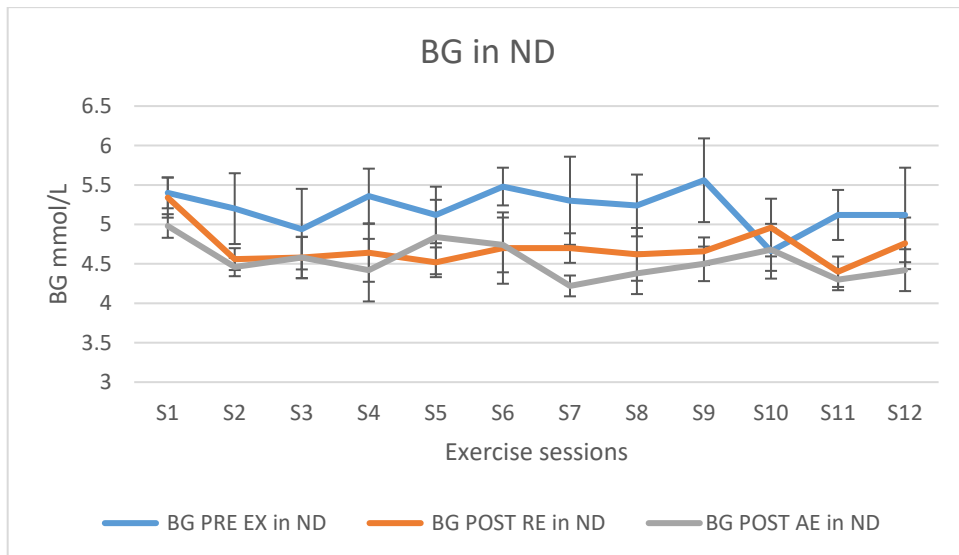


Figure 26: BG changes across the whole exercise sessions in ND group.

4.7.4 Chronic effect of BG

There is a reduction in the mean BG values of each group of volunteers across 12 exercise sessions especially session 8 and session 11, while it increased in session 12 (see figure 24, 25). In T2D, comparisons are made between sessions 1 (S1) and 12 (S12) before exercise, after resistance training and after aerobic training. Before exercise BG was in S1 (7.7 ± 0.6) and then decreased in S12 (7.0 ± 0.6). The P value were $P=0.210$. After resistance exercise in S1 BG was (7.5 ± 0.6) and then decreased in S12 to (6.7 ± 0.5), $P=0.164$. Finally after AE, in S1 the BG was (6.5 ± 0.3) and decreased in in S12 to (5.7 ± 0.4), $P=0.072$. P values all show no significant reduction in the T2D group. There was an improvement after AE when comparing S1 and S12 but insignificant. After AE, when comparing Pre S1 and post S12, a high reduction in BG is visible but this is still not significant because the P value is slightly higher than 0.05. The reason for the lack of significant results may be due to two reasons that we know within the data source. One T2D volunteer had HbA1c = 7.5 % (58 mmol/mol) and his random BG was usually 14 mmol/l, which needs medical intervention but there was no anti-hyperglycaemic medication prescribed for him. The second reason, was that another of the T2D volunteers had ran out of medication for the last 2 weeks of the exercise program; which affects the BG improvement. Looking at the BG in ND when comparing between S1 & S12 in Pre Ex, Post RE, & Post AE. Before exercise, BG was in S1 (5.4 ± 0.19) and decreased in S12 to (5.1 ± 0.59), $P=0.66$. After resistance exercise, BG in S1 was (5.3 ± 0.25) then decreased in S12 to (4.8 ± 0.32), $P=0.23$. After aerobic exercise, BG was in S1 (5.0 ± 0.15) then decreased in S12 to (4.4 ± 0.27), $P=0.03$. These P values show that the only significant reduction when comparing (S1 & S12) after aerobic training. In T2D and ND performing moderate intensity exercise, the increment in peripheral glucose uptake is matched by an equivalent increase in hepatic glucose production (Borghouts et al., 2002). The current study shows that BG level was largely reduced after AE more than RE in both T2D and ND groups, which shown before in previous study by Yardley et al. (2013) who compared the effect of performing RE then AE with AE then RE on BG (Yardley et al., 2013).

4.7.5 Acute effect of BG in session 1

In T2D as in figure 24, the result of BG before exercise, after resistance exercise and after aerobic exercise was presented as mean \pm SEM. In session one the BG pre exercise was (7.7 \pm 0.6), while it was decreased after RE to (7.5 \pm 0.6) and finally decreased more after aerobic to (6.5 \pm 0.3) which shows reduction in the very short term (acute as in during the very first session on untrained bodies) in BG in response to exercise. P values by comparing pre exercise and post RE (P=0.596), by comparing pre Ex and post AE (P=0.039) which shows significant reduction of BG after AE. In ND, (figure 25) was as the following, before exercise (5.4 \pm 0.19), after resistance BG decreased slightly to (5.3 \pm 0.25), and after aerobic it decreases more to (5.0 \pm 0.15). P value by comparing pre Ex and post RE was (P=0.878) while by comparing pre Ex and post AE was (P=0.157) which shows no significant reduction after both RE and AE exercise in ND group. Previous study found that BG has been improved after combination exercise of AE and RE (Prior et al., 2015). In this study, the finding show that the significant reduction of BG was only after AE in T2D.

4.7.6 Blood Pressure

BP changes during 12 sessions of combination exercise:

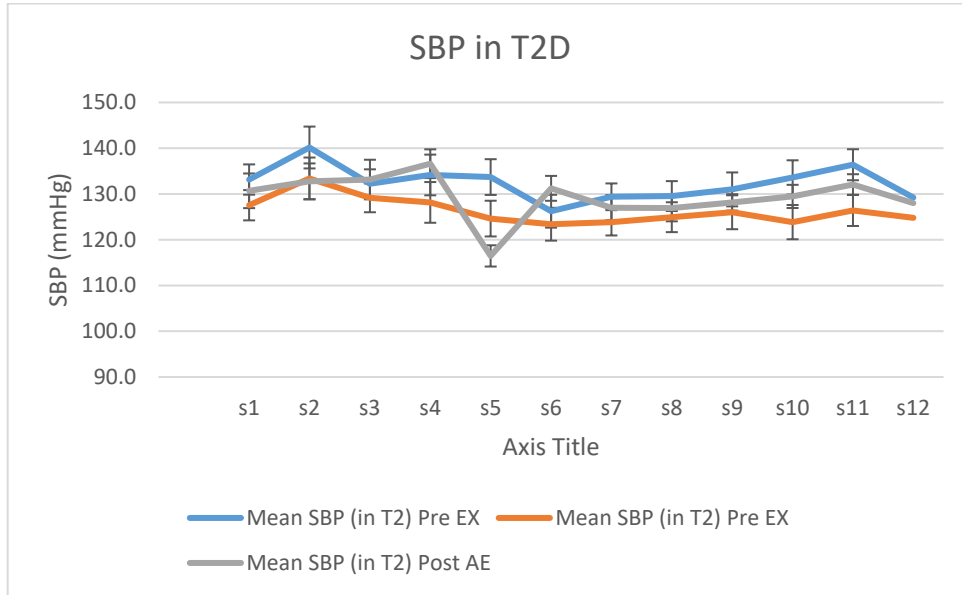


Figure 27: SBP across the whole exercise session in T2D.

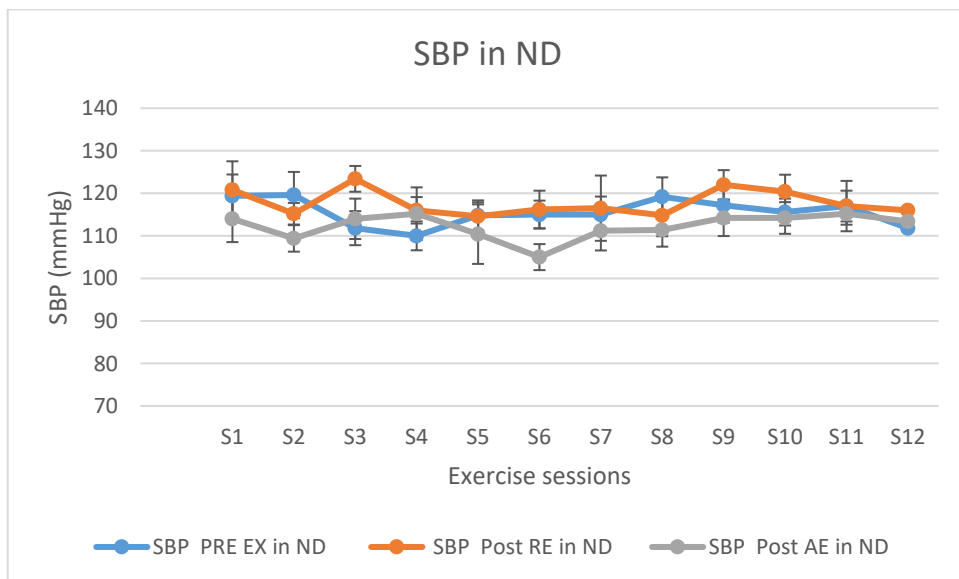


Figure 28: SBP across the whole exercise session in ND.

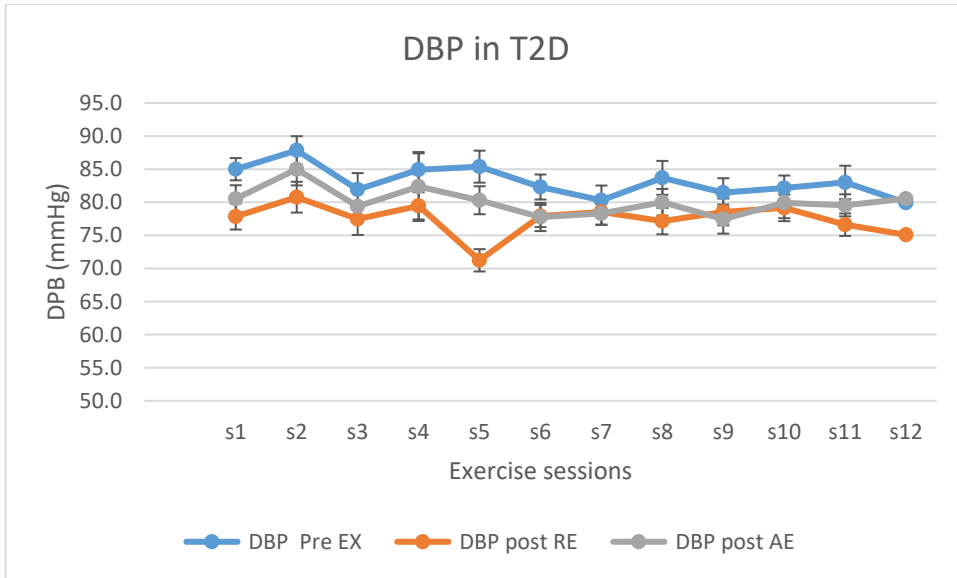


Figure 29: DBP across the whole exercise sessions in T2D.

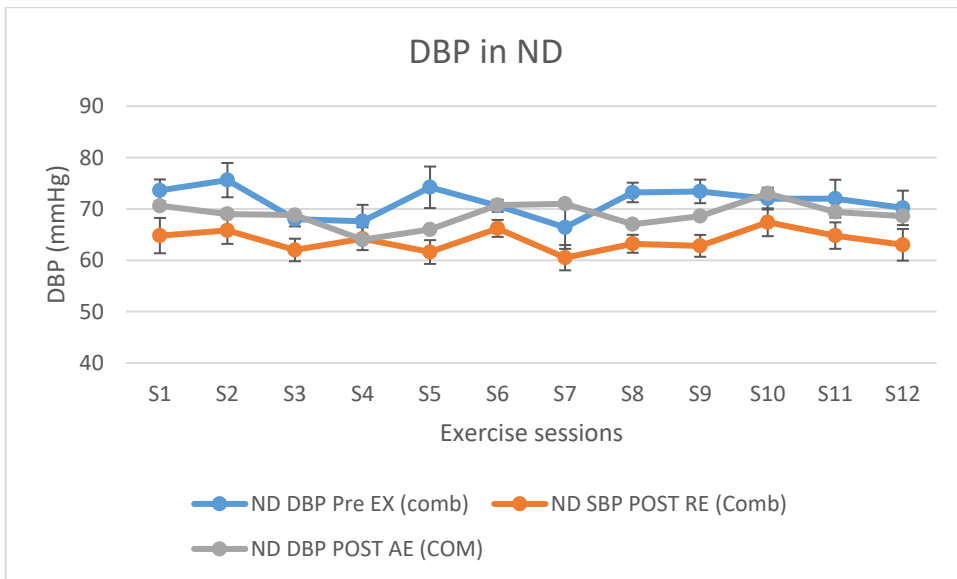


Figure 30: DBP across the whole exercise sessions in ND.

The SBP results for T2D are shown in figure 26. SBP before exercise in session one was (133.6 ± 4.5) and decreased in S12 to (128.2 ± 3.4). It shows no significant reduction when comparing (S6 & S12, $P=0.801$) and in comparing (S1 & S12, $P=0.131$). Finally, after AE exercise the result of SBP shows significant changes only after S12, in S1 was (129.5 ± 2.8) which decreased in S12 to (124.2 ± 2.2), $P=0.036$). Figure 28 shows the results for DBP in T2D. Before exercise, DBP changes as the following, in S1 was (86.6 ± 2.1) and decreased in S12 to (79.9 ± 2.5), $P=0.010$ which shows significant reduction when comparing S1 and S12. Moreover, after aerobic training the reading was in S1 (82.5 ± 1.8),) and in S12 (79.6 ± 1.7), $P=0.963$. This result presented no significant improvement. In this study group T2D, some of the volunteers had hypertension and had been stabilised on medication, although one of them had high blood pressure readings despite antihypertensive medication. Due to this increment of BP for this volunteer the results shows no significant reduction. In the ND, SBD results as shown in figure 27. Before exercise, SBP in S1 was (119.4 ± 3.5) then decreased in S12 to (111.8 ± 5.9), $P=0.30$. Finally, after aerobic exercise the SBP in S1 was (114.0 ± 4.6) then decreased in S12 to (113.4 ± 2.6), $P=0.83$). DBP in ND results as presented in figure 29. DBP before exercise in S1 was (73.6 ± 2.1) which decreased in S12 to (70.2 ± 3.4), $P=0.12$. After aerobic DBP in S1 was (70.6 ± 1.0) and decreased in S12 to (68.6 ± 3.6), $P=0.60$. In ND the reduction in SBP and DBP was not significant that's might be due to that these group are healthy and has no hypertension so their BP reading is normal before and after exercise. Hypertension plays role in the development of the macrovascular and microvascular complications (Frontoni et al., 2014; North and Palmer, 2015; Takao et al., 2014). The reduction of BP helps in reduced cardiovascular mortality and improved renal outcome (Lovshin and Zinman, 2014; Takao et al., 2014). In the study, the BP has improved but not as significantly as found in previous studies after the combination of AE and RE (Sigal et al., 2007; Laoutaris et al., 2013). The reduction in this study was only significant in SBP after AE. Moreover, recent studies show that the BP decreases after AE which is accompanied with weight reduction and accompanied by an improvement in the vascular system (Eskandar, Katayon and Ali, 2015).

4.7.7 Heart rate

HR changes during 12 sessions of combination exercise

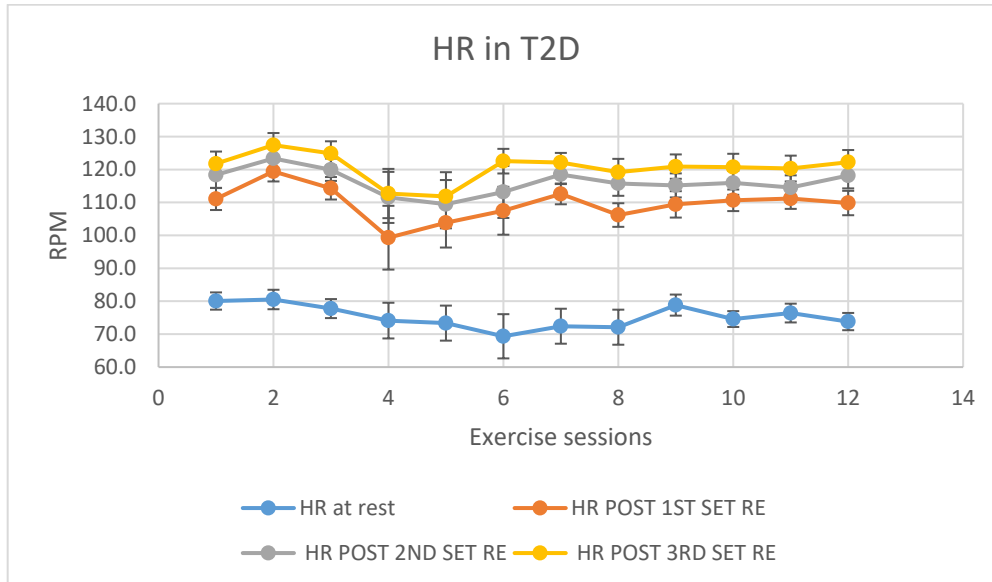


Figure 31: HR changes across the whole exercise sessions in T2D.

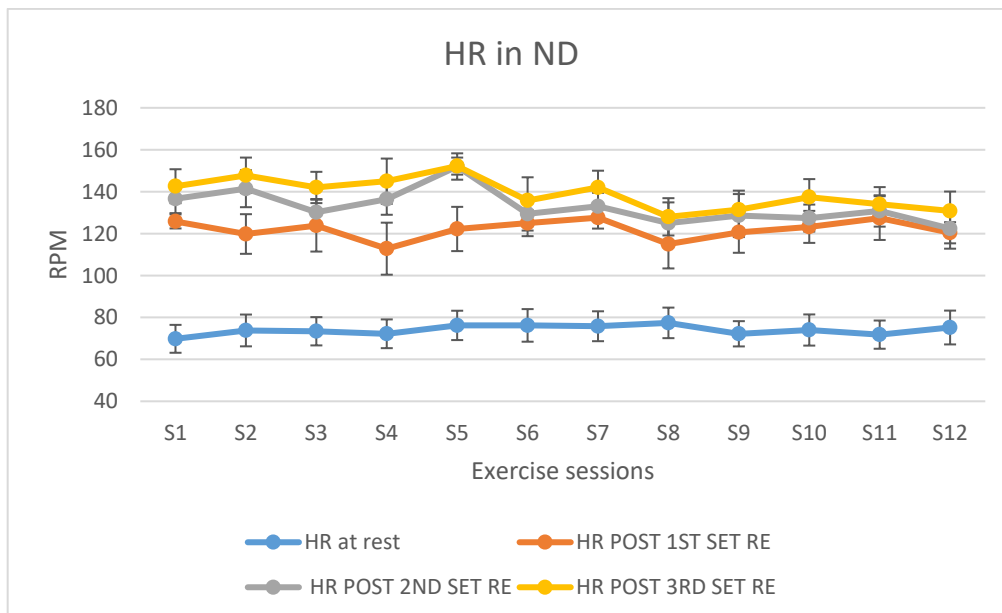


Figure 32: HR changes across the whole exercise sessions in T2D.

Figure shows HR improvement in T2D. At rest, HR was in S1 (80.1 ± 2.60) and decreased in S12 (73.8 ± 2.6), $P=0.023$, it shows significant reduction when comparing S1 with S12. The results show a reduction in HR in T2D patients before exercise and after the 1st set of RE but this reduction was not significant. After the 2nd set of resistance, there was a reduction across the 11th exercise session, however, in the 12th session the HR increase slightly. In the 3rd set of RE, the HR fluctuated while the increments were greater but showed no improvement. Figure shows ND result, resting HR in S1 was (69.8 ± 6.7) which increased in S12 to (75.2 ± 8.1), $P=0.433$). The HR shows reduction during 12th session after 1st, 2nd and 3rd set of resistance, nevertheless this reduction was not significant. As shown in the result, present study showed improvement in the resting and that was significant in T2D. These findings are in agreement with a previous study which found that a combination of AE and RE exercise programme in T2D had a good effect across all exercise sessions on the resting HR (Yavari et al., 2012)

4.7.8 Lactate

Lactate changes in T2D during 12 sessions of combination exercise

Table 12: lactate changes across (12 sessions) for T2D group. Data expressed as Means± SE, % of changes and P value:

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Pre Ex	2.5±0.3	2.8±0.6	2.5±0.5	2.9±0.6	2.6±0.4	2.5±0.5	2.1±0.4	2.3±0.2	2.4±0.5	2.7±0.3	2.1±0.4	2.3±0.5
Post Ex	10.7±2.3	10.0±2.6	10.9±2.3	6.3±1.4	6.6±1.1	5.3±0.5	5.5±0.4	5.6±0.4	4.9±0.3	4.7±0.7	4.8±0.8	5.1±0.6

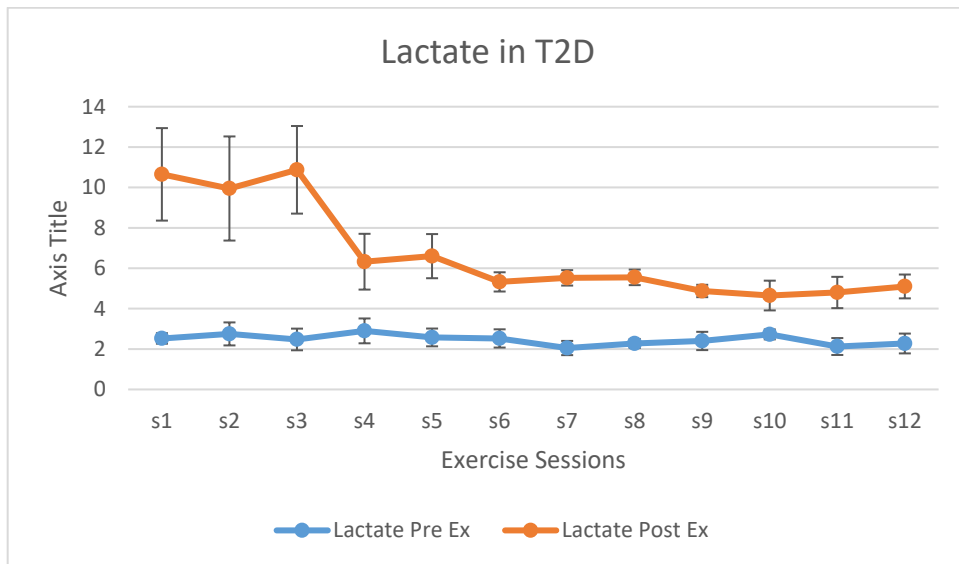


Figure 33: Lactate changes across the whole exercise sessions in T2D

The data for lactate have been taken from T2D only because we only received the lactate kits after ND finished thier programme. As in section 5.7, table (22), the level of lactate before exercise in S1 was (2.5 ± 0.3), in S6 was (2.5 ± 0.5) and decreased in S12 to (2.3 ± 0.5). P values as the following, by comparing (S1 & S6, $P= 1.000$), (S6 & S12, $P= 0.792$), and finally by comparing (S1 & S12, $P=0.516$). After exercise, in S1 lactate was (10.7 ± 2.3), then decreased in S6 to (5.3 ± 0.5) and in S12 to (5.1 ± 0.6). P values are (S1 & S6, $P= 0.077$), (S6 & S12, $P=0.574$) and (S1 & S12, $P=0.111$). The lactate level was improved more after exercise but this reduction was not significant. It has been found in a systemic review by Asano et al. that the effect of aerobic exercise on lactate threshold is not enough studied and until now inconclusive (Asano et al., 2014). High plasma lactate found in T2D, insulin resistance and obese subjects (Consitt et al., 2016). Lactate plays role in inhibiting glycolysis and insulin signalling which inhibits insulin-stimulated glucose uptake (see section 2.9) (Consitt et al., 2016).

4.7.9 Respiratory exchange ratio (RER):

Table 13: RER changes across (12 sessions) for T2D and ND groups. Data expressed as Means \pm SE, % of changes and P value:

RER	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
T2D	0.91 \pm 0.04	0.86 \pm 0.03	0.86 \pm 0.05	0.91 \pm 0.04	0.97 \pm 0.02	0.94 \pm 0.04	0.93 \pm 0.04	0.91 \pm 0.03	0.86 \pm 0.05	0.91 \pm 0.04	0.91 \pm 0.03	0.93 \pm 0.03
ND	0.92 \pm 0.04	0.90 \pm 0.04	0.93 \pm 0.03	0.94 \pm 0.04	0.92 \pm 0.04	0.94 \pm 0.03	0.91 \pm 0.04	1.00 \pm 0.05	1.00 \pm 0.04	0.96 \pm 0.04	0.92 \pm 0.04	0.96 \pm 0.04

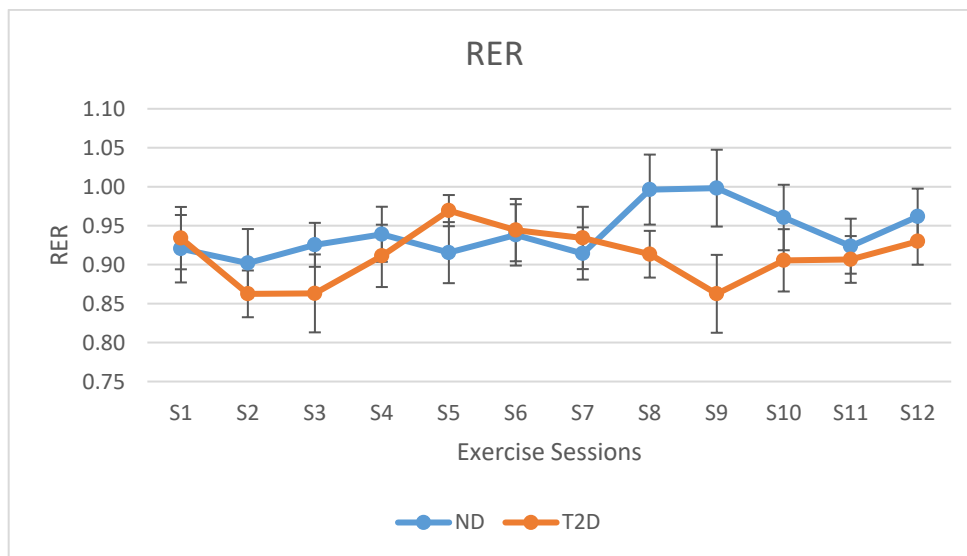


Figure 34: RER in both groups during 12 sessions

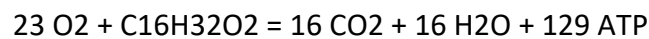
RER is the ratio of carbon dioxide (CO₂) volume that released from the lung to the oxygen (O₂) enter and used by the lungs at the same time. The volume of consumed oxygen per minute is termed as VO₂, while the volume of produced carbon dioxide is VCO₂ (Jeukendrup and Wallis, 2005). Thus, the ratio of VCO₂/VO₂ is the RER. The normal range of RER is (0.7- 1.0) which demonstrates the following types of substrate oxidation,; 0.7 shows fat oxidation, 1.0 shows carbohydrate (CHO) oxidation, and in between these values it shows oxidation of both CHO and fat (Ferrannini, 1988). In the human body, the main source of energy is produced by carbohydrate (CHO) and fat metabolism (Jeukendrup and Wallis, 2005). CHO and fat catabolises to water, CO₂ and energy. CHO oxidation requires less O₂ molecules than fat oxidation.

The equation of CHO molecules oxidation is:



In this equation there are 6 molecules of CO₂ which produced for every 6 molecules of O₂ consumed through CHO oxidation which gives ratio of 1.0.

The equation of fat molecules oxidation is:



In this equation the production of 16 molecules of CO₂ for every 23 molecules of consumed O₂ through fat oxidation gives ratio of 0.7 (Jeukendrup and Wallis, 2005). This results of this study shows that RER readings for T2D group are started in S1 (0.91±0.04) and become in S12 (0.93±0.03) and for ND are between (0.92±0.04) and (0.96±0.004), which represent that during 20 min of AE exercise CHO are mainly oxidised. These findings from the decline of BG after to AE exercise during the whole exercise sessions shows that during our study CHO are mainly oxidized. These finding supported by Asano et al., (2014) and explained that during exercise, metabolic stress increased carbohydrate oxidation, increased O₂ consumption after exercise which keep increasing fat oxidation at rest during recovery period after exercise and improving glucose tolerance and insulin sensitivity (Asano et al., 2014).

4.7.10 1RM & RPE

4.7.10.1 1RM

T2D improvement in 1RM during 12 sessions of combination exercise

Table 14: 1RM improvement comparing Pre S1 and Post S12 for T2D. Data expressed as Means± SE, % of changes and P value:

	1RM Pre Ex S1	1RM Post Ex S6	1RM Post Ex S12	P value
Squat	32.5±4.1		44.5±5.6	0.002
Chest	28.1±2.0		34.0±2.9	0.001
Back	51.8±3.8		67.6±4.0	0.000
Tricep	30.2±2.6		45.3±2.9	0.000
Bicep	28.2±2.1		35.9± 2.7	0.001

ND 1RM improvement during 12th sessions

The available data for 1RM only before the 1st session of exercise and after the 6th session of exercise for ND on combination exercise.

Table 15: 1RM improvement comparing 1RM Pre S1 and Post S6 for ND. Data expressed as Means± SE, % of changes and P value:

	1RM Pre Ex S1	1RM Post Ex S6	P value
Squat	45.0± 8.4	52.0± 6.4	0.024
Chest	28.0± 4.6	30.0± 5.7	0.186
Back	46.0± 4.0	54.0± 2.4	0.023
Tricep	24.0± 2.4	30.0± 3.2	0.076
Bicep	24.0± 2.4	28.0± 2.0	0.185

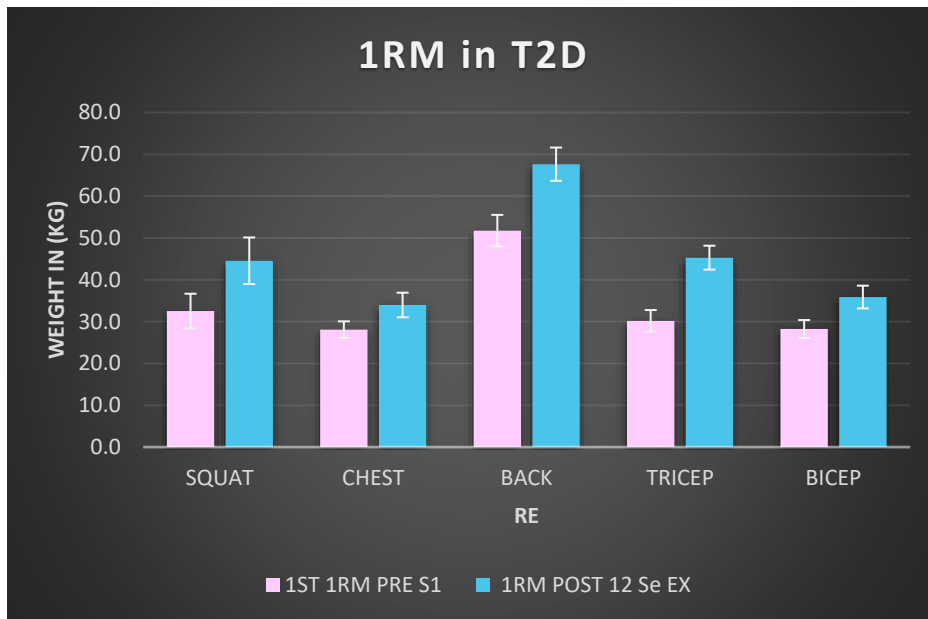


Figure 35: 1RM in T2D before S1 after S6 and after S12.

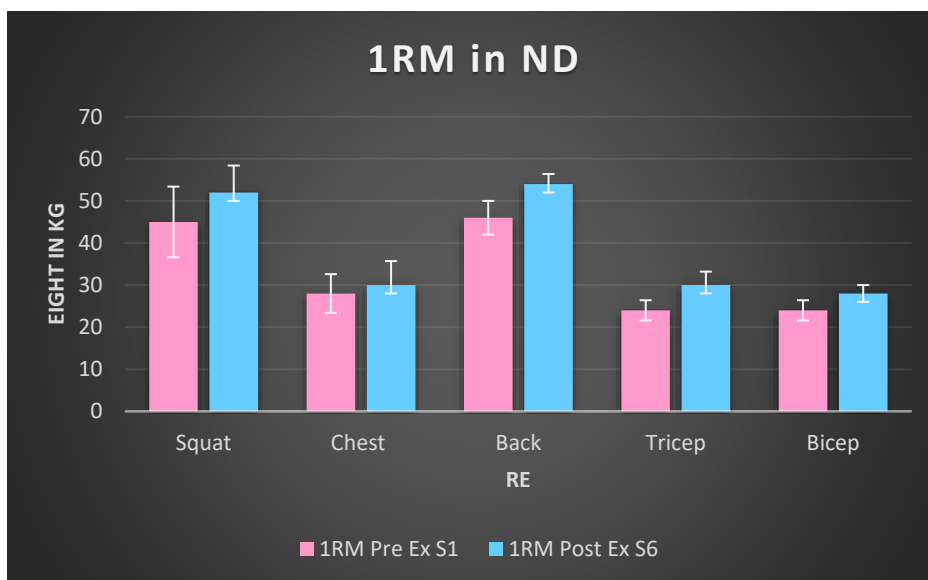


Figure 36: 1RM in ND before S1 and after S6.

Figure (34) represent the result of 1RM in T2D which was as the following; when comparing the 1RM before S1 and after S12 squat was (32.5±4.1) and increased to (44.5±5.6), P= 0.002 which is significant. Chest was (28.1±2.0) and increased to (34.0±2.9), P=0.001. Back was (51.8±3.8) and increased to (67.6±4.0), P=0.000. Tricep was (30.2±2.6) and increased to (46.0± 3.7), P= 0.000. Bicep was (28.2±2.1) and increased to (35.9± 2.7), P=0.001. P values shows significant improvement in the T2D fitness in the whole steps of resistance exercise which illustrate the benefit of

combination exercise on these group. Figure 35, shows the results of ND 1RM before S1 and 1RM after S6. Squat was (45.0 ± 8.4) and increased to (52.0 ± 6.4), $P= 0.02$. Chest was (28.0 ± 4.6) and increased slightly to (30.0 ± 5.7), $P=0.18$. Back was (46.0 ± 4.0) and increased to (54.0 ± 2.4), $P=0.02$. Triceps was (24.0 ± 2.4) and increased to (30.0 ± 3.2), $P=0.07$. Bicep was (24.0 ± 2.4) and increased slightly to (28.0 ± 2.0), $P=0.18$. The significant improvement in 1RM in ND was in squat, back and bicep, while in chest and triceps the increment was not significant.

4.7.10.2 RPE

RPE changes during 12 sessions of combination exercise

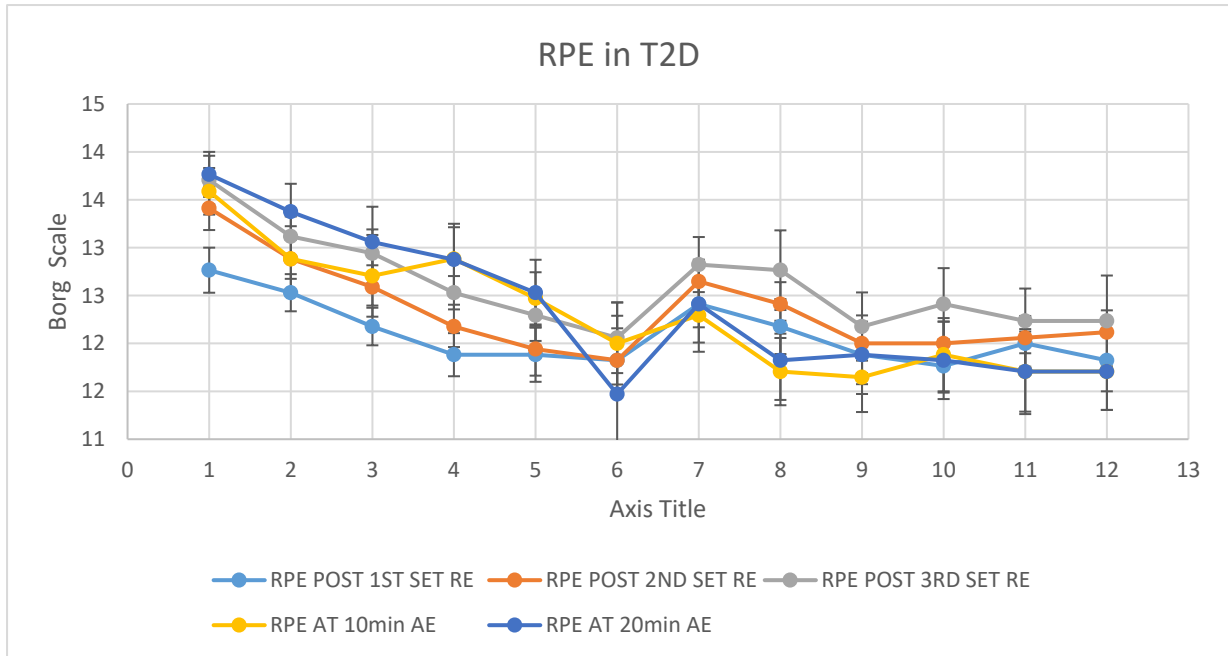


Figure 37: RPE across the whole exercise sessions in T2D.

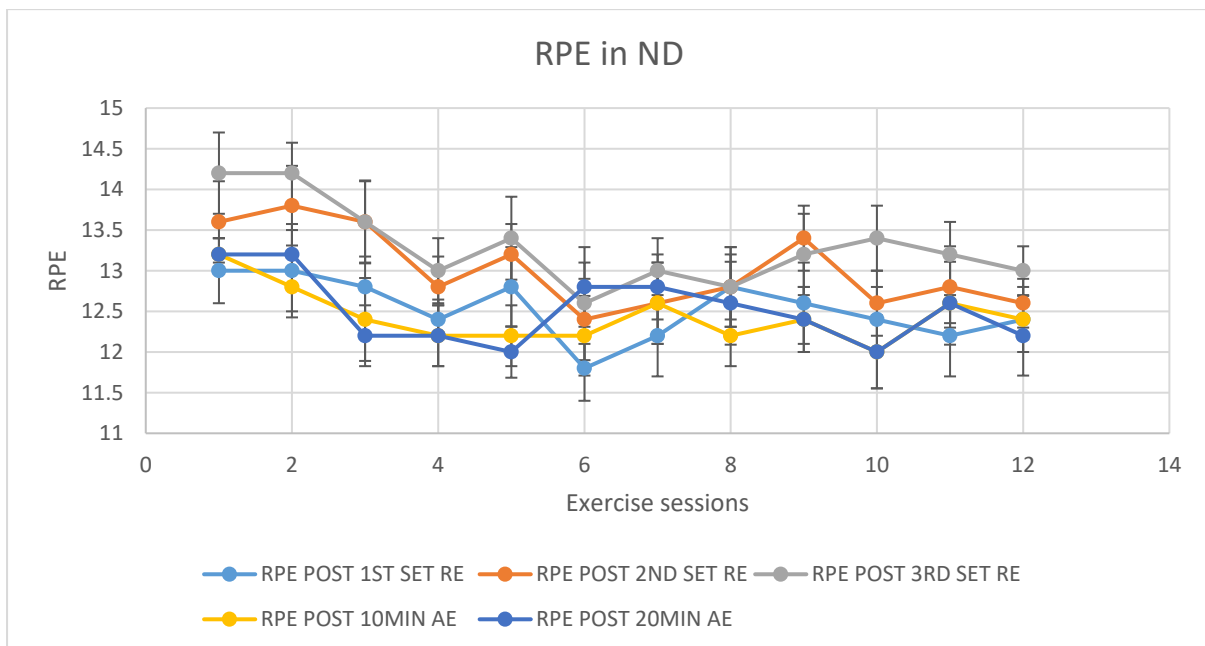


Figure 38: RPE across the whole exercise sessions in ND.

Figure 36 shows mean \pm SEM for RPE in T2D in 5 occasions, after 1st, 2nd and 3rd set of resistance training, and after 10 and 20 minutes of aerobic exercise. The results show reduction in these 5 occasions across 12th exercise sessions. After the 1st set of resistance, RPE in S1 was (12.8 \pm 0.24) and decreased in S6 to (11.8 \pm 0.33) and in S12 to (11.8 \pm 0.32). P value was (S1 & S6, P=0.024), (S6 & S12, P=1.00), and (S1 & S12, P=0.034). After 2nd set of resistance, RPE decreased significantly when comparing S1 & S12, P=0.011. After the 3rd set of resistance, RPE decreased in S12, P=0.011). After 10 minute of AE, RPE decreased in S12, P=0.002. After 20 minute of aerobic, RPE decreased also in S12, P=0.001. The improvement in T2D fitness can be presented in their RPE which shows significant reduction across the whole exercise session. Their feeling of the difficulty of the exercise became more less during the 12th sessions which illustrates a very good improvement in their body fitness. Figure 37 shows mean \pm SEM for RPE for ND in 5 occasions, after 1st, 2nd and 3rd set of resistance training, and after 10 and 20 minutes of aerobic exercise. As in T2D the results show reduction in these 5 occasions across 12th exercise sessions. Post 1st set of resistance, RPE decreased in S12, P=0.503 which not significant. Post 2nd set of resistance, RPE also decreased in S12, (P=0.19) not significant as well. Post 3rd set of resistance, RPE decreased significantly in S12, P=0.07. While post 10 minute of aerobic, RPE decreased but not significantly, P=0.141. Finally, post 20 minute of aerobic, RPE was decreased in S12, P=0.093 which also not significant. There was a reduction in RPE but significant in T2D, not in ND group, which demonstrates that the level of exercise was not as hard as compared to the position before the commencement of the exercise programme. In a previous study, greater RPE was associated with higher lactate, higher heart rate, and patients diagnosed with hypertension (Huebschmann et al., 2015). Their conclusion shows that in older women the exercise effort was greater with T2D than controls (Huebschmann et al., 2015).

4.7.11 Lipid profile

Lipid profile at baseline and after 6 weeks of exercise

Table 16: Lipid profile changes among T2D group. Data expressed as Means± SE, and P value.

	Pre 1st Se Ex	Post 12th Se Ex	P value
TC	4.44± 0.40	4.40± 0.35	0.867
HDL	1.27± 0.21	1.25± 0.14	0.884
TRG	1.58± 0.22	1.58± 0.31	1.000
LDL	2.25± 0.38	2.48± 0.26	0.767
Non-LDL	2.96± 0.31	3.16± 0.27	0.482
LDL/HDL	2.42± 0.48	2.10± 0.20	0.403

Table 17: Lipid profile changes among ND group. Data expressed as Means± SE, and P value.

	Pre 1st Se Ex	Post 12th Se Ex	P value
TC	3.94± 0.26	3.85± 0.40	0.756
HDL	1.50± 0.19	1.55± 0.17	0.301
TRG	1.49± 0.27	1.28± 0.36	0.246
LDL	2.17± 0.10	2.32± 0.27	0.621
Non-LDL	2.67± 0.22	2.63± 0.34	0.891
LDL/HDL	1.57± 0.12	1.69± 0.32	0.780

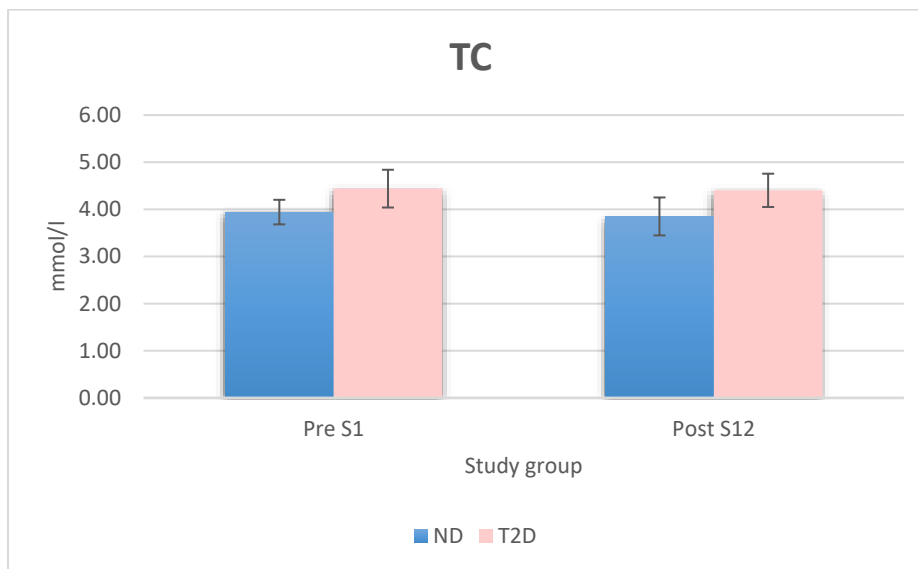


Figure 39: TC changes in both groups

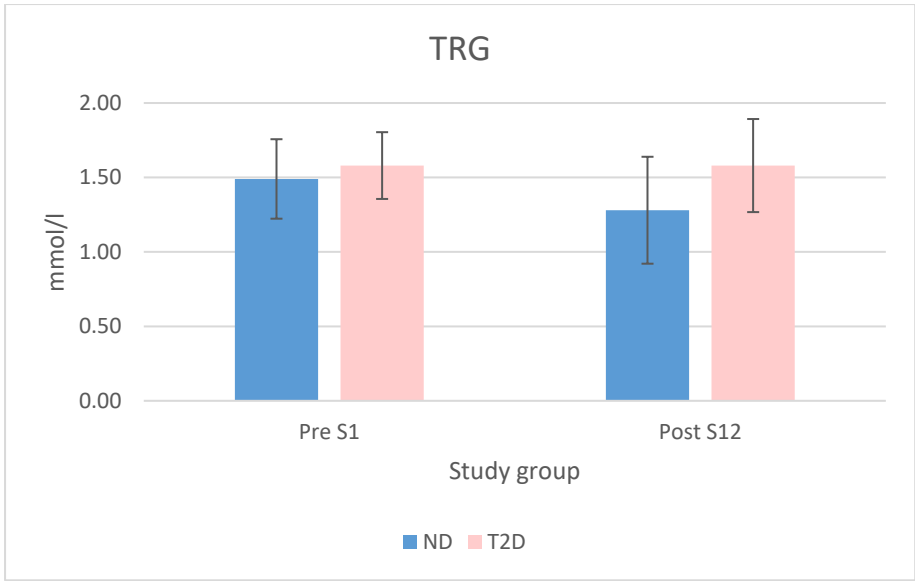


Figure 40: TRG changes in both groups

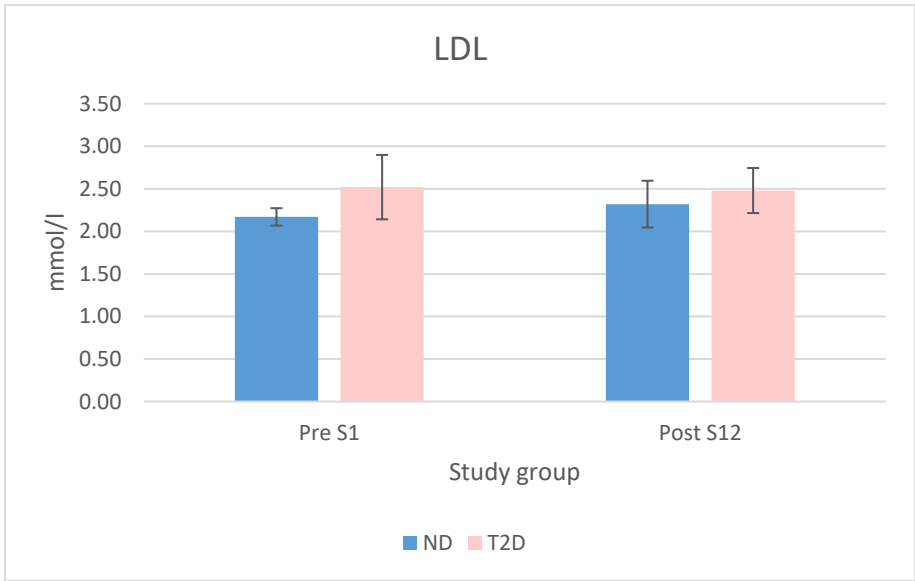


Figure 41: LDL changes in both groups

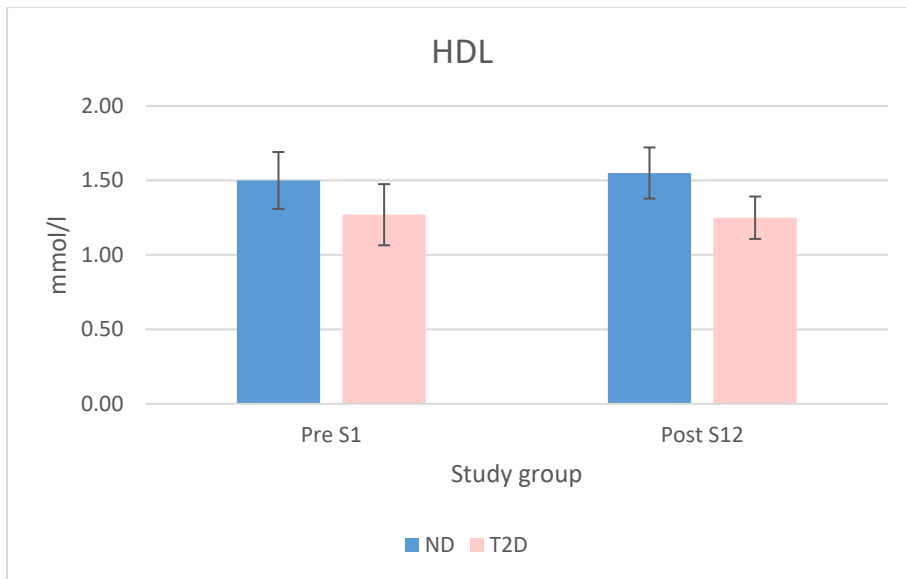


Figure 42: HDL changes in both groups

As shown in (figure38, 39, 40 and 41), in T2D there was a minimal reduction in TC ($P=0.867$), however HDL ($P=0.884$) had been slightly decreased and LDL ($P=0.767$) slightly increased while no changes in TRG ($P=1.000$). Most T2D volunteers use statins which plays role in improving the lipid profile results. All ND volunteer has normal level of lipid profile TC ($P=0.756$), LDL ($P=0.621$), and TRG ($p=0.246$) which shows minimum reduction. While HDL ($P=0.301$) increased slightly. Sigal et al., (2007) has found previously insignificant changes in lipid values which is similar to the current study.

4.8 Conclusion

In conclusion, T2D combination exercise was shown to have a significant effect on HbA1c, and the anthropometric variables (weight, waist, BMI and lung capacity). This finding is in agreement with that reported by Sigal et al., (2007) and Yavari et al., (2012) where the effects of aerobic and resistance training, alone and in combination were shown to have effects on glycaemic control, specifically on HbA1c in T2D patients, although the combined exercise were shown to have greatest effect. In addition, the present study shows a reduction in BG after performing resistance exercise then aerobic exercise when compared to aerobic exercise then resistance exercise. Thus, the order of the exercise is seen to have an effect on glycaemic control, this finding correlates with that reported in the studies of Yardley et al., (2013). Furthermore, the present study showed that combination exercise is valuable in improving insulin sensitivity in T2D where OGTT shows a very significant improvement of BG level in both groups, which was higher in T2D. The studies conducted by Davidson et al., (2009) corroborate these findings by inferring that combination exercise decreases insulin resistance, thus improve insulin sensitivity (Davidson et al., 2009). Other variables observed in T2D included SBP and DBP, and minimal reduction was observed with slight increment at S12. However, the effect on the mean was due to persistent hypertension of one of the volunteers. More so, Resting HR improved significantly after 12th sessions of exercise which is also reported in studies conducted by Maiorana et al., (2002). However, HR reduction after resistance exercise in the present study was minimal which increase slightly at S12. The improvement in 1RM after the six weeks' exercise was significant in T2D for the five different RE exercises working upper and lower muscle group (Chest, Squat, Back, Biceps and Triceps) which illustrates the benefit of combination exercise on body fitness. In ND there was a significant improvement only in squat and back, not in the chest, triceps, and bicep exercise. Similar findings were demonstrated by previous study as both back and squat induced improvements in sprint performance (Seitz, Trajano and Haff, 2014). Many factors can affect results observed such as diet, adherence to anti-diabetes and anti-hypertensive medication and other medications. The improvement in the control group in lipid and HbA1c, BMI and OGTT represent their adherence to medication and diet (Jorge et al., 2011). However, the reduction in lipid profile in this study was not significant, which could be due to the normal range of lipid profile recorded for the ND group prior to the main exercise. Also, the lipid profile in T2D was not elevated before the main study, and individuals were using statin which

makes the result invalid in term of the effect of exercise as an improvement, however, previous study by Gordon et al., (2008) and Kelley and Kelley, (2007) showed that combination of conventional exercise and yoga, and aerobic exercise, respectively, have effects on lipid profile of adults with T2D. In addition, there was a reduction in lactate, however, it was not statistically significant. Although, the study of Crawford et al., (2010) associated plasma lactate with the prevalence of T2D and inferred that the measurement of this variable can be used as a biomarker in observing the decreased oxidative capacity associated with insulin resistance. Finally, this study recommends that the combination exercise programme of resistance and aerobic is valuable for T2D because it has the advantages skeletal muscle strength which is supported by the improvement in 1RM. Previous study showed similar finding that high load resistance exercise improved muscle strength (Schoenfeld et al., 2015). It is also shows improvements in glycaemic control as found also different studies before, however, this study was conducted for 6 weeks which no one else has done it before. Moreover, the new finding in the current study that no other researcher has conduct it before are the GLP-1 comparison within T2D group who are using different medications (chapter 6) and the inflammatory markers which also compare between T2D and ND as well as within T2D who are using different medication (chapter 7).

Chapter 5 The effect of moderate combination exercise (cardio “aerobic” and resistance) on the insulin sensitivity in T2D and ND subjects

5.1 Research question:

Is the insulin sensitivity (IS) improved after 6 weeks of moderate combined exercise? Do the improvements correlate with medication type?

5.2 Aim:

The aim of the study is to investigate the effects of a combined exercise programme AE and RE on blood glucose and improve IS in T2D and ND. Moreover, to assess IS and insulin resistance (IR) by different indices. This study also aimed to assess the IS and IR after 6 weeks of combination exercise and see the effect of different medication on IS in T2D by using different assessment methods.

5.3 Insulin resistance (IR)

Insulin is a peptide hormone which enhances the growth cells and stimulates lipogenesis, glycogen and protein synthesis and prevents glycogenolysis, lipolysis and protein breakdown (Antuna-Puente et al., 2011; Mann and Bellin, 2016). The definition of IR is when the insulin has minimum biological effect on the target tissues (Henríquez et al., 2013). It is considered as the main defect underlying T2D pathogenesis and associated with obesity and METs (Henríquez et al., 2013). It was found in T2D that the pancreas increases secretion of insulin where the subject becomes IR (Lee et al., 2010). In previous studies cardiovascular disease, diabetes, hypertension, hypertriglyceridemia, obesity and physical inactivity have a strong correlation with IR (Dube et al., 2012; Lorenzo et al., 2015; Verheggen et al., 2016; Lee et al., 2010). According to Lee et al., in 1998, a World Health Organization (WHO) consultation report recommended term for this condition, which is “metabolic syndrome” (Lee et al., 2010). Generally, IR starts many years before the diagnosis of T2D and can be considered as a genetic factor (Tangvarasittichai, 2015). Insulin deficiency and IR are the main features of the T2D. Abdominal obesity, modern sedentary lifestyle, cause disturbance in adipokines secretion and then insulin resistance (adipokines will explain more lately in chapter 7). It has

been stated that the accumulation of intramuscular fat leads to insulin resistance and developed T2D (Asano et al., 2014). Recently, several mechanisms have been proposed to explain how obesity and/or FFA can interfere with insulin signalling (Boden, 2011). As shown previously in section (2.7.6) additional glucose from diet enters the Krebs cycle in the mitochondria and produces pyruvate then Acetyl CoA then citrate which converts again to Acetyl CoA and via enzyme HMG CoA reductase this can produce cholesterol (see figure 3) (Mann and Bellin, 2016). Acetyl CoA is able to convert to FFA which then combine to glycerol in cytoplasm to procedure TRG and store it in adipose tissues (Morgantini et al., 2014). The accumulation of TRG causes IR (Boden, 2011). Many substances stored in adipose tissue such as FFA, resistin, TNF and interleukin 6 (Boden, 2011). All these substances can lead to IR when infused in high amount in the body. Nevertheless, these substances should meet 3 criteria to consider as having physiological link between obesity and insulin resistance. The first criteria is that the substance elevated in the blood of obese people. The second that when the blood level of this substance elevated this caused elevation in IR. The third criteria is that when the low blood level of this substance decreased it caused reduction in the IR. Therefore, FFA is only can meet these 3 criteria in human subjects (Boden, 2011). In obesity, FFA levels found elevated in plasma due to the enlargement in adipose tissue mass which secretes more FFA. The clearance of FFA might reduce as well. Furthermore, in plasma if the FFA levels are high that prevent the anti-lipolytic action of insulin which will promote high production rate of FFA into the circulation (Boden, 2011). In the body after infusing lipid emulsion, there is an acute elevation of FFA into circulation which reduces insulin stimulated glucose uptake mainly in skeletal muscle. IR occurs within 2-4 of infusion and then disappear after 4 hours of elimination (Boden, 2011). Elevated FFA have been found caused an impairment in insulin signalling which leads to deficiency in stimulated glucose transport and/or phosphorylation. The elevation of FFA intracellularly cause reduction in GLUT4 translocation to the plasma membrane which decreases the sensitivity of insulin receptors (IRS) to insulin and then insulin resistance (Ceriello and Motz, 2004; Nolan et al., 2015; Boden, 2011). It is known that plasma FFA are move into cells and oxidize to produce ATP or store as TRG. As a result, high FFA levels in the circulation cause accumulation of TRG intracellular (Boden, 2011). High levels of FFA, glucose and insulin stimulates over secretion ROS, which correlates with oxidative stress and causes acceleration of the onset of T2D (Tangvarasittichai, 2015).

The mechanism of insulin resistance occurred in the mitochondria by enhancing the superoxide release on mitochondrial electron transport chain (Ceriello and Motz, 2004; Tangvarasittichai, 2015; Boden, 2011). That by over production of FFA and glucose in the circulation leads to high TG in the muscle and adipose tissue which increased the secretion of AcetylCoA. AcetylCoA then enters the citric acid cycle and elevates the generation of electron donor (NADH) which increases membrane potential (H). High production of NADH that is unable to degenerate by oxidative phosphorylation, the proton gradient in the mitochondrial increases and single electrons are transported to oxygen which causes the production of free radicals superoxide (Ceriello and Motz, 2004; Tangvarasittichai, 2015; Boden, 2011). FFA consider as a pro-inflammatory mediator that activated the nuclear factor- κ B (NF- κ B). NF- κ B is a protein complex and plays an important role in regulating the immune response to infection, so irregulation of NF- κ B has been associated with cancer, inflammatory and autoimmune diseases (Tangvarasittichai, 2015). NF- κ B leads to overproduction of ROS and minimise glutathione production (Tangvarasittichai, 2015). It has been proves that vitamin E supplementation prevents the activation NF- κ B by FFA (Tangvarasittichai, 2015). Antioxidants help in protection β -cell from the oxidative stress and inhibit its toxicity, inhibit activation of NF- κ B and minimise the production of AGEs. Examples of antioxidants are zinc, N-acetyl cysteine (NAC), aminoguanidine and α -phenyl-tert butylnitrone (Tangvarasittichai, 2015). In the normal physiological conditions, antioxidants in the cell reduce the level of ROS (Tangvarasittichai, 2015). In hyperglycaemia in T2D, the increased level of glucose stimulates oxidative mechanisms and minimizes the antioxidant defence system (Tangvarasittichai, 2015). In addition to which the body fails to counteract the elevated generation of reactive oxygen species (ROS) (Tiwari, 2013). This leads to an imbalance between ROS and its protection, and this causes oxidative stress and cellular injury (Verdile et al., 2015). Although ROS performs several regulatory functions in cells, uncontrolled or overproduction of ROS has adverse effects (Verdile et al., 2015). It should be noted that ROS levels are increased in cases of obesity, mainly abdominal obesity (Tangvarasittichai, 2015). Oxidative stress mediates IR and impaired intolerance glucose tolerance as well as the development of diabetes, which consequently leads to atherosclerotic complications as well as micro- and macrovascular complications (Tiwari, 2013; Verdile et al., 2015).

Reactive nitrogen species (RNS) and ROS have negative regulation effects on the process of insulin regulation associated with the prevalence of resistance to insulin and T2D (Verdile et al., 2015). The elevation of oxidative stress acts as a risk factor in causing β -cell dysfunction, IR, dyslipidemia, impaired glucose tolerance and finally developing T2D (Tangvarasittichai, 2015). Hyperglycemia leads to increased production of hydrogen peroxide (H_2O_2), as well as the down-regulation of the gene that expresses catalase “antioxidant enzyme” (Patel et al., 2013). Oxidative stress in T2D causes various adverse impacts on the physiology of cells. These include lipid peroxidation. ROS target lipids, where hydroperoxides cause toxicity on cells via degradation. Reactions between hydroperoxides with copper and iron form malondialdehyde (MDA), which damages cell membranes. Change in the structure and metabolism of lipids is reported in diabetes specifically in cases of vascular complications (Tiwari, 2013). Increased MDA levels in individuals with diabetes suggests that injury due to peroxidation is implicated in diabetic complications. Increased lipid peroxidation indicates a drop in non-enzymatic and enzymatic antioxidants' defence mechanism (Saddala et al., 2013). Secondary complications of diabetes such as neural disorders and atherosclerosis are induced by lipid peroxidation (Ramesh et al., 2012). Consistently, an increase in lipid peroxidation shows a close association with high levels of glucose in patients with diabetes (Bandeira et al., 2012). Proteins are also potentially targeted by ROS (Tiwari, 2013). The product of protein oxidation is carbonyls, and these are biomarkers of the existence of oxidative stress (Tiwari, 2013). An increase in the level of content of protein carbonyl (PC) and malondialdehyde (MDA) are reported in the cells and plasma of the patients with diabetes. PC is a marker of protein oxidative damage, while MDA is a marker of lipid oxidative damage (Deokar, Jagtap and Yerawar, 2016). An increase in PC and MDA among the patients with diabetes are important in the pathogenesis of diabetes complications (Deokar, Jagtap and Yerawar, 2016). Catalase is another biomarker of oxidative tension in T2D. Shortage of catalase causes the β cells in the pancreas to experience oxidative stress through the production of excess ROS, which leads to dysfunction in β cells and eventually diabetes (Tiwari, 2013). Furthermore, superoxide dismutase (SOD) is involved in protecting against histological and cellular damage due to ROS (Patel et al., 2013). (Wang et al., 2011) have indicated that when SOD is over-expressed, it prevents diabetes by overcoming oxidative stress, minimising (ROS), and increasing antioxidant enzymes. Finally, oxidative stress also affects glutathione (GSH) levels in patients with diabetes by lowering the GSH level (Calabrese et al., 2012). Indeed, the

complications associated with lowered GSH in the plasma include inflammation and hyperlipidemia in patients suffering from diabetes (Das, Vasan and Sil, 2012). The accumulation of FFA leads to the inhibition of glucose oxidation, glycogen synthesis, and elevate the production of advanced glycation end product (AGE) (Nolan et al., 2015). Elevation of FFA and glucose levels has been found to stimulate inflammatory markers as a result of elevation of oxidative stress, ROS and reduced antioxidants (Tangvarasittichai, 2015). Pro-inflammatory markers are detected in different diseases such as T2D, atherosclerosis, cancer and aging (Tangvarasittichai, 2015). Numerous clinical trials have found that some antioxidants medication could improve insulin sensitivity in insulin-resistant subjects, such as glutathione vitamin C, or vitamin E (Ceriello and Motz, 2004; Tangvarasittichai, 2015). If there is mild postprandial hyperglycaemia, hyperinsulinaemia and insulin resistance, this can be considered as an impaired glucose-tolerant state (Codario, 2011). This conclude that high fat diet and obesity are linked to insulin resistance, inflammation and T2D.

5.4 Factors stimulated by insulin resistance

Many factors are stimulated by IR such as enhanced lipolysis i.e. adipose cells produce free fatty acids, inhibition of glucose transport and phosphorylation (Antuna-Puente et al., 2011; Codario, 2011). Moreover, reduction of glucose oxidation rate and glycogen synthesis, increased apolipoprotein B secretion and hepatic lipase activity (Antuna-Puente et al., 2011; Codario, 2011). As a result, high levels of free fatty acids prevent insulin secretion from the beta cells, which leads to decreased IS in the muscles and liver. IR and T2D are characterized by dyslipidaemia which is a main risk factor for CVD (Tangvarasittichai, 2015). Eating high fat diet, creates a chylomicron rich in TG which triggers elevation of hepatic FFAs and VLDL TG-rich particles secretion. That influence metabolism of HDL-C by exchange with TG- rich lipoproteins via cholesteryl ester transfer protein to release particles of HDL consists of high concentrations of TG (Tangvarasittichai, 2015). Moreover, hepatic lipase hydrolysed the particles of HDL-TG to HDL and TG. The HDL molecule is very small which facilitates its excretion from the kidney. This elevation of TG and reduction of HDL leads to dyslipidaemia (Tangvarasittichai, 2015).

5.5 Assessment of methods and indexes of insulin sensitivity

IR linked to obesity, dyslipidemia, hypertension, atherosclerotic vascular disease, stroke and coronary heart disease and plays crucial role in the pathophysiology of diabetes (Patarrão, Lautt and Macedo, 2014; Gutch et al., 2015). The β -cell secretes Insulin and C-peptide at equivalent amount in the plasma. Therefore, the level of insulin secretion can be estimated from plasma C-peptide level which is a marker of insulin secretion (Dickson et al., 2016). Nevertheless, peripheral insulin has shorter half-life than C-peptide which affect accuracy of assessing insulin secretion by measuring C-peptide (Ajmera et al., 2013). Thus, it is very essential to measure IS and IR in human (Patarrão et al., 2014; Gutch et al., 2015). Berson and Yalow won the Nobel Prize by discovering a technique to measure blood insulin concentration in the late 1950s (Lee et al., 2010). Recently, there are several methods and indexes to assess IS and IR (Patarrão et al., 2014; Gutch et al., 2015). These methods assist in investigating the pathophysiology and epidemiology and the effect of therapeutic agents and indeed the best way of assessing the progression in human from normal glucose tolerance (NGT) to T2D is by measuring the IS (Davidson, Docherty and Chase, 2014) (Patarrão et al., 2014). There are diverse glucose tolerance tests have been used to diagnose prediabetes and diabetes subjects. Examples of these diagnostic tests, intravenous glucose tolerance test (IGTT), oral glucose tolerance test (OGTT) and meal glucose tolerance test (MTT). It is used to assess IS, glucose effectiveness (*SG*), β -cell function and insulin secretion (Ajmera et al., 2013). Furthermore, different physiology-based indexes are used as well for diagnosis e.g. Matsuda, HOMA, QUICKI and CIGMA (Ajmera et al., 2013). Currently, the best consistent methods to quantify IR are hyperinsulinemic euglycemic clamp (HIEC) and intravenous glucose tolerance test (IGTT) (Gutch et al., 2015). Different indices have been used in different situation and so for clinical uses the best indices that are applicable be use are HOMA, QUIKI and Matsuda, while in the epidemiological studies the suitable indices are McAuley, Belfiore, Avignon, Cederholm, HES and Stumvoll (Gutch et al., 2015).

5.6 Methods of insulin sensitivity/resistance assessment

5.6.1 Hyperinsulinemic Euglycemic Clamp (HIEC)

The HIEC test is established by DeFronzo, it is known as the “gold standard” test (Muniyappa, Madan and Quon, 2015; Patarrão et al., 2014; Davidson et al., 2014). This test needs 2.5- 4 hours to be done as well as it needs to draw up to 20 blood samples (Davidson et al., 2014). In this test, insulin should be infused to fasting individuals at an infusion rate from 5 – 120 mU/m²/min (dose per body surface area per minute, through 180 minutes). The advantage of HIEC is that it quantifies the disposal of the whole body glucose at a certain level of insulinemia in steady-state condition, so direct measure insulin at steady state condition (Gutch et al., 2015). HIEC has some limitations as it is expensive, needs intravenous infusion, requires many workers who had very good experience, frequent blood samples and time consuming.

Equation: IS Clamp = $MG \times \Delta I$

M is normalized for G (steady-state blood glucose concentration)

ΔI is (the difference between fasting and steady-state plasma insulin concentrations)

5.6.2 Insulin tolerance test (ITT)

ITT is a good method to measure insulin resistance and has a good correlation with the frequently sampled intravenous glucose tolerance test (Sin et al., 1996). It is a valid and useful test for evaluating IS in diabetes individual, even after insulin treatment (Okita et al., 2014). It can be conducted by infusion intravenous insulin at rate 0.1 IU/kg for fasting individuals. Blood samples collected at 9 times points (2 samples before infusion and 7 after). After half an hour, intravenous glucose should be injected to avoid any hypoglycaemia. IS can be measured in the individuals as the faster the decrease in blood glucose, the better the IS. The equation is to measure the linear decline in plasma glucose (KITT) by dividing 0.693 by the plasma glucose half-life (the reduction by 50% from baseline):

Equation: $KITT = 0.693 / t_{1/2} \times 100$

5.6.3 Insulin suppression test (IST)

IST methods developed by Shen et al. in 1970 and later amended by Harano et al. which is quantifies directly IS and IR (Patarrão et al., 2014; Muniyappa et al., 2015; Bonadonna et al., 2018; Kim et al., 2016). This test is used to suppress insulin and glucagon endogenous secretion. The IST conducted by the infusion of somatostatin (250 µg/h) or octreotide “somatostatin analog” (25 µg bolus then intravenous infusion 0.5 µg/min) (Patarrão et al., 2014; Muniyappa et al., 2015). Intravenous glucose (240mg/m²/min) and intravenous insulin (25 mU/m²/min) infused at the same time over 3 hours. Every 30 min the blood samples should be taken over 2.5 hours. The steady state of plasma insulin (SSPI) and glucose (SSPG) started from 150 minutes to 180 minutes of IST, thus the blood samples should be then taken every 10 minutes at that time (Patarrão et al., 2014; Muniyappa et al., 2015). The advantage of (SSPI) and (SSPG) that it needs few numbers of workers, less technically demanding than HIEC and measures directly the metabolic action of insulin. IST has some limitations such as it needs intravenous infusion, expensive and time consuming (Patarrão et al., 2014; Muniyappa et al., 2015).

5.6.4 Continuous infusion of glucose with model assessment (CIGMA)

CIGMA methods needs intravenous infusion of glucose, then IS should be measured near steady state of insulin and glucose concentrations (Patarrão et al., 2014). CIGMA acts as postprandial insulin and glucose concentration (Patarrão et al., 2014). This test quantify IS, glucose tolerance and β-cell function, thus it is used as diagnostic test in clinical setting (Ajmera et al., 2013; Patarrão et al., 2014).

5.6.5 Minimal model analysis of frequently sampled intravenous glucose tolerance test (FSIVGTT)

Bergman, Cobelli and colleagues have been developed the FSIVGTT model in 1979 (Patarrão et al., 2014). Intravenous bolus glucose should infused to fasting individual at (0.3g/kg bw) over 2 min, then after 20 min an insulin (4 mU/kg/min) should infused over 5 min. In the modified FSIVGTT mode, infuse calcium channel blocker like tolbutamide after 20 min of glucose administration to stimulate the secretion of endogenous insulin (Patarrão et al., 2014;

Muniyappa et al., 2015). Blood samples collected at 2 time points (0 and 180 min after the administration of glucose) (Patarrão et al., 2014; Muniyappa et al., 2015). This model has two equations, one to quantify plasma insulin dynamics and the other one to assess plasma glucose dynamics (Patarrão et al., 2014) (Patarrão et al., 2014; Muniyappa et al., 2015). It has some advantages over HIEC, because it is easier, the IV bolus doesn't need adjustment of infusion and does not required steady state condition. Moreover, the assessment of (*SI*), (*IG*) and β -cell function can be easily achieved from a single dynamic test (Patarrão et al., 2014; Muniyappa et al., 2015). However, FSIVGTT model requires multiple sampling, IV injection, time consuming (3 hours), and requires extensive labor workers similar to IST and HIEC (Patarrão et al., 2014; Muniyappa et al., 2015).

5.6.6 Oral Glucose Tolerance Test (OGTT)

The OGTT is very simple test and probably the most common test that has been used as diagnostic test in the clinical practice (Ajmera et al., 2013; Patarrão et al., 2014; Muniyappa et al., 2015). Fasted subjects have to drink oral glucose load (75g), then blood samples at (0, 30, 60, and 120 minutes). Comparing OGTT with HIEC, IST and FSIVGTT it demonstrates that the OGTT mimics the physiological conditions of glucose and insulin dynamics more than other tests (Patarrão et al., 2014; Muniyappa et al., 2015). The advantages of OGTT over HIEC and FSIVGTT that it is simple, and cheap so in clinical setting it is considered the test of choice (Patarrão et al., 2014; Muniyappa et al., 2015). OGTT has some limitations, such as the data obtained not showing enough evidence of glucose and insulin dynamics action. However, using OGTT data in some indices gives best prediction of IS and β -cell function (Patarrão et al., 2014; Muniyappa et al., 2015). It can be adapted to give more data points.

5.6.7 Meal Tolerance Test (MTT)

MTT has been developed by some authors to assess IS, glucose effectiveness and insulin secretion (Patarrão et al., 2014). A meal (liquid or solid) consists of CHD, protein and fat is ingested by fasting subjects and then glucose and insulin measurement should be taken during 2 hours (Patarrão et al., 2014). MTT does not have an artificial post-load hypoglycaemia which presented an advantage to the test. Using different models to quantify (*SI*), (*IG*), insulin secretion, β -cell function and incretins is achievable (Patarrão et al., 2014).

5.6.8 Rapid Insulin Sensitivity Test (RIST)

RIST is new test which have been developed and used in human, mice, cats, and rats. Insulin is infused to the subjects by (50mIU/kg) over 5 minutes and then check glucose samples after 1, and 2 minutes, as well as take arterial venous blood samples at 5 minutes time (Patarrão et al., 2014). After that start intravenous infusion of glucose to avoid hypoglycaemia (Patarrão et al., 2014).

5.7 Simple surrogate indexes for insulin sensitivity/resistance

5.7.1 Homeostasis Model Assessment (HOMA)

HOMA is used to assess insulin resistance and β -cell function from basal (fasting) glucose and insulin concentration; and it was established in 1985 by Matthews et al. (Gutch et al., 2015; Patarrão, Lutt and Macedo, 2014; Muniyappa, Madan and Quon, 2015; Abbasi et al., 2018). The glucose level are controlled by insulin-dependent hepatic glucose production, whereas the level of insulin depend on the response of pancreatic β -cell to glucose concentrations. Therefore, any defect in β -cell function leads to a lower response to glucose-stimulated insulin secretion. Similarly, the reduced suppressive effect of insulin on hepatic glucose production shows insulin resistance (Patarrão et al., 2014). HOMA has some advantages such as it is a simple index, shows fasting steady state of glucose and insulin levels, requires only one fasting blood sample, less cost, minimum needs of technical expertise and minimally invasive (Gutch et al., 2015; Patarrão et al., 2014; Muniyappa et al., 2015). HOMA is a durable tool and widely used in clinical setting, research and epidemiological studies (Gutch et al., 2015; Patarrão et al., 2014; Muniyappa et al., 2015).

Equation by Matthews et al.: $IR_{HOMA} = (I0 \times G0) / 22.5$

5.7.2 Quantitative insulin sensitivity check index (QUICKI)

QUICKI quantifies the fasting blood glucose and insulin concentrations mainly in obese and diabetes in a linear association of insulin sensitivity and glucose clamp (Gutch et al., 2015). QUICKI advantages is that it is accurate index of IS, reliable, requires only one fasting blood samples, less cost, minimum needs of technical expertise and minimally invasive. The limitation here are that because of inter laboratory differences in insulin assay, QUICKI requires varying normal levels to be developed for each laboratory (Gutch et al., 2015; Patarrão et al., 2014; Muniyappa et al., 2015).

Equation: $QUICKI = 1 / (\log I0 + \log G0)$

5.7.3 McAuley index

The McAuley index requires different data such as fasting blood insulin concentration and TRG (Gutch et al., 2015; Abbasi et al., 2018). This index is used to quantify IR in normoglycemic subjects. This index showed an advantage of given best result of IR by using both fasting insulin and TRG. The limitation is that it is a laborious method and only applicable for epidemiological studies (Gutch et al., 2015).

$$\text{Equation: McAuley} = e^{(2,63 - 0,28 \ln(I0) - 0,31 \ln(TAG0))}$$

5.7.4 Matsuda index

Matsuda index was developed by Matsuda and DeFronzo (Gutch et al., 2015). It is considered as a novel index which quantifies the whole body IS index (WBISI) from OGTT in a simple way (Gutch et al., 2015). The data consist of the collected blood samples while fasting and then during the 2 hours of the test which shows peripheral tissues and hepatic IS. It measures insulin (mIU/l) and glucose (mg/dl) concentrations at 4 times points (0, 30, 60 and 120 minutes) (Gutch et al., 2015). This index had some advantage of predicting T2D onset, by using data of blood insulin and glucose concentration at 30 minutes of OGTT to measure IS with $(\Delta I_{0-30}/\Delta G_{0-30}$ or $\Delta I_{0-120}/\Delta G_{0-120})$ and $ISI_{(Matsuda)}$ or modified $ISI_{(Matsuda)}$ (Patarrão et al., 2014; Gutch et al., 2015). Moreover, it is represent hepatic and peripheral tissue sensitivity to insulin (Gutch et al., 2015).

$$\text{Equation: Matsuda} = 10000/\text{sqr}(\text{fasting I} * \text{Fasting G}) * (\text{Mean I} * \text{Mean G})$$

5.7.5 Belfiore index

Primarily, Belfiore index is used to measure IS. It requires blood samples of basal insulin and glucose concentration (fasting state) as well as the mean value of the insulin and glucose level during the OGTT test (Gutch et al., 2015). This index compares the defined normal reference value with specific time points for insulin and glucose values at (0-1-2 h or 0-2 h) (Gutch et al., 2015). This formula can be possible quantify IS which located between 0 and 2, so in individuals who have normal IS it will be around 1 (Patarrão et al., 2014). Any index higher than 1.27 represents a pathological IR (Gutch et al., 2015). The advantage of Belfiore that it represented the values of insulin and glucose concentration at basal state (fasting) as well as

the mean concentration during OGTT. The requirement of multiple blood samples give limitation to this index (Gutch et al., 2015).

$$\text{Equation: Belfiore} = 2 / (\text{AUC (I(mIU/ml))} * \text{AUC(G(mg/dl))}) + 1$$

5.7.6 Cederholm index

Cederholm index developed by Cederholm and Wibell. Mostly, it shows the peripheral IS and glucose uptake in skeletal muscle during OGTT because of the main role of peripheral tissue in the disposal of glucose (Patarrão et al., 2014; Gutch et al., 2015). This index requires blood samples in two time points (0 - 2 h) during OGTT. In normal non-obese subjects, the value of IS around $79 \pm 14 \text{mgI}^2/\text{mmol/mIU}/\text{min}$. While in obese, pre-diabetes and T2D the value is less (Patarrão et al., 2014).

$$\text{Equation: Cederholm} = 75000 + (\text{G0} - \text{G120}) * 1.15 * 180 * 0.19 * \text{m}/120 * \text{Gmean} * \log(\text{Imean})$$

5.7.7 Avignon index

Avignon index has been developed by Avignon et al. in 1999, which required collecting blood samples at basal "fasting state" (Sib), after 2 hours of OGTT (Si2h) and then measure the IS at these two time points Sib and Si2h as well as measuring the average of Sib and Si2h which is (SiM) (Patarrão et al., 2014; Gutch et al., 2015). Avignon is a simple test which achieve glucose tolerance and insulin sensitivity in single test (Gutch et al., 2015).

$$\text{Equation: Avignon} = 10 / (\text{I0(mU/L)} * \text{G0(mmol/L)} * \text{VD})$$

5.7.8 Stumvoll index

Stumvoll et al developed this index which quantify IS during the OGTT by measuring plasma insulin and glucose concentration, BMI and age (Patarrão et al., 2014; Gutch et al., 2015). This laborious techniques requires multiple sampling at (0, 1 and 2h) of the OGTT (Patarrão et al., 2014; Gutch et al., 2015). It has some advantage that it gives accurate result by using demographic data (age, BMI and sex) as well as the plasma glucose and insulin concentration (Gutch et al., 2015).

Equations with or without demographic data:

$$\text{Stumvoll} = 0.222 - 0.00333 \times \text{BMI} - 0.0000779 \times \text{I120} - 0.000422 \times \text{age}$$

$$\text{Stumvoll} = 0.156 - 0.0000459 \times \text{I120} - 0.000321 \times \text{I0} - 0.00541 \times \text{G120}$$

5.8 Effects of exercise on the insulin sensitivity

Regular exercise shows benefit in reducing and reversing T2D (Dube et al., 2012). Therefore, regular exercise consider as the best choice for the prevention of diabetes (Dube et al., 2012). IR defined if the individual has these values; Matsuda <4.3 McA ≤ 5.8 , HOMA ≥ 2.6 and QUICKI ≤ 0.33 . While IS defined when the subject has Matsuda ≥ 4.3 McA > 5.8 , HOMA < 2.6 and QUICKI > 0.33 (Menik and Palangasinghe, 2006; Gutch et al., 2015). It has been found that IS improved by exercise because it has insulin sensitizing properties (Ortega et al., 2014; Jorge et al., 2011). When muscle contracted during exercise it helps GLUT-4 to transfer to cell membrane (Kjobsted et al., 2017; Jorge et al., 2011). Insulin-stimulated glucose uptake has been improved in skeletal muscle after a single bout of exercise in both IR and ND muscle (Kjobsted et al., 2017). It has been found that there is a link between improving IS and improving inflammatory markers which explain the association of IR and the presence of low grade inflammation (Verheggen et al., 2016).

A study by Whyte et al. 2013 was aimed to see the effect of single session of sprint interval training (SIT) and a single extended sprint (ES) on metabolic biomarkers and IS (Whyte et al., 2013). The participants were men, overweight/obese (n=10). All of them go through three 2 days randomized trial. The first OGTT was performed before the 1st day of exercise and before that the metabolic measurement has been taken to assess resting metabolic rate and rate of fat and carbohydrate oxidation. On the first day, the volunteers divided in three groups (CON= control), (SIT= four maximal 30-s sprints, with recovery period of 4.5 min in between each set), and (SE= single maximal extended sprint. On the second day, all individual should go through OGTT test to measure FBG, 2hrs post glucose load and blood pressure (Whyte et al., 2013). The plasma samples used to analyse lipid profile and glucose and insulin concentration by using enzyme-linked immunosorbent assay (ELISA). Moreover, IS index was measured by using Matsuda method and assess insulin resistant by Homeostasis Model Assessment (HOMA-IR) method (Whyte et al., 2013). The results shows improvement in ISI when comparing to CON, it was significantly higher in ES group by (44.6%, $p= 0.022$) as well as HOMA-IR was significantly lower by (31.8%, $p=0.043$). In the other hand, the differences between CON and SIT in ISI (1.5% higher, $p=0.208$) and HOMA-IR (18% lower, $p=0.556$) was not significant. The BP results show no significant changes in both systolic and diastolic BP

between groups (Whyte et al., 2013). In conclusion, the main outcome was the significant improvement in ISI and fat oxidation in the SE group (Whyte et al., 2013).

Older men and women (50-80 years) were participating in a study by Prior et al., in Baltimore, Maryland (Prior et al., 2015). All participants had diabetes or CVD and planned to do 6 months AE. OGTT have been taken at baseline and then after 2 weeks of stopping last AE session. AE individuals were used motorized treadmills 3 times weekly, each session take 45 min (Prior et al., 2015). IS was measured by using HIEC after fasting for 12 hours. Muscle biopsies have been taken from the right vastus lateralis immediately before HEIC and 2 hrs after infuse insulin to quantify GS activation, glycogen content and CS activity. Immunohistochemistry were used to assess capillaries in the muscle. Furthermore, GLUT4 was identified to compare basal sample and 2 hrs post insulin infusion. Subjects used indirect calorimetry while exercising on treadmill to calculate Vo₂max. The changes in IS after 6 months increased by 25% and contributes in the enhancement of GLUT4 and skeletal muscle CD (Prior et al., 2015). It shows a correlation between the reduction of inflammatory cytokine, improve glucose phosphorylation and the sustained improvement of IS (Prior et al., 2015). This conclude that after AE the elevation of skeletal muscle CD is higher and that correlate to improvement in the metabolism of glucose and glucose transporter (Prior et al., 2015). Many factors contribute to improve IS after aerobic exercise such as elevated GLUT-4 appearance in skeletal muscle, AMPK expression and insulin activation of glycogen synthesis (Prior et al., 2015).

Another study was conducted by Fenicchia et al. (2004) to assess the influence of acute and chronic resistance training on glucose and insulin responses to an oral glucose tolerance test in women with T2D (Fenicchia et al., 2004a). Participants performed RE session for 50 minutes for 3 non-consecutive days per week for 6 weeks. Baseline measurement for body compositions has been taken before first exercise session and then at the end of 6 weeks. The OGTT was completed in 3 occasions, first one before starting the exercise programme, the second one after the acute exercise and finally the third one after the last session in week 6. (Fenicchia et al., 2004b) shows the results after 6 weeks in the body composition, in diabetic group the fat mass decreased significantly ($P < 0.01$), but, this reduction was not significantly matched by an improvement in glucose AUC ($r = 0.3$, $P > 0.05$). The result of OGTT shows that the first test before starting the RE was significantly higher in diabetic group (9.1 ± 1.3

mmol/L) than the control group (5.3 ± 0.7 mmol/L, $P < 0.05$). In the control group, the results showed no differences after exercise. This study suggested that there was no chronic effect on the insulin sensitivity after 6 weeks of RE (Fenicchia et al., 2004b).

This study aimed to assess the insulin sensitivity (IS) after 6 weeks of combination exercise and see the effect of different medication on IS in T2D by using different assessment methods.

5.9 Assessment of insulin sensitivity from OGTT:

Methods that directly assess insulin sensitivity and resistance are expensive, complex and invasive (Lorenzo et al., 2015). Accordingly, researchers developed number of surrogates indices to quantify IS and IR from OGTT alone or with other metabolic variables (Lorenzo et al., 2015). The aim of this study also is to assess insulin sensitivity by different methods. Three indices has been used in this study to assess IS values, HOMA-IR, QUICKI and Matsuda index. As discussed previously (section 5.5) these three indices are the most useful in clinical practice. It has also strong correlation with HIEC the gold standard method in assessing IS, while in this study OGTT method has been used instead. Ortega, 2014 found that Matsuda IS index represent both peripheral and hepatic insulin sensitivity (Ortega et al., 2014). In this study T2D and ND participants have been conducted in doing moderate combination exercise twice a week for 6 weeks to assess the insulin sensitivity from OGTT in three occasions, before the volunteer starts 1st exercise session, after 1st exercise session and then finally after finishing 12th exercise session.

5.10 Method

5.10.1 Study design

In each exercise session the participant performed a combined exercise program consists of 30 min of resistance exercise followed by 20 min moderate cycling. This is done twice a week for 6 weeks. At the beginning of each session the participants have to stretch-up for 11 steps of stretching, then he/she must cycle for five minutes to warm up. The RE consist of 3 sets, in each set the volunteer performed (squat, chest, back, biceps and triceps) 10 times. Blood samples collected at base line after these sessions (S1, S2, S4, S6, S8, S10 and S12), centrifuge and refrigerate the plasma to be analysed later by a commercially-available enzyme-linked immunosorbent assay (ELISA) with <0.01 cross reactivity with pro-insulin to measure insulin level as explained before in (section 3.9.1).

5.10.2 Intervention group

The intervention group are T2D (n=11) of five sub-groups and ND (n=8) who are doing combination exercise.

Intervention group are classified in five groups

- 1- T2D volunteers with no medication.
- 2- T2D volunteers on Metformin only.
- 3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4-I).
- 4- T2D volunteers on Metformin and SGT2-I.
- 5- Non-diabetes (ND).

Medication	Number of Volunteers
Pravastatin 40mg OD	1
Atorvastatin 20mg OD	3
Metformin 500 mg BD	3

Metformin 850 TDS	1
Metformin 1000 mg BD	2
Sitagliptin 50 mg OD (DPP4-I)	1
Sitagliptin 100mg OD (DPP4-I)	2
Empagliflozin 10 mg OD	2

5.11 Results and discussion:

The data of T2D and ND participants have been used to analyse insulin level by ELISA and BG by using FPP during OGTT. Data are analysed by using Excel 2010 and SPSS (version 22), Values were expressed as mean with standard error of mean. Pre and post exercise samples were compared using a paired-samples t-test. The level of statistical significance ($P < 0.05$).

5.11.1 OGTT and ISI in T2D and ND

5.11.1.1 Results of BG and insulin in T2D and ND

Table 18: BG level from OGTT comparisons between pre-exercise, post 1st session of exercise and post 12th session of exercise for T2D on combination exercise. Data expressed as Means \pm SEM and P value.

Time point (Minutes)	Pre Ex (1 st OGTT)	Post S1 (2 nd OGTT)	Post S12 (3rd OGTT)
0	8.7 \pm 0.6	8.1 \pm 0.9	6.9 \pm 0.2
30	14.4 \pm 1.2	13.4 \pm 1.9	12.1 \pm 0.7
60	17.3 \pm 1.4	15.6 \pm 2.1	14.2 \pm 0.9
120	15.1 \pm 1.4	12.1 \pm 1.9	10.2 \pm 1.0
AUCs	30.55 \pm 2.5	27.6 \pm 3.6	24.4 \pm 1.9

P value by comparing (Pre Ex & Post S1) = 0.000 which is < 0.05

P value by comparing (Pre Ex & Post S12) = 0.000 which is < 0.05

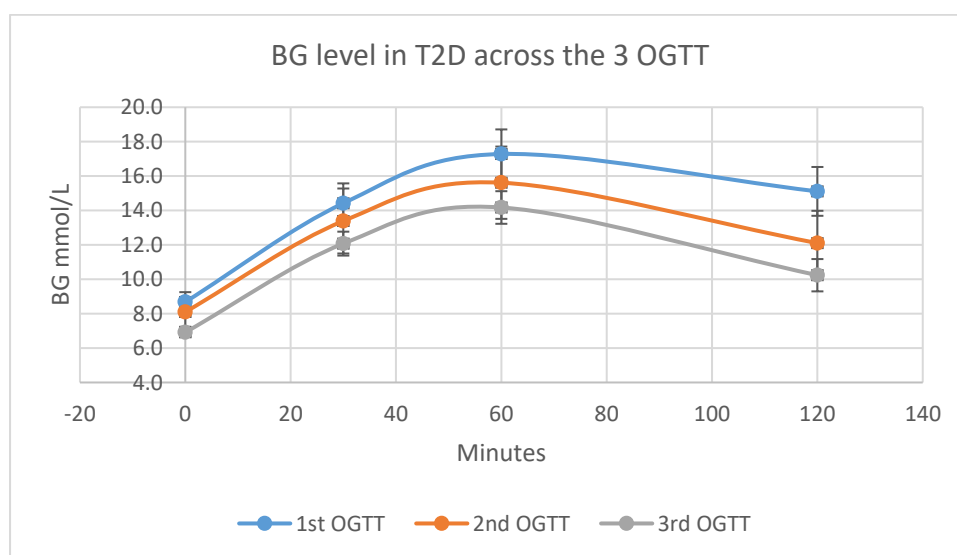


Figure 43: BG level during the 3 OGTT in T2D

Table 19: Insulin concentration (mU/L) from OGTT comparisons between pre-exercise, post 1st session of exercise and post 12th session of exercise for T2D on combination exercise. Data expressed as Means± SEM and P value.

Time point (Minutes)	Pre Ex (1 st OGTT)	Post S1 (2 nd OGTT)	Post S12 (3rdOGTT)
0	55.75± 12.31	45.50± 12.19	26.89± 9.21
30	221.34± 71.65	229.34± 69.83	68.24± 24.76
60	226.17± 82.88	168.44± 36.78	66.84± 24.16
120	183.20± 57.28	180.87± 62.73	63.98 ± 21.68
AUCs	94.5± 31.6	85.2± 24.0	30.1± 10.7

P value by comparing (Pre Ex & Post S1) = 0.363

P value by comparing (Pre Ex & Post S12) = 0.031

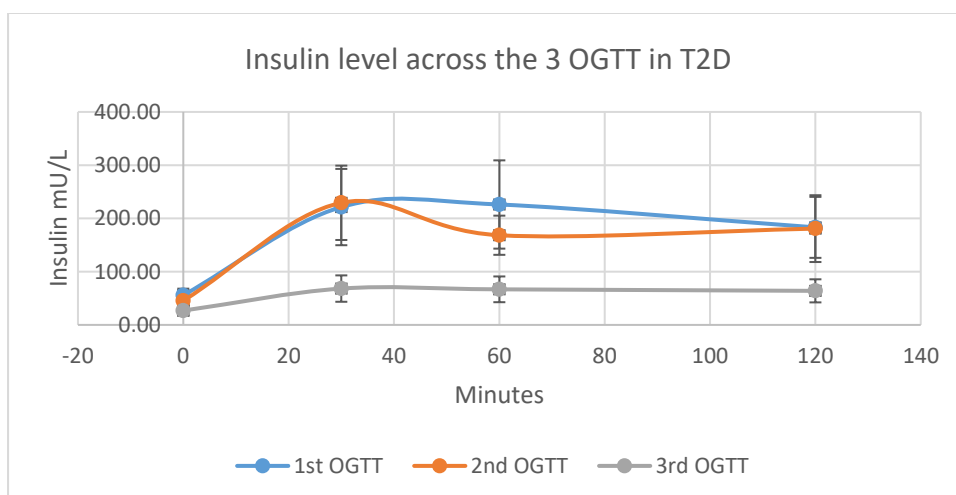


Figure 44: Insulin level during the 3 OGTT in T2D

Table 20: BG level from OGTT comparisons between pre-exercise, post 1st session of exercise and post 12th session of exercise for ND on combination exercise. Data expressed as Means± SEM and P value:

Time point (Minutes)	Pre Ex (1 st OGTT)	Post S1 (2 nd OGTT)	Post S12 (3rdOGTT)
0	5.3± 0.2	4.7±0.5	4.8± 0.3
30	9.4±1.9	7.4±1.8	9.5± 2.6
60	8.2±2.3	8.2±2.1	9.0± 2.7

120	6.0±0.9	5.3±0.7	4.7± 0.4
AUCs	14.9± 3.2	13.±3.0	14.4±3.2

P value by comparing (Pre Ex & Post S1) = 0.055

P value by comparing (Pre Ex & Post S12) = 0.175

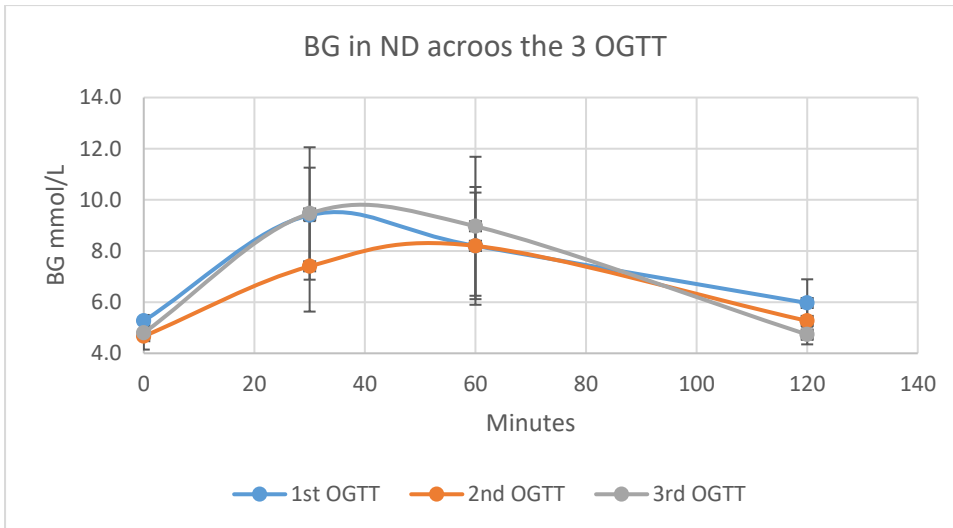


Figure 45: BG level during the 3 OGTT in ND

Table 21: Insulin concentration from OGTT comparisons between pre-exercise, post 1st session of exercise and post 12th session of exercise for ND on combination exercise. Data expressed as Means± SE and P value

Time point (Minutes)	Pre Ex (1st OGTT)	Post S1 (2nd OGTT)	Post S12 (3rdOGTT)
0	6.21± 1.72	5.52± 1.13	3.78± 0.95
30	49.22± 9.55	49.03± 11.24	45.95± 18.10
60	60.50± 21.82	48.95± 27.47	55.64± 26.74
120	32.38± 8.82	18.88± 4.25	14.15± 2.11
AUCs	21.5± 6.0	18.4± 6.9	18.4± 7.7

P value by comparing (Pre Ex & Post S1) = 0.162

P value by comparing (Pre Ex & Post S12) = 0.148

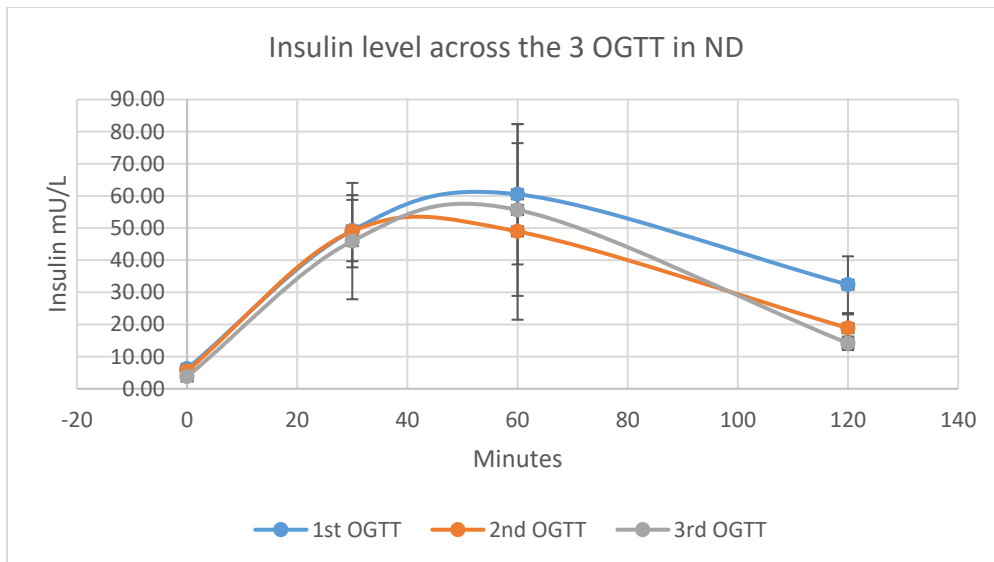


Figure 46: Insulin level during the 3 OGTT in ND

The FBG was different in each test throughout the 3 OGTT. Most of T2D and ND participants came to the next OGTT with lower FBG that's why it was unable to control their FBG and keep it within same level. The shape of the BG curve represent the information of number of metabolic factors such as IS, insulin secretion and hepatic glucose sensitivity (Chung et al., 2017; Cree-Green et al., 2018; Tschritter et al., 2003). In T2D, usually BG reached a maximum level between 30 and 90 minutes followed by a decrease until 120 minutes. On the other hand, BG in ND usually peaked at 30 or 60 minutes (Chung et al., 2017). In the current study, figure 42 shows BG curve which means that these individuals have T2D and less IS and the peak started between 60 and 90 minutes and then began to decrease till 120 minutes. P values shows significant reduction in BG after S1 and after S12 as well. It has been stated previously that delay in BG peak time indicated increase in BG and decrease in insulin sensitivity and secretion (Wang et al., 2018). In clinical setting, BG peak at 60 minutes might needs also screening for another metabolic abnormalities such as hepatic steatosis in obese subjects (Cree-Green et al., 2018). The AUC of BG for the 1st OGTT was (30.55±2.5 mmol/L/h) then decreased in the 2nd OGTT finally decreased more in the 3rd OGTT to (24.4±1.9 mmol/L/h), this reduction demonstrates improvement in the IS in T2D. Figure 44 represents the data of BG for ND, curve starts the 1st peak before 30 minutes in the 2nd OGTT. While in 1st and 3rd OGTT the peak was at 30 minutes. Finally, the 3rd OGTT shows the highest reduction in BG which illustrates the significant effect of chronic exercise on BG (P <0.05). The AUC for

the 1st OGTT was (14.9±3.2 mmol/L/h) and became (13.9±3.0 mmol/L/h) in the 2nd OGTT which slightly increased in the last OGTT to (14.4±3.2 mmol/L/h) but still less than baseline.

Insulin level in T2D as represented in figure 43, the 1st OGTT showed first peak of insulin at 60 minutes. While in the 2nd and 3rd OGTT showed maximum elevation at 30 minutes then decreased slightly at 60 min. The highest reduction has been found at 120 min in the 1st and 3rd OGTT while increased in the 2nd OGTT and that improved more by the 3rd OGTT (P=0.031). The AUC for insulin in the 1st OGTT was (94.5±31.6 mU/L/h) while this decreased slightly in the 2nd OGTT to (85.2±24.0 mU/L/h) and decreased more in the last OGTT to (30.1±10.7 mU/L/h). In ND (figure 45), the insulin elevated at 30 minutes while the maximum peak was at 60 min and it was higher in the 1st and 3rd OGTT and then decreased by 120 min and shows the highest reduction in the 3rd OGTT but not significant. Furthermore, AUC was (21.4± 6.0 mU/L/h) at baseline which decreased slightly in the 2nd OGTT and similar in the 3rd OGTT (18.4± 7.7 mU/L/h). This result in contrast to the result that found previously by Fenicchia et al., which shows no effect on BG after 6 weeks of RE exercise nevertheless, the effect was after the acute bout of exercise only (Fenicchia et al., 2004). It has been found in his study that there was no effect on insulin after both acute and chronic exercise. The study duration of Fenicchia et al., is similar to the current study however, the current study consists of combination exercise which could be the reason behind the significant results in BG reduction after chronic exercise.

5.11.1.2 IS in the both T2D and ND

Table 22: Insulin sensitivity in T2D by using 3 different indices (Values expressed as mean \pm SEM) and P value

	OGTT (Matsuda index)		OGTT (HOMA-IR)		OGTT (QUICKI)	
	T2D	ND	T2D	ND	T2D	ND
Pre Exercise (baseline)	16.27 \pm 3.45	100.82 \pm 17.10	16.66 \pm 2.85	1.47 \pm 0.27	0.27 \pm 0.01	0.36 \pm 0.01
Post 1st Exercise session (acute)	20.71 \pm 4.03	133.04 \pm 12.47	11.25 \pm 2.81	1.15 \pm 0.21	0.22 \pm 0.03	0.38 \pm 0.01
P value	0.285	0.284	0.134	0.140	0.251	0.130
Post 12th Exercise session (Chronic)	32.65 \pm 6.64	155.01 \pm 18.12	8.83 \pm 2.19	0.80 \pm 0.11	0.29 \pm 0.01	0.40 \pm 0.01
P value	0.017	0.156	0.016	0.054	0.057	0.032

This study calculated ISI in T2D and ND subjects by three different indices (as described in section 5.9). If the values of Matsuda <4.3 McA ≤ 5.8 , HOMA ≥ 2.6 and QUICKI ≤ 0.33 so it considered that subjects had IR (Menik and Palangasinghe, 2006; Gutch et al., 2015). Table 37 displays all the three indices and demonstrates that there is an improvement in IS in both groups. Firstly, the Matsuda index show minor improvement in IS in T2D after the first exercise session. In T2D it was (16.27 \pm 3.45) then increased after S1 to (20.71 \pm 4.03), and (P=0.285). While the chronic effect of six weeks of exercise represents more increments in IS after (32.65 \pm 6.64) and (P= 0.017) which considered as a significant improvement (see Table 22). In the ND group, the Matsuda also illustrates some improvement in IS as it was (100.82 \pm 17.10) and increased after session one to (133.04 \pm 12.47), (P= 0.284). It is also elevated more to (155.01 \pm 18.12), (P= 0.156) though no significant result was found. Previous studies have found a significant improvement in IS using Matsuda index (Whyte, Gill and Cathcart, 2010).

Secondly, HOMA-IR also displays slight reduction in IR in T2D after the 1st session from (16.66±2.85) to (11.25±2.81) and (P= 0.134). This reduction was higher after six weeks of chronic exercise (8.83±2.19) and (P= 0.016) which also considered as a significant improvement. In ND, the improvement in IR was higher than T2D. HOMA-IR was (1.47±0.27) before performing any exercise and decreased to (1.15±0.21) and (P= 0.140). The reduction was higher after 6 weeks and decreased to (0.80±0.11), (P= 0.054) which is nearly significant. Similar studies of combination exercise on T2D was conducted and HOMA-IR displays a significant reduction after combination exercise compared to AE or RE alone (Jorge et al., 2011). Moreover, a significant improvement in HMOA-IR after diet and RE in obese women (Antuna-Puente et al., 2009). Another study states that a sing maximal extended sprint improved IS significantly by using HOMA-IR (Whyte et al., 2013). In contrast, Ortega et al., (2014) demonstrates insignificant improvement in HOMA-IR post exercise (Ortega et al., 2014). The reason behind the insignificant of Ortega study might be that the exercise only one bout and the current study is 6 weeks of exercise.

Finally, QUICKI as well demonstrates an improvement in IS in T2D after session 12 only while no improvement found after the 1st exercise session. This value was (0.27±0.01) as base line and then decreased to (0.22±0.03) and (P=0.251). Moreover, this increased again after S12 higher than baseline to (0.29±0.01) and (P= 0.057) which states a very good improvement and it is nearly significant. While in ND participants, QUICKI shows improvement after 1st and 12th sessions, it was (0.36±0.01) and then increased after S1 to (0.38±0.01) and (P=0.130). Furthermore it is increased more after S12 to (0.40±0.01) and (P=0.032) which is significant. This result is in agreement with previous study which assess the IS after diet and RE, QUICKI improved significantly after intervention in obese women (Antuna-Puente et al., 2009).

5.11.2 Results of OGTT and ISI in T2D subgroups according to the medication types

5.11.2.1 BG and insulin in T2D subgroups

1- T2D with no medication

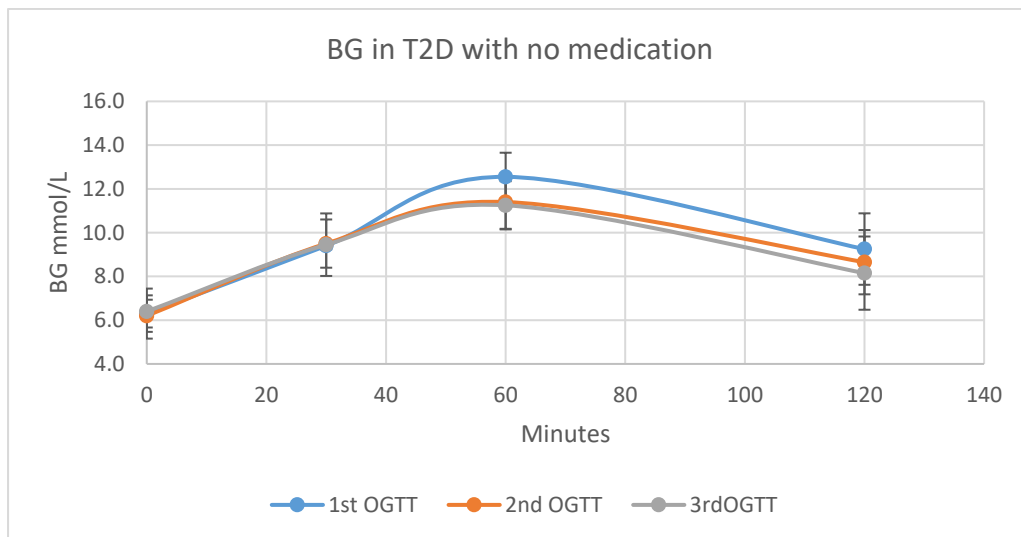


Figure 47: BG in T2D with no medication

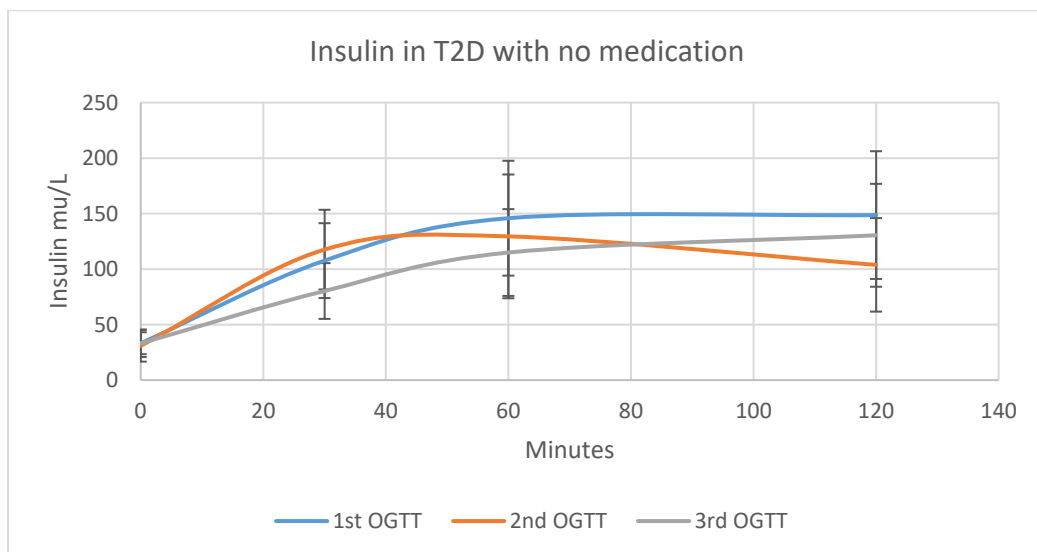


Figure 48: Insulin in T2D with no medication

In the subgroup of T2D who are not using any medication, AUC of BG was (21.0 ± 2.6 mmol/L/h) at baseline which decreased to (20.9 ± 2.9 mmol/L/h) in the 2nd OGTT and finally in the last OGTT decreased more to (19.1 ± 1.9 mmol/L/h). Moreover, insulin AUC shows also reduction from (57.4 ± 19.9 mU/L/h) to (52.4 ± 20.0 mU/L/h) and then further reduction in the last OGTT to (46.2 ± 15.6 mU/L/h).

2- T2D on metformin only

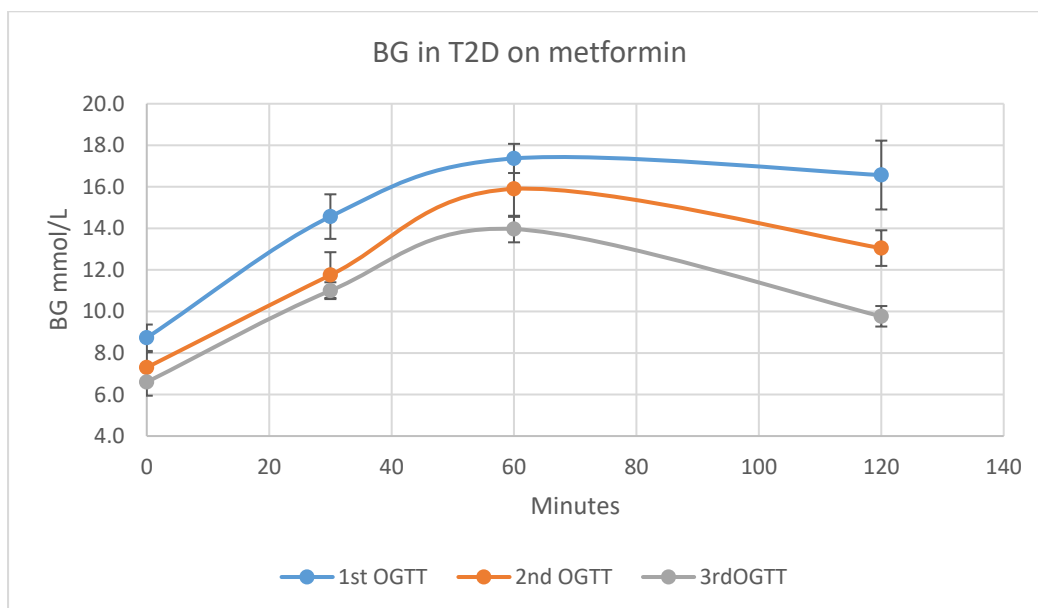


Figure 49: BG in T2D on metformin only

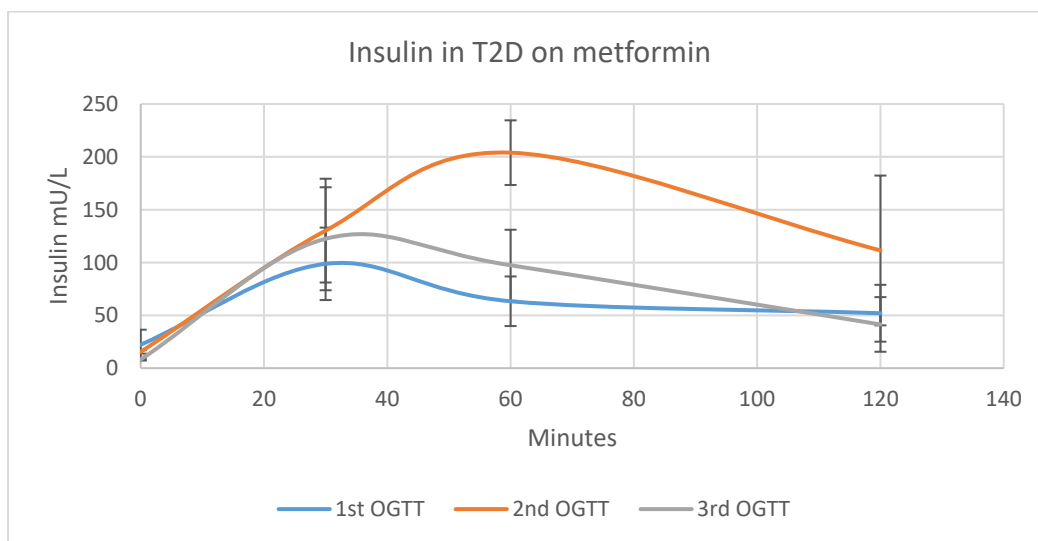


Figure 50: Insulin in T2D on metformin only

This group of T2D on metformin demonstrated reduction in AUC of BG which was $(31.5 \pm 3.6$ mmol/L/h) at baseline and then decreased in the 2nd OGTT to $(27.0 \pm 3.7$ mmol/L/h) and decreased more in the last OGTT to $(24.1 \pm 2.8$ mmol/L/h). However, AUC of insulin shows no reduction during the 12th exercise sessions which increased from $(33.2 \pm 13.1$ mU/L/h) to $(66.3 \pm 19.3$ mU/L/h) after 1st exercise and decreased to $(40.8 \pm 16.0$ mU/L/h) after the 3rd OGTT but still higher than baseline.

3- T2D on metformin and DPP4-I

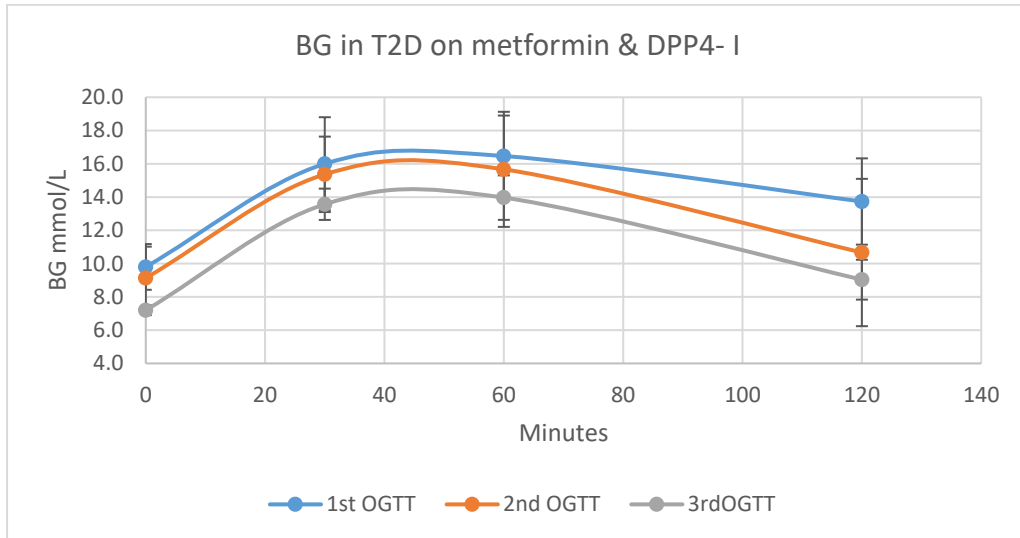


Figure 51: BG in T2D on metformin & DPP4-I

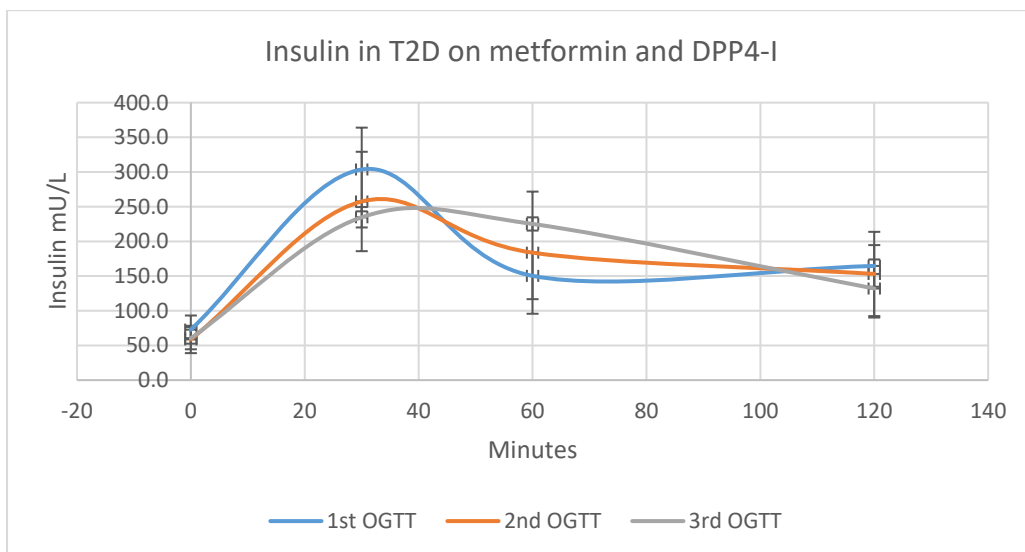


Figure 52: Insulin in T2D on metformin & DPP4-I

The AUC of this group illustrates reduction in BG and insulin nevertheless, the reduction in insulin AUC was higher in the 2nd OGTT than the 3rd. AUC of BG was (29.9±5.2 mmol/L/h) and decreased to (27.6±7.2 mmol/L/h) in the 2nd OGTT and to (23.7±3.6 mmol/L/h) in the 3rd OGTT. Insulin was (95.4±19.9 mU/L/h) before exercise then decreased to (91.2±33.3 mU/L/h) and increased slightly to (92.7±8.9 mU/L/h) but still higher than baseline.

4- T2D on metformin and SGT2-I

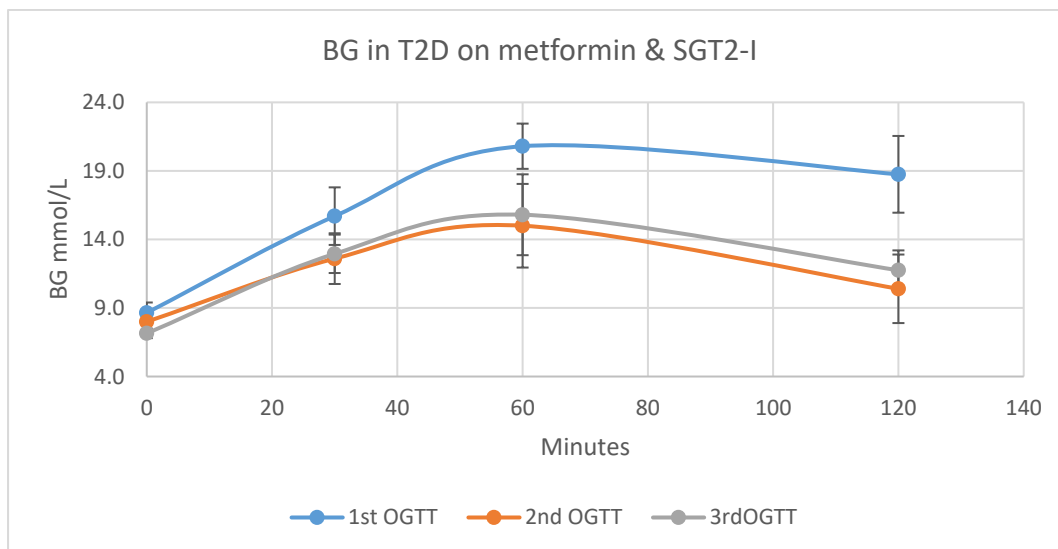


Figure 53: BG in T2D on metformin and SGT2-I

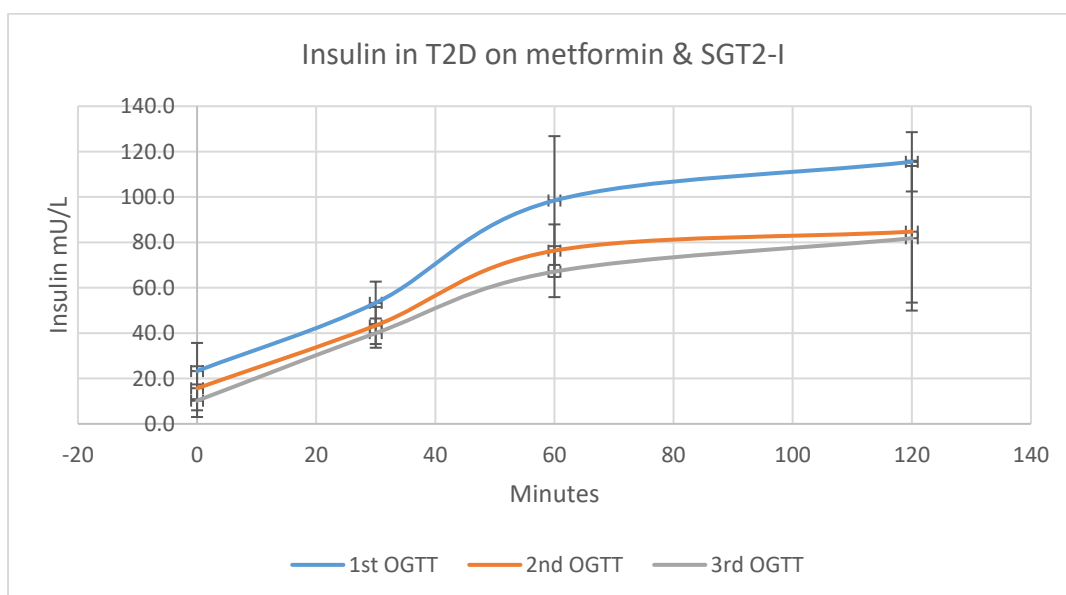


Figure 54: Insulin in T2D on metformin & SGT2-I

AUC in BG in this subgroup was $(35.0 \pm 5.6 \text{ mmol/L/h})$ and decreased in the 2nd OGTT to $(25.8 \pm 6.2 \text{ mmol/L/h})$ then increased slightly to $(26.8 \pm 4.0 \text{ mmol/L/h})$ but still less than baseline. Insulin shows reduction in AUC from $(36.9 \pm 8.4 \text{ mU/L/h})$ to $(28.3 \pm 6.7 \text{ mU/L/h})$ in the 2nd OGTT then to (25.5 mU/L/h) in the 3rd OGTT.

5.11.2.2 IS in T2D subgroups

Table 23: Insulin sensitivity in T2D by Matsuda index according to medication type (Values expressed as mean \pm SEM)

	No medication	Metformin only	Metformin & DPP4-I	Metformin & SGT2-I
Pre Exercise (baseline)	7.43 \pm 1.75	24.42 \pm 9.73	10.92 \pm 1.49	18.34 \pm 5.21
Post 1st Exercise session (acute)	24.96 \pm 12.94	24.84 \pm 3.05	15.04 \pm 8.99	25.92 \pm 0.58
P value	0.285	0.814	0.640	0.350
Post 12th Exercise session (Chronic)	22.37 \pm 7.26	51.02 \pm 12.19	11.71 \pm 2.75	54.80 \pm 9.88
P value	0.151	0.151	0.621	0.081

By comparing medication types, Matsuda index in T2D group that have no medication was (7.43 \pm 1.75) at baseline and then increased to (24.96 \pm 12.94), (P=0.285) after 1st OGTT which then decreased slightly after 6 weeks to (22.37 \pm 7.26), (P=0.151). In T2D with metformin, Matsuda before exercise was (24.42 \pm 9.73) and became (24.84 \pm 3.05), (P=0.814) which increased more after six weeks of exercise to (51.02 \pm 12.19), (P=0.151). The result of Matsuda in T2D using metformin and DPP4-I was (10.92 \pm 1.49) and increased to (15.04 \pm 8.99), (P=0.640) after 1st exercise session and finally become (11.71 \pm 2.75), (P=0.621) after six weeks. The best improvement was in T2D participants who were using metformin and SGT2-I which shows that Matsuda level was (18.34 \pm 5.21) and then increased after 1st exercise session to (25.92 \pm 0.58), (P=0.350), this elevated more after 12th exercise session to (54.80 \pm 9.88), (P=0.081) which shows that it is close to be significant.

Table 24: Insulin sensitivity in T2D by HOMA-IR index according to medication type (Values expressed as mean \pm SEM)

	No medication	Metformin only	Metformin & DPP4-I	Metformin & SGT2-I
Pre Exercise (baseline)	26.12 \pm 4.49	10.93 \pm 7.65	16.42 \pm 5.73	12.92 \pm 1.68
Post 1st Exercise session (acute)	10.88 \pm 4.24	2.91 \pm 1.52	22.39 \pm 7.37	8.89 \pm 0.44
P value	0.008	0.389	0.559	0.190
Post 12th Exercise session (Chronic)	11.01 \pm 3.90	2.33 \pm 0.32	17.52 \pm 4.44	4.54 \pm 1.10
P value	0.003	0.374	0.878	0.204

The results of HOMA-IR index in T2D who were on no medications was (26.12 \pm 4.49) at baseline and decreased after 1st exercise session to (10.88 \pm 4.24), (P=0.008) which shows a significant improvement in IS while after six weeks of exercise HOMA-IR level decreased to (11.01 \pm 3.90), (P=0.003) which is very significant. This significant improvement could be because this group has just diagnosed as T2D and had the lowest HbA1c therefore the exercise intervention plays a role in improving IR in this group. In T2D group who were used metformin only, HOMA-IR was (10.93 \pm 7.65) and decreased to (2.91 \pm 1.52), (P= 0.389), while after six weeks it became (2.33 \pm 0.32), (P= 0.374). Moreover, HOMA-IR in T2D who used metformin and DPP4-I was (16.42 \pm 5.73) and increased to (22.39 \pm 7.37), (P= 0.559) and then become (17.52 \pm 4.44), (P= 0.878). The result in T2D who used metformin and SGT2-I was (12.92 \pm 1.68) and decreased to (8.89 \pm 0.44), (P= 0.190) which decreased more to (4.54 \pm 1.10), (P= 0.204).

Table 25: Insulin sensitivity in T2D by QUICKI index according to medication type (Values expressed as mean \pm SEM)

	No medication	Metformin only	Metformin & DPP4-I	Metformin & SGT2-I
Pre Exercise (baseline)	0.246 \pm 0.003	0.297 \pm 0.023	0.267 \pm 0.009	0.265 \pm 0.005
Post 1st Exercise session (acute)	0.283 \pm 0.014	0.305 \pm 0.005	0.257 \pm 0.012	0.295 \pm 0.005
P value	0.093	0.705	0.580	0.205
Post 12th Exercise session (Chronic)	0.280 \pm 0.010	0.337 \pm 0.007	0.260 \pm 0.010	0.330 \pm 0.10
P value	0.038	0.195	0.635	0.144

Finally QUICKI as well illustrates a significant improvement after six weeks in T2D who used no medication. QUICKI was (0.246 \pm 0.003) and increased to (0.283 \pm 0.014), (P= 0.093) and decreased more to (0.280 \pm 0.010), (P= 0.038) which is a significant improvement in IS. The improvement in this had similarity to HOMA-IR and the reason behind the significant result might be because this group had the lowest HbA1c as explained before and still had good β -cell function which benefits in the improvement of IS. In the metformin group, QUICKI was (0.297 \pm 0.023) and increased after 1st exercise session to (0.305 \pm 0.005), (P=0.705) which increased more after six weeks to (0.337 \pm 0.007), (P=0.195). Furthermore, in metformin and DPP4-I group the level was (0.267 \pm 0.009) and decreased after 1st session to (0.257 \pm 0.012), (P= 0.580) and after 12th session (0.260 \pm 0.010), (P= 0.635). In metformin and SGT2-I group, QUICKI was (0.265 \pm 0.005) and increased after 1st session to (0.295 \pm 0.005), (P= 0.205) and then increased more after the 12th exercise session to (0.330 \pm 0.10), (P=0.144).

Recent study states that assessing insulin resistance by using Matsuda “4 or 5 time point “is valuable and better than other indices that only use fasting time point (Lorenzo et al., 2015). It is applicable to use during pharmacological intervention, exercise and weight loss and may

be attractive in these because it is cheaper than the HIEC method (Lorenzo et al., 2015). It has been proved that indices of ISI quantified from IVGTT and OGTT are valid surrogates of HIEC (Ortega et al., 2014). Matsuda indices calculated from OGTT, however, illustrates good correspondence with the HIEC (Ortega et al., 2014). Other researchers have found that results from HOMA could be affected more by reason of variation in fasting insulin more than QUICKI (Antuna-Puente et al., 2008). Ortega compared OGTT and IVGTT and concluded that the values of ISI quantified from IVGTT were more appropriate than OGTT for identifying the acute effect of aerobic exercise and IVGTT was also more reproducible (Ortega et al., 2014). The oral glucose has an extra effect compared with IV glucose by stimulating GI hormones and influence metabolism in the liver. OGTT results in release of incretins that enhance insulin secretion therefore OGTT might afford more physiological conditions for estimation of β -cell function than IVGTT (Hayashi et al., 2013). These hormones interact with the oral glucose and affect its absorption as well as affect insulin secretion which cause variability within individuals BG and insulin concentration (Ortega et al., 2014).

5.12 Conclusion:

The results show correlation between Matsuda, HOMA-IR and QUICKI in the improvement of IS. T2D participants show some IR at baseline when assessing IS and IR by using these three indices. This resistance improved across the whole exercise sessions which illustrates improvement by the end of six weeks but this was only significant after six weeks when using Matsuda and HOMA-IR indices. On the other hand, ND volunteers have no IR before performing any exercise therefore the improvement in IR was only significant in QUICKI after 6 weeks of exercise comparing to T2D group. In the group that had no medication for diabetes the results states that the combination exercise was very useful in improving IS and IR in these subjects as found by HOMA-IR and QUICKI indices. HOMA-IR might be more sensitive to the variation in fasting insulin than QUICKI so QUICKI might provide more accurate results. Moreover, previous research state that Matsuda is more valued during intervention such as exercise and weight loss. In conclusion, Matsuda could be more applicable in this study while it is using 4 time point not only fasting values. OGTT also is cost effective comparing with HIEC. However, OGTT has some limitation that the absorption of BG and BI affected by gastric hormone and cause intra-subject variation in their concentration. Diet and adherence to antidiabetic medication also could affect the IS results (Jorge et al., 2011).

5.12.1 Limitations:

- 1- Use of OGTT rather than the gold standard of glucose clamp technique (HIEC).
- 2- Only few volunteers have been recruited.
- 3- No control group.
- 4- No specific diet has been followed.

5.12.2 Future work:

- 1- Arrange to use HIEC method which available in hospitals to give valid results.
- 2- Recruit more participants as intervention and control group.
- 3- Assess GLP-1 during OGTT.

Chapter 6 The effect of moderate combination exercise aerobic and resistance on the incretin hormone (GLP-1) in T2D subjects

6.1 Research questions:

Is the incretin hormone improved after 6 weeks of moderate combined exercise? Do the improvements correlate with medication type?

6.2 Aim:

Few studies have been conducted to investigate the effect of exercise on incretin hormones especially GLP-1. Thus, the aim of this study is to investigate the effect of combination exercise programme (cardio “aerobic” and resistance) on GLP-1 levels in T2D participants. In the last few years, incretin has become an important hormone in the treatment of T2D, therefore, it is very important to assess GLP-1 level in T2D subjects.

6.3 Incretin hormones and DDP-IV:

Incretins are peptide hormones that are secreted by the entero-endocrine cells in the gastrointestinal tract such as L and K cells (Fava, Dong and Wu, 2016; Rhee et al., 2014). The common incretin hormones that have been identified include glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (Nauck and Meier, 2016). GIP is secreted by the K-cells in duodenal part of the small intestine whereas GLP-1 is secreted by the L-cells of the large and small intestine (Nauck *et al.*, 2011). Recent research reported that GLP-1 is also produced by the pancreatic α -cells (Fava, Dong and Wu, 2016). The hypothesis of stimulating pancreatic secretion by the gut endocrine was reported as a breakthrough in medical sciences (Friedman, 1954). This is also true for understanding the role of incretins in regulating the secretion of insulin and glucagon, thus regulating the blood glucose (Kim and Egan, 2008; Ahrén, Carr and Deacon, 2010; Mudaliar and Henry, 2012; Lastya, Saraswati and Suastika, 2014; Rehfeld, 2018). There are numerous incretin hormones, however, the present study will focus on GLP-1 because it plays a vital role in T2D (Spellman, 2011; Lastya, Saraswati and Suastika, 2014). It is noteworthy to mention that GLP-1 is a metabolic hormone which stimulates a decrease in BG and is reported that intra-islet GLP-1

may exert insulinotropic and glucagonostatic effects by paracrine and/or autocrine actions (Fava, Dong and Wu, 2016). GLP-1 is said to exert its action by increasing insulin levels which is released from pancreatic β -cells after food consumption before the BG begins to rise, thus decreasing β -cell apoptosis, enhance β -cell proliferation and neogenesis (Fava, Dong and Wu, 2016; Kayaniyil et al., 2016; Campbell and Drucker, 2013). This hormone reduces the rate of absorption of nutrients into the bloodstream by reducing gastric emptying which can reduce appetite and food intake in both normal and obese individuals through the nervous system (Kim and Egan, 2008; Fava, Dong and Wu, 2016). It also reduces glucagon release from α pancreatic cells which play an important role in blood glucose regulation (Fava, Dong and Wu, 2016). On the other hand, in hyperglycaemia the high level of glucagon suppresses the release of GLP-1 but the mechanism underlying this suppression is not fully understood (Nauck et al., 2011).

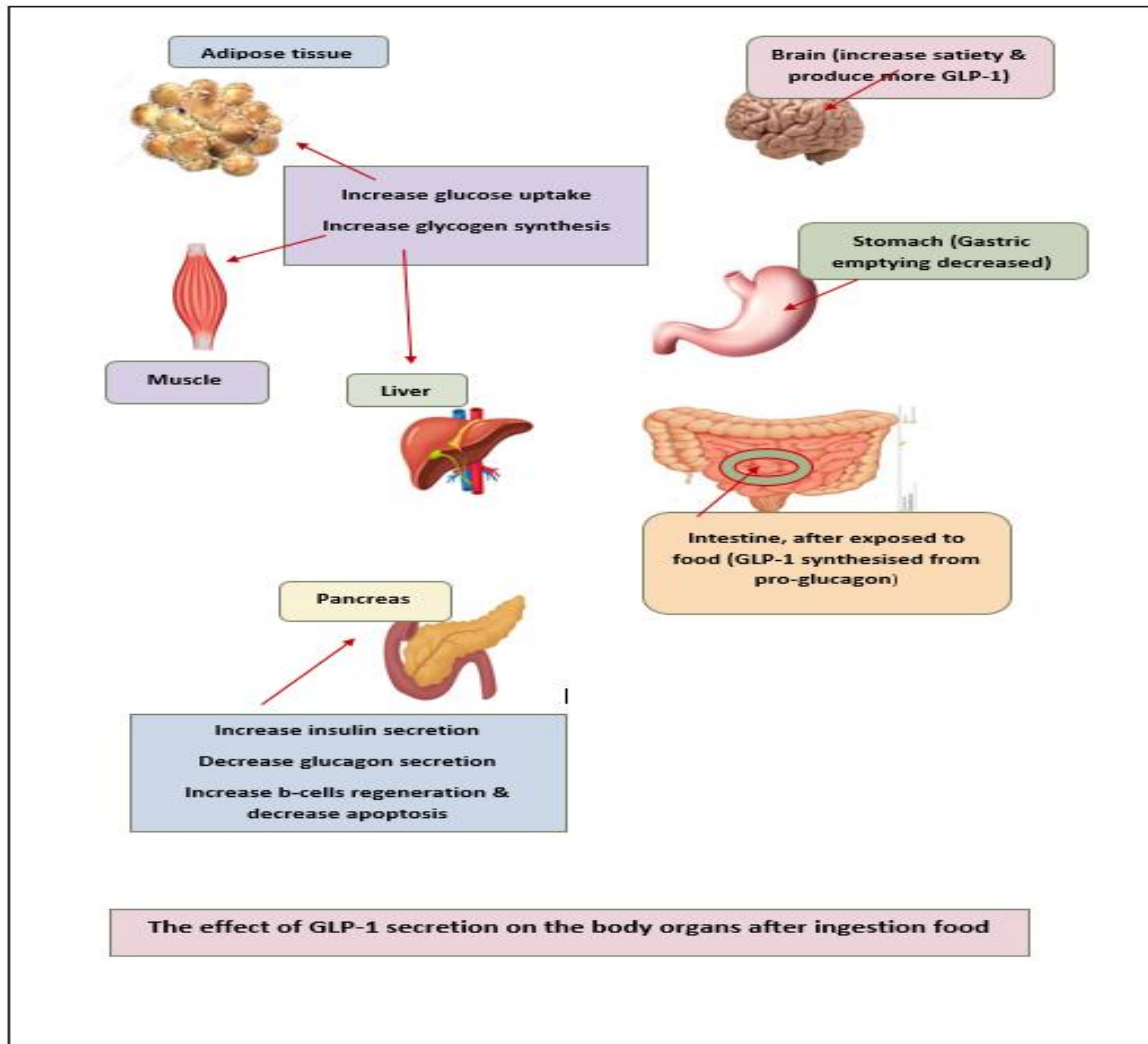


Figure 55: Physiological effects of GLP-1 after food ingestion

In the basal state, the level of GLP-1 is (5-15 pmol/l) while, in postprandial or after oral glucose the level becomes (20-60 pmol/l) (Nauck et al., 2011). After a meal, GLP-1 starts to elevate after 10 to 15 minutes and reaches a peak in two hours then decreases slowly for hours until it reaches a baseline (Nauck *et al.*, 2011). When GLP-1 binds to a single GLP-1 receptor it shows various physiological functions which play a role in regulating glucose homeostasis (see figure 54) (Holst, 2013). Therefore, any deficiency in GLP-1 can play a major role in pathophysiology of type 2 diabetes (Spellman, 2011). In the case of obesity the secretion of GLP-1 decreases with increasing appetite (Hussein et al., 2014). The levels of GLP-1 are decreased in the T2D individual (Szablewski, 2011). However, in such a case the body retains the capability to respond to this hormone. So, administration of exogenous GLP-1 can control

hyperglycaemia (White, 2008). At high glucose levels, GLP-1 and GIP lead to a glucose dependent increase in insulin secretion. In case of obesity, GLP-1 level was indirectly associated to the status of insulin resistance (Hussein et al., 2014).

It has been found that some neurotransmitters such as gastrin-releasing peptide and acetylcholine trigger the release of GLP-1 which proves that intramural enteric nervous system contributes in mediating the upper gut signal (Nauck et al., 2011). The GLP-1 level is not decreased in case of gut resections which demonstrate that GLP-1 secretion is started immediately when L-cell exposed to the food in the upper part of small intestines, not in the lower part of large intestine and ileum (Nauck et al., 2011). Interestingly, the level of GLP-1 after ingestion of food was found higher than GIP level possible because the required velocity for the movement of gastric secretion to stimulate K-cells in the duodenum is less than the one required to stimulate L-cells at many distal locations (Nauck et al., 2011). The incretin system has been a common intervention for treating T2D for many years (Kayaniyil et al., 2016). Several studies have indicated the beneficial aspects of GLP-1 approaches in managing T2D as sufficient via controlling cardiovascular conditions like blood pressure, patient body weight, protein metabolism that is individually created by GLP-1 consequences from glycemic control and atherosclerosis markers (Satoh-Asahara et al., 2013; Petrie, 2013). After food ingestion, the level of GLP-1 is elevated in the circulation and enter capillaries of endothelial cell, then degraded by DPP-4 which is located there. Approximately third to fourth level of the original GLP-1 level reaches the portal vein, then transported to the liver (Holst, 2013). The role of GLP-1 in decreasing appetite and regulating glucose metabolism occurs after food intake in the intestinal L cells which release GLP-1. First, GLP-1 binds to receptors inside the gut which in turn activate and send a signal to CNS to suppress appetite. Secondly, GLP-1 absorption occurs through the blood circulation system, then enters the pancreatic β cells and regulates pancreatic secretion (Holst, 2013). Medication such as metformin is shown to affect the secretion of GLP-1 by increasing its level in human and rodent L cells but not the GIP (Campbell and Drucker, 2013). The underlying mechanism for the increase of GLP-1 by metformin is not fully understood (Campbell and Drucker, 2013). In normal physiological conditions, the enzyme Dipeptidyl Peptidase 4 (DPP-4) degrades GLP-1 and GIP (Holst, 2013). For that reason, GLP-1 is not useful as pharmacological agent, however, injectable GLP-1 receptor agonists are resistant to degradation by DPP-4 enzymes thus preserving the effects

of GLP-1 analogue, e.g. exenatide (Byetta,Bydureon®) and liraglutide (Victoza®). Other inhibitors of DPP-4 are those that increase serum levels of GLP-1 and enhance insulin secretion e.g. sitagliptin (Januvia®) and vildagliptin (Galvus®) (ADA, 2018). Furthermore, evidence from clinical trials showed that exenatide and liraglutide have significant effect in reducing the body weight of participant with and without diabetes (Holst, 2013). In addition, Exenatide was reported to have anti-inflammatory effect on the CNS, pancreas and cardiovascular system by suppression pro-inflammatory cytokines (Campbell and Drucker, 2013). Cross-sectional studies have indicated that serum postprandial concentrations of GLP-1 are considerably lower among obese individuals and this increased when weight was lost (Holst, 2013). These reduction might be due either to the elevation of free fatty acid which lowers responsiveness of L-cells to carbohydrate or to the insulin resistance in these obese subjects (Holst, 2013). In summary, literature have presented the vital role of GLP-1 in diabetes, thus it levels was studied in the present study.

6.4 Effect of exercise on GLP-1 level:

Few research studies have studied the effect of exercise on GLP-1 level and most reported that exercise generally elevates GLP-1 plasma levels in T2D and at different intensity (Ueda, Nakahara and Miyamoto, 2013). The mechanism of this increase is not fully clear, however, it is important to understand it so to design an exercise program which could be used as preventive therapy for obesity and its complications (Ueda et al., 2013). A study of assessing appetite after exercise has proven that the GLP-1 increment after exercise was due to the physiological suppression of appetite (Beaulieu et al., 2014). A review by Hazell et al., (2016) compared the effect of exercise intensity from different studies on GLP-1 and hypothesized that the potential mechanisms on how the level of GLP-1 changes after exercise could be the variation of BG and blood insulin level, the cytokine release or muscle metabolism (Hazell, 2016).

Recently a Canadian study was piloted to see the effect of exercise intensity on GLP-1 in healthy active female (Hallworth et al., 2017), where participants were divided into three groups based on training type namely sprint interval training (SIT), moderate intensity continues training (MICT), and no exercise control (CTRL). The exercise study was scheduled to last for 10 days and blood samples were collected before and immediately after each exercise session. Samples were transferred into EDTA tubes 90 mins after exercise, then protease inhibitor cocktail was added to prevent GLP-1 degradation. A visual analogue scale was used to assess hunger at each sampling time points (Hallworth et al., 2017). The literature concluded that GLP-1 showed significant elevation after postprandial exercise in participants of SIT and MICT grouped when compared to the control, however, there was no differences in the increased GLP-1 level between SIT and MICT group (Hallworth et al., 2017).

Another recent study was conducted on endurance-trained males (Holliday and Blannin, 2017). Result illustrated a significant elevation of GLP-1 level immediately after exercise in 30min and also in 45 min cycling exercise ($P=0.001$) (Holliday and Blannin, 2017). In addition, Howe et al., (2016) studied the effect of acute intense exercise on appetite and guts hormones one of which was GLP-1. To achieve this, 15 eligible trained endurance female athletes (18-40 years) participants were recruited. The exercise programme consisted of two randomised trials which were 7 days apart, namely moderate intensity exercise (MIE) and

high intensity exercise (HIE) at 60% and 85% VO_{2MAX} , respectively. The effect of intense exercise on GLP-1 was shown to significantly increase after 60 min(s) (Howe *et al.*, 2016).

It is important to mention that some researchers reported that neither intense nor moderate exercise has effect on GLP-1. An example of such study is that conducted by Kawano *et al.*, (2013) which was a randomised control trial focusing on the effect of weight-bearing on GLP-1 and hunger rate, and participants were healthy non-obese sedentary young men. The bicycle ergometer was considered as non-weight bearing exercise while rope skipping as weight-bearing exercise. GLP-1 and hunger rating were monitored before, during and after exercise (Kawano *et al.*, 2013). Although data presented by Kawano *et al.*, (2013) showed that hunger was suppressed during and after exercise for short period of time, there was no increase in GLP-1 level.

In regards to T2D, Eshghi, Bell and Boulé, (2013) conducted a crucial study that aimed at studying the effects of aerobic exercise on the plasma incretins among T2D individuals. The study was motivated by the fact that there are very few studies on the effect of exercise on incretins on the secretion of insulin. Eshghi and colleagues aimed at examining the effects of aerobic exercise and/or metformin on the levels of incretin hormones, their reason on including metformin was due to the fact that is largely used among diabetic. The randomised crossover study concluded that exercise has an effect on incretin hormone level including GLP-1, however, the level of GLP-1 was seen to be higher among participants on metformin in both pre-lunch and post-lunch sessions when compared to participants in the placebo group (Eshghi *et al.*, 2013). Another study with similar results as Eshghi *et al.*, (2013) is that reported by O'Connor *et al.*, (2006), where their findings showed prolonged aerobic exercised showed a significant increment of incretin hormones level (GLP-1 and GIP) in non-diabetic individuals, while no significant effect was observed in T2D participants.

Shaabani, Abolfathi, and Alizadeh (2016) also looked into the effect of exercise on GLP-1 via a randomised control study. In their study, participants recruited were female T2D (n=20, age 33-53 years) which were divided into exercise and control group. The exercise conducted was running on a treadmill (VO_{2max} 55-80%) for 5 days a week for the duration of 4 weeks, and each session was 30 min long which was increased slightly to 60 min in week 4. Blood samples were collected at baseline and after the 4th week of exercise. ELISA kit was used in analysing GLP-1 and insulin. The result showed that aerobic exercise has no significant effect on GLP-

1, BG, insulin and BMI ($P > 0.05$) compared to control (Shaabani et al., 2016). This study, the studies by Eshghi et al., (2013) and that of O'Connor et al., (2006) are important because they focus on T2D while other studies were on athletes, healthy and obese subjects.

Although literature on the effect of exercise on GLP-1 and T2D was reviewed, it is important to also look at the literature on the effects of exercise and GLP-1R agonist on GLP-1 level. The study by Mensberg et al., (2017) has been considered as the first clinical trial that combined exercise and a GLP-1R agonist to assess GLP-1 hormone level in the body. The following parameters namely HbA1c, FBG, body weight, insulin, C-peptide, glucagon, and incretins hormones were all assessed at baseline and after the programme. Participants were T2D (> 18 years) who are treated with diet and/or metformin and are randomly grouped into 2 groups namely exercise-placebo and exercise-liraglutide (Victoza[®]) group. Each exercise session was designed for a duration of one hour and conducted three times weekly for 16 weeks. Two trained exercise physiologists assigned as supervisors during the exercise programmes. Participants received daily injectable dose (0.1ml) of liraglutide or placebo for the first week then 0.2ml for the second week and 0.3ml for the third week and continue using this dose until the end of the programme. The results published showed that GLP-1 level in the placebo group decreased from (20.1 ± 8.2) to (18.1 ± 5.7) , while liraglutide group showed an elevated level of GLP-1 level (18.7 ± 3.9) to 19.3 ± 5.6). Moreover, HbA1c was shown to have greatly reduced in liraglutide group from (66.0 ± 16.0) to (44.0 ± 8.0) and slightly decreased from (64.0 ± 13.0) to (61.0 ± 17.0) in the placebo group. The study concluded that the combination of exercise and GLP-1R agonist has better effect on both HbA1c and GLP-1 level, suggesting that this could be a suitable therapeutic option for obese individuals with T2D who are on diet and/or metformin (Mensberg et al., 2017).

Recent study by Tura et al., (2017) stated that there is some incretin resistance in individuals with T2D who are overweight. The study was conducted with two groups including 8 T2D and 8 normal glucose tolerant participants and both groups received 25, 75, 125 g oral glucose tolerance test with corresponding isoglycemic glucose infusion and a mathematical model was used obtain β -cell sensitivity to incretins. Although GLP-1 response was seen to be higher in the insulin sensitive participants, BMI was lower. Despite the secretion of GLP-1, it was seen to have improper action in T2D pointing to the possibility of GLP-1 resistance. (Tura et al., 2017).

T2D, obesity and exercise are linked to elevated IL-6 level while contracting skeletal muscle is also associated with the release of interleukin-6 (IL-6). The latter is said to stimulate the secretion of GLP-1 from the intestine and the pancreas (Ellingsgaard *et al.*, 2011).

From the above reviewed literature, it is recommended that combine exercise can be used to control diabetes and increase insulin sensitivity in T2D patients, however, further research should be conducted to provide more options and evidence, since some literature present contradictory evidence. Despite the wide range of studies on incretin, only few studies have been conducted on T2D participants and still there is a need to compare the effect of exercise and different types of pharmacological therapy on GLP1. In this study, the researcher are going to analyse new thing which no researcher before have demonstrate it. The finding of T2D have been used to compare between those who are using different types of medication and doing exercise with those who are not using any medication just doing exercise; and analyse the effect of that on GLP-1 level in each group.

6.5 Method:

6.5.1 Study design

In each exercise session, volunteers performed a combined exercise program consisting of 30 mins of resistance exercise followed by 20 mins moderate cycling twice a week for 6 weeks. At the beginning of each session, the volunteers started with 11 steps of stretching, then cycled for five minutes to warm up. Blood samples were collected at baseline after (S1, S2, S4, S6, S8, S10, and S12), then centrifuged and refrigerated to be analysed later by a commercially available enzyme-linked immunosorbent assay (ELISA) with <0.01 cross reactivity with GLP-1 kit as explained in section 3.9.2.

6.5.2 Intervention group

The intervention groups were T2D participants (n= 11) and categorised into three groups based on medication namely T2D volunteers on metformin only, T2D volunteers on metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4 I) and T2D volunteers on metformin and SGT2-I.

Medication	Number of Volunteers
Pravastatin 40mg OD	1
Atorvastatin 20mg OD	3
Metformin 500 mg BD	3
Metformin 850 TDS	1
Metformin 1000 mg BD	2
Sitagliptin 50 mg OD (DPP4-I)	1
Sitagliptin 100mg OD (DPP4-I)	2

Empagliflozin 10 mg OD	2
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6.6 Results and discussion:

The GLP-1 data were processed using Excel 2010 and SPSS (version 22), values were expressed as mean with standard error of mean. Pre and post exercise samples were compared using a paired-samples t-test with statistical significance of $P < 0.05$.

Table 26: Age, weight and HbA1c in T2D participants.

	Pre Ex	Post S12
Age (years)	49 ± 3.11	
Weight (KG)	96.00 ± 9.4	93.04 ± 8.7
HbA1c	7.45 ± 0.7 % (58 ± 8.1 mmol/mol)	6.4 ± 0.4 % (46.5 ± 4.5 mmol/mol)

6.6.1 The incretin result among the whole T2D:

Table 27: GLP-1 level during the 1st session of exercise where results shows acute effect, Data expresses as mean ± SEM and P value:

GLP-1 (pmol/L)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	9.7 ± 2.1	10.4 ± 2.0	8.7 ± 0.9	0.594

Table 28: GLP-1 level during the 12 sessions of exercise where results shows chronic effect, Data expresses as mean ± SEM and P value:

GLP-1 (pmol/L)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	9.7 ± 2.1	8.7 ± 0.9	7.7 ± 1.5	8.6 ± 1.3	9.6 ± 1.0	7.7 ± 1.1	9.6 ± 1.7	7.0 ± 1.0	0.333

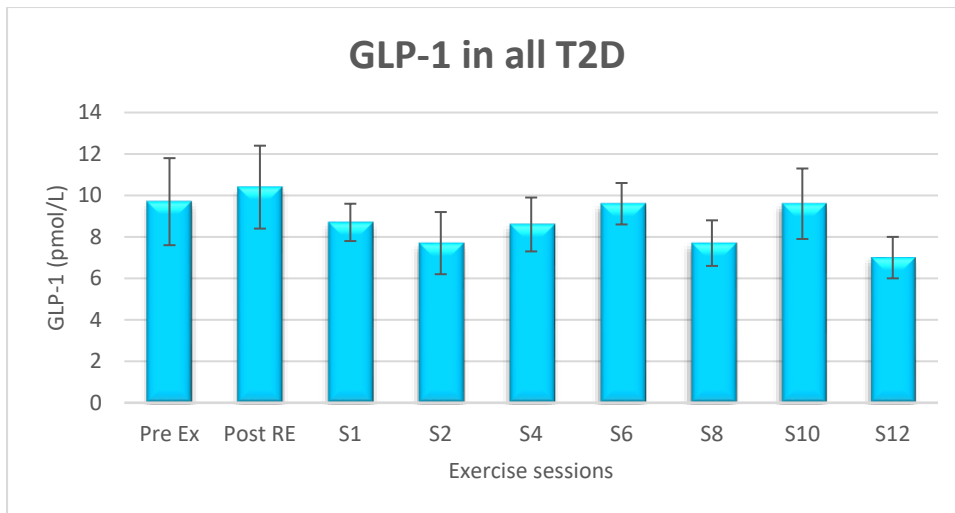


Figure 56: GLP-1 level during the 12 sessions of Exercise in the whole 3 groups of T2D, showing a downward trend as opposed to what was reported in literature.

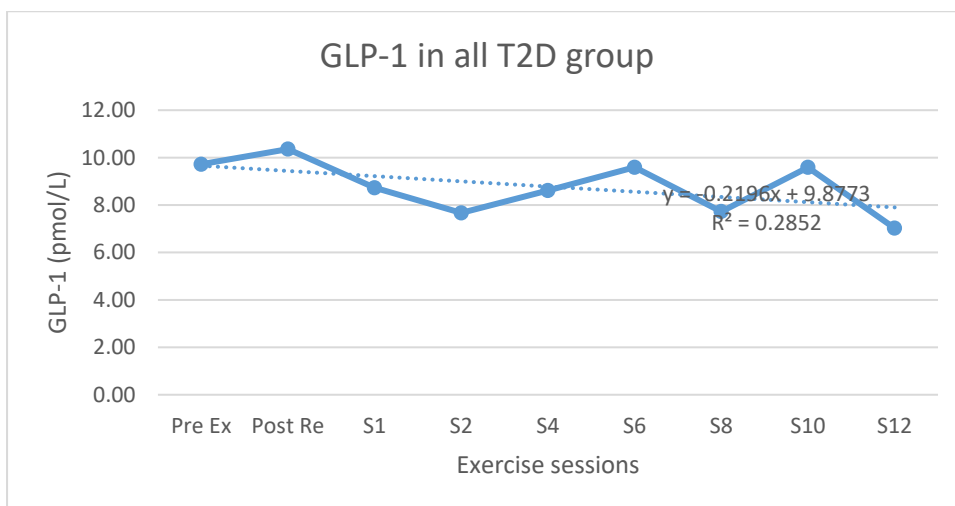


Figure 54a: This is a regression with normalised intervals, showing a slight downward trend but poor relationship over the 6 weeks (where not all sessions were measured)

The result obtained from the combination of three groups as in (figure 54), shows a slight reduction in the trend of the means between session one and session 12. GLP-1 was (9.7 ± 2.1) at baseline and decreased to (8.7 ± 0.9) , ($P = 0.594$) after the 1st session. This decreased slightly more after the 12th session to (7.0 ± 1.0) , ($P = 0.333$), illustrating that exercise has statistically insignificant effect on GLP-1 hormone as measured in this group, although there is a downward trend.

6.6.2 Results according to medication type:

Table 29: GLP-1 level during the 1st session of Exercise (Metformin group), where results shows acute effect. Data expresses as mean \pm SEM and P value:

GLP-1 (pmol/L)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	11.6 \pm 3.3	11.4 \pm 2.8	9.6 \pm 1.7	0.444

Table 30: GLP-1 level during the 12 sessions of Exercise (Metformin group) where results shows chronic effect. Data expresses as mean \pm SEM and P value:

GLP-1 (pmol/L)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	11.6 \pm 3.3	9.6 \pm 1.7	7.1 \pm 2.2	9.9 \pm 2.2	8.1 \pm 1.3	6.1 \pm 0.7	7.7 \pm 2.1	5.2 \pm 0.9	0.092

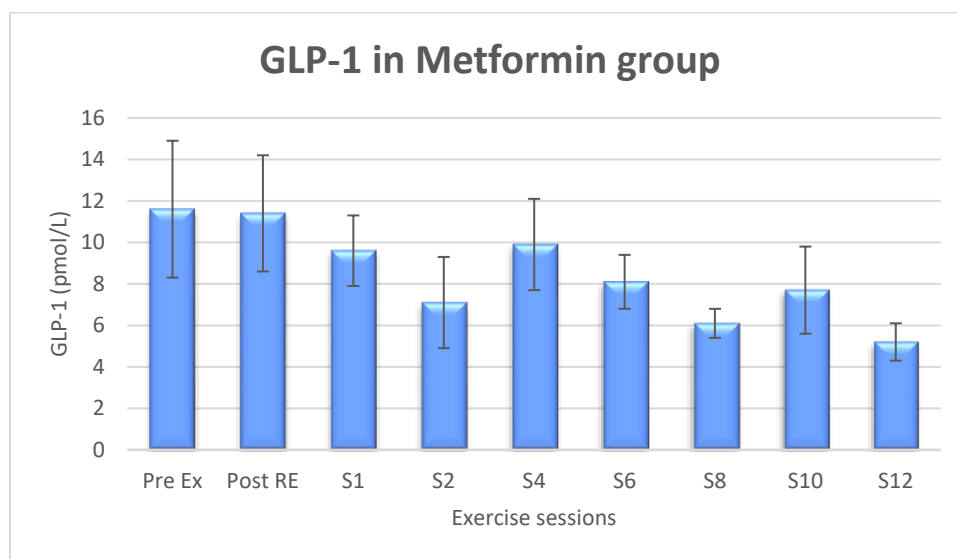


Figure 57: GLP-1 level in Metformin group during the whole exercise sessions

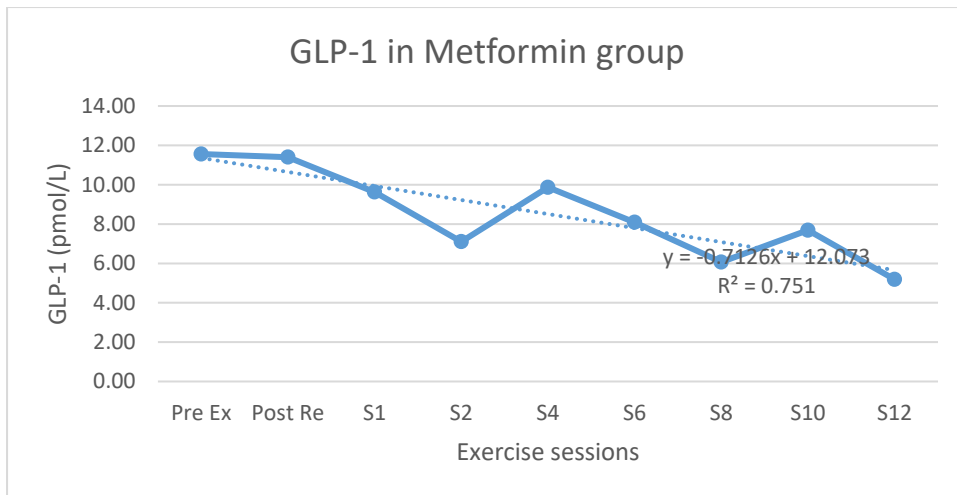


Figure 55a: This is a regression with normalised intervals, showing a strong downward trend and stronger relationship over the 6 weeks (where not all sessions were measured)

Interrogating this result for the effect of individual adjunct drugs in regards to metformin, a reduction in GLP-1 level was more evident in the metformin group among the three groups. Before the 1st session, GLP-1 was (11.6 ± 3.3) and decreased to (9.6 ± 1.7) ($P=0.444$) after S1. This decreased further after S12 to (5.2 ± 0.9) , ($P=0.092$) but no significant reduction was found in the chronic and acute effect of exercise when assessing the trend of means over the 12 sessions.

Table 31: GLP-1 level during the 1st session of Exercise (Metformin and DPP4-I group) where results shows acute effect, Data expresses as mean \pm SEM and P value:

GLP-1 (pmol/L)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	11.8 \pm 4.1	12.2 \pm 6.0	7.2 \pm 1.3	0.347

Table 32: GLP-1 level during the 12 sessions of Exercise (Metformin and DPP4-I group) where results shows chronic effect, Data expresses as mean \pm SEM and P value:

GLP-1 (pmol/L)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	11.8 \pm 4.1	7.2 \pm 1.3	5.8 \pm 2.1	6.8 \pm 0.4	8.9 \pm 1.3	6.2 \pm 0.7	9.6 \pm 5.3	6.8 \pm 0.2	0.423

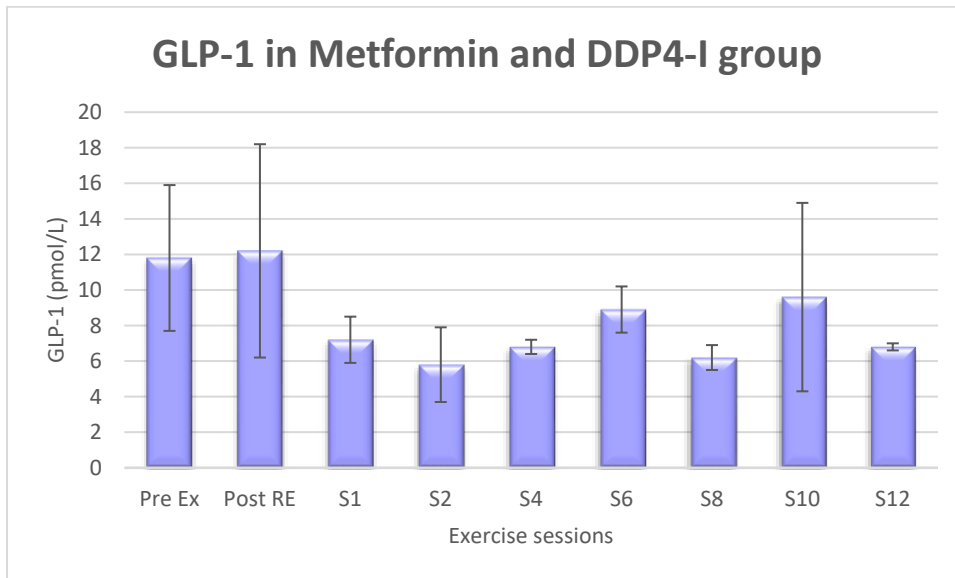


Figure 58: GLP-1 level in Metformin and DPP4-I group during the whole exercise sessions

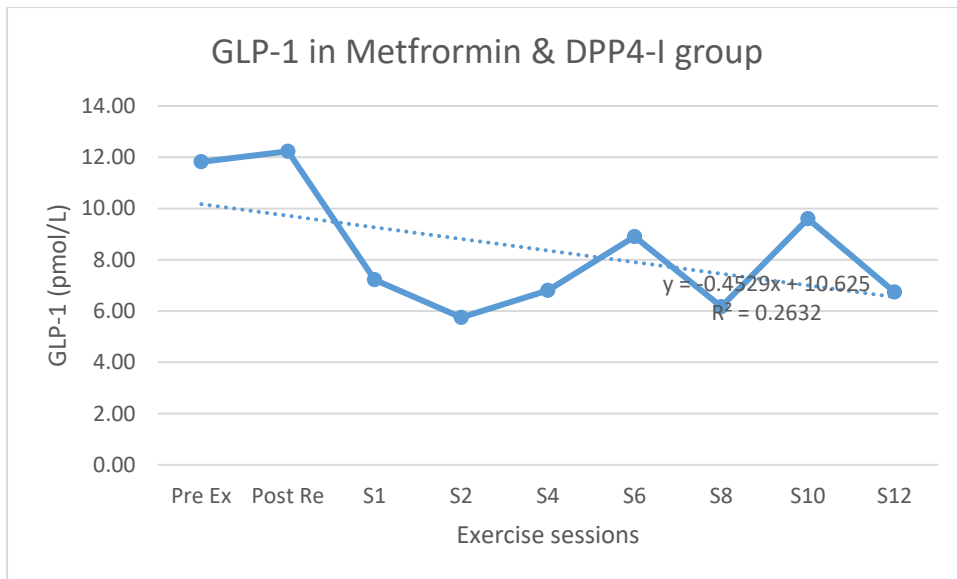


Figure 56a: This is a regression with normalised intervals, showing a very slight downward trend and very poor relationship over the 6 weeks (where not all sessions were measured)

In the 2nd group (Metformin and DPP4-I group), a reduction also occurred thus GLP-1 decreased from (11.8 ± 4.1) to (7.2 ± 1.3) , ($P = 0.347$) after S1. This reduction was slightly more after the 12th session (6.8 ± 0.2) , ($P = 0.423$).

Table 33: GLP-1 level during the 1st session of Exercise (Metformin and SGT2-I group) where results shows acute effect, Data expresses as mean \pm SEM and P value:

GLP-1 (pmol/L)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	3.9 \pm 1.5	6.4 \pm 2.4	8.4 \pm 1.2	0.345

Table 34: GLP-1 level during the 12 sessions of Exercise (Metformin and SGT2-I group) where results shows chronic effect, Data expresses as mean \pm SEM and P value:

GLP-1 (pmol/L)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	3.9 \pm 1.5	8.4 \pm 1.2	10.7 \pm 4.6	7.9 \pm 3.6	13.3 \pm 0.3	12.6 \pm 0.9	13.4 \pm 0.8	11.0 \pm 0.8	0.196

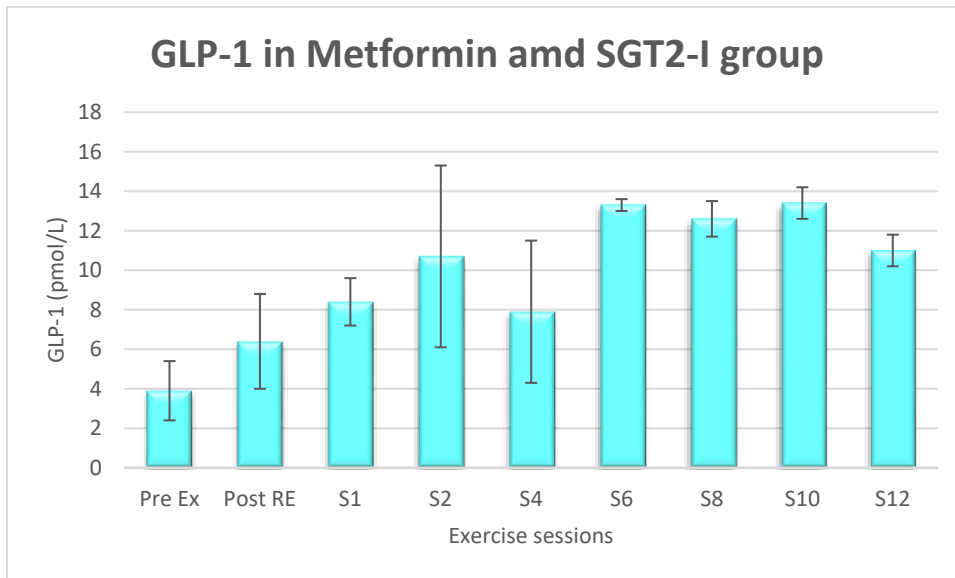


Figure 59: GLP-1 level in Metformin and SGT2-I group during the whole exercise sessions

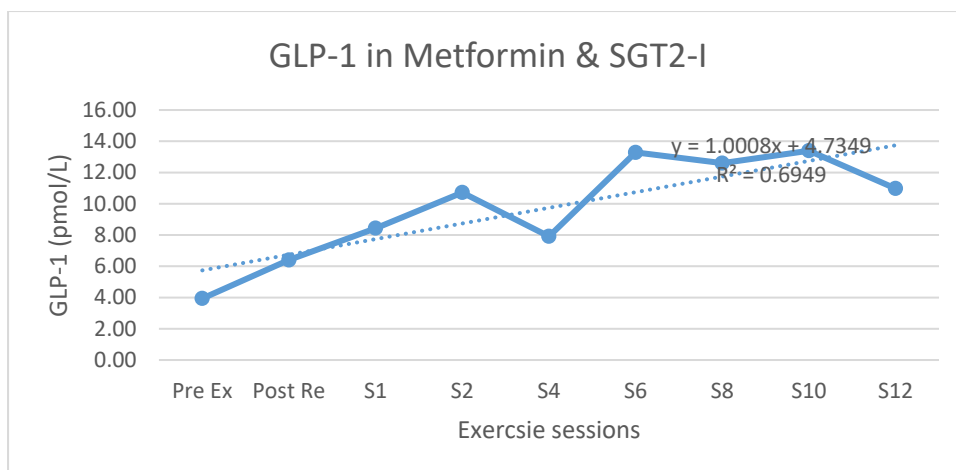


Figure 57a: This is a regression with normalised intervals, an upward trend but not a very convincing relationship over the 12 sessions, although the R2 value is much better than the one above over the 6 weeks (where not all sessions were measured)

In the third group, the trend was reversed. In this set (Metformin and SGT2-I group), the result shows elevation in GLP-1 after both acute and chronic effect, however, the elevation after 12th sessions was higher. GLP-1 in this group was (3.9±1.5) and increased to (8.4±1.2), (P=0.345) after S1 and then increased more to (11.0±0.8), (P=0.196) after 6 weeks of exercise. The result demonstrates that the combination of metformin and SGT2-I is associated with the elevation of GLP-1 hormone. The improvement in GLP-1 in the last group could be a result of an improvement in HbA1c as mentioned in previous literature published by Mensberg et al., (2017) where a correlation between GLP-1 and HbA1c was reported. In the present study, the highest reduction of HbA1c among all T2D groups was observed in the metformin and SGT2-I group and it reduced from (77 mmol/mol) to (58 mmol/mol).

The combination of metformin with SGT2 inhibitors plus exercise is an improvement in diabetes control, despite the statistically ambivalent result. It is clear that in this group, there may be a trend that is worth further investigation and should be examined with other relevant factors.

6.7 Conclusion:

SGLT2-I and metformin are shown to affect individuals' weight and the reduction in weight could be due to improved level of GLP-1. Since research showed that there is a correlation between increased level of GLP-1 and weight reduction. As seen in the study of Hussein et al., (2014) which showed lower GLP-1 levels in obese individuals and negatively correlated GLP-1 with BMI and waist circumference, however, no significant correlation was noticed between GLP-1 levels and age or sex. Some studies state that no significant correlation was noticed between GLP-1 levels and age or sex (Hussein et al., 2014). Thus, the present study didn't correlate GLP-1 level with age or sex. Another factor that might also affect the level of GLP-1 during six weeks is eating prior to the sessions, because Nauck et al., (2011) showed that age, body weight and fasting state could affect GLP-1 level. In addition, insulin resistance can also impair the secretion of GLP-1 (Nauck et al., 2011). Thus, suggesting that there are several factors that could affect the response of GLP-1 in both T2D and ND. However, the study exercise training programme used in the present study showed no significant differences in serum GLP-1 levels which could be due to the small sample size or other unknown factors.

In summary, according to outcomes of other studies, it looks that assessment of variations in serum GLP-1 levels need more studies with longer duration and higher number of participants to show valid results.

6.7.1 Limitations:

- 1- Only few volunteers were recruited after we received the specific tube for collecting blood samples.
- 2- No data of GLP-1 was collected from ND group due to high price of special tubes that was used to collect blood sample (BD P800).
- 3- No specific diet has been followed.

6.7.2 Future work:

Conduct another study with larger sample size and analyse GLP-1 in ND group as well with longer time follow up to obtain conclusive results.

Chapter 7 The effect of moderate combination exercise cardio “aerobic” and resistance on the inflammatory markers in T2D and ND subjects

7.1 Research questions:

Do inflammatory markers improve after 6 weeks of exercise? Do the improvements correlate with medication type?

7.2 Aim:

The aim of the study is to investigate the effects of a combined exercise programme (cardio “aerobic” and resistance) on the inflammatory markers in T2D and ND.

7.3 Inflammatory markers:

Cytokines are protein molecules that play main roles in operating the body immune system (Vijayaraghava and Doreswamy, 2017). They are secreted by different kind of cells into the circulation, the reticulo-endothelial, the adipose tissue and the musculoskeletal systems. Their release into circulation in response to different stimuli e.g. infections, antigens, mental stress and exercise (Vijayaraghava and Doreswamy, 2017). Adipose tissue is the main site for storage of extra energy in the form of TG. It consists of multiple cell types such as pre-adipocytes, adipocytes, immune cells and endothelial cells (Jung and Choi, 2014). Obesity causes adipose tissue dysfunction which leads to metabolic complication (Abate, 2013). There are two kind of adipose tissue which have two opposite function; white adipose tissue (WAT) and brown adipose tissue (BAT). The role of WAT is to store extra energy in form of TG while BAT is responsible for dissipation of energy by heat production (Saely, Geiger and Drexel, 2012). WAT can be considered as a disseminated endocrine organ that consists of various cells such as adipocyte, macrophage, T lymphocyte and endothelial cells (Feijóo-Bandín et al., 2016). WAT plays a role in the metabolism and regulation of lipid and energy homeostasis. The distribution of WAT in the body consists of subcutaneous adipose tissue (SAT) which accumulates under the skin of abdominal, femoral and gluteus area and also in visceral adipose tissue (VAT) which located in the visceral of abdominal cavity specifically in the mesentery (Feijóo-Bandín et al., 2016). Various studies found that SAT is associated with a cardioprotective phenotype and VAT was contributed to the development of insulin

resistance, T2D, dyslipidaemia and cardiovascular disease (CVD) (Feijóo-Bandín et al., 2016). The large mass of VAT augments the synthesis of triacylglycerol-rich lipoproteins (Kishida, Funahashi and Shimomura, 2014). These occur because VAT venous blood drains through the portal vein into liver and secretes large quantities of FFA that may then locate in the skeletal muscle and pancreas. This enhances glucose production and decreased the dispose of glucose in the liver (Kishida, Funahashi and Shimomura, 2014; Feijóo-Bandín et al., 2016). SAT plays a role in energy long-term storage and preservation of IS in infants and in adolescence (Kishida, Funahashi and Shimomura, 2014). Although, consuming large amounts of food in these ages leads to obesity. Furthermore, BAT is a special tissue which converts energy to heat when activated by the sympathetic nervous system following signals from the hypothalamus. It has been believed that BAT only found in infants but recently research proved that BAT also found in adults (Betz and Enerback, 2015; Saely, Geiger and Drexel, 2012). BAT plays a central role in IS in humans and rodents (Betz and Enerback, 2015). BAT is a thermogenic connective tissue that preserves body central temperature at a constant 37 °C even in moderately cold weather. The differences between WAT and BAT is that WAT generally contains only a huge lipid droplet and have only a few mitochondria while BAT consist of numerous lipid droplets in each cell and are full with mitochondria (Betz and Enerback, 2015). High amount of BAT in the body is associated with lower body weight (Saely, Geiger and Drexel, 2012). In humans BAT is found in fat tissues in the neck, thorax, and abdomen, intestines, heart, lungs, kidneys, and adrenals (Lee, Swarbrick and Ho, 2013). Production of an interplay of metabolic active proteins such as pro-inflammatory cytokines (TNF and IL-6) and adipokines or adipocytokines (leptin, resistin and adiponectin) (Abate, 2013).

7.3.1 C - reactive protein (CRP)

William Tillet and Thomas Francis discovered CRP in 1930, at the Rockefeller Institute for Medical Research, New York (Shrivastava et al., 2015). CRP is a 206- amino acid member of the short pentraxin family and mostly synthesized in the hepatic cells. Its production is controlled by cytokines, mainly interleukin 6 (IL-6) (Salazar et al., 2014; Tanaka and Kishimoto, 2014). The normal median level of CRP is 0.8 mg/L, while during acute infection the level of CRP elevated to about 10,000-fold and then after that it became very low level (Shrivastava et al., 2015). CRP is a non-specific marker for inflammation such as infection (Salazar et al.,

2014; Liu et al., 2016; Dasgupta and Wahed, 2013a). It plays a role in body defence against infective diseases (Shrivastava et al., 2015). An increase in CRP level has been associated with IR, higher BMI, elevated WC, T2D and MetS, as well as different chronic inflammatory disease such as CVD, certain rheumatoid condition and cancer (Kaur, 2014; Salazar et al., 2014). The level of CRP in obese individuals is reported to be elevated when compared to insulin sensitive individuals. CRP is good independent indicator of cardiovascular disease (CVD) which is associated with MetS (Kaur, 2014; Aggarwal et al., 2015; Hayashino et al., 2014). Lukic et al. states that the level of CRP increased in obese T2D individuals with hypertension when compared to obese subjects with T2D (Lukic et al., 2014). Research shows an association between CRP and atherosclerosis and CRP are said to bind lipoproteins especially low-density (LDL) and very low-density lipoproteins (VLDL) which are found in in atherosclerotic plaque (Shrivastava et al., 2015). It contributes to atherosclerosis by enhancing the activation of endothelial cell generation of foam cell inside the wall of artery (Hayashino et al., 2014). Furthermore, increased CRP is associated with coronary heart disease (CHD), peripheral artery disease (PAD) and Ischemia, myocardial infarction (MI), stroke and sudden cardiac death (Shrivastava et al., 2015). It was reported that neuropathy is associated with high level of CRP (Grossmann et al., 2015). Interestingly, T2D subjects who are using GLP-1 receptor agonist showed reduction in CRP level which reduced complications such as atherosclerosis and nephropathy (Grossmann et al., 2015).

7.3.2 Interleukin (IL-6)

In humans, IL-6 is a protein synthesized by T cells and macrophages at the sites of inflammation to stimulate immune response in case of infection, burn, trauma or tissue injury (Tanaka, Narazaki and Kishimoto, 2014). Then it transfers in the blood to liver and stimulates hepatic secretion of CRP (Tanaka, Narazaki and Kishimoto, 2014). IL-6 is also induced by TNF and is produced widely including in striated (skeletal) muscle and visceral tissue (Liu et al., 2016; Kaur, 2014). IL-6 receptors are found in the hypothalamus and is important in appetite regulation. IL-6 normal value is 0.5-15 pg/ml (Alecú et al., 1998). In healthy subjects, IL-6 has vital roles such as regulating glucose metabolism, increasing the release of leptin, increasing lipolysis and suppressing the activity of lipoprotein lipase in adipocyte (Kaur, 2014; Lukic et

al., 2014). On the other hand, IL-6 affects the function of pancreatic β -cells as well as interfering with insulin signalling and also, its reduction linked to increase high-density lipoprotein (HDL) (Liu et al., 2016; Kaur, 2014). High level of IL-6 associated with Mets, but the mechanisms by which IL-6 decrease HDL levels are unclear (Feingold and Grunfeld, 2015). The anti-inflammatory effect of IL-6 inhibits TNF from affecting insulin signalling which could prevent MetS and hyperglycaemia (Brown et al., 2015). The action of IL-6 is altered according to the secreting tissue and its action is controversial because it acts as anti-inflammatory and pro-inflammatory cytokine (Jung and Choi, 2014; Brown et al., 2015). Dual action might be due to either individual variation of training status or methodological differences in exercise intensity or duration (Brown et al., 2015). Therefore IL-6 secreted acutely after muscle contracted during exercise and this is useful in the metabolism because it acts in this case as anti-inflammatory cytokine by an inhibition of TNF- α and by stimulating IL-1ra (IL-1 receptor antagonist) (Karstoft and Pedersen, 2016). IL-6 is increased with obesity and T2D, as it linked to IR and high level of IL-6 activates the secretion of CRP in the liver (Liu et al., 2016; Kaur, 2014). High mass of adipose tissue linked to highly secreted pro-inflammatory cytokine and cause insulin resistance (Jung and Choi, 2014; Pedersen and Fischer, 2007). The studies by Lukic et al. showed that the level of IL-6 is higher in T2D obese and HTN individuals compared to T2D obese subjects (Lukic et al., 2014). While the increase of muscle mass that strongly associated with the secretion of anti-inflammatory cytokine and has beneficial effect on metabolism (Pedersen and Fischer, 2007). Also, it has been found that the administration of IL-6 to human body increases insulin-stimulated glucose disposal as well as FA oxidation, and therefore it has been suggested that IL-6 and its receptors might be useful as a targets for the treatment of some metabolic diseases (Pedersen and Fischer, 2007).

7.3.3 Tumor Necrosis Factor (TNF)

TNF is a pro-inflammatory cytokine and a produced mostly by macrophages and monocytes (Liu et al., 2016; Feijóo-Bandín et al., 2016). TNF enhances the production of CRP and is positively correlated with IR and T2D (Liu et al., 2016; Kaur, 2014). An increase in TNF is associated with elevated level of TG, LDL, body weight and WC as well as decrease HDL. However, a decreased level of TNF is associated with high levels of HDL (Feijóo-Bandín et al., 2016). A positive correlation between TNF and T2D is reported, where plasma level of TNF can be used to obtain the status of IR (see figure 49) (Liu et al., 2016). Primarily, the production

of TNF is enhanced by IL-18 (IL-1 family member), and in turn, TNF enhances the release of IL-6 and CRP (Liu et al., 2016). The normal level of TNF 0-16 pg/ml (Alecú et al., 1998).

7.3.4 Leptin

Leptin was the first adipokine, discovered by Friedman in 1994. It is a 167 aminoacid polypeptide, with production on chromosome 7 (Feijóo-Bandín et al., 2016; Li and Li, 2016). Friedman called the newly discovered hormone “leptin” (Greek = thin) (Li and Li, 2016). Leptin is mainly secreted in WAT and can be secreted by skeletal muscle, liver, kidneys, ovary, placenta, bone marrow and heart. It mainly acts in the central nervous system and plays essential role in decreasing appetite and enhancing the consumption of energy by acting on hypothalamus (Mechanick, Zhao and Garvey, 2018; Li and Li, 2016; Feijóo-Bandín et al., 2016). In women, leptin levels are higher than in men which might be due to the greater ratio of body fat in female (Mechanick et al., 2018). Elevated leptin levels are associated with decreased energy intake and increased energy utility, thus decreased storage as adiposity (Mechanick et al., 2018). Paradoxically, the level of leptin was shown to increase in obese subjects and decrease when the weight is lost, suggesting that these subjects have leptin resistance (Kaur, 2014; Feijóo-Bandín et al., 2016). Leptin resistance is linked to obesity because the plasma level of leptin was shown to associate with the mass of WAT. The receptors of leptin found in WAT, kidneys, liver, skeletal muscle, pancreas, ovaries, heart and brain (Kaur, 2014; Feijóo-Bandín et al., 2016). Leptin activates macrophages and monocyte which regulate inflammation by releasing pro-inflammatory cytokines such as TNF and IL-6. Elevation of leptin (leptin resistance) is also associated with insulin resistance, rheumatoid arthritis, Alzheimer’s and cancer as well as increased risk of stroke and MI (Feijóo-Bandín et al., 2016). Various studies showed that leptin stimulates macrophage and leukocyte into endothelial wall. This cascade increase the risk of atherosclerotic plaque by enhancing platelet activation and formation of plaques in the arteries, also by enhancing the secretion of pro-inflammatory markers such as IL-6, TNF or reactive oxygen species (ROS) which cause oxidative stress (OS) then endothelial dysfunction and finally atherosclerosis (Feijóo-Bandín et al., 2016). Although leptin is a vasodilator, its elevation is associated with hypertension because it stimulates peripheral vascular resistance (Kaur, 2014; Feijóo-Bandín et al., 2016). The effects relate to hyperglycaemic states in MetS and T2D are interesting. A study conducted by Meek, describes the role of leptin in controlling hyperglycaemia in uncontrolled

insulin-deficient diabetes (uDM) (Meek and Morton, 2016). In an insulin resistant diabetes rodent model, the administration of leptin reduces severe insulin resistance and improve diabetes associated with leptin deficiency. The mechanism of glucose lowering effect of leptin acts by decrease consumption of food and elevate the glucose urinary excretion or increase β -cell recovery. Furthermore, it normalizes the secretion of glucose from the liver and the peripheral tissue uptake of glucose (Meek and Morton, 2016). It has been found that leptin would be a useful anti-obesity medication (Mechanick et al., 2018). Therapeutic treatment of leptin has been approved in case of lipodystrophy. The action of leptin on lowering glucose in T1D and T2D rodents gives possibility in using it in future as adjunct therapy to insulin in diabetes subjects (Meek and Morton, 2016).

7.3.5 Resistin

Resistin is an adipokine with a similar molecular structure to adiponectin, it is a protein 12.5 kDa of molecular weight and is produced in human by macrophages and pre-adipocytes (Feijóo-Bandín et al., 2016). In obese individuals, the resistin level is increased but the mechanism is unknown (Feijóo-Bandín et al., 2016). The mechanism of the association between resistin and obesity, T2D, MetS and CVD is also still unknown (Abate et al., 2014). Some studies were illustrated that the association between obesity component in MetS and resistin is controversial, but others show a correlation between resistin and BMI or WC (Abate et al., 2014). Resistin thus has a crucial role in MetS pathogenesis (Abate et al., 2014). The expression of resistin in the pancreatic β -cell is greater in T2D than in healthy people and resistin correlates positively with CRP and IL-6 in T2D subjects (Abate et al., 2014). Its elevation is also associated with the complication of T2D such as neuropathy, nephropathy, atherosclerosis and gout (Abate et al., 2014). The level of resistin is reported to increase with the increment of OS and myocardial injury (Feijóo-Bandín et al., 2016).

7.3.6 Cystatin C (CysC)

Cystatin C is a 13 kDa protein consisting of 122-amino acid, belonging to the family of cysteine protease inhibitors and produced by different nucleated cells (Javanmardi et al., 2015; Shetty et al., 2017). It is also called microprotein (Javanmardi et al., 2015). Cystatin C (CysC) considered as alternative markers for glomerular filtration rate (GFR) which can be detected in urine and blood samples (Di Somma and Marino, 2019). The plasma level of CysC increased

in case of kidney function declined and it is a precise test for kidney function (Di Somma and Marino, 2019). In early stage chronic kidney disease (CKD), CysC is superior to serum creatinine as a marker of kidney function (Čabarkapa, 2015). Elevated level of CysC linked to increased cardiovascular risk in general population or preeclampsia in pregnant women. Some factors could affect the level of CysC such as the use of glucocorticoids, smoking, malignancy, inflammation, adiposity or thyroid dysfunction (Inker and Levey, 2014; Čabarkapa, 2015). When compared with serum creatinine, CysC is not influenced by gender, race, age, diet and muscle mass (Javanmardi et al., 2015; Král et al., 2016). While creatinine is strongly dependent upon the body composition, age, gender, ethnicity and GFR (Grubb, 2011). As a result, CysC has been recommended as the best reliable predictor for both CKD, atherosclerosis and high risk of CVD (Kobayashi et al., 2017). CVD and CKD represent similar pathophysiologic bases such as metabolic abnormalities, endothelial dysfunction, chronic inflammation and OS (Satoh-Asahara et al., 2011). The mechanism of the elevation of CysC in CVD is unclear, the justification is only that CVD is linked to renal impairment (Magnusson et al., 2016). The mechanism of this correlation is also unknown (Magnusson et al., 2016). CysC elevation correlate with abdominal obesity and IR developing in T2D (Schmid et al., 2014). The normal level reported is between 0.52 and 0.98 mg/L (Král et al., 2016). An increase in the level of plasma CysC is associated with the decline in GFR in diabetes compared to healthy subjects (Javanmardi et al., 2015). In T2D, CysC is a useful marker compared to serum creatinine or microalbuminuria in predicting diabetes nephropathy (Javanmardi et al., 2015; Shetty et al., 2017).

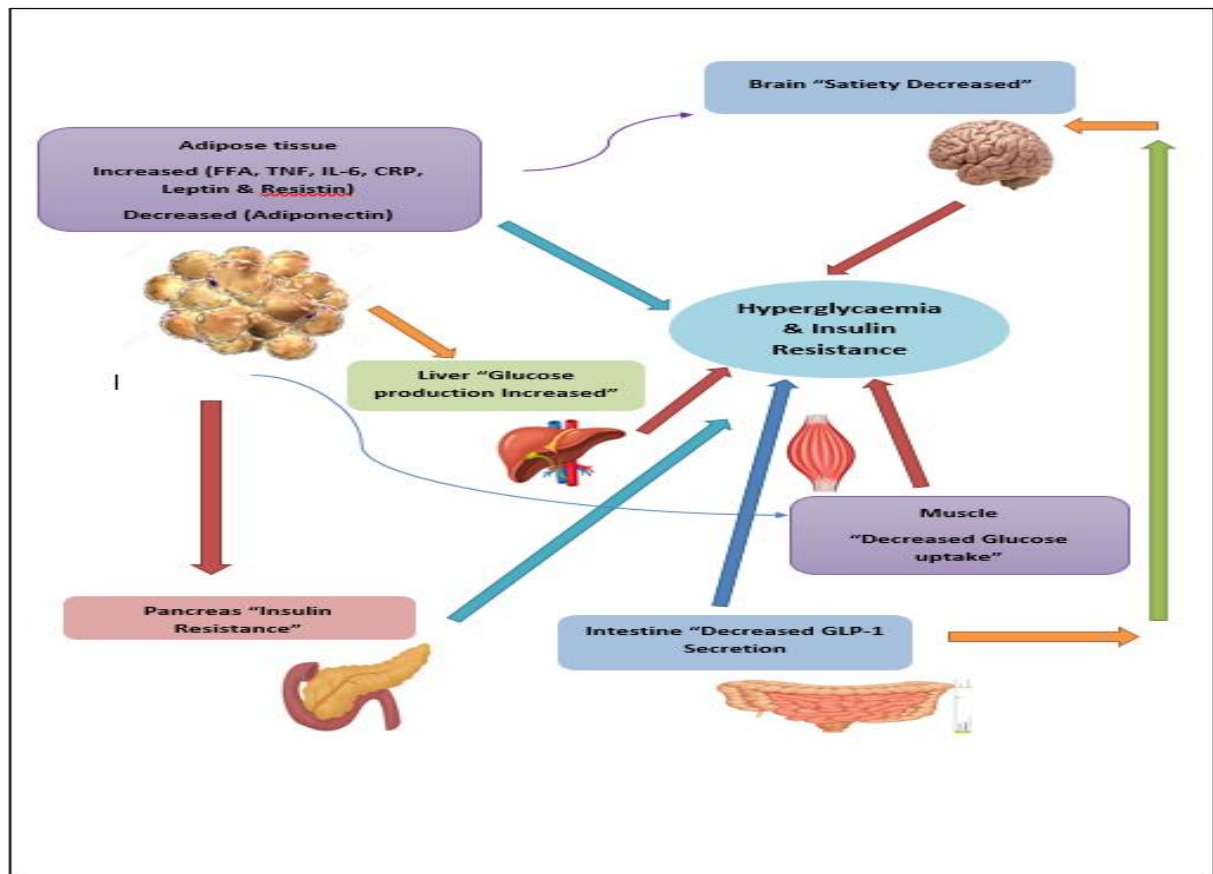


Figure 60: Cytokines disturbance in adipose tissue and diabetes

7.4 Inflammatory markers of diabetes

Markers and other factors demonstrate inflammation play a vital role in T2D development (Wang et al., 2013a). The high level of these biomarkers stimulate IR and are associated with high risk in T2D (see figure 59) (Liu et al., 2016). In previous studies, chemokines, cytokines and white blood cell count have been identified as inflammatory factors that can anticipate the development of T2D (Esser et al., 2014). A meta-analysis and systematic review confirmed this by demonstrating that increased levels of CRP and IL-6 considerably relate to a higher risk of developing T2D (Wang et al., 2013b; Esser et al., 2014). In T2D and obesity, the pancreas, the adipose tissue, and the skeletal muscle cells responsible for producing pro-inflammatory cytokines, e.g. include IL-1 β , tumor necrosis factor (TNF- α), and IL-6 (Wada and Makino, 2013; Esser et al., 2014). These cytokines facilitate insulin resistance by interfering with the insulin signalling in the tissues (Esser et al., 2014). In non-obese, adipocyte secrete anti-inflammatory adipokine such as adiponectin., while in case of obesity the genes responsible for encoding

inflammatory factors are up-regulated and that cytokines and chemokines are overproduced in distended adipose tissues (Ouchi et al., 2011). Thus by the macrophage content of adipose tissue increased in correlation with adipocyte size and body mass, and expression of pro-inflammatory cytokines generally come from macrophages. As the quantity of macrophages increased in adipose tissue, obesity convert these cells from an anti-inflammatory to a pro-inflammatory state (Jung and Choi, 2014). Therefore, improving insulin sensitivity via weight loss leads to a decline in the expression of pro-inflammatory genes (Esser et al., 2014). These pro-inflammatory cytokines correlate with the extent of obesity and they are related to the resistance of insulin and systemic inflammation (Esser et al., 2014). Inflammation exists in the pancreatic islets of individuals suffering from T2D as demonstrated by the existence of higher levels of pro-inflammatory chemokine and cytokine levels, and increased death of β cells (Donath and Shoelson, 2011). Furthermore, there is an increase in the manifestation and localised release of IL-1 β , which is a pro-inflammatory cytokine in the pancreatic islet of diabetic individuals (Verdile et al., 2015; Esser et al., 2014). Obesity, resistance towards insulin, and T2D are linked to chronic inflammation (Sell, Habich and Eckel, 2012).

Several studies illustrate, that there is a link between greater risk of increasing CVDs and adipose tissue pathological function. That risk increases by producing different chemical mediators to act in paracrine, endocrine and autocrine (Feijóo-Bandín et al., 2016). As stated above, biomarkers such as adiponectin, IL-6, CRP and TNF linked to pathogenesis of T2D which could help in the development of new treatment for T2D (Liu et al., 2016).

7.5 Effect of exercise on the inflammatory markers

The value of exercise is closely linked to the anti-inflammatory effect. Physical exercise is linked to a decline in TNF-, IL-6, and CRP (Teixeira de Lemos et al., 2012; Orsatti et al., 2014; Phillips et al., 2012). However, evidence suggests that the intensity and duration of exercise and muscle damage after acute exercise could influence IL-6 response to acute exercise (Reihmane and Dela, 2014). Physical exercise has a known impact in cytokines released by cells in the muscle. The production of TNF was directly inhibited by physical exercise via the production of IL-6 from the exercising muscles (Fisman and Tenenbaum, 2010). In this case, IL-6 is an anti-inflammatory factor whose concentration increases in the plasma after physical exercise which illustrates its dual action (Fisman and Tenenbaum, 2010). Several studies have

focused on determining the anti-inflammatory effects of inflammatory markers by chronic exercises. Different inflammatory markers have been found to demonstrate different response to training activities. Basically, various studies on the effect of exercise on inflammatory markers demonstrate that exercise and training have a potential of reducing the level of circulation of the inflammatory markers in T2D as it is an inflammatory disease (Hamer et al., 2014). The majority of studies have shown that cardiac rehabilitation and chronic physical exercises have indicated a possible anti-inflammatory effect (Orsatti et al., 2014). The anti-inflammatory impact of exercise in T2D is concerned with reducing levels of inflammatory cytokines and increasing the anti-inflammatory cytokines (Teixeira de Lemos et al., 2012; Hamer et al., 2014).

In a 12-month follow-up study, it was revealed that an increase from moderate to vigorous exercise was linked to a decline in IL-6 and CRP, independently of changes in BMI among the T2D individuals (Herder et al., 2009). Consistently, aerobic exercise training resulted in a declined CRP and that a decrease in central adiposity partly mediated this effect (Vieira et al., 2009). Campbell et al., (2009) reported similar results in a 12-month trial where it was shown that moderate exercise training led to a decrease in CRP only in women with obesity at baseline and in those whose body fat loss was over 2%. Other studies however found that there is no changes in CRP level after exercise (Nygaard et al., 2017; Mendham et al., 2011). A systemic review concluded that exercise is a good therapeutic option to improve adiponectin and leptin level in over weight and obese individual (Yu et al., 2017). As such, this acts as an independent factor affecting the inflammatory markers and inhibiting physical activity. On the other hand, cross-sectional studies demonstrated that physical activity is related to reductions in the circulating levels of TNF and IL-6 (Bongers et al., 2017). Patients with heart failure complications were found to have a reduced skeletal muscle TNF after 6-month exercise training. Although, serum levels were not affected in this same group (Brown et al., 2015). Exercise training promotes bioavailability and minimises circulating markers that are found in the endothelial dysfunction (Verheggen et al., 2016). Some previous studies demonstrate that exercise improve CysC (Eskandar, Katayon and Ali, 2015). Positive effects of aerobic exercise have been noticed in improving cardiac and kidney function by improving CysC. While Serin et al. (2009) found that 6 weeks submaximal exercise has no effect on renal function (Eskandar, Katayon and Ali, 2015). A study by Eskandar et al. (2015) has stated that

aerobic exercise improve FBG, SBP and lipid profile significantly, however the effect on CysC, WC and DBP was not significant, before exercise CysC was (0.62 ± 0.29 ng/mL) and after exercise become (0.61 ± 0.25 ng/mL) (Eskandar, Katayon and Ali, 2015). Resistance training was found to induce an improvement in CRP, lipid profile and alterations of muscle strength in postmenopausal obese women (Orsatti et al., 2014). As such, the findings of the study concluded that 8-week resistance training is useful in improving inflammation levels, glycemic profiles, strength and muscle mass for individuals with obesity. The main weakness of the Orsatti study is that it used a relatively small sample size in the study. Recruiting women was a significant strength for this study because they are not usually involved in resistance training (Orsatti et al., 2014). Resistin is an adipocyte that is linked to obesity and inflammation which is linked to obesity disorders like IR and diabetes mellitus. In order to identify the effect of serum resistin and physical exercise for men, Yahya et al. (2014) used 14 men who were exposed to a 40 minutes running that was exercised in moderate intensity (Yahya et al., 2014). The study concluded that exercise test for a particular single session with moderate intensity does not have significant effect on resistin in sedentary obese men. This insignificant result might be due to small sample size for the study (Yahya et al., 2014). CRP is linked to a high severity of atherosclerosis. The evidence is insufficient to demonstrate the effect of resistance training on CRP (Hayashino et al., 2014). A meta-analysis of randomized control trials by Hayashino et al., (2014) found that exercise is beneficial in improving CRP and IL-6 in subjects with T2D as well as in subjects with diabetes it might be a good therapeutic option to reduce inflammatory markers (Hayashino et al., 2014). The study by Ramel et al. (2015) was established to found out the response of CRP to resistance exercise in adult individuals. The intervention was set as a 12-week resistance training (Ramel et al., 2015). The study concludes that the level of CRP decreased significantly after enduring 12 weeks exercise activity for all subjects exhibiting abnormal high CRP, which is important in reducing the possibility of future diseases. The result of this study was not significant to the hypothesis created in this particular study (Ramel et al., 2015). In addition, additional RCT of 8 weeks of walking exercise was conducted on 30 men aged (51.56 ± 11.28) years and divided into two groups; control group and intervention group (Nuri et al., 2016). The results illustrate improvement of VO_2 peak and reduction in body fat percentage ($P < 0.05$). However, the level of leptin and IR has no significant improvement (Nuri et al., 2016). It has been concluded that that 8 weeks of aerobic

exercise improved body fat percentage and VO₂peak, nevertheless it has no effect on serum leptin or IR (Nuri et al., 2016).

The anti-inflammatory impact of exercise in T2D individuals has been demonstrated in the literature. Eckardt et al. (2014), suggested that regular exercise reduces inflammatory marker levels and protects against diseases caused by inflammation (Eckardt et al., 2014; Esser et al., 2014). In conclusion, this part shows the connection that exists between T2D and inflammation by highlighting the inflammatory markers linked to this disease. Therefore, regular exercise should be a critical part of the various strategies used in managing T2D. The plan in this study is to start 6 weeks combination exercise to measure the effect of exercise on the inflammatory markers in T2D and ND, and to see the effect of pharmacological treatment on these markers which no one else has conduct it before.

7.6 Method:

7.6.1 Study design

Each exercise session will consist of a combined exercise protocol of 30 min of resistance exercise followed by 20 min moderate cycling twice a week for 6 weeks. Volunteer should stretch-up for 11 steps of stretching, then the volunteer must cycle for five minutes to warm up. Blood samples collected at base line after S1, S2, S4, S6, S8, S10 and S12. Blood samples centrifuge and refrigerate to be analysed by The Evidence Investigator™ Biochip Array technology (Randox, UK) is a Multiplexing ELISA technology which described previously in section 3.10.

7.6.2 Intervention group

The intervention group are T2D (n= 13) and ND (n=8) who are doing combination exercise.

Intervention group are classified in four groups

- 1- T2D volunteers with no medication.
- 2- T2D volunteers on Metformin only.
- 3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4 I).
- 4- Non-diabetes (ND).

Medication	Number of Volunteers
Pravastatin 40mg OD	1
Atorvastatin 20mg OD	3
Metformin 500 mg BD	3
Metformin 850 TDS	1
Metformin 1000 mg BD	2
Sitagliptin 50 mg OD (DPP4-I)	1

Sitagliptin 100mg OD (DPP4-I)	2
Empagliflozin 10 mg OD	2

7.7 Results and discussion:

The plasma obtained from T2D and ND volunteers was used to analyse for inflammatory markers with the aid of Randox machine in this study. Data was analysed by using Excel 2010 and SPSS (version 22), Values were expressed as mean with standard error of mean. Pre and post exercise samples were compared using a paired-samples t-test and the level of statistical significance was act at ($P < 0.05$).

7.7.1 Results among the two groups (ND and T2D)

7.7.1.1 CRP

Table 35: Changes in CRP level was observed during the 1st exercise session (acute effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

CRP (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	21.2±5.5	26.3±8.4	23.1±6.0	0.268
ND	7.4±2.5	6.2±1.7	6.9±1.9	0.784

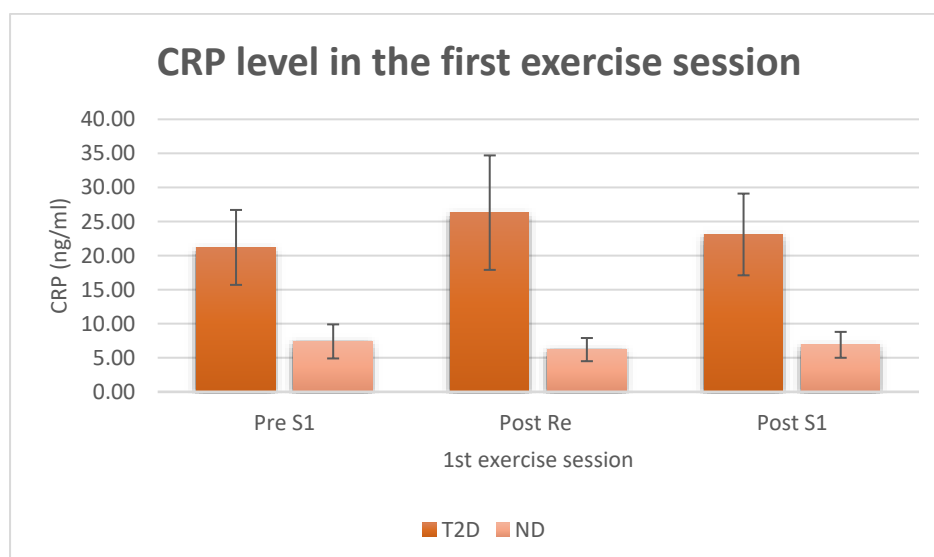


Figure 61: CRP level in T2D and ND in the 1st exercise session

Table 36: Changes in CRP level was observed during the 12 exercise sessions (chronic effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S12 was obtained.

CRP (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	21.2±5.5	23.1±6.0	16.3±4.9	11.1±4.5	15.1±3.5	19.0±5.5	15.3±5.3	14.7±5.5	0.014
ND	7.4±2.5	6.9±1.9	7.2±1.4	5.3±0.95	4.7±0.5	5.9±1.4	4.4±0.4	5.1±1.1	0.396

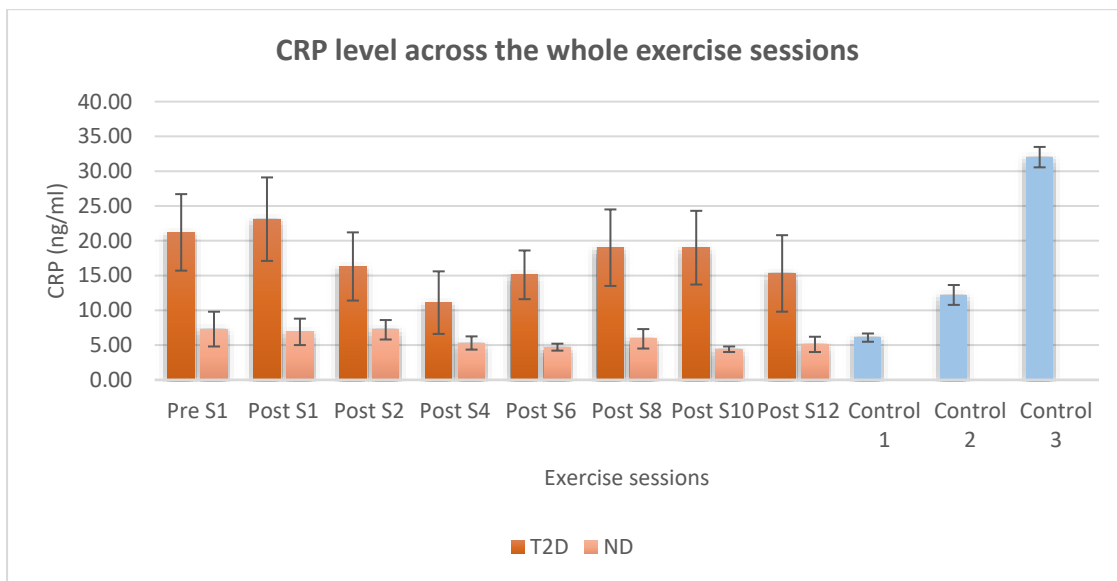


Figure 62: CRP level in T2D and ND across the whole exercise sessions

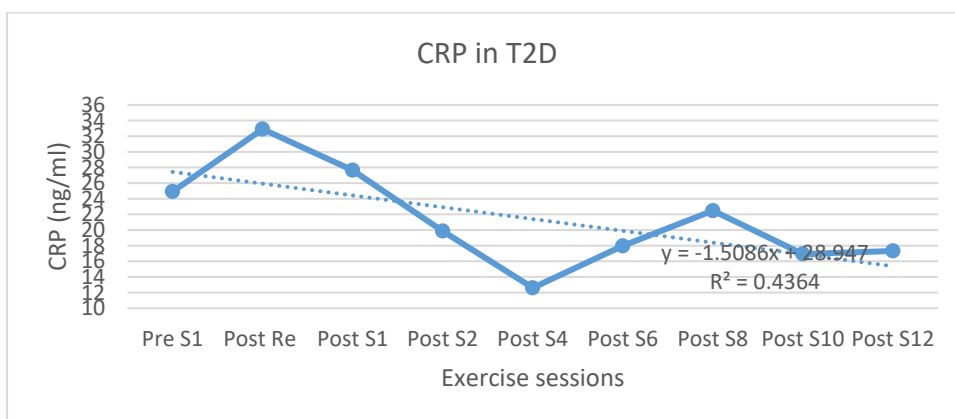


Figure 60a: This is a regression with normalised intervals, representing a downward trend and a poor relationship over the 6 weeks (where not all sessions were measured)

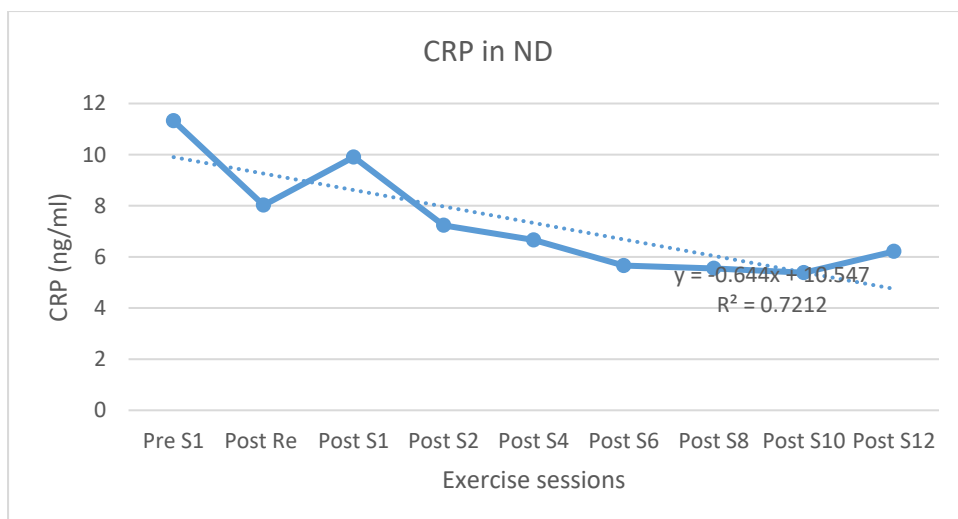


Figure 60b: This is a regression with normalised values, showing a slight downward trend with a strong relationship over the 12th sessions (where not all sessions were measured)

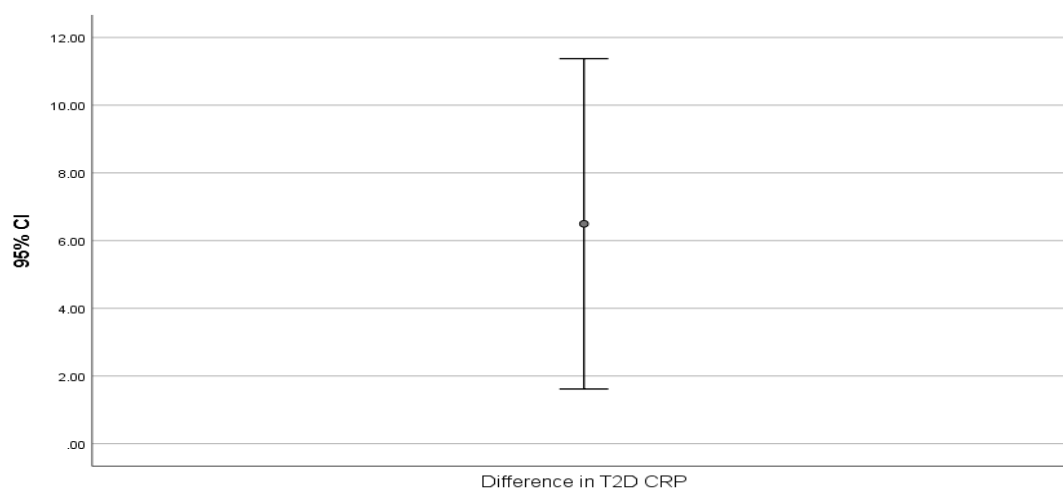


Figure (60c): T2D CRP shows CI 95% higher than zero which mean this difference is significant even if there are overlap in SEM

In T2D individuals, an elevation in the level of CRP (figure 60) was observed after the 1st exercise session from 21.2±5.5 to 23.1±6.0 ng/ml (P=0.268) and this level decreased significantly after 12th exercise session to 14.7±5.5 ng/ml (P=0.014) (figure 61). Moreover, combination of exercise and the use of Metformin improve CVD risk factors such as CRP (Malin and Braun, 2016). In our study, 14 volunteers out of 17 were using Metformin in combination with exercise which explain this significant improvement. In ND, the results of

CRP after the acute effect of exercise was observed to have decreased from 7.4 ± 2.5 to 6.9 ± 1.9 ng/ml ($P=0.784$). However, this decreased more after the 6th week exercise session to 5.1 ± 1.1 ng/ml ($P=0.396$) which was not significant. In T2D, the results showed that CRP increased after acute effects of exercise and finally decrease after 6 weeks of exercise while in ND CRP decreased after acute and chronic effect of exercise. CRP is reported to have increased after acute effect of exercise due to muscle injury in untrained adults (Brown et al., 2015). The reduction in CRP by exercise is reported to minimise T2D risk (Malin and Braun, 2016). A cross sectional comparison and longitudinal exercise studies were conducted and showed acute elevation of CRP and reduction after long term exercise which is similar to this study (Kasapis and Thompson, 2005; Fernandes et al., 2011). Furthermore, different intervention study illustrates that there is a reduction in CRP level after 12 weeks of resistance exercise program (Ramel et al., 2015).

7.7.1.2 IL-6

Table 37: Changes in level of IL-6 was observed during the 1st exercise session (acute effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

IL6 (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	1.79±0.4	2.34±0.4	2.61±0.5	0.007
ND	0.64±0.1	0.71±0.1	1.51±0.4	0.074

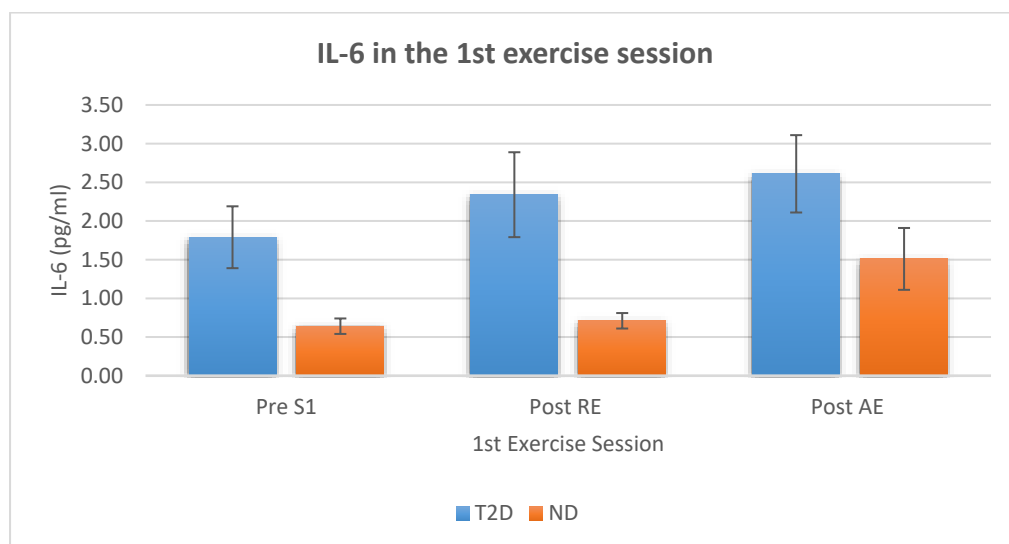


Figure 63: IL-6 level in T2D and ND across the 1st exercise session

Table 38: Changes in IL-6 level was observed during the 12 exercise sessions (chronic effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S12 was obtained.

IL6 (pg/ml)	Pre Ex	Post S1	S2	S4	S6	S8	S10	S12	P value
T2D	1.79±0.4	2.61±0.5	2.20±0.5	2.20±0.4	3.10±0.7	2.39±0.4	2.21±0.5	3.88±1.9	0.282
ND	0.64±0.1	1.51±0.4	1.14±0.3	1.16±0.3	1.18±0.3	0.79±0.1	0.94±0.2	1.10±0.5	0.126

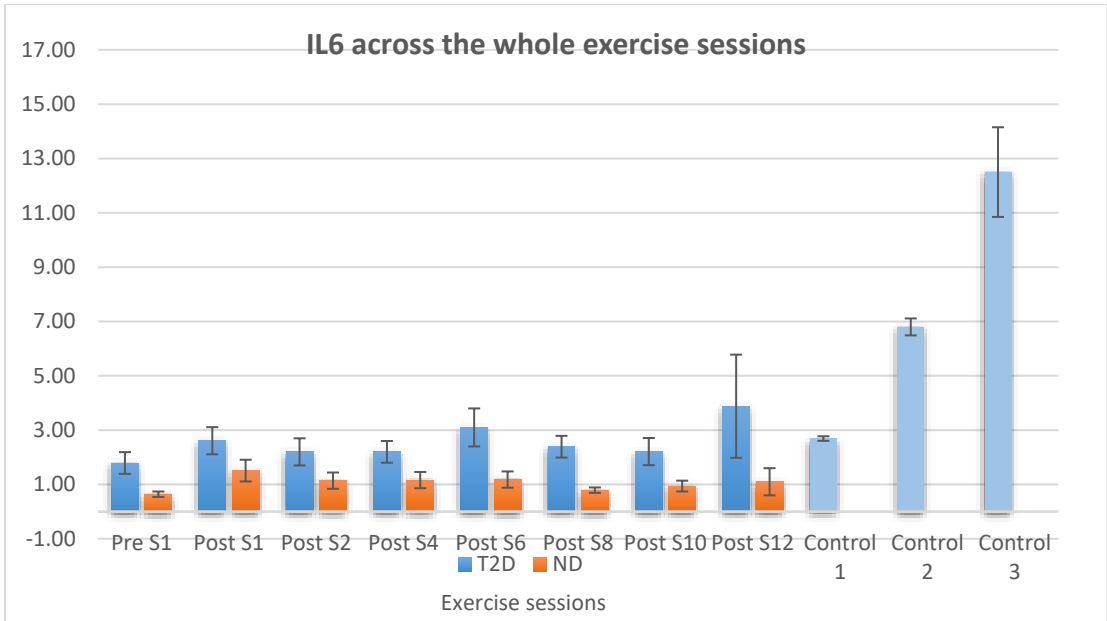


Figure 64: IL-6 level in T2D and ND across the whole exercise sessions

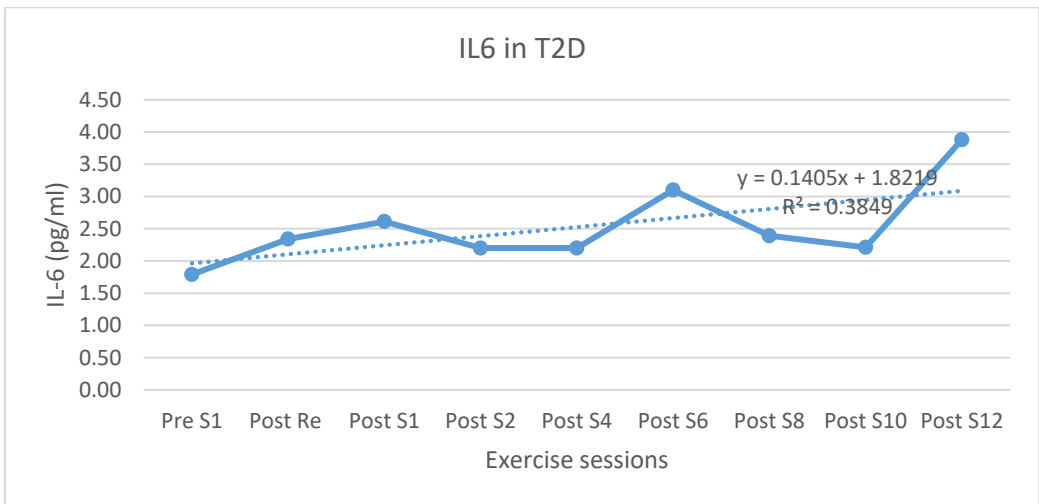


Figure 63a: This is a regression with normalised intervals, an upward trend over the 6 weeks (where not all sessions were measured)

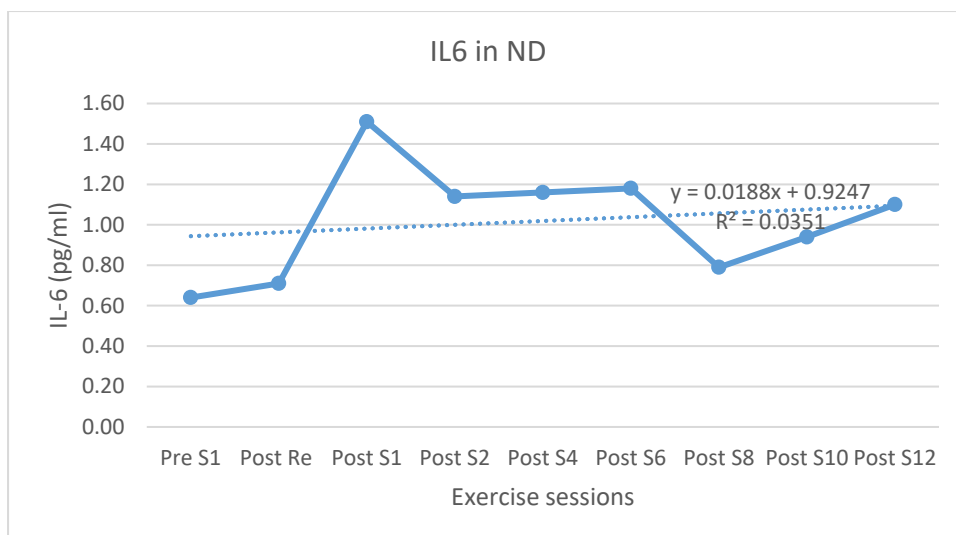


Figure 63b: This is a regressions with normalised intervals, showing a slight upward trend but not a very strong relationship (where not all sessions were measured)

The data obtained (in figure 62) for IL-6 shows an increment from 1.79 ± 0.4 to 2.61 ± 0.5 pg/ml ($P=0.007$) after the first exercise session which shows that the acute effect of combination of exercise is significant. Similar affect was recently reported by Eshghi et al. and a significant elevation of IL-6 was reported ($P=0.03$) (Eshghi et al., 2017; Bongers et al., 2017). Moreover, IL-6 level was observed to increase with continuous performing regular exercise (figure 63), an increase from 1.79 ± 0.4 to 3.88 ± 1.9 pg/ml ($P=0.002$), this significant change suggests that chronic exercise increases the level of IL-6 higher than acute exercise. It was mentioned previously that IL-6 is linked to T2D and obesity but it is higher in T2D subject with HTN (Lukic et al., 2014). Our finding shows that most of our volunteer had HTN and higher BMI than ND which suggest that is the main reason for increasing IL-6 with exercise. Previous study reported similar finding that there is significant improvement in insulin sensitivity however, no improvement in the IL-6 level after exercise (Verheggen et al., 2016). In ND, the results for IL-6 show elevation after the 1st exercise session from 0.64 ± 0.1 to 1.51 ± 0.4 pg/ml, ($P=0.074$). Moreover, this level is found to remain high after 12th exercise session and a value of 1.10 ± 0.5 pg/ml is obtained ($P=0.126$). It was clarified that T2D, obesity and Exercise are linked to elevate IL-6 level (Ellingsgaard et al., 2015). A recent study also states that chronic moderate aerobic exercise lead to elevation in IL-6 (Eshghi et al., 2017).

7.7.1.3 TNF

Table 39: Changes in TNF- α level was observed during the 1st exercise session (acute effect) in T2D and ND. Data is express as mean \pm SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

TNFA (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	8.76 \pm 3.2	8.20 \pm 2.6	8.06 \pm 2.7	0.489
ND	4.07 \pm 0.8	4.44 \pm 0.8	4.36 \pm 0.8	0.059

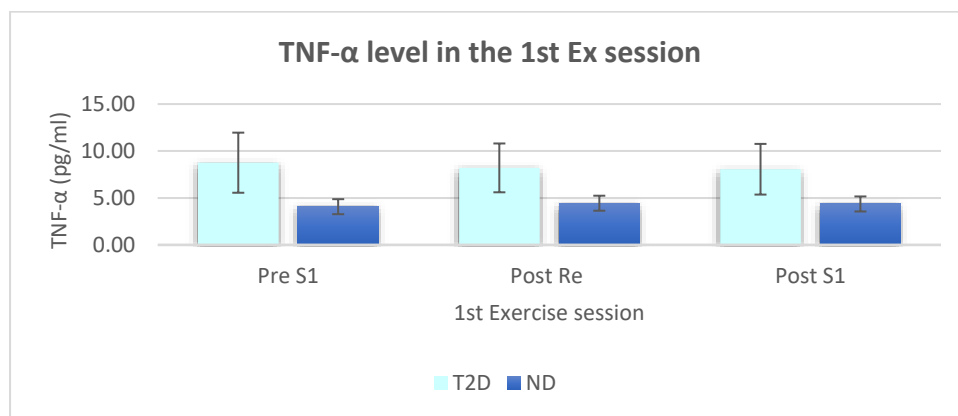


Figure 65: TNF- α level in T2D and ND in the 1st exercise session

Table 40: Changes in TNF- α level was observed during the 12 exercise sessions (chronic effect) in T2D and ND. Data is express as mean \pm SEM and P value for the effect Pre Ex (S1) and after S12 was obtained.

TNFA (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	8.76 \pm 3.2	8.06 \pm 2.7	5.97 \pm 0.7	8.37 \pm 2.7	8.55 \pm 2.7	7.05 \pm 2.1	5.82 \pm 0.79	7.85 \pm 2.4	0.423
ND	4.07 \pm 0.8	4.36 \pm 0.8	4.15 \pm 0.5	4.18 \pm 0.6	4.12 \pm 0.7	4.11 \pm 0.6	4.07 \pm 0.5	4.11 \pm 0.5	0.890

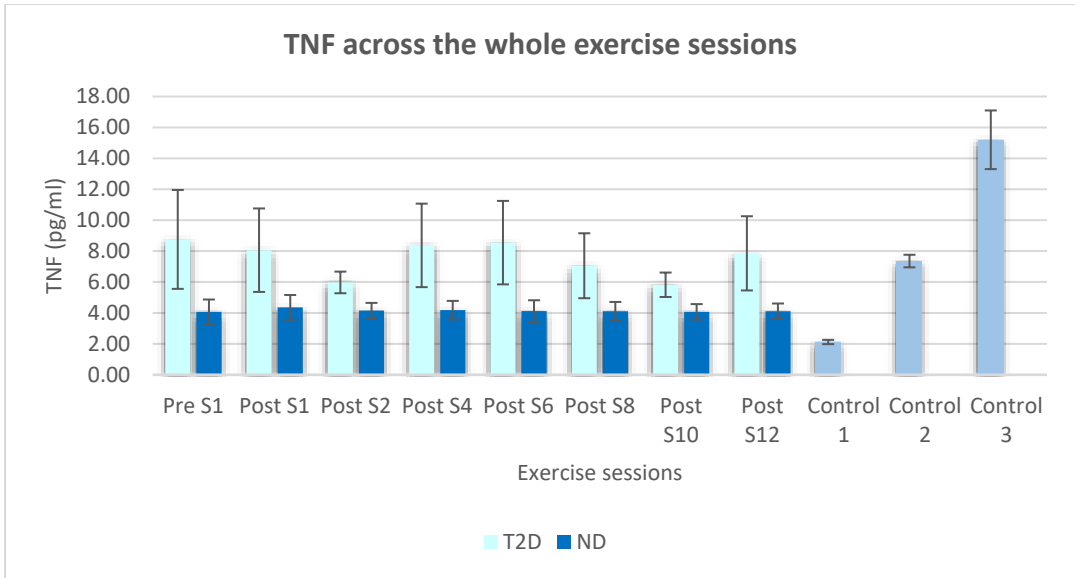


Figure 66: TNF-α level in T2D and ND across the whole exercise sessions

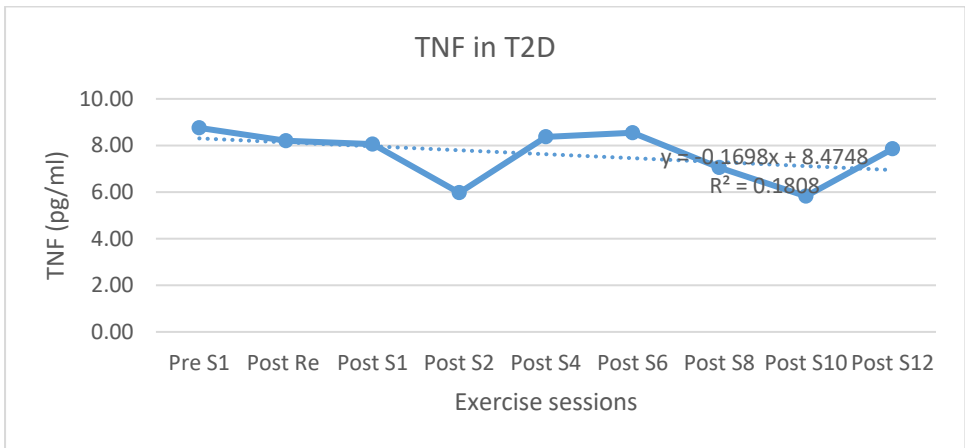


Figure 65a: This is a regression with normalised intervals, representing a very slight downward trend and not strong relationship (where not all sessions were measured)

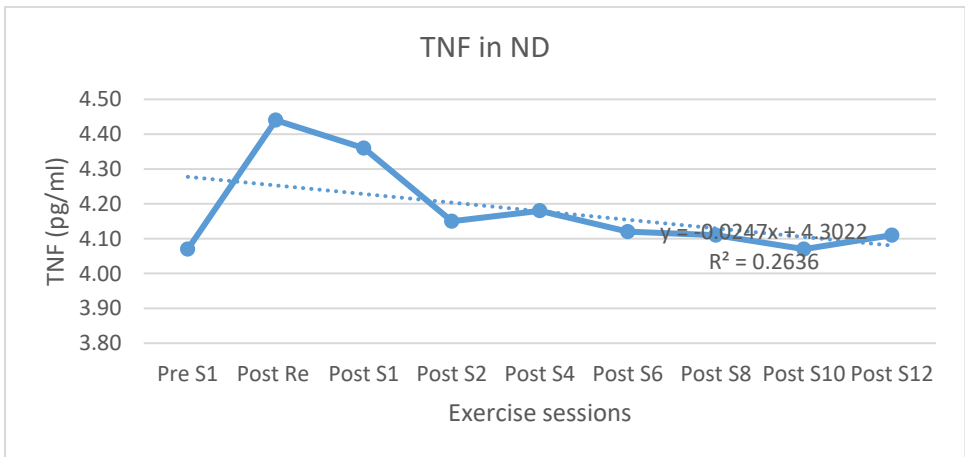


Figure 65b: This is a regressions with normalised intervals, a very slight upward trend with a poor relationship (where not all sessions were measured)

The value of TNF- α before exercise was recorded as 8.76 ± 3.2 pg/ml and then decreased slightly to 8.06 ± 2.7 pg/ml ($P=0.489$) after the acute effect of 1st exercise session (figure 64). Moreover, this level more slightly decreased after 6 weeks of exercise to 7.85 ± 2.4 pg/ml ($P=0.423$) (figure 65). In ND, there was a closely significant effects on TNF- α after 1st exercise session, data obtained was from 4.07 ± 0.8 to 4.36 ± 0.8 pg/ml ($P=0.059$). However, after 6 weeks of exercise a slight elevation of 4.11 ± 0.5 pg/ml was observed ($P=0.890$). A study by Matthew Bouchonville shows similar finding to our finding which improve TNF- α after acute effect but the result is not significant as well (Bouchonville et al., 2014).

7.7.1.4 Leptin

Table 41: Changes in leptin level was observed during the 1st exercise session (acute effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

LEPT (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	7.95±1.8	8.77±2.6	9.15±2.4	0.707
ND	8.24±3.3	7.55±2.8	7.01±2.4	0.384

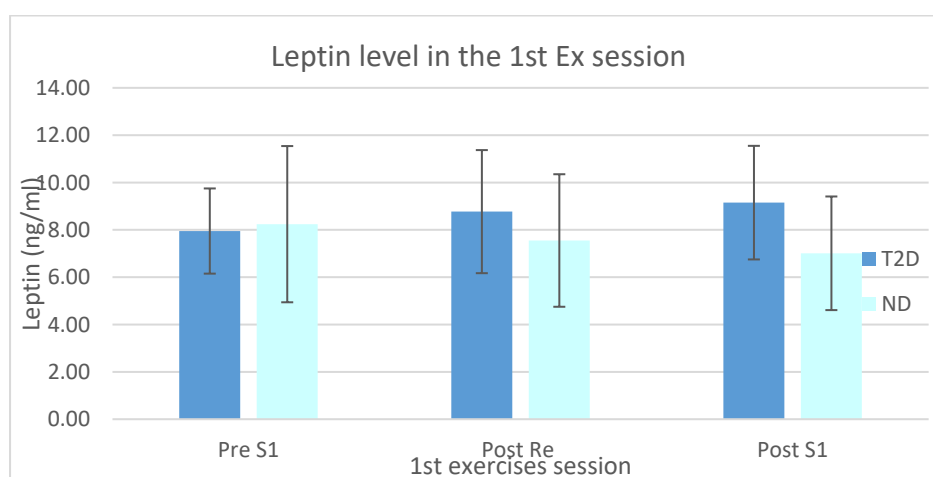


Figure 67: Leptin level in T2D and ND in the 1st exercise session

Table 42: Changes in leptin level was observed during the 12 exercise sessions (chronic effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S12 was obtained.

LEPT (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	7.95±1.8	9.15±2.4	10.48±2.5	10.42±3.6	11.68±3.6	10.52±3.6	11.85±3.3	7.25±1.8	0.522
ND	8.24±3.3	7.01±2.4	8.91±4.1	8.49±5.9	7.39±3.0	7.30±3.7	6.33±2.6	7.21±3.2	0.536

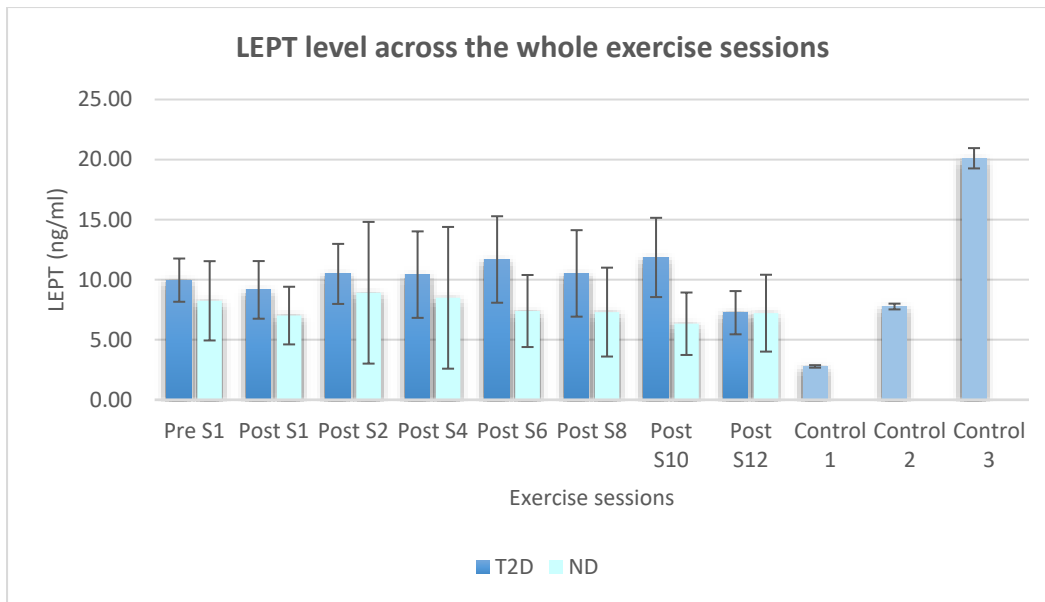


Figure 68: Leptin level in T2D and ND across the whole exercise sessions

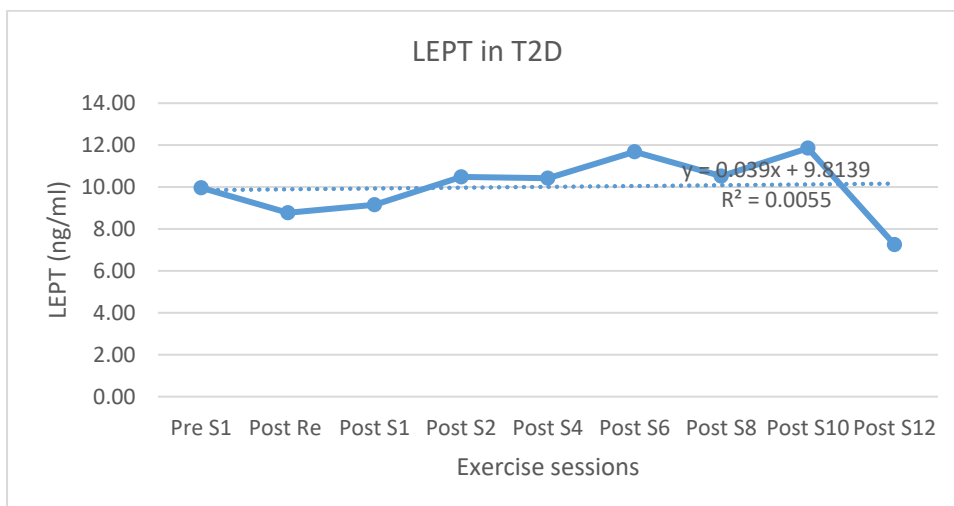


Figure 67a: This is a regression with normalised intervals, a downward trend over the 6 weeks (where not all sessions were measured)

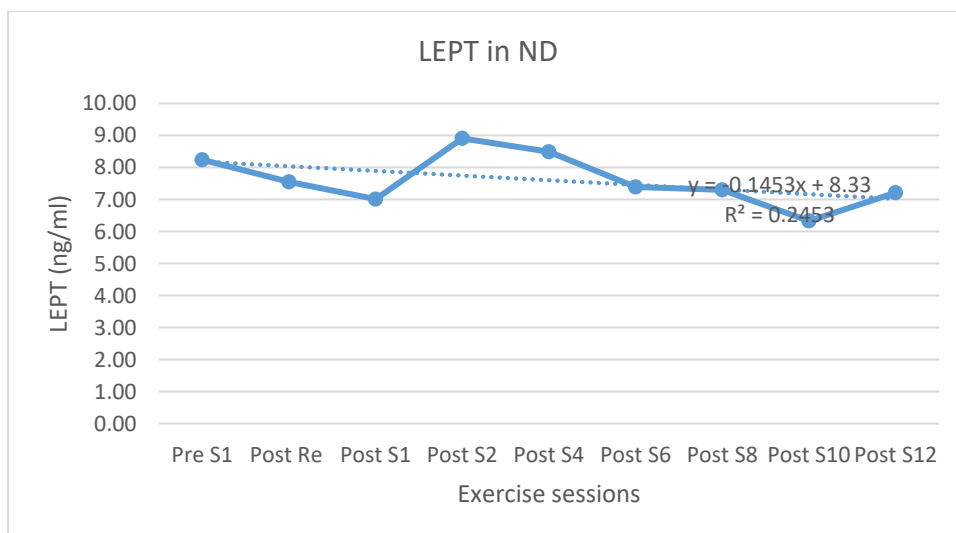


Figure 67b: This is a regression with normalised intervals, showing a strong relationship (where not all sessions were measured)

As represents in (figure 66) leptin levels increased after 1st exercise session from 7.95±1.8 to 9.15±2.4 ng/ml (P=0.707), the observed increase could be a result of the acute effect of combination of exercise. This increases during the whole exercise sessions and then decreased after S12 to 7.25±1.81 ng/ml (P=0.522) (figure 67). Leptin resistance is also linked to insulin resistance and rheumatoid arthritis, from 1st exercise session to 10th exercise session its level stays slightly high which might be due to leptin resistance (Feijóo-Bandín et al., 2016). Our finding shows improvement in insulin sensitivity (see section 6.1.7) as well as slight reduction in IL-6 which illustrate that the chronic effect of exercise shows improvement in insulin and leptin resistance. This finding was illustrated in a systemic review (8 studies and 231 T2D participants) which reported that reduction in leptin after combination exercise was not significant (Hayashino et al., 2014). Another systemic review showed that exercise stimulates catecholamines release which affects serum leptin level (Yu et al., 2017). On the other hand, the present study showed that the level of leptin slightly decreased in session one from 8.24±3.3 to 7.01±2.4 ng/ml (P=0.536), then increased after 12th sessions to 7.21±3.2 ng/ml (P=0.693) in ND but still lower than the baseline. Recent randomised control study by Nuri et al, found that there was no significant effect of exercise on leptin level (Nuri et al., 2016).

7.7.1.5 Resistin

Table 43: Changes in Resistin level was observed during the 1st exercise session (acute effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

RETN (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	3.23±0.9	3.71±1.1	2.52±0.3	0.832
ND	2.60±0.47	2.79±0.54	2.64±0.46	0.710

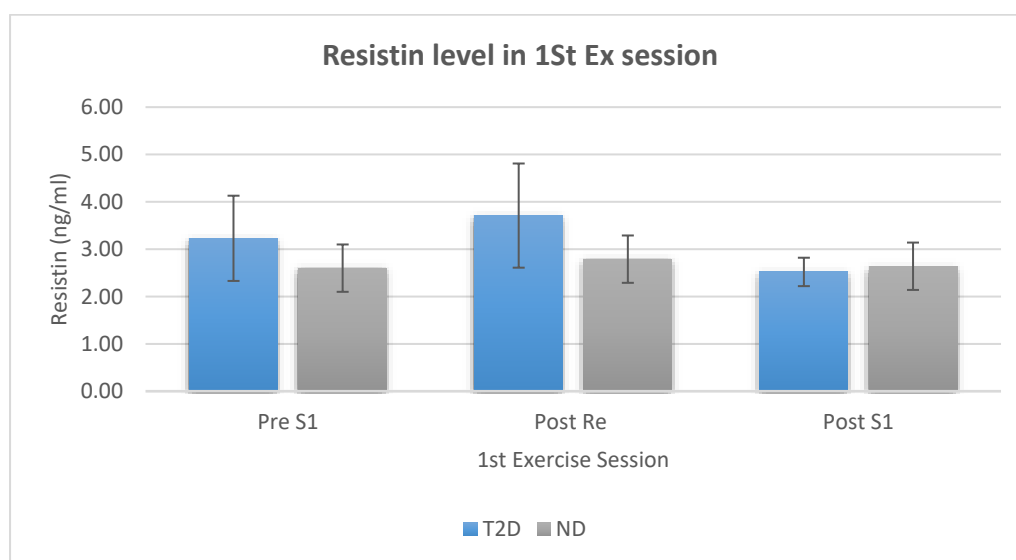


Figure 69: Resistin level in T2D and ND across the 1st exercise session

Table 44: Changes in Resistin level was observed during the 12 exercise sessions (chronic effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S12 was obtained.

RETN (ng/ml)	Pre S1	S1	S2	S4	S6	S8	S10	S12	P value
T2D	3.23±0.9	2.52±0.3	4.40±1.3	4.33±1.2	4.67±1.5	3.83±1.5	4.45±1.4	3.79±1.5	0.320
ND	2.60±0.5	2.64±0.5	2.34±0.3	2.59±0.6	2.53±0.4	2.26±0.2	2.19±0.2	2.92±0.6	0.197

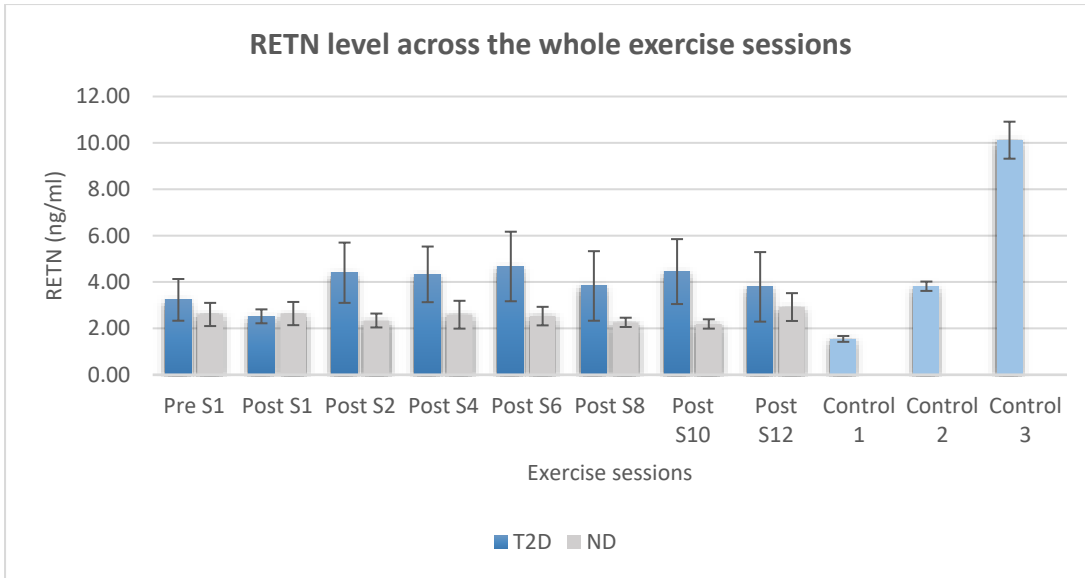


Figure 70: Resistin level in T2D and ND across the whole exercise sessions

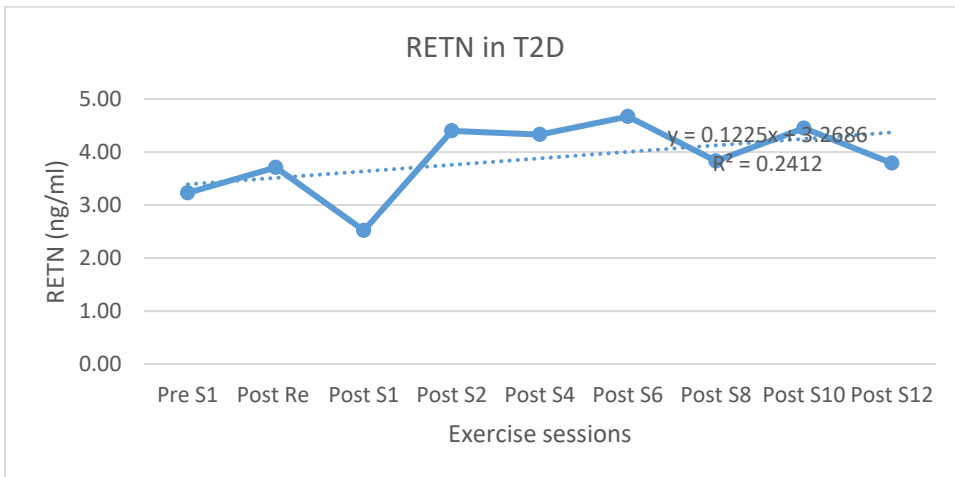


Figure 69a: This is a regression with normalised intervals, showing a very slight upward trend and a poor relationship over the 12th sessions (where not all sessions were measured)

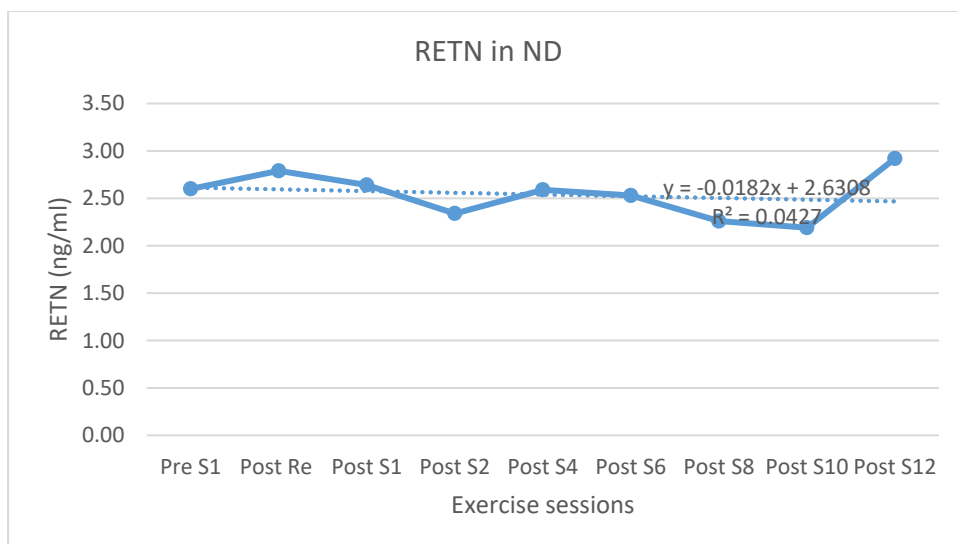


Figure 69b: This is a regression with normalised intervals, a very slight upward trend with no relationship over 6 weeks (where not all sessions were measured)

Resistin level in T2D before exercise was recorded as 3.23 ± 0.9 ng/ml which decreased after 1st session of exercise to 2.52 ± 0.3 ng/ml ($P=0.832$) (figure 68). While figure 69 represents the chronic effect of 6 weeks combination of exercise shows an insignificant increase in its level to 3.79 ± 1.5 ng/ml ($P=0.320$). On the other hand, the systemic review from 7 studies showed that there was no significant reduction in resistin among 250 T2D participants (Hayashino et al., 2014). In ND, the result of resistin shows no notable changes after the acute effect of combination exercise from 2.60 ± 0.47 to 2.64 ± 0.46 ng/ml ($P=0.710$). And there was very slight elevation after the chronic effect up to 2.92 ± 0.6 ng/ml ($P=0.197$); nevertheless, it is not significant effect. Other studies found no effect of exercise on the resistin level (Yahya et al., 2014).

7.7.1.6 Cystatin C

Table 45: Changes in cystatin C level was observed during the 1st exercise session (acute effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

CYSC (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	5.9±1.5	6.4±1.5	7.3±1.8	0.404
ND	3.1±1.6	3.5±1.6	2.8±1.3	0.513

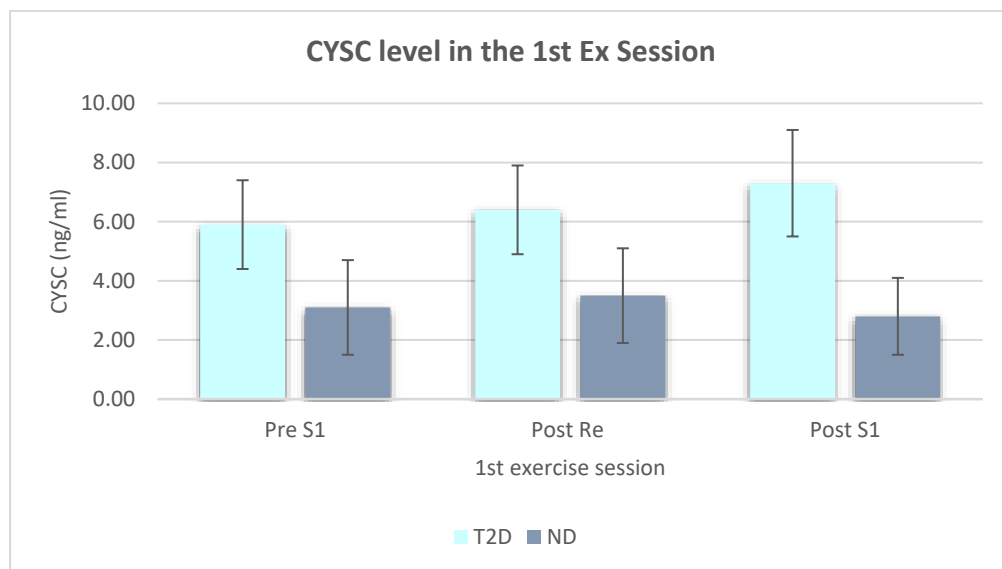


Figure 71: Cystatin C level in T2D and ND in the 1st exercise session

Table 46: Changes in cystatin C level was observed during the 12 exercise sessions (chronic effect) in T2D and ND. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S12 was obtained.

CYSC (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	5.9±1.5	7.3±1.8	6.8±1.9	7.9±1.8	6.3±1.7	8.3±1.9	6.3±1.6	6.6±1.9	0.400
ND	3.1±1.6	2.8±1.3	3.7±2.4	3.4±2.0	4.1±2.1	3.2±1.6	3.1±1.7	4.1±2.8	0.446

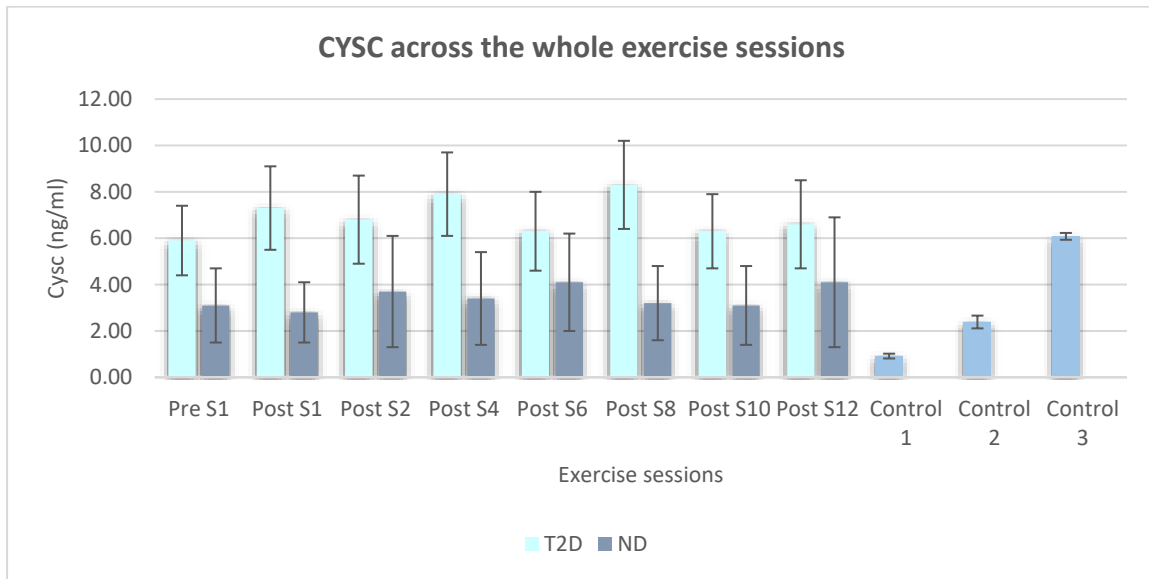


Figure 72: Cystatin C level in T2D and ND across the whole exercise sessions

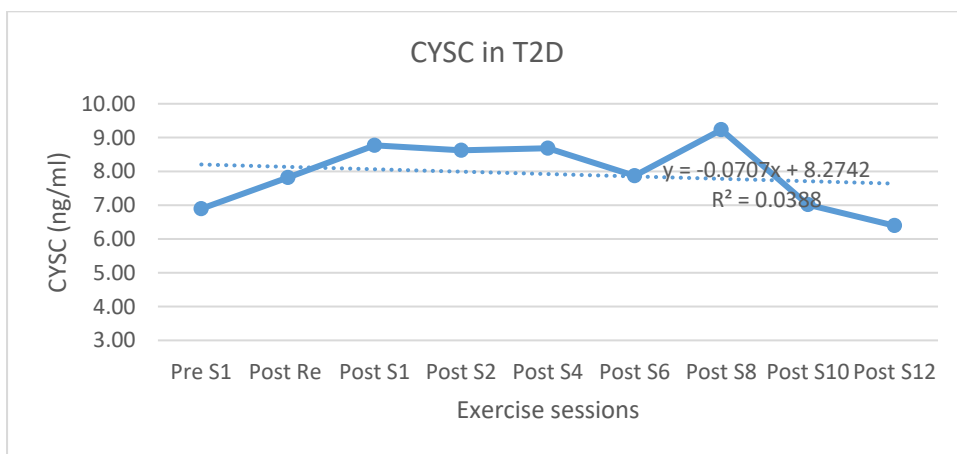


Figure 71a: This is a regression with normalised intervals, showing a very slight downward trend with a strong relationship over the 12th sessions (where not all sessions were measured)

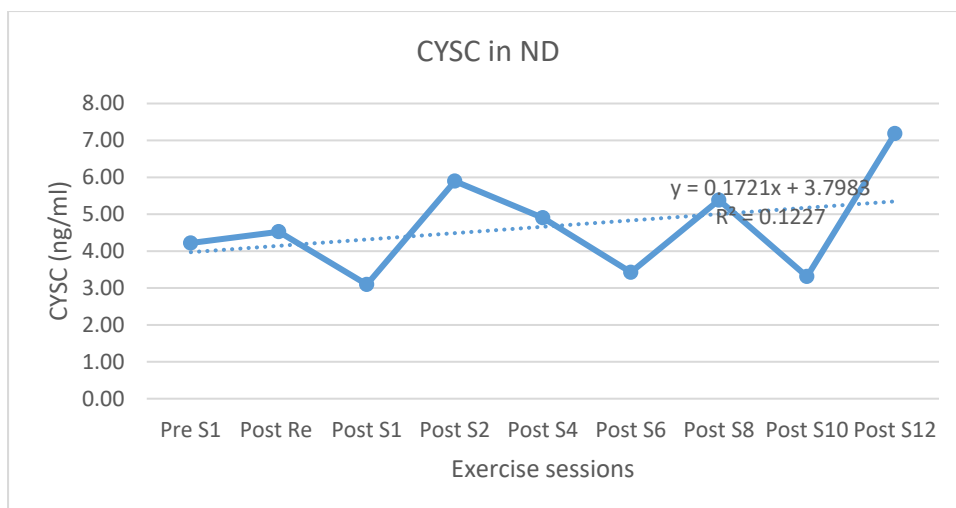


Figure 71b: This is a regression with normalised intervals, representing an upward trend with poor relationship over 6 weeks (where not all sessions were measured)

CysC levels in T2D was observed to have increased after the 1st session from 5.9 ± 1.5 ng/ml to 7.3 ± 1.8 ($P=0.404$) (figure 70) and decreased after 12 exercise session to 6.6 ± 1.9 ng/ml ($P=0.732$) but still higher than baseline (figure 71). Similar findings were reported by a recent study which states that the acute effect of exercise (treadmill walk) could result in minor kidney injury (elevate CysC) (Bongers et al., 2017). However, another study illustrates that prolong walk decreases this level of Cystatin C (Bongers et al., 2017). On the other hand, in ND, the results of CysC obtained showed reduction after 1st day of exercise from 3.1 ± 1.6 to 2.8 ± 1.3 ng/ml ($P=0.513$), however, after 12th exercise sessions the level increased to 4.1 ± 2.8 ng/ml ($P=0.446$). These data obtained suggest that the exercise has no effect on CysC values in both T2D and ND. Recent study illustrate similar finding, that eight weeks of aerobic exercise alone has no significant effect on CysC on females with KT (Eskandar, Katayon and Ali, 2015).

7.7.2 Results between the three T2D groups:

7.7.2.1 CRP

1-T2D with no medication for diabetes:

Table 47: Changes in CRP level was observed during the 1st exercise session (acute effect) in T2D subjects with no pharmacological intervention. Data is express as mean± SEM and P value for the effect Pre Ex (S1) and after S1 was obtained.

CRP (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	36.28±14.46	38.15±13.36	27.99±19.58	0.403

Table 48: Changes in CRP level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S12 was obtained.

CRP (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	36.28±14.5	27.99±19.6	18.44±13.5	23.93±15.6	14.09±7.9	24.50±16.3	22.36±18.6	45.46±20.1	0.644

2-T2D on metformin only.

Table 49: Changes in CRP level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S1 was obtained.

CRP (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	18.7±8.2	29.6±20.9	22.5±11.4	0.327

Table 50: Changes in CRP level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S12 was obtained.

CRP (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	18.7±8.2	22.5±11.4	17.6±10.9	15.4±8.1	15.5±8.2	18.2±9.1	16.8±6.9	13.6±5.8	0.122

3-T2D on metformin and DPP4-I

Table 51: Changes in CRP level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S1 was obtained.

CRP (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	14.27±5.52	17.30±6.60	24.51±0.50	0.517

Table 52: Changes in CRP level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S12 was obtained.

CRP (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	14.27±5.52	24.50±0.050	13.91±4.98	6.59±3.58	18.14±4.46	18.32±9.86	8.44±4.34	7.97±0.84	0.638

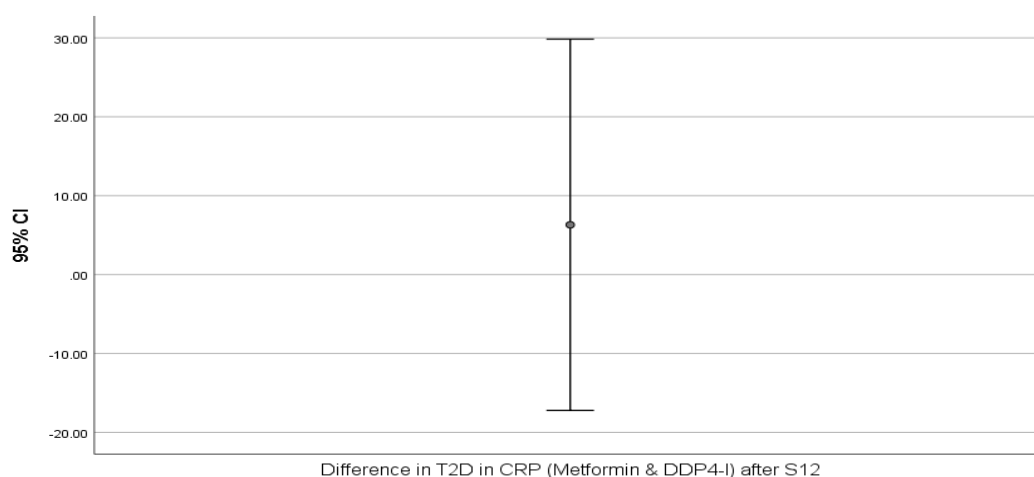


Figure 73: T2D CRP in metformin and DPP4-I group shows CI 95% lower than zero which means this difference is not significant even if the chart shows the highest elevation after S1 and highest reduction after S12

In T2D the type of medication might have different effect on the inflammatory markers. The first group haven't receive any medication for diabetes, in the first exercise session CRP shows reduction from 36.3 ± 14.5 to 28.0 ± 19.6 , and ($P= 0.403$). While after session 12 CRP has been increased to 45.5 ± 20.1 and ($P=0.644$). In metformin group, CRP increased after acute effect of exercise from 18.7 ± 8.2 to 22.5 ± 11.4 and ($P= 0.327$). However, this decreased after 12 sessions to 13.6 ± 5.8 and ($P= 0.122$). The last group was metformin and DPP4-I group, the acute effect in the first exercise session showed elevation from 14.27 ± 5.52 to 24.51 ± 0.50 and ($P= 0.517$). While this decreased after 6 weeks of exercise to 7.97 ± 0.84 and ($P= 0.638$).

7.7.2.2 IL-6

1- T2D volunteers with no medication.

Table 53: Changes in IL-6 level was observed during the 1st exercise session (acute effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S1 was obtained.

IL-6 (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	3.04±1.21	2.98±1.19	3.47±1.39	0.336

Table 54: Changes in IL-6 level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S12 was obtained.

IL-6 (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	3.04±1.21	3.47±1.39	3.20±1.75	2.91±1.18	4.17±1.22	2.88±0.86	3.16±1.72	9.41±5.63	0.459

2- T2D volunteers on Metformin only.

Table 55: Changes in IL-6 level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S1 was obtained.

IL-6 (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	1.37±0.50	1.93±0.36	1.53±0.61	0.361

Table 56: Changes in IL-6 level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S12 was obtained.

IL-6 (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	1.37±0.50	1.53±0.605	1.61±0.35	2.41±0.24	2.02±0.54	0.10±0.10	1.66±0.29	1.68±0.04	0.666

3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4- I).

Table 57: Changes in IL-6 level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S1 was obtained.

IL-6 (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	0.99±0.18	1.63±0.36	2.08±0.84	0.277

Table 58: Changes in IL-6 level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S12 was obtained.

IL-6 (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	0.99±0.18	2.08±1.50	1.50±0.13	2.10±0.53	1.44±2.16	0.98±0.58	2.68±0.30	1.50±0.40	0.161

IL-6 showed very slight elevation in the group who haven't receive any pharmacological treatment from 3.04±1.21 to 3.47±1.39 and (P= 0.336). On the other hand, this increased after 12 exercise sessions to 9.41±5.63 and (P= 0.459) but not significant. This IL-6 slightly increased in metformin group after 1st session from 1.37±0.50 to 1.53±0.61, (P= 0.361) and to 1.68±0.04, (P= 0.666) after 6 weeks of exercise. In the last group (metformin and DPP4-I), IL-6 increased after acute effect of exercise from 0.99±0.18 to 2.08±0.84, (P= 0.277) and then to 1.50±0.40, (P= 0.161) after 12th sessions.

7.7.2.3 TNF

1- T2D volunteers with no medication.

Table 59: Changes in TNF level was observed during the 1st exercise session (acute effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S1 was obtained.

TNF (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	6.30±0.32	5.97±0.13	5.74±0.12	0.662

Table 60: Changes in TNF level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S12 was obtained.

TNF (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	6.30±0.32	5.74±0.12	5.51±0.38	5.62±0.15	6.35±0.78	5.58±0.32	5.74±0.53	5.76±0.50	0.349

2- T2D volunteers on Metformin only.

Table 61: Changes in TNF level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S1 was obtained.

TNF (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	5.28±1.50	4.83±1.14	4.72±1.06	0.424

Table 62: Changes in TNF level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S12 was obtained.

TNF (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	5.28±1.50	4.72±1.06	4.64±0.80	4.56±0.81	4.58±0.72	4.27±0.61	4.34±0.61	4.52±0.10	0.368

3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4 I).

Table 63: Changes in TNF level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin DPP4-I). P value for the effect Pre Ex (S1) and after S1 was obtained.

TNF (pg/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	18.43±9.68	19.27±8.55	21.26±9.69	0.570

Table 64: Changes in TNF level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin DPP4-I). P value for the effect Pre Ex (S1) and after S12 was obtained.

TNF (pg/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	18.43±9.68	21.26±9.69	8.43±0.98	17.23±7.71	15.37±7.42	16.54±6.05	9.92±0.34	19.56±6.45	0.646

TNF represent slight reduction in the first group without any pharmacological treatment from 6.30±0.32 to 5.74±0.12, (P= 0.662). In the last exercise session TNF was 5.76±0.50, (P= 0.349) and showed no significant changes. Similarly, in the metformin group the reduction not significant after 1st and 12th exercise sessions, it was 5.28±1.50 and slightly decreased to 4.72±1.06, (P= 0.424) after the 1st session then to 4.52±0.10, (P= 0.368) after the 12th session. Nevertheless, the last group (metformin and DPP4-I) demonstrated slight elevation after 1st session from 18.43±9.68 to 19.56±6.45 and P= 0.646.

7.7.2.4 Leptin

1- T2D volunteers with no medication.

Table 65: Changes in leptin level was observed during the 1st exercise session (acute effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S1 was obtained.

LEPT (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	6.46±3.52	5.59±3.12	6.04±3.01	0.21

Table 66: Changes in leptin level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S12 was obtained.

LEPT (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	6.46±3.52	6.04±3.34	6.04±3.01	4.84±2.68	6.00±3.10	6.84±4.13	6.12±2.77	6.45±3.25	0.980

2- T2D volunteers on Metformin only

Table 67: Changes in leptin level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S1 was obtained.

LEPT (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	12.32±4.59	10.88±3.56	11.86±3.38	0.769

Table 68: Changes in leptin level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S12 was obtained.

LEPT (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	12.32±4.59	11.86±3.38	11.36±4.26	13.22±3.77	14.69±3.17	14.83±8.61	12.73±6.84	12.91±6.12	0.764

3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4 I).

Table 69: Changes in leptin level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S1 was obtained.

LEPT (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	13.29±3.31	7.64±1.71	9.47±2.35	0.880

Table 70: Changes in leptin level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S12 was obtained.

LEPT (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	13.29±3.31	7.64±1.71	9.47±2.41	9.87±1.44	16.21±4.22	10.95±2.87	16.46±2.06	9.89±2.48	0.403

Leptin in the group without any medication shows very slight reduction after S1 which was (6.46±3.52) and became (6.04±3.01), and P= 0.210. While after S12 became (6.45±3.25), P=0.980 which shows no changes. Moreover, metformin group represent similar changes to the previous group, which reduce very slightly after S1 fro (12.32±4.59) to (11.86±3.38), and P= 0.769. After S12 became (12.91±6.12), and P=0.764 which considered not significant. The last group (metformin and DPP4-I) also displays reduction after S1 from (13.29±3.31) to (9.47±2.35), and P=0.880 which illustrate no significant results as well. After S12 this become (9.89±2.48), and P=0.403 which is represent no significant effect.

7.7.2.5 Resistin

1- T2D volunteers with no medication.

Table 71: Changes in Resistin level was observed during the 1st exercise session (acute effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S1 was obtained.

RETN (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	3.96±0.37	2.87±0.13	3.58±0.10	0.744

Table 72: Changes in Resistin level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S12 was obtained.

RETN (ng/ml)	Pre S1	S1	S2	S4	S6	S8	S10	S12	P value
T2D	3.96±0.37	3.58±0.10	3.44±0.27	4.34±0.21	5.83±1.21	3.96±0.73	6.00±0.91	4.64±0.20	0.129

2- T2D volunteers on Metformin only.

Table 73: Changes in Resistin level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S1 was obtained.

RETN (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	2.50±0.63	2.44±0.51	2.42±0.41	0.795

Table 74: Changes in Resistin level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S12 was obtained.

RETN (ng/ml)	Pre S1	S1	S2	S4	S6	S8	S10	S12	P value
T2D	2.50±0.63	2.42±0.41	2.05±0.17	2.94±0.39	2.39±0.27	2.38±0.51	2.46±0.46	2.54±0.68	0.535

3- T2D volunteers on Metformin and Dipeptidyl Peptidase 4 inhibitor (DPP4 I).

Table 75: Changes in Resistin level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S1 was obtained.

RETN (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	6.67±2.45	8.69±2.42	3.32±0.66	0.686

Table 76: Changes in Resistin level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S12 was obtained.

RETN (ng/ml)	Pre S1	S1	S2	S4	S6	S8	S10	S12	P value
T2D	6.67±2.45	3.32±0.66	8.69±2.13	7.44±2.56	9.23±3.29	13.96±4.37	8.51±3.74	14.13±4.45	0.543

Resistin result in the first group with no medication after S1 demonstrates no significant reduction which reduced from (3.96±0.37) to (3.58±0.10), P=0.744. In contrast, after S12 this level increased to (4.64±0.20), and P=0.129. Furthermore, metformin group shows no significant changes. After S1 resistin decreased very slightly from (2.50±0.63) to (2.42±0.41), and P=0.795. After S12 this become very slightly higher than the base line (2.54±0.68), P=0.535 which still not significant. Resistin level in the metformin and DPP4-I decreased after S1 from (6.67±2.45) to (3.32±0.66), and P=0.686 however P value still not significant. While after S12 this became (14.13±4.45), P=0.543 which is not significant as well.

7.7.2.6 Cystatin C

1-T2D with no medication for diabetes:

Table 77: Changes in cystatin C level was observed during the 1st exercise session (acute effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S1 was obtained.

CYSC (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	11.19±3.10	10.55±3.83	11.64±4.15	0.735

Table 78: Changes in cystatin C level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with no pharmacological intervention. P value for the effect Pre Ex (S1) and after S12 was obtained.

CYSC (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	11.19±3.10	11.64±4.15	12.25±4.90	13.06±4.20	12.02±4.12	13.17±4.07	7.64±2.67	11.30±3.97	0.920

2-T2D on metformin only:

Table 79: Changes in cystatin C level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S1 was obtained.

CYSC (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	5.2±1.9	5.3±2.6	5.7±1.7	0.743

Table 80: Changes in cystatin C level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin only). P value for the effect Pre Ex (S1) and after S12 was obtained.

CYSC (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	5.2±1.9	5.7±1.7	4.6±2.8	7.4±2.0	4.7±2.7	8.8±3.2	7.9±3.2	7.7±3.4	0.217

4-T2D on metformin and DPP4-I:

Table 81: Changes in cystatin C level was observed during the 1st exercise session (acute effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S1 was obtained.

CYSC (ng/ml)	S1			P value
	Pre Ex	Post RE	Post AE	
T2D	2.94±0.61	5.38±1.55	1.37±1.37	0.182

Table 82: Changes in cystatin C level was observed during the 12 exercise sessions (chronic effect) in T2D subjects with pharmacological intervention (Metformin and DPP4-I). P value for the effect Pre Ex (S1) and after S12 was obtained.

CYSC (ng/ml)	Pre Ex	S1	S2	S4	S6	S8	S10	S12	P value
T2D	2.94±0.61	1.37±1.37	5.10±1.97	3.27±0.91	3.60±0.84	3.88±2.14	3.12±0.14	1.81±0.69	0.030

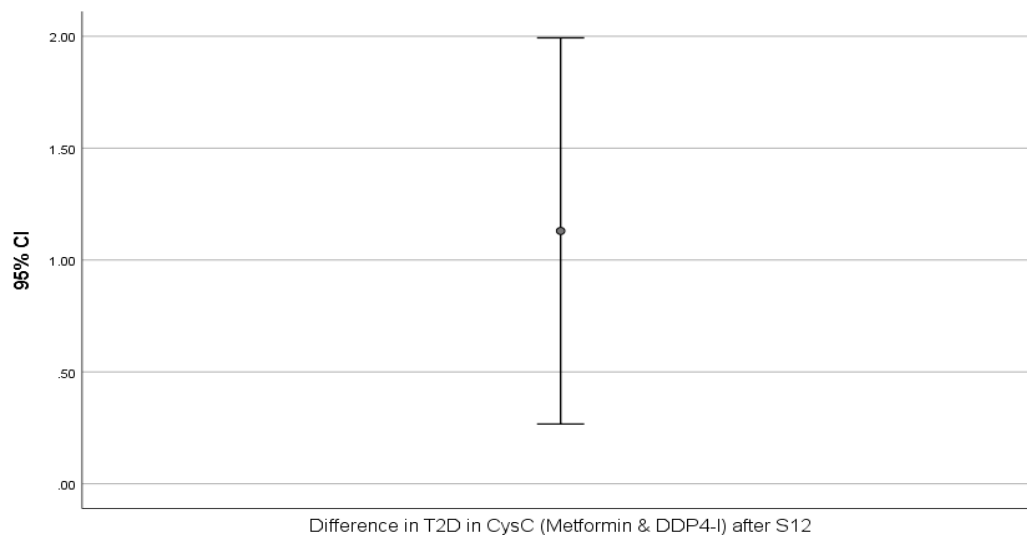


Figure 74: T2D CysC shows CI 95% higher than zero which mean this difference is significant even if the reduction seems not significant

CysC results in the first group who haven't receive any treatment showed very slight elevation after 1st session from 11.19±3.10 to 11.64±4.15, (P= 0.735) and this changed to 11.30±3.97, (P= 0.920) after last exercise session. Similar elevation in the metformin group after 1st

exercise session from 5.2 ± 1.9 to 5.7 ± 1.7 , ($P = 0.743$). While this increased more after the 12th exercise session to 7.7 ± 3.4 , ($P = 0.217$). On the other hand, CysC decreased in the metformin and DPP4-I group after 1st exercise session from 2.94 ± 0.61 to 1.37 ± 1.37 , ($P = 0.182$) and then changed after the last session to 1.81 ± 0.69 , ($P = 0.030$) which showed very significant improvement.

7.8 Conclusion:

Literature correlates elevated CRP level with increased risk of diabetes mellitus among adults (Prestes et al., 2009) and its level is increased in individuals with T2D (Thomsen et al., 2010), hence associated with IR and the dysfunction of endothelial cells and is used as a predictor biomarker of cardiovascular events since it links atherosclerosis and inflammation (Kajitani et al., 2010). In the present study, acute effects of exercise resulted in an increase in the level of CRP which then decrease after 6 weeks of exercise in both groups and the change was significant in T2D. This finding correlate with that published by Balducci *et al.*, (2010) and (Kim, 2014) suggesting that exercise decrease the level of CRP in T2D.

The function of IL-6 in insulin resistance in T2D has been emphasized in the field of research over the years (Glund and Krook, 2007; Glund et al., 2007; Jiang et al., 2013; Kim, 2014), this is because proinflammatory cytokines, for example, resistin and IL-6 were shown to be associated with certain metabolic disorders including T2D (Hotamisligil, 2006). The study of Ellingsgaard et al., (2011) proposed that IL-6 plays a positive role in improving IS via exercise, consequently improving the functions of β -cells and the presence/circulation of GLP-1. In the present study, both acute and chronic effect of exercise resulted in the elevation of IL-6 in both T2D and ND participants, however, the increment still insignificant. However, the study of Kim, (2014) reported a significant decrease in the level of IL-6 among participants exposed to circuit training when compared to aerobic exercise. Combination exercise is reported to greatly decrease IL-6 levels (Hopps, Canino and Caimi, 2011), however, the levels were increased in the present study. This observation on IL-6 mentioned in this study could be due to different reasons one of which is the type/mode of exercise used.

Furthermore, the variable TNF- α was observed in the present study, and literature shows that intense exercise results in increased level of TNF- α which subsequently increases the level of anti-inflammatory cytokines including the TNF- α in ND individuals (Pedersen and Febbraio, 2005; Petersen and Pedersen, 2005). However, in T2D the level of TNF- α is increased (Thomsen et al., 2010) and some research look into how this increased level can be decreased with exercise. In the present study, there was an insignificant reduction in the level of TNF in T2D while ND shows slight elevation. However, Hopps, Canino and Caimi, (2011) reported that the use of combined exercise greatly decrease the level of TNF- α which was supported by studies conducted by (You and Nicklas, 2006; Balducci et al., 2010; de Salles et al., 2010).

Another fluctuating variable observed in the present study is leptin. The role of leptin in regulating energy balance has made it an important research variable and in the present study its level was observed where the levels decreased after acute effects, then decreased more after the last exercise sessions in T2D. Moreover, leptin decreased in ND after acute effect and the 12th exercise sessions. Likewise, a decrease in leptin level was reported by Ishii et al., (2001) and Hayashino et al., (2014).

Another variable studied in the present study is resistin, a protein known for its ability to increase insulin resistance. Research has shown that the stimulation of resistin is in concomitant with inflammatory molecules such as IL-6 and TNF- α , and has an important function in endothelial cells and macrovascular diseases (Schwartz and Lazar, 2011; Codoñer-Franch and Alonso-Iglesias, 2015). Another vital role of resistin is its correlation to insulin resistance in T2D (McTernan et al., 2003; Kusminski, McTernan and Kumar, 2005; Tokuyama et al., 2007), and its level was observed in the present study. In the present study, resistin increased in T2D and didn't change in ND after 1st exercise session, however, it was increased in both groups after 12th exercise sessions although the increment is not significantly. Most studies reported a decreased level of resistin after exercise training (Kadoglou et al., 2007; Balducci et al., 2010; Wenning et al., 2013), however the study by Giannopoulou et al., (2005) reported that resistin level increased after exercise which correlates with the present study, while Hayashino et al., (2014) reported that no changes was observed in the levels of resistin. Marcelino-Rodríguez et al., (2017) deduced an inverse association of resistin with exercise.

Moreover, the level of CysC prior to exercise training in T2D was increased and decreased after 1st session and also after 6 weeks in T2D, but the level was observed to increase in ND, yet this was not significant. When the result was analysed in T2D according to medication type all these results have no significant changes comparing the three group only CysC in metformin and DPP4-I group represents significant reduction after 6 weeks of combination exercise with $P < 0.05$. Various studies reported that exercise lowers the levels of inflammatory markers. However, results are inconsistent, representing different modes, durations and intensities of exercise which might have different effects on inflammatory markers. Furthermore, underlying chronic disease such as arthritis, hypertension or acute infection as well as obesity might interfere in the improvement of these markers, and also the medication types as well might play a role in the changes of these inflammatory markers.

7.8.1 Limitations:

- 1- Only few volunteers were recruited for both T2D and ND.
- 2- No data for control group.
- 3- No specific diet has been followed.

7.8.2 Future work:

Conduct another study with larger sample size and control group of both T2D and ND.

Chapter 8

8.1 Previous studies which used ELISA in the analysis:

ELISA	No ELISA
Shaabani et al., (2016)	Prior et al., (2015)
Eshghi et al., (2017)	Fenicchia et al., (2004)
Verheggen et al., (2016)	Tura et al., (2017)
Jorge et al., (2011)	Bongers et al., (2017)
(Kawano et al., 2013).	
Whyte et al., (2013)	
Hallworth et al., (2017)	
Holliday and Blannin, (2017)	
Herder et al., (2009)	
Mendham et al., (2011)	
(Nuri et al., 2016)	
Nygaard et al., (2017)	

8.2 General conclusion:

It is well known that exercise is beneficial for the human health and literature showed that it plays vital role in the management of diabetes to control BG and improve HbA1c to avoid any serious complication. In this study, the reduction in HbA1c was significant in both group as represented above in chapter 5. Furthermore, it has been found a significant effect on the anthropometric variables (weight, waist, BMI and lung capacity) in T2D group. In ND, the significant results was on in lung capacity. Resting HR improved significantly in T2D after 12th sessions of exercise. However, SBP, DBP and lipid profile show insignificant reduction especially in T2D. Moreover, exercise is useful to strength the muscle thus improving insulin sensitivity as stated above in chapter 6. T2D participants show some insulin resistance at baseline which improved after six. It was significant when using Matsuda and HOMA indices. While ND have no IR before starting exercise program thus the improvement in IR was only significant in QUICKI after six weeks of exercise comparing to T2D. It is also important to improve the secretion of GLP-1 which is recommended for future trials. In the present study, GLP-1 was seen to have improved only in metformin and SGT2-I group (chapter 7). SGLT2 inhibitors and metformin has been found affecting the body weight and it might be the reason behind the improvement of GLP-1 level. Finally, there is insignificant effect of exercise on the inflammatory markers in both groups only in T2D IL-6 shows significant elevation after S12 and CRP represent significant reduction after S12. However, within T2D group (metformin and DDP4-I) there was a significant reduction in CysC after S12. In general, combination exercise shows improvements in the human health but that need more comparison with AE alone or RE alone to prove that it is superior.

9. References:

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11. Appendices:

11.1 Appendix 1 (Consent form for T2D)



Leicester School of Pharmacy
Faculty of Health & Life Sciences

CONSENT FORM Patient Identification

Number:.....

Title of Project: The Use of a Cardio and Resistance Exercise Programme to Assess Immunological and Physiological Parameters in Type 2 Diabetes Mellitus Volunteers.

Name of Principal Investigator: Prof M J Taylor, Dr T Sahota, Dr P Tomlins, Mr K Chauhan, Dr R Furmonaviciene, Mr B Alharbi, Mrs N Alsubaie and Mr A Hill.

PLEASE SIGN INITIALS IN BOX

1. I confirm that I have read and understood the Participant Information Sheet dated (01/07/2017) for the above study. I have had the opportunity to consider the information, ask questions and 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.

Name of Participant

Date

Signature

Name of Researcher

Date

Signature

11.2 Appendix 2 (Consent form for ND)



Leicester School of Pharmacy
Faculty of Health & Life Sciences

CONSENT FORM Patient Identification Number for this trial:.....

Title of Project: The Use of a Cardio and Resistance Exercise Programme to Assess Immunological and Physiological Parameters in Volunteers.

Name of Principal Investigator: Prof M J Taylor, Dr T Sahota, Dr P Tomlins, Mr K Chauhan, Dr R Furmonaviciene, Mr B Alharbi, Mrs N Alsubaie and Mr A Hill.

PLEASE SIGN INITIALS IN BOX

1. I confirm that I have read and understood the Participant Information Sheet dated (01/07/2017) for the above study. I have had the opportunity to consider the information, ask questions and 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.

Name of Participant

Date

Signature

Name of researcher

Date

Signature

11.3 Appendix 3 (information sheet for T2D)



Leicester School of Pharmacy
Faculty of Health & Life Sciences

Participant Information Sheet

Date:01/07/2017

Title of Project: The Use of a Cardio and Resistance Exercise Programme to Assess Immunological and Physiological Parameters in Type 2 Diabetes Mellitus Volunteers.

Principal Investigators: Prof M J Taylor, Dr T Sahota, Dr P Tomlins, Dr R Furmonaviciene, Mr K Chauhan, Mr B Alharbi, Mrs N Alsubaie and Mr A Hill.

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part. If you do wish to take part please complete the slip at the end of this sheet.

What is the purpose of the study?

As part of the growing research into Diabetes Mellitus the School of Pharmacy at De Montfort University are working to investigate the effects of a combined exercise programme (cardio and resistance) on blood glucose, metabolic and immunological parameters that could help you with the management of diabetes and increase your insulin sensitivity.

This research involves a combination of two types of exercises and this is where we need your help. We would like to invite you 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, to possibly manage your blood glucose level more effectively and increase insulin sensitivity.

We will keep your information strictly confidential and nobody other than the research team will have access to your 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 have I been invited?

You have been invited to take part in this study because you are at risk or have Type 2 Diabetes and are aged between 18 and 60.

Do I have to take part?

It is up to you to decide. We will answer any questions you have about the study and go through this information sheet. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive from your doctor or hospital.

What will happen to me if I take part?

Preliminary procedures

Before enrolling in the study you will be asked to attend a screening visit where we will:

- Discuss and complete confidential questionnaires regarding your health, family history and physical activity level.
- Measure your blood pressure and heart rate.
- Measure your height and weight
- Provide an opportunity for you to ask questions.
- Familiarise you with equipment to be used in the study and teach you how to use the recumbent ergometer bike and how to lift the weights safely on the multi-gym machine.

- This session will also be used to determine the intensity of exercise during cycling on the recumbent ergometer bike using Heart Rate Reserve (HRR). Also, in this orientation session we will use predicted one repetition maximum (1RM) to determine how much weight for different muscles you should lift in the resistance exercise session later.

These preliminary procedures will enable us to determine whether you are suitable to safely participate in the study or not.

Main experimental trials

The main experimental trial will involve 2 x 2 hour exercise sessions a week for a 6 or 12 week period, (exercise session includes rest and final observation of volunteer). We would conduct these trials over the duration of your degree study (3 years) so we can assess the impact of exercise over a longer period of time.

Blood glucose levels will be monitored before, during and after each session, using a standard finger prick test. In addition, Cholesterol, High density lipoprotein, low density lipoprotein and Triglyceride will be monitored before, and after each exercise programme using a finger prick test.

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 predicted one-repetition maximum strength 1-RM) using upper and lower muscle groups followed by 20 min moderate cycling at 50 – 60% of pre-determined heart rate reserve (HRR). Heart rate (HR) and rate of perceived exertion (RPE) will be taken in a different time points throughout the exercise trial. We will also perform an Oral Glucose Tolerance test to assess insulin sensitivity at the beginning and end of the programme and blood samples will be screened for other immunological parameters. If you are taking any statin medication then you may also be asked to provide urine samples for analysis in a parallel study- We may also ask you to provide a saliva sample for a further study which assesses microbes present in saliva.

Incentives

We would like to offer an incentive of a subsidised gym membership at the De Montfort University's QEII Leisure Centre on completion of 100% of the dates agreed. This is a thank you for your participation but also to help you maintain the healthy exercise regimen until the following year's assessment.

What are the possible benefits of taking part?

As a result of being involved in this study you will receive health and fitness information about yourself including fitness tests and body measurement. You may also witness a decrease in your Hba1c levels and an increase in your insulin sensitivity as well as other general improvements to your health.

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 you with feedback about the main study findings and also about your own results and would be delighted to explain our findings and discuss possible implications with you.

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 you will only participate if it is safe for you to do so. However, if you have any concerns at any time about any aspect of the way you have been approached or treated during the course of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact details below), and the normal De Montfort University complaints mechanisms will be available to you.

Will my taking part in the study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the University, will have your name and address removed so that you cannot be recognised from it.

What will happen if I don't want to carry on with the study?

While we do not expect the programme to cause you to become upset if this does happen then you will have the option to pause or stop your participation immediately, you may continue only if you wanted to. If you withdraw from the study, we will destroy all your identifiable data, but may use the data collected up to your 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 you will not come to any harm if you take part. However, approval means that the Committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

You will be given a copy of this information sheet and a signed consent form to keep for your records.

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

Prof M Joan Taylor on 01162 506 317 or mjt@dmu.ac.uk,

Dr Tarsem Sahota on 01162 506 220 or ssahota@dmu.ac.uk

Exercise Physiology Laboratory HB1.29 Hawthorn Building Tel.No: 01162 506 220

Thank you for taking the time to read this Volunteer Information Sheet

11.4 Appendix 4 (information sheet for ND)



Leicester School of Pharmacy
Faculty of Health & Life Sciences

Participant Information Sheet

Date:01/07/2017

Title of Project: The Use of a Cardio and Resistance Exercise Programme to Assess Immunological and Physiological Parameters in Volunteers.

Principal Investigators: Prof M J Taylor, Dr T Sahota, Dr P Tomlins, Dr R Furmonaviciene, Mr K Chauhan, Mr B Alharbi, Mrs N Alsubaie and Mr A Hill.

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

Ask us if there is anything that is not clear or if you would like more information. Please take your time to decide whether or not you wish to take part. If you do wish to take part please complete the slip at the end of this sheet.

What is the purpose of the study?

As part of the growing research into Diabetes Mellitus the School of Pharmacy at De Montfort University are working to investigate the effects of a combined exercise programme (cardio and resistance) on blood glucose, metabolic and immunological parameters that could help you with the management of diabetes and increase your insulin sensitivity.

This research involves a combination of two types of exercises and this is where we need your help. We would like to invite you 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, to possibly manage your blood glucose level more effectively and increase insulin sensitivity.

We will keep your information strictly confidential and nobody other than the research team will have access to your 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 have I been invited?

You have been invited to take part in the study as a volunteer who has no history of Diabetes Mellitus and are aged between 18 and 60.

Do I have to take part?

It is up to you to decide. We will answer any questions you have about the study and go through this information sheet. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive from your doctor or hospital.

What will happen to me if I take part?

Preliminary procedures

Before enrolling in the study you will be asked to attend a screening visit where we will:

- Discuss and complete confidential questionnaires regarding your health, family history and physical activity level.
- Measure your blood pressure and heart rate.
- Measure your height and weight
- Provide an opportunity for you to ask questions.
- Familiarise you with equipment to be used in the study and teach you how to use the recumbent ergometer bike and how to lift the weights safely on the multi-gym machine.
- This session will also be used to determine the intensity of exercise during cycling on the recumbent ergometer bike using Heart Rate Reserve (HRR). Also, in this orientation session we will use predicted one repetition maximum (1RM) to determine how much weight for different muscles you should lift in the resistance exercise session later.

These preliminary procedures will enable us to determine whether you are suitable to safely participate in the study or not.

Main experimental trials

The main experimental trial will involve 2 x 2 hour exercise sessions a week for a 6 or 12 week period, (exercise session includes rest and final observation of volunteer). We would conduct these trials over the duration of your degree study (3 years) so we can assess the impact of exercise over a longer period of time.

Blood glucose levels will be monitored before, during and after each session, using a standard finger prick test. In addition, Cholesterol, High density lipoprotein, low density lipoprotein and Triglyceride will be monitored before, and after each exercise programme using a finger prick test.

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 predicted one-repetition maximum strength 1-RM) using upper and lower muscle groups followed by 20 min moderate cycling at 50 – 60% of pre-determined heart rate reserve (HRR). Heart rate (HR) and rate of perceived exertion (RPE) will be taken in a different time points throughout the exercise trial. We will also perform an Oral Glucose Tolerance test to assess insulin sensitivity at the beginning and end of the programme and blood samples will be screened for other immunological parameters. If you are taking any statin medication then you may also be asked to provide urine samples for analysis in a parallel study. We may also ask you to provide a saliva sample for a further study which assesses microbes present in saliva.

Incentives

We would like to offer an incentive of a subsidised gym membership at the De Montfort University's QEII Leisure Centre on completion of 100% of the dates agreed. This is a thank you for your participation but also to help you maintain the healthy exercise regimen until the following year's assessment.

What are the possible benefits of taking part?

As a result of being involved in this study you will receive health and fitness information about yourself including fitness tests and body measurement. You may also witness a decrease in your Hba1c levels and an increase in your insulin sensitivity as well as other general improvements to your health.

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.

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All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the University, will have your name and address removed so that you cannot be recognised from it.

What will happen if I don't want to carry on with the study?

While we do not expect the programme to cause you to become upset if this does happen then you will have the option to pause or stop your participation immediately, you may

continue only if you wanted to. If you withdraw from the study, we will destroy all your identifiable data, but may use the data collected up to your withdrawal.

Who has reviewed the study?

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You will be given a copy of this information sheet and a signed consent form to keep for your records.

Contact for Further Information

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Prof M Joan Taylor on 01162 506 317 or mjt@dmu.ac.uk,

Dr Tarsem Sahota on 01162 506 220 or ssahota@dmu.ac.uk

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Thank you for taking the time to read this Volunteer Information Sheet