EVALUATION OF THE ACTIONS OF DIOSMIN – HESPERIDIN COMBINATION ON HIV- PROTEASE INHIBITOR-INDUCED HYPERGLYCEMIA IN *Rattusnorvegicusalbinus*

BY

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DECLARATION

I hereby declare that this is my original work and it has not been submitted anywhere else for academic qualification in any learning institution.

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DEDICATION

To my son Jesse, daughter Lensa and all medical researchers

ABSTRACT

Glucose intolerance is impaired glucose uptake by peripheral tissues due to insulin resistance and is usually associated with chronic hyperglycemia. The condition often leads to overt diabetes mellitus [DM] - a metabolic disorder that is characterized by the body's inability to lower blood glucose to normal levels. Available reports show that medication with HIV protease inhibitors [PIs] causes insulin resistance and hence glucose intolerance in HIV- positive patients. This study evaluated the actions of the flavonoids (Diosmin[DIOS] and Hesperidin[HES]) on HIV protease inhibitor (HIV-PIs) induced hyperglycemia in *Rattusnorvegicusalbinus* (wistar rats). Seventy two Wistar rats were randomly assigned into six groups of twelve rats each. Each group was given a specific treatment with HIV protease inhibitors and or flavonoids for 42 days. The blood glucose, HbA1c and insulin levels were measured analyzed.The Control and the ATV/RTV+DIOS/HES treated groups had a significant difference only once at the end of week one of the treatment period (diff. =0.625 mmol/L, p=0.014). The Control group, DIOS/HES and the ATV/RTV+DIOS/HES treated groups did not show any significant differences in their group mean blood glucose levels from the second week of treatment to the end of the study. The ATV/RTV (5.95±2.15 mmol/L on week 3), LPV/RTV (5.14±0.92 mmol/L on week 3) and LPV/RTV+DIOS/HES (5.30±1.72 mmol/L on week 3) treated groups had significantly elevated group mean blood glucose levels on the third week of the study compared to the control group (3.88±0.42 mmol/L on week3). The HIV-Protease inhibitor treatments increased blood insulin levels (grand mean of 3.58±2.27µIU/mL at baseline (day 0) to 20.32±2.27 µIU/mL on day 42, $p<0.001$). The HIV-protease inhibitors increased group mean HbA1c levels with the LPV/RTV treated group increasing from 4.67± 0.16 percent on day 0 to 5.45±0.77 percent on day 42 ($p<0.01$) and ATV/RTV treated group mean HbA1c level increasing from 4.52±0.16 percent on day 0 to 5.13±0.53 percent on day 42 ($p<0.01$) while the DIOS/HES group mean HbA1c levels decreased significantly (4.73±0.16 percent at day 0 to 3.35±0.48 percent on day 42, $p<0.001$). The results demonstrated that the co-administration of ATV/RTV with DIOS/HES significantly reduced the blood glucose elevating effects of the ATV/RTV combination. The co-administration of the flavonoids with HIV-PIs also reduced the blood HbA1c levels resulting from HIV-PIs treatment.

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ACRONYMS AND ABBREVIATIONS

AIDS - Acquired Immunodeficiency syndrome

Akt - Threonine/serine protein kinase

AMPK - 5'-AMP –activated protein kinase

ANOVA- Analysis of Variance

ATV - Atazanavir

Bax - Bcl2 associated X protein [apoptosis]

Bcl2-B - cell lymphoma2 [apoptosis]

DM - Diabetes Mellitus

ERK - Extracellular signal-regulated kinase/extracellular receptor kinase.

FFA - Free fatty acid

FOX (Forkhead box) -A family of transcription factors regulating gene expression for growth, longevity and cell differentiation.

GLUT- Glucose transporter

HAART- Highly active antiretroviral therapy

HIV– Human immunodeficiency virus

HSP- Heat shock protein (antioxidant and chaperone)

IDDM- Insulin dependent diabetes mellitus

IRS- Insulin receptor substrate

LSD- Least significant difference

LPV – Lopinavir

MAPK - Mitogen activated protein (Serine/threonine protein kinase)

MTOR- Mammalian target of ripamycin (member of PI3K)-Inhibition leads to immunosuppression.

MMOL – Millimole (mmol)

NIDDM- Non-insulin dependent diabetes mellitus

PDPK1- 3- phosphoinostide-dependent protein kinase-1(serine/threonine protein kinase)

PKA- Protein kinase A (cAMP dependent kinase)

PPAR- Peroxisome proliferator-activated receptor

RTV – Ritonavir

SGLT- Sodium–coupled glucose transporter

SD – Standard deviation

SREBP – Sterol regulatory element- binding protein

TZD– Thiazolidinediones

OPERATIONAL DEFINITIONS

ATAZANAVIR – An HIV- protease inhibitor

DAFLON™ TABS- Tablets containing the micronized flavonoids Diosmin and Hesperidin. This combination is a used to treat venous insufficiency.

DIOSMIN - Flavonoid component of Daflon™ tablets.

DIABETES MELLITUS – An endocrine disorder characterized by the body's inability to secrete adequate amounts of insulin or the target cells becomes unresponsive to insulin (insulin resitance).

FLAVONOIDS- Naturally occurring polyphenolic compounds found in many fruits and vegetables.

GLUCOSE INTOLERANCE- Impaired glucose tolerance

GLYCOSURIA-The presence of glucose in urine.

HbA1c – Glycated hemoglobin

HESPERIDIN - Flavonoid component of Daflon™ tablets.

HIV-PROTEASE INHIBITOR- Antiretroviral drugs which work by inhibiting the enzyme HIV protease.

HYPERGLYCEMIA- Abnormally elevated blood glucose levels in blood.

INSULIN RESISTANCE- Interference with insulin signal transduction in the target cells.

LOPINAVIR – An HIV- protease inhibitor

RITONAVIR – An HIV- protease inhibitor

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Hyperglycemia is sustained high blood glucose due to impairment of glucose metabolism and transport in the body. It may becaused by insulin resistance, particularly by peripheral tissues like skeletal muscle and adipose tissue due to interference in the insulin signal transduction pathway that leads to the translocation glucose transporters (e.g. GLUT-4) to the cell membrane to allow for the inward movement of glucose into these cells(Carr, Samaras, Burton, et al., 1998; Murata et al., 2000; Schütt et al., 2004). This leads to chronic hyperglycemia which may in turn lead to overt Diabetes Mellitus (Bhowmik et al., 2013).Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia caused by such factors as autoimmune reactions against β-cells of the islets of Langerhans.Other causes include genetic factors leading to a decline in insulin production. Environmental factors such as obesity, excessive junk food (high- energy[carbohydrate] and high-fat fast food) consumption, sedentary and stressfullifestyles and aging have long been established as risk factors in the onset of diabetes mellitus(Ozougwu, 2013).HIV/AIDS is a chronic infection that occurs when individuals contract the Human Immunodeficiency Virus (HIV) (Barré-Sinoussi, 1996; Gallo & Montagnier, 2003; Sharp & Hahn, 2011; Weiss, 1993). If untreated HIV infection progresses to full blown acquired immunodeficiency syndrome (AIDS) which inevitably leads to death (González-Scarano & Martín-García, 2005; Gran et al., 2008; Zolopa et al., 2009). To prevent this progression of HIV infection to AIDS, patients are treated with antiretroviral drugs (ARVs). Kenya is one of the four HIV high burden countries with an estimated 1.5million people living with HIV/AIDS and about 70,000 new infections among adults and 6600 among children

respectively, annually(NACC, 2014). According to the 2015 Kenya AIDS response progress report by the Kenya National AIDS council, about 900,000 people of the 1.5million Kenyans living with HIV/AIDS were on antiretroviral drugs (ARVs) using the highly active antiretroviral therapy (HAART) model.Currently, using the HAART model, management of HIV/AIDS involves administration of a combination of at least two classes of antiretroviral drugs in the treatment regimen (HAART). The use of at least three drugs from two classes of ARVs is necessary to prevent the development of resistance by the HIV to these drugs. The main classes of antiretrovirals include: reverse transcriptase inhibitors (RTIs), HIV protease inhibitors (PIs), penetration inhibitors and HIV integrase inhibitors. Each of these classes of ARVs is associated with a number of adverse effects.HIV protease inhibitors-the focus of this study have such adverse effects that include induction of insulin resistance that may lead to chronic hyperglycemia that may turn into overt DM(Hammer et al., 2008; Singh et al., 2013; Testa et al., 2016; Zhuang et al., 2002). So far these side effects have bothered patients treated with these drugs and hence the need for a search of ways to manage these unwanted /side effects. Flavonoids are naturally occurring polyphenolic compounds that have been shown to antagonize insulin resistance and hyperglycemia(Babu et al., 2013; Bahadoran et al., 2013; Grassi et al., 2015; Yamagata, 2018). In this study, these compounds (flavonoids) are administered to animals(wistar rats) on HIV-Protease inhibitors to find out if they can counter the insulin resistance and the resulting hyperglycemia caused by the HIV-protease inhibitors.

1.2 Problem Statement

HIV- protease inhibitors (HIV-PIs) are key drugs in HAART in the management of HIV/AIDS patients and have led to a remarkable reduction in mortality and morbidity amongst HIV/AIDS patients. However, they are now known to cause a number of serious side effects such as

lipodystrophy, lipoatrophy, and hyperlipidemia and insulin resistance leading to glucose intolerance, hyperglycemia and altered plasma lipid profiles.Hyperlipidemia is characterized by increased triglycerides and fatty acid plasma levels(Palella et al., 1998; Paterson et al., 2000). Lipodystrophy is the abnormal distribution of body fat characterized by truncal obesity and a buffalo hump. Lipoatrophy is fat wasting particularly from the face and the limbs, and is characterized by decreased leptin levels in blood (Baril et al., 2005; Carr, Samaras, Burton, et al., 1998; Estrada et al., 2006; Koutkia & Grinspoon, 2004).Insulin resistance and the accompanying hyperglycemia in patients on HIV protease inhibitors is a syndrome of elevated insulin levels which is however ineffective in reducing blood glucose levels. This is due to interference with the signal transduction pathway in the target cells particularly thoseinvolving the glucose transporters (GLUT-4) in skeletal muscle and adipose tissue(Calderhead et al., 1990; Cheatham et al., 1996; Kozka et al., 1991; Murata et al., 2000, 2002).

Flavonoids are polyphenolic organic compounds found in plants, fruits and vegetables that have been found to exhibit blood sugar level lowering effects by various cellular and molecular mechanisms.They are thought to act by their antioxidant action, which action is protective to β-cells of the pancreatic islets of Langerhans. There is evidence that they scavenge free radicals thus reducing oxidative stress on the β-cells resulting in increased insulin secretion (Coskun et al., 2005; Vessal et al., 2003). In a nutshell, the flavonoids promote the peripheral utilization of glucose (increase sensitivity of cells to insulin), inhibit glucose absorption from the small intestine and enhance insulin secretion(Jadhav & Puchchakayala, 2012).Flavonoids also act by targeting molecular pathways in the β-cells, hepatocytes, adipocytes and skeletal muscles cells. These compounds also reduce apoptosis of the β-cells by their antioxidant action(Oladele et al., 2010).

The problem

The main purpose of this study was to assess if this adverse effect –hyperglycemia, induced by HIV- protease inhibitors due to insulin resistance can be ameliorated by the co-administration of flavonoids–specifically the Diosmin /Hesperidin combination together with the HIV protease inhibitors- lopinanvir/ritonavir combination and atazanavir/ritonavir combination in animal model (Wistar rats).

1.3 Objectives

1.3.1 Main Objective

To evaluate the effect of Diosmin – Hesperidin combination on HIV-protease inhibitor-induced hyperglycemiain Wistar rats (*Rattusnorvegicusalbinus*).

1.3.2 Specific Objectives

1. To determine theblood glucose levels in Wistarrats (*Rattusnorvegicusalbinus*)before and after treatment with HIV- protease inhibitors(Lopinavir/ritonavir or Atazanavir/ritonavir).

2. To asses the effect of the administration of the Diosmin-Hesperidin combination on blood glucose levels in *Rattusnorvegicusalbinus*treated with HIV-protease inhibitors (Lopinavir/Ritonavir (LPV/RTV) or Atazanavir/Ritonavir (ATV/RTV)).

3. To evaluatethe effect of the administration of the Diosmin-Hesperidin combination on blood insulin levels in HIV-protease inhibitor (LPV/RTV or ATV/RTV) treated rats (*Rattusnorvegicusalbinus*).

4.To find outthe effect of the administration Diosmin-Hesperidin combination on the blood glycated hemoglobin (HbA1c) levels in HIV-protease inhibitor (LPV/RTV or ATV/RTV)treated rats (*Rattusnorvegicusalbinus*).

1.4 Study hypotheses

1.4.1 Hypothesis 1

H_0: There is no difference in the group mean blood glucose levels in all Wistar rat (*Rattusnorvegicusalbinus*) groups before and after treatment with HIV- protease inhibitors (Lopinavir/ritonavir or Atazanavir/ritonavir).

H_0: $\mu 1=\mu 2=\mu 3=\mu 4=\mu 5=\mu 6$

1.4.2 Hypothesis 2

H_0: There is no significant difference in the group blood glucose means after the administration of Diosmin-Hesperidin combination in the rats (*Rattusnorvegicusalbinus)* treated with HIV-protease inhibitors (LPV/RTV or ATV/RTV).

H_0: $\mu 1=\mu 2=\mu 3=\mu 3=\mu 4=\mu 5=\mu 6$

1.4.3 Hypothesis 3

H_0:There is no significant difference in the group mean blood insulin levels in the rats administered with the Diosmin-Hesperidin combination on blood insulin levels in HIV-protease inhibitor (LPV/RTV or ATV/RTV) treated rats (*Rattusnorvegicusalbinus*).

H_0:$\mu 1=\mu 2=\mu 3=\mu 4=\mu 5=\mu 6$

1.4. 4 Hypothesis 4

H_0: There is no a significant difference in the group mean HbA1c levels among the ratsafter the administration of the Diosmin-Hesperidin combination in HIV-protease inhibitor (LPV/RTV or ATV/RTV) treated rats (*Rattusnorvegicusalbinus*).

H_0:$\mu 1=\mu 2=\mu 3=\mu 4=\mu 5=\mu 6$

1.5 Justification of the Study

The HIV-protease inhibitors(PIs) cause some serious adverse effects such as insulin resistance that may manifest as hyperglycemia.Long-term exposure to HIV-protease inhibitors interferes with glucose metabolism which seems mainly to be due to insulin resistance and impaired insulin secretion by β-cells of the pancreas(Chandra et al., 2009; Schütt et al., 2004). This study is important in the sense that currently in Kenya and worldwide millions of HIV/AIDS patients are on highly active antiretroviral therapy (HAART).HIV- protease inhibitors (PIs)which form an important pillarof HAART can precipitate insulin resistance leading to chronic hyperglycemia whichin turn can lead to overt diabetes mellitus(Carr, Samaras, Burton, et al., 1998). Any effort that can generate new ways of countering these adverse effects without producing more complications and adverse effects is worthwhile. This why this study was designed; to establish if the flavonoidsdiosmin and hesperidin(naturally occurring and safe)when co-administered with protease inhibitors can reduce these adverse effects.

Empirical literature

So far, studies done have focused separately on either the hyperglycemic effects of HIV-protease inhibitors or the antihyperglycemic effects of flavonoids on diabetic subjects. For

instancein a study by in Taiwan(Hsu et al., 2017), Diosmin was demonstrated to alleviate hyperglycemia in type 1 diabetic rats. A similar study in Japan(Akiyama et al., 2009) also demonstrated the antihyperglycemic effects of hesperidin on rats. In 2004,Schutt(Schütt et al., 2004), working in Germany confirmed that chronic exposure of Beta cells of langerhans to HIV-protease inhibitors led to hyperglycemia as a result of development of insulin resistance.A recent overviewin India(Marella, 2017), emphasized that it is now a recognized fact that flavonoids are potent antidiabetic agents. A case report from Nigeria (Bakari et al., 2007)clearly shows how antiretroviral therapy in apatient led to the development of diabetes mellitus in that patient.Researchers working in India have demonstrated that diabetes mellitus can be one of the consequences of HAART(Kalra et al., 2011).However, so far no study has assessed the effect of hesperidin and diosmin on HIV- proteaseinhibitor inducedhyperglycemia. The presence of this knowledge gap formed the basis and the justification of this study.

1.6 Scope of the Study

The scope of this study was limited to assessing the effect of two of the most commonly used HIV-protease inhibitor combination in Kenya (Lopinavir+Ritonavir and Atazanavir+Ritonavir) on blood glucose levels, blood insulin levels and HbA1c levels of Wistar rats by these HIV-protease inhibitors. The studyalso assessed the effect of the flavonoids combination of Hesperidin and Diosmin on the hyperglycemia induced by these HIV-protease inhibitors on Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Diabetes is a growing metabolic disorder characterized by chronically elevated blood glucose (hyperglycemia).This has been further complicated by iatrogenic hyperglycemiadue to glucose intolerance in patients who are on ARVs, particularly the HIV- protease inhibitors. This literature review is aimed at crystallizing some of these side effects and how the administration of flavonoids has been demonstrated to treat hyperglycemia in animal and human subjects and if this efficacy can be extended to HIV-PI induced hyperglycemia.

Diabetes mellitus is a disease that is characterized by the inability of the body to lower blood glucose levels to normal levels(Ozougwuet al,2013).HIV-protease inhibitors (HIV-PIs) are a cornerstone in HAART, however they are associated with a number of adverse effects such as chronic hyperglycemia that can lead to overt diabetes mellitus(Testa et al., 2016).Some of the notable adverse effects include lipodystrophy, lipoatrophy, insulin resistance that may lead to hyperglycemiaand lastlyhyperlipidemia(Finkelstein et al., 2015; Gkrania-Klotsas & Klotsas, 2007). Lipodystrophy is the abnormal redistribution of body fat with a marked accumulation of fat in the abdominal region.Lipoatrophy is body fat wasting accompanied by the loss of body weight.Insulin resistance is the disruption of insulin hormone signal transduction at insulin receptors leading to hyperglycemia. Hyperlipedemia is the abnormal lipid profile that is observed in patients who are on HIV- protease inhibitors due to acute increase in de novo lipogenesis caused by inhibition of glucose -6- phosphatesynthetase(Nijveldt et al., 2001). The main characteristic is elevated blood triglycerides(TGs) and free fatty acids(FFAs)(Bodasing & Fox,

2003; Carr, Samaras, Chisholm, et al., 1998).Peripheral insulin resistance is a prominent side effect of HIV protease inhibitors such as Nelfinavir and others(Carr, Samaras, Burton, et al., 1998). HAART and HIV protease inhibitors are also associated with the metabolic syndrome- which is a group of metabolic and non-metabolic disorders characterized by truncal obesity, elevated plasma lipid levels, hypertension, hyperglycemia and the development of cardiovascular disease(CVD)(Estrada et al., 2006; Petoumenos & Worm, 2011).HIV-protease inhibitors can acutely decrease glucose stimulated insulin release (within 1 hour) from mouse β-cells(Murata et al., 2000; Petoumenos & Worm, 2011).Flavonoids such as Quercetin and Rutin antagonize insulin resistance by scavenging free radicals which cause oxidative stress and damage to β-cellsand have even been noted to exert antidiabetic effects instreptozotocin- induced diabetes inrats (Calderhead et al., 1990; Cheatham et al., 1996; Coskun et al., 2005; Vessal et al., 2003).

2.2 Glucose metabolism in man

2.2.1 Sources of dietary glucose

The main source of dietary glucose is the digestion of carbohydrate foods that are consumed either as naturally occurring foods such as fruits and grains or as added sugar – sugar that is added to food during manufacture, preparation or preservation. The high consumption of added sugar has been associated with obesity, coronary heart disease, diabetes mellitus and metabolic syndrome(Azaïs-Braesco et al., 2017; Hu & Malik, 2010; Johnson et al., 2013; Ruiz et al., 2017; Ruiz & Varela-Moreiras, 2017).

2.2.2 Digestion and absorption of carbohydrates

Carbohydrates provide 40-80% of the total energy consumption. For this to happen complex carbohydrates (table 1) must be broken down into simple sugars such as glucose during the

process of digestion (figure 1)that takes place in the gut(Gibney et al., 2017; Lowe, 2007; Weaver et al., 2014).A number of factors determine the rate of carbohydrate digestion-the most important of which is the structure of the carbohydrate i.e. its complexity, the degree of cooking or processing, the presence of fiber, the protein or lipid content of the food, the presence of enzyme inhibitors (Jean-Marie, 2018)and the rate of gastric emptying(Foundation, 2016a, 2016b).The digestion and absorption of carbohydrates may fail due to lack of monosaccharide transporters inthe wall of the small intestine or lack of enzymes required for digestion of carbohydrates. The digestion of carbohydrates begins in the mouth with salivary amylase, proceeds in the duodenum with pancreatic amylase and is completed on the brush border (as shown in Table 1 and figure 1) of the small intestine with enzymes such as sucrase,maltase,lactase,(British Nutrition Foundation, 2019; Foundation, 2016a)

Table-1: Types of carbohydrates and sites of digestion

Class	Degree of polymerisation	Examples	Site of digestion
Monosaccharides	1	Glucose	Small intestine
		Fructose	Small intestine
Disaccharides	2	Sucrose	Small intestine
		Lactose	Small intestine
Polyols	1-2	Xylitol	Predominantly large intestine
		Erythritol	Small intestine
Oligosaccharides	3-9	Maltodextrin	Small intestine
		Inulin	Large intestine
		Fructo-oligosaccharides	Large intestine
Polysaccharides	≥10	Starch	Predominantly small intestine
		Non-starch polysaccharides	Large intestine

(Adopted from the(British Nutrition Foundation, 2019; Foundation, 2016b)

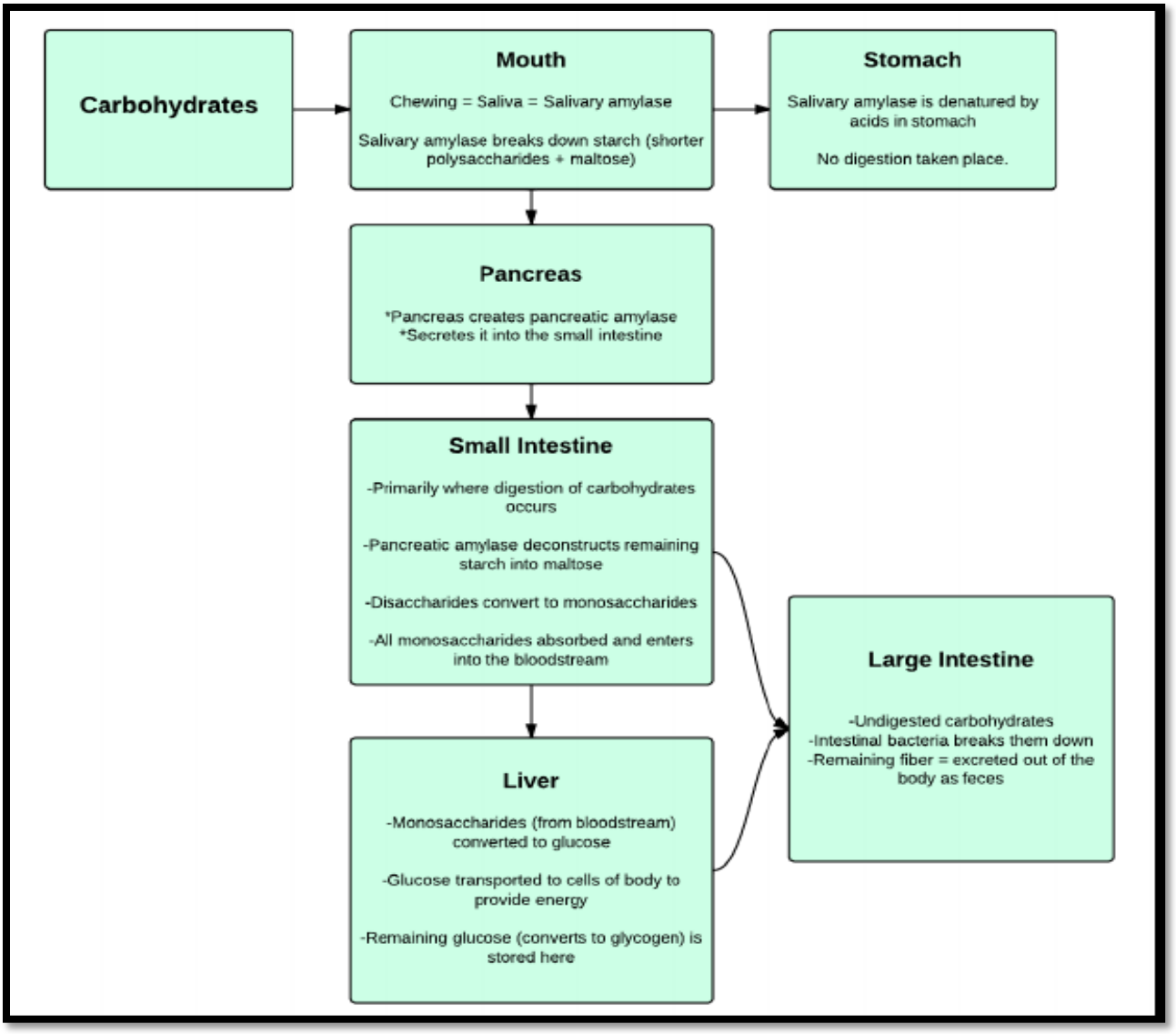


Figure 1: Flowchart of the digestion of carbohydrates (Adopted from Jenny Kim, 2014)

2.2.3 The role of various hormones in glucose metabolism and blood level regulation

Glucose metabolism is quite complex and is regulated by various hormones as displayed in table 2.These include insulin, glucagon, cortisol, catecholamines (particularly epinephrine), thyroxine, growth hormone,ghrelin,cholecystokinin (CCK),incretin hormones(glucose–dependent insulinotropic hormone(GIP) and glucagon-like peptide (GLP), adiponectin and others(Qaid

&Abdelrahman, 2016). There is an inverse relationship between insulin and ghrelin blood levels. This in turn affects blood glucose levels because insulin (figure 3) has a direct effect on the peripheral utilization of glucose particularly by skeletal muscles and adipose tissue. Ghrelin is secreted by the oxyntic cells of the stomach and is known to increase food intake and adiposity. Ghrelin is a growth hormone secretagogue acting through the growth hormone secretagogue receptor (GHSR).Growth hormone has short term and long term effects on carbohydrate and lipid metabolism and it has diabetogenic effect(Aguirre et al., 2016; Bonert & Melmed, 2017; Qaid & Abdelrahman, 2016)Growth hormone can reduces the amount of insulin secreted and attenuates its effects on the liver(Iglesias et al., 2012; Neely et al., 1992; Piatti et al., 1999; Rizza et al., 1982; Rufinatscha et al., 2018; Vélez et al., 2018). The hormone cholecystokinin (CCK) promotes satiety by slowing gastric emptying. This in turn affects the amount of glucose that can be absorbed from the gastrointestinal tract and consequently blood glucose levels(Clifton et al., 2009; Keogh et al., 2010).Blood cortisol levels are partly dependent on blood glucose levels implying that energy supply is necessary to cope with stress(Kirschbaum et al., 1997).

Thyroid hormone tends to increase the rate of carbohydrate metabolism and pancreatic functions. Hyperthyroidism is known to promote hyperglycemia(A. M. Cohen et al., 1977; Hage et al., 2011; Hwang et al., 1996; Jibhkate et al., 2014; Sun et al., 2017). Adiponectin, secreted by the adipose tissue has important insulin sensitizing properties. Thyroid hormone and adiponectin tend to reduce body fat through thermogenesis and lipid oxidation (Achari & Jain, 2017; Ahima et al., 2006; Gerozissis, 2008; Kleinridders et al., 2014).Glucagon is produced by the α-cells of the pancreas. It increases glycogenolysis and antagonizes glycogenesis.It acts synergistically with catecholamines. It also decreases the expression of GLUT-2 glucose transporters (Aronoff et al., 2004; Jiang & Zhang, 2003; Ramnanan et al., 2011; Taborsky, 2010)

Table 2: Mechanisms of key glucose metabolism regulators

	Glucose production	Glucose utilization	Lipolysis
Insulin	↓	↑	↓
Glucagon	↑	–	–
Epinephrine	↑	↓	↑
Cortisol	↑	↓	↑
Growth hormone	↑	↓	↑
FFA	↑	↓	–

(Adopted from(Shrayyef & Gerich, 2010))

The incretin hormones GIP and GLP-1 also play a significant role in the regulation of blood glucose levels indirectly through their action on insulin secretion. Glucagon like peptide (GLP-1) is synthesized by the L cells of the intestines. It suppresses glucagon production by the α-cells of the islets of Langerhans (Campbell & Drucker, 2013; Drucker, 2006; Holst et al., 2009; Kazafeos, 2011). Glucose- dependent insulinotropic hormone (GIP) is produced by the L cells of the small intestines. It increases the secretion of insulin (Fukami et al., 2013; Maemondo et al., 2010; Sim et al., 2018). All these hormones together with insulin are essential for tightly controlling blood glucose concentrations especially after consuming foods rich in carbohydrates.After digestion of carbohydrates into simple sugars such as glucose and fructose- they are absorbed into the general circulation by glucosetransporters (glucose transporters (GLUTs) and sodium dependent glucose transporters (SGLTs), figure 2).

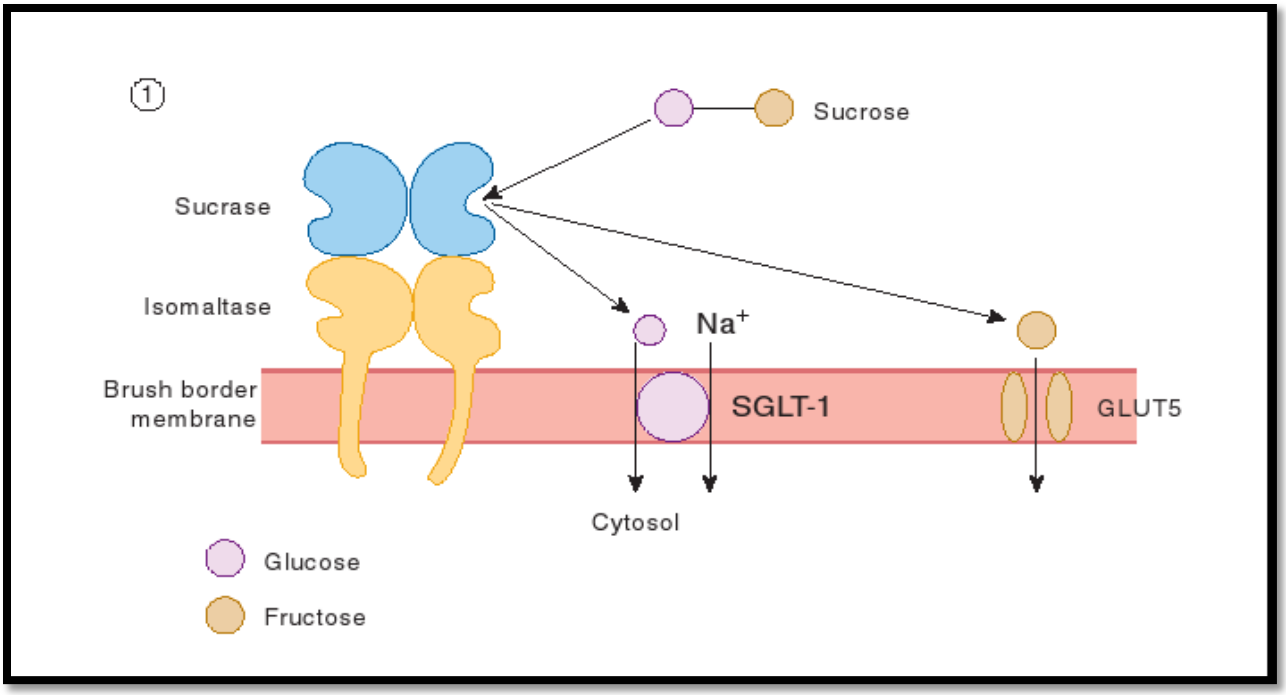


Figure 2: The absorption of glucose across the cell membrane of the enterocytes(Barret et al., 2012)

2.2.4: Why do cells need glucose transporters ?

Glucose transporters are required because the cell membrane is impermeable to glucose. This is because glucose being virtually lipid insoluble cannot penetrate the plasma membrane. This difficulty is overcome by glucose transporters (GLUTs) - integral proteins that are located on the plasma membrane for this purpose (Carayannopoulos et al., 2000a; Devaskar & Mueckler, 1992) The molecular actions of insulin leading to the translocation of glucose transporters to the cell membrane is displayed in figure 3, figure 4 and table 3.

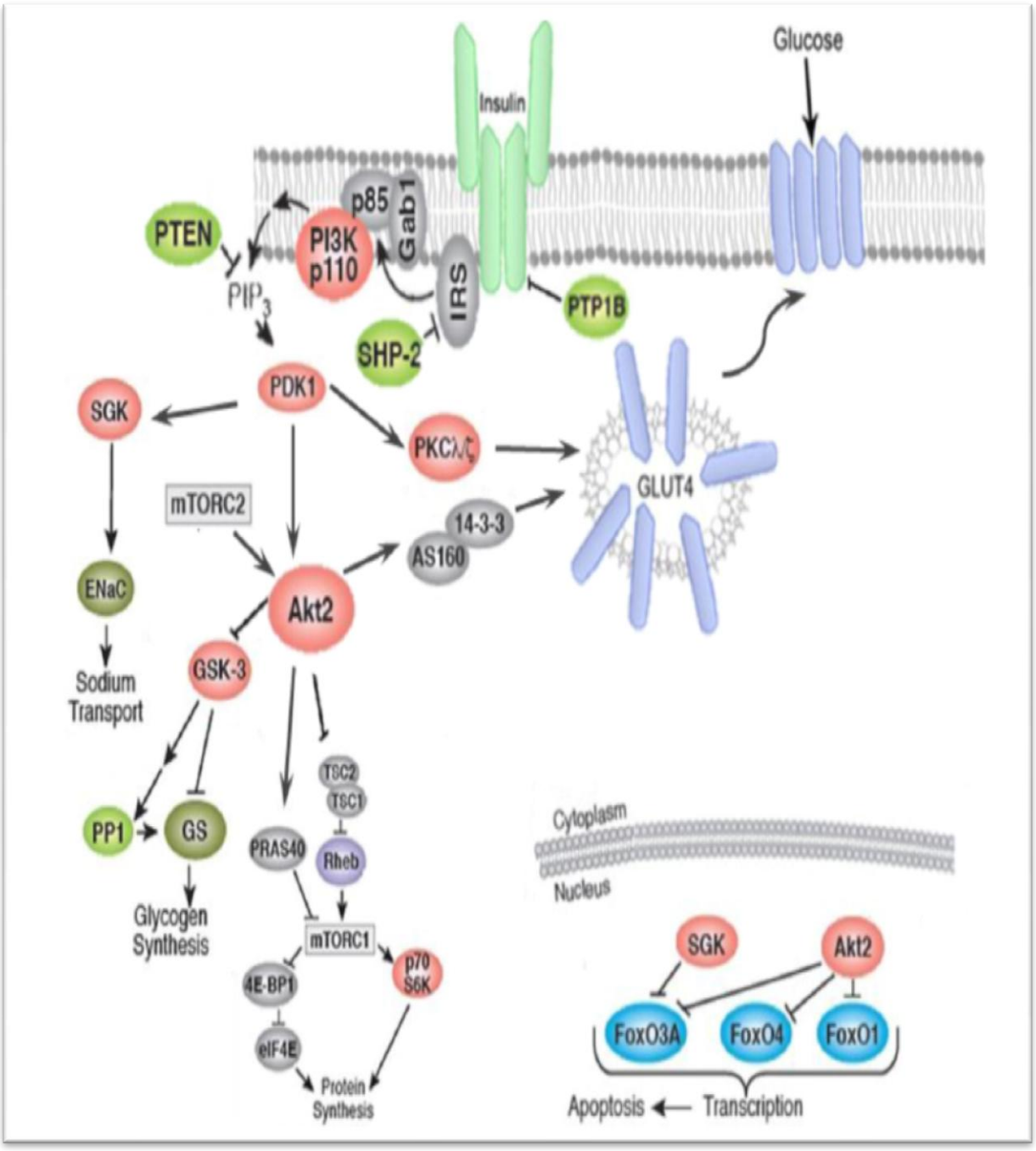


Figure 3: Effect of insulin on GLUT4 glucose transporters and mechanism of action of flavonoids.Attachment of insulin leads to activation of the insulin receptor(IR), causing phosphorylation of downstream adaptors like the insulin receptor substrate(IRS). After phosphorylation of its tyrosine residue, IRS activates many molecules such PI3K by the activation of the Akt/PKB and the PKC ζ pathways. Activation Akt results in glycogenenesis. The activation of Akt also leads to the movement of GLUT4 to the cell membrane resulting in the inward movement of glucose into the cell (Hajiaghaalipour et al., 2015)

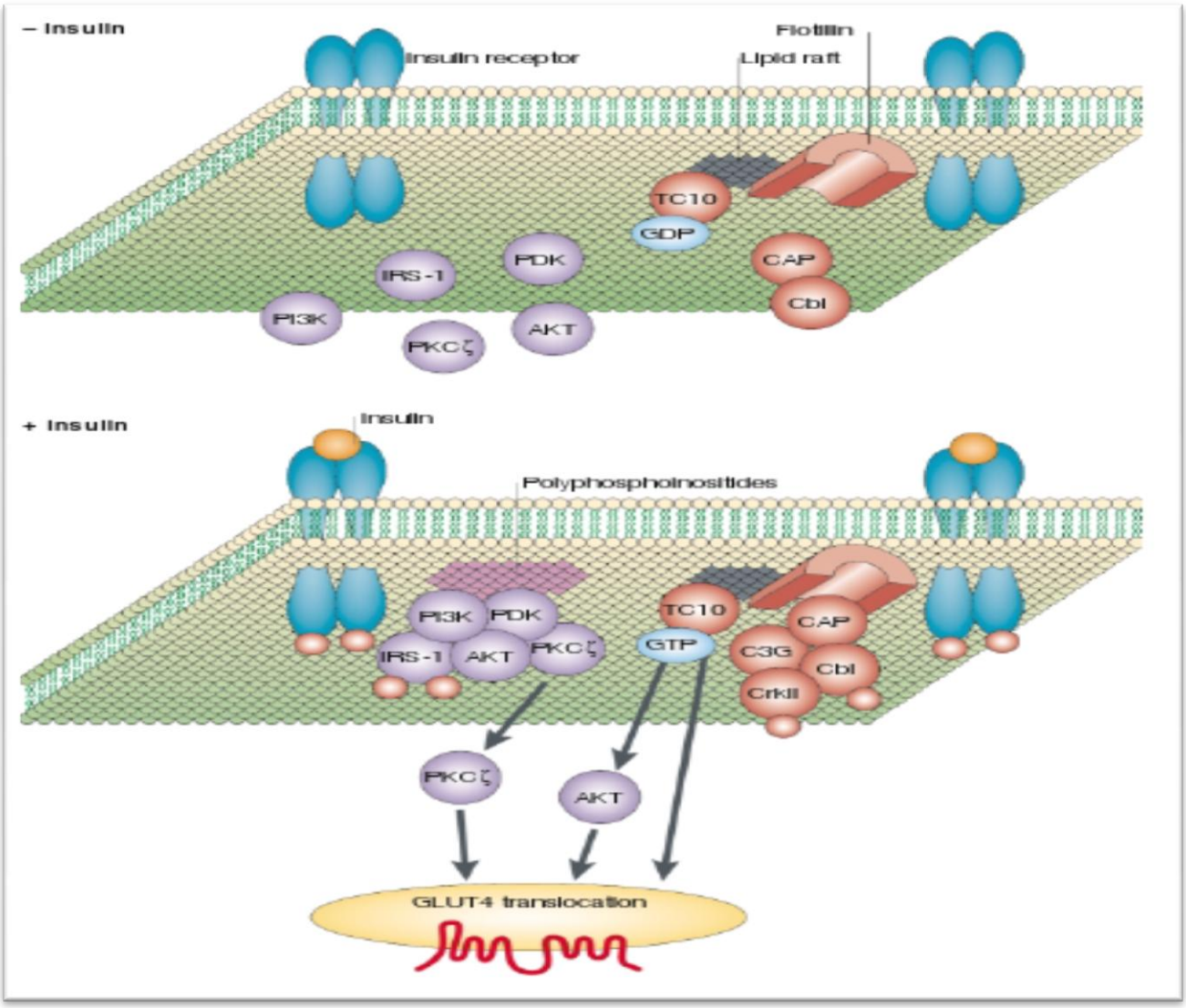


Figure 4: Insulin signaling pathways (Bryant et al., 2002)

Glucose transporters are divided into two broad groups:

1. Sodium–dependent glucose transporters (SGLTs). Six subtypes have been identified i.e. SGLT1 – SGLT 6. These transporters rely on the sodium gradient created by the Na^+-K^+ATPase to transport glucose into the cell by secondary active transport. They are mainly located on the proximal portion of the renal tubules for the reabsorption of sodium and glucose, and also in the small intestine for the absorption of glucose following digestion of complex carbohydrates

Table 3: Sodium- independent glucose transporters and their characteristics

Isoform	Previous name	Class	Main tissue localization	Insulin sensitive?	Functional characteristics (transport)	Present in skeletal muscle?*	Present in white adipose tissue?*
GLUT1	–	I	Erythrocytes, brain, ubiquitous	No	Glucose	Yes	Yes
GLUT2	–	I	Liver, pancreas, intestine, kidney	No	Glucose (low affinity); fructose	No	No
GLUT3	–	I	Brain	No	Glucose (high affinity)	No	Yes (m)
GLUT4	–	I	Heart, muscle, WAT, BAT, brain	Yes	Glucose (high affinity)	Yes	Yes
GLUT5		II	Intestine, testes, kidney	No	Fructose; glucose (very low affinity)	Yes	Yes
GLUT6	GLUT 9	III	Brain, spleen, leucocytes	No	Glucose	No	n.d.
GLUT7		II	n.d.	n.d.	n.d.	n.d.	n.d.
GLUT8	GLUT X1	III	Testes, brain and other tissues	No (yes in blastocytes)	Glucose	Yes (m)	Yes (m)
GLUT9	GLUT X	II	Liver, kidney	n.d.	n.d.	No	n.d.
GLUT10		III	Liver, pancreas	No	Glucose	Yes (m)	n.d.
GLUT11†	GLUT 10	II	Heart, muscle	No	Glucose (low affinity); Fructose (long form)	Yes (m)	No
GLUT12	GLUT 8	III	Heart, prostate, muscle, small intestine, WAT	Yes	n.d.	Yes	Yes
HMIT		III	Brain	n.d.	H^+–myo-inositol	No (m)	Yes (m)

(Adopted from (Wood & Trayhurn, 2003), *BAT-brown adipose tissue, WAT-white adipose tissue, nd-not determined, HMIT (H^+-coupled myo-inositol transporter))*

(Carayannopoulos et al., 2000a, 2000b; Devaskar & Mueckler, 1992). The second group of glucose transporters is the sodium-independent glucose transporters (GLUTs).Thirteen isoforms have been identified: GLUT1 – GLUT12 and HMIT. Their characteristics and distribution are shown above (Table 3).

Glucose is carried into pancreatic β-cells of the islets of Langerhans through GLUT-2 and its oxidation leads to insulin secretion.Insulin is essential in the regulation blood glucose levels by:

(i) Enhancing the uptake of glucose into peripheral tissues such as skeletal muscle, adipose tissue and the kidneys- through translocation of GLUT4 vesicles to the plasma membrane to allow for the entry of glucose into these cells.

(ii) Enhancing glucose utilization or storage in the liver by promoting glycogenesis.

(iii) Switching off lipolysis and enhancing lipogenesis in white adipose tissue (Chen et al., 2013; Lukačínová, Mojžiš, Beňačka, Keller, et al., 2008; Mahmoud et al., 2015; Miranda Pedroso et al., 2019). If the level of glucose in blood goes below normal concentrations, the hormone glucagon is secreted by the α-cells of the pancreatic islets. Glucagon elevates blood glucose levels by: (i) Promoting glycogenolysis and gluconeogenesis in the liver. (ii) Accelerating lipolysis and production of free fatty acids (FFAs) from adipose tissue which is then released into blood for energy generation.

Failure to tightly regulate blood glucose levels due to inadequate production of insulin by the pancreas and other factors such as obesity can easily lead to diabetes mellitus (Alam et al., 2014; Babu et al., 2013; Hossain et al., 2016; Ravishankar et al., 2013; Yu et al., 2016). Inadequate secretion of insulin leads to type two diabetes mellitus (T2DM), also known as non-insulin dependent diabetes mellitus (NIDDM), which is normally treated by lifestyle changes and oral hypoglycemic agents. The other type of diabetes is characterized by complete failure of the β -cells to produce insulin and leads to the development of type one diabetes (T1DM) or insulin dependent diabetes mellitus (IDDM) which can only be treated by administration of exogenous human insulin. The four important classes of oral hypoglycemic drugs are sulphonylureas (e.g. tolbutamide, glipizide, chlorpropamide), biguanides (e.g. metformin), thiazolidinediones(TZDs) e.g. pioglitazone, rosiglitazone and α-glucosidase inhibitors (e.g. acarbose) are mainly used for the management of T2DM.In addition, some of the more recently approved drugs such as

glucagon-like peptide-1 agonists, dipeptidyl peptidase-IV (DPP-IV) inhibitors and amylin analogues are also used in the management of type -2 diabetes. Due to adverse effects of these drugs and simply alleviating symptoms while not targeting the cause of the disease, there have been persistent efforts to identify potential compounds that can "cure" Diabetes Mellitus, for example by stimulating β-cell regeneration and preventing apoptosis of the β-cells, leading to a return of endogenous control of glucose homeostasis(Ralph A. DeFronzo, 2004; Esposito et al., 2012; Hajiaghaalipour et al., 2015; Olokoba et al., 2012; Stumvoll et al., 2005; Zheng et al., 2018).

Naturally occurring plant compounds are attractive candidates because they are abundant in nature, inexpensive to produce and may have fewer side-effects than currently used pharmacotherapeutic agents / compounds.Flavonoids are among such candidate molecules(Babu et al., 2013; Chen et al., 2013; Jaeger et al., 2018; Lukačínová, Mojžiš, Beňačka, Keller, et al., 2008; Salib et al., 2013; Sreelatha & Inbavalli, 2012). HIV- protease inhibitors are among the first line drugs of the highly active anti-retroviral therapy (HAART) and are used in combination with reverse transcriptase inhibitors, integrase inhibitors, etc. Their main drawback is the high incidence of adverse effects such as induction of insulin resistance, lipodystrophy and alterations in lipid metabolism. These side effects can be very troublesome to some of the patients on these drugs (Anuurad et al., 2010; Lee et al., 2005; Moran et al., 2016; Pokorná et al., 2009; Wensing et al., 2010).Insulin resistance has been implicated in the hyperglycemia associated with HIV – Protease inhibitors used in the management of HIV/AIDS. These drugs also cause impairment of the ability to lower glucose by precipitating insulin resistance (Carr et al., 2008; Lien & Feinglos, 2005; Woerle et al., 2003a). HIV-protease inhibitors acutely inhibit the insulin responsive glucose transporter- GLUT4, resulting in peripheral insulin resistance coupled with decreased

insulin secretion by the β-cells(Hruz, 2011; Hruz et al., 2008; Koster et al., 2003; Murata et al., 2000; Rudich et al., 2001). In Kenya, the most commonly used HIV-protease inhibitors in HAART are Lopinavir with Ritonavir, Atazanavir with ritonavir and Darunavir. Ritonavir is normally combined with the other HIV protease inhibitors to protect them from metabolizing enzymes in the liver (CYP450 super family) hence its commonly referred to as a pharmacokinetic booster(MOH, 2014; Nacc/Nascop, 2012; Nichols et al., 2008)

2.3 Incidence and prevalence of hyperglycemia in patients on HIV protease inhibitors

According to a study done in Greece(Tsiodras et al., 2000), patientson HIV- protease inhibitors developed hyperglycemia and it was established that there is an association between HIV-protease inhibitor use and hyperglycemia (glucose intolerance) in patients taking these drugs. However the prevalence of insulin resistance is more common at 50% of HIV –protease inhibitor treated patients(R. A. DeFronzo, 2010; Gutierrez & Balasubramanyam, 2012; Kalra & Agrawal, 2013; Kent et al., 2015).Increased incidence of DM has been observed in patients on HIV-protease inhibitors (Brown et al., 2005; Janssens et al., 2007; Kalra et al., 2011; Kalra & Agrawal, 2013). A study by a number of researchers observed that routine use of HIV-protease inhibitors was linked to the development of hyperglycemia due to insulin resistance induced by the HIV protease inhibitors(Calza et al., 2004; Lee et al., 2005; Tebas, 2008). From a cross-sectional comparative studyin Ethiopia, 7.9% of patients on HIV-PIs developed hyperglycemia(Abebe et al., 2014). A multicenter investigation by Todd Brown(Brown et al., 2005) found that the prevalence of DM in patients on HIV-PIs was higher compared to those who were HIV negative and unexposed to HIV-PIs, establishing that the incidence of DM in the exposed group was four times higher compared to HIV negative peopleunexposed to the HIV-PIs. From these studies and investigations, it is apparent that there is a strong link between

HAART/HIV- protease inhibitors use and the occurrence of hyperglycemia in patients on HAART incorporating HIV- protease inhibitors.

2.4 Hyperglycemia and HbA1c

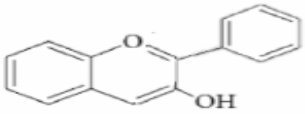
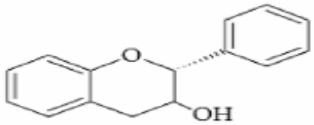
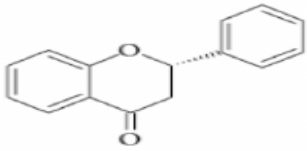
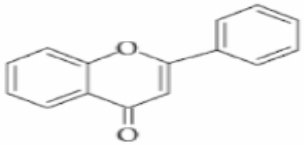
HbA1c (glycated hemoglobin) is a marker of chronic hyperglycemia(Berg, 2013). HbA1c is formed when glucose binds covalently to the β-chain of adult hemoglobin(Al-Ghamdi, 2004; Di Angelantonio et al., 2014; Weykamp et al., 2009; Wong et al., 2017).Insulin resistance is characterized by chronic hyperglycemia.HbA1c isa measure of the ratio between glycated hemoglobin (HbA1c) and total hemoglobin HbA1 expressed as a percentage. There is a direct correlation between blood glucose levels and HbA1c. Higher blood glucose leads to higher HbA1c(Nathan et al., 2008; Sayed et al., 2018). HbA1c levels above 6.5% are indicativeof diabetes mellitus(Joint United Nations Programme on HIV/AIDS, 2013).Normal HbA1c ranges between 4.5% and 5.7%. HbA1c levels are now used as an important screening tool for diabetes and pre-diabetes(Bonora & Tuomilehto, 2011; Kaur et al., 2019; Mahajan & Mishra, 2011)

2.5 Flavonoids

The flavonoids are naturally occurring polyphenolic phytochemicals based on the flavone nucleus(Hajiaghaalipour et al., 2015; Lukačínová, Mojžiš, Beňačka, Keller, et al., 2008). Flavonoids can also be defined simplyas naturally occurring phenolic compounds(Babu et al., 2013). They are found in plants - mainly fruits and vegetables. There are various types of flavonoids as displayed intable 4. Flavon-3-ols – include epicatechin, catechin, epicatechingallate, gallocatechin, epigallocatechin and epigallocatechin gallate.These are found in teas, grapes, leguminous plants, and other fruits. Flavonones - include naringin, naringenin, and hesperidin. These are found mainly in citrus fruits, and grapefruits. Anthocyanidins - are

found in berries, fruits, vegetables and red wine. These include Cyanidin, delphinidin,malvidin, peonidin, pelargonidin and petunidin. Flavonols - these are found in most plants and include kaempferol, quercetin, fisetin, isorhamnetin and myricetin.In nature they exist as glycosides. Flavones - Include apigenin and luteolin and are found in many herbs (see table 4).

Table-4: Examples and sources of some flavonoids

Group of Flavonoid	Dietary sources	Compounds	Chemical formula
Anthocyanidins 	Fruits (e.g., Cherry and berries) vegetables and red wine	Cyanidin Delphinidin Malvidin Pelargonidin Peonidin Petunidin	$C_{15}H_{11}O_6$ $C_{15}H_{11}O_7$ $C_{17}H_{15}O_7$ $C_{15}H_{11}O_5$ $C_{16}H_{13}O_6$ $C_{16}H_{13}O_7$
Flavan-3-ols 	Tea, Fruits, Cocoa and Chocolate	Catechin Epicatechin Procyanidins	$C_{15}H_{14}O_6$ $C_{15}H_{14}O_6$ $C_{30}H_{26}O_{12}$
Flavanones 	Citrus fruits (e.g., grapefruits, lemons, and oranges)	Naringin Naringenin Hesperidin	$C_{27}H_{32}O_{14}$ $C_{15}H_{12}O_5$ $C_{28}H_{34}O_{15}$
Flavones 	Fruit skins, red wine, buckwheat, red pepper, and tomato skin, celery, parsley and many herbs	Apigenin luteolin	$C_{15}H_{10}O_5$ $C_{15}H_{10}O_6$

Adopted from:(George et al., 2017; Hajiaghaalipour et al., 2015; Lukačínová, Mojžiš, Beňačka, Rácz, et al., 2008)

Isoflavones- are found in soy. The main isoflavones are daidzein and genistein.According to Lucacinova*et al* (2008) administration of the flavonoids -quarcetin and chrysin significantly

lowered serum glucose in alloxan-induced diabetes in rats thus pointing to these natural compounds as potential treatments and possibly a cure for Diabetes Mellitus.

2.6 The antihyperglycemic actions of flavonoids

The flavonoid compounds and other phytochemicals are thought toameliorate diabetes mellitus and the accompanying hyperglycemia by their antioxidant action which is cytoprotective and to some extent regenerates the β- cells of the Islets of Langerhans(Al-Ishaq et al., 2019; Alberti & Zimmet, 1998; Gosslau et al., 2018; Hajiaghaalipour et al., 2015; Ismail-Beigi, 2012; Marín-Peñalver et al., 2016; Vancampfort et al., 2016). These compounds are also thought to inhibit the sodium coupled glucose transporter (SGLT), α-amylase and sucrase activity in the small intestine which tend to reduce the postprandial surge in blood glucoselevels(figures 5&6, table 5). Other mechanisms include direct stimulation of β-cells of Langerhans to release insulin(Al-Ishaq et al., 2019; Feng et al., 2019; Hussain & Marouf, 2013; J. Li et al., 2019).It is also thought that flavonoids interfere with renal reabsorption of glucose leading to loss of glucose in urine(Lukačínová, Mojžiš, Beňačka, Keller, et al., 2008; Mahmoud et al., 2015; Salib et al., 2013)

Flavonoids act through a number of other mechanisms to counter hyperglycemia. Flavonoids scavenge free radicals and inhibit peroxidation.This prevents oxidative stress and protects β-cells leading to increased insulin secretion (Coskun et al., 2005; Sellamuthu et al., 2013). These compounds may also act on the cell cycle and initiate DNA repair mechanisms(George et al., 2017). Flavonoids also increase peripheral glucose utilization, decrease glucose transporter activity from the intestine and enhance insulin secretion(Jadhav & Puchchakayala, 2012; Mahmoud et al., 2015). It is also postulated that flavonoids may be acting on various molecular targets, signaling pathways in the β-cells and have been shown to regenerate damaged β-cells in

the pancreas(Diatewa et al., 2004; Jiraungkoorskul & Jiraungkoorskul, 2015; Lavle et al., 2016).These compounds (flavonoids) have also been shown to regulate different signal transduction pathways(figures 5&6,table 5) in skeletal muscle,hepatocytes,adipocytes,reduce apoptosis of β-cells, enhance insulin secretion and reduce insulin resistance(Bergman, 2013; Del Prato, 2009; Oladele et al., 2010; Saini, 2010; Y. Zick, 2003; Yehiel Zick, 2001)

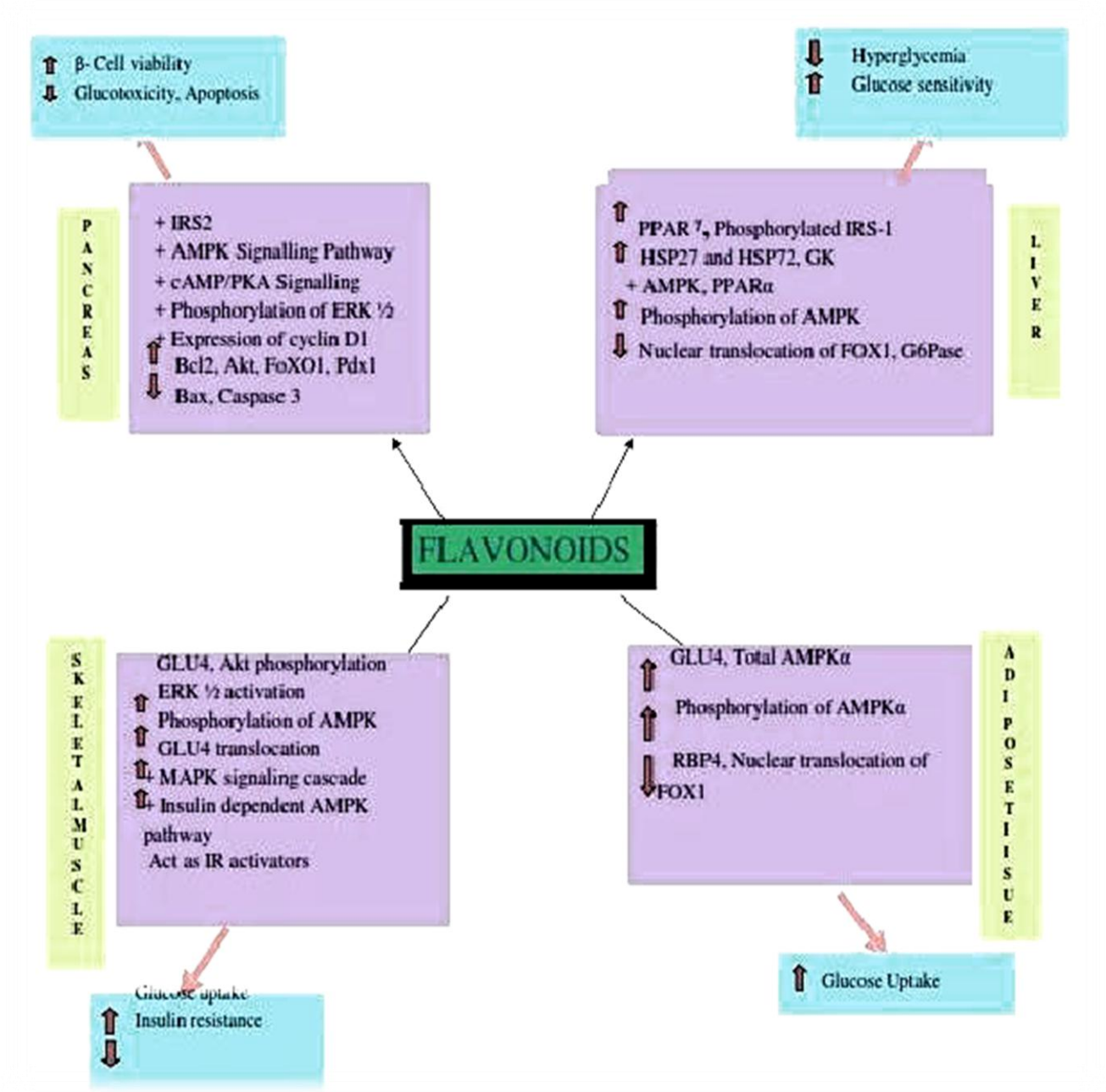


Figure5: Effect of flavonoids on glucose metabolism(Lavle et al., 2016)

The various molecular mechanisms and actions of flavonoids are shown in figure 5 above, table 5 and figure 6 below.

Table 5: Glucose pathways targeted by flavonoids

Flavonoids	Glucose transporter isoforms	Pathways/target molecules	Experimental model	Targets	Comments
Anthocyanins					
Anthocyanin	GLUT-4	IRS1, PI3k/AKT pathway, Anti-inflamtroy pathway	HFD-treated mice	Liver	Suppressed reactive oxygen species, restored the impairment of PI3k/AKT pathway, suppressed the JNK, NF- κB and IKKβ activation.
Anthocyanin	GLUT-4	AMPK pathway, glucose uptake	*In vitro*	Adipocyte 3T3-L1, C2C12 muscle cells and β TC-tetcells	Enhanced glucose uptake, insulin-like activities, insulin-sensitizing properties, PPARγ agonist activity, increased insulin secretion.
Anthocyanin	GLUT-4	Glucose uptake	STZ-induced diabetic rats	Heart, skeletal muscle, pancreatic tissues and serum	Antioxidant activity, prevent pancreatic apoptosis, decreased glucose levels, Increased insulin secretion, activated insulin receptor phosphorylation and increased GLUT-4 expression.
Anthocyanin	GLUT-4	AMPK, insulin sensitivity, PPAR	diabetic mic	White adipose tissue, skeletal muscle, and the liver	hyperglycemia and insulin sensitivity *via* activation of AMPK, upregulation of glucose transporter 4 in WAT and skeletal muscle, suppression of glucose production and inactivation of acetyl-CoA carboxylase in the liver.
Cyanidin 3-glucoside	GLUT-4	Antiinflamtroy pathway, glucose uptake, GLUT 4 regulation,	KK-Ay diabetic mice	White adipose tissue	Ameliorated hyperglycemia and insulin sensitivity, upregulated the GLUT 4,downregulated the inflammatory adipocytokines (TNF-α and MCP-1).
Cyanidin 3-glucoside	GLUT-4	Antiinflamtroy pathway, modulating the JNK/FoxO1 signaling pathway	C57BL/Ks db/db	White adipose tissue and blood	Lowered fasting glucose levels, improved the insulin sensitivity, reduced inflammatory cytokines (TNF-α, IL-6, and MCP-1).
Cyanidin 3-Glucoside	GLUT-4	Glucose uptake	*In vitro*	Adipocyte 3T3-L1	Insulin-like activities, increased adipocyte glucose uptake, GLUT-4 expression and translocation, increased nuclear PPARg activity, improve insulin resistance.
Flavon-3-ols					
Catechin	GLUT-4	Enhanced GLUT4 mRNA and protein expression	STZ-induced diabetic rats	Liver, muscle and blood	Hypo-glycemic, Glucose oxidizing and insulin mimetic activities.
(−)-epicatechin(EP)	GLUT-4	Glucose uptake, PI3K	*In vitro*	3T3-L1 adipocytes	Promote the translocation of GLUT-4 through activation of PI3K, increased phosphorylation of PKCλ/ζ.
(-)-epigallocatechin (EGC)	GLUT-4	Glucose uptake, PI3K	*In vitro*	3T3-L1 adipocytes	Promote the translocation of GLUT-4 through activation of PI3K, increased phosphorylation of PKCλ/ζ.
(−)-epigallocatechin-3-gallate (EGCG)	GLUT-4	Suppressed JNK pathway	*In vitro and* obese KK-ay mice,HFD-induced obese rats	Adipocytes tissue, 3T3-L1 adipocytes	Decreased JNK phosphorylation and promoted GLUT-4 translocation.
(−)-epigallocatechin-3-gallate (EGCG)	GLUT-4	AMPK, insulin signaling pathway	*In vivo*	Skeletal muscle and adipose tissue	Activated AMPK pathway, improving insulin signaling pathway, decrease oxidative stress, membrane translocation and Ser307 phosphorylation of IRS-1, increase in Ser473 phosphorylation of Akt and GLUT-4 translocation in skeletal muscle and adipose tissue.

(George et al., 2017; Hajiaghaalipour et al., 2015; Lukačínová, Mojžiš, Beňačka, Rácz, et al., 2008)

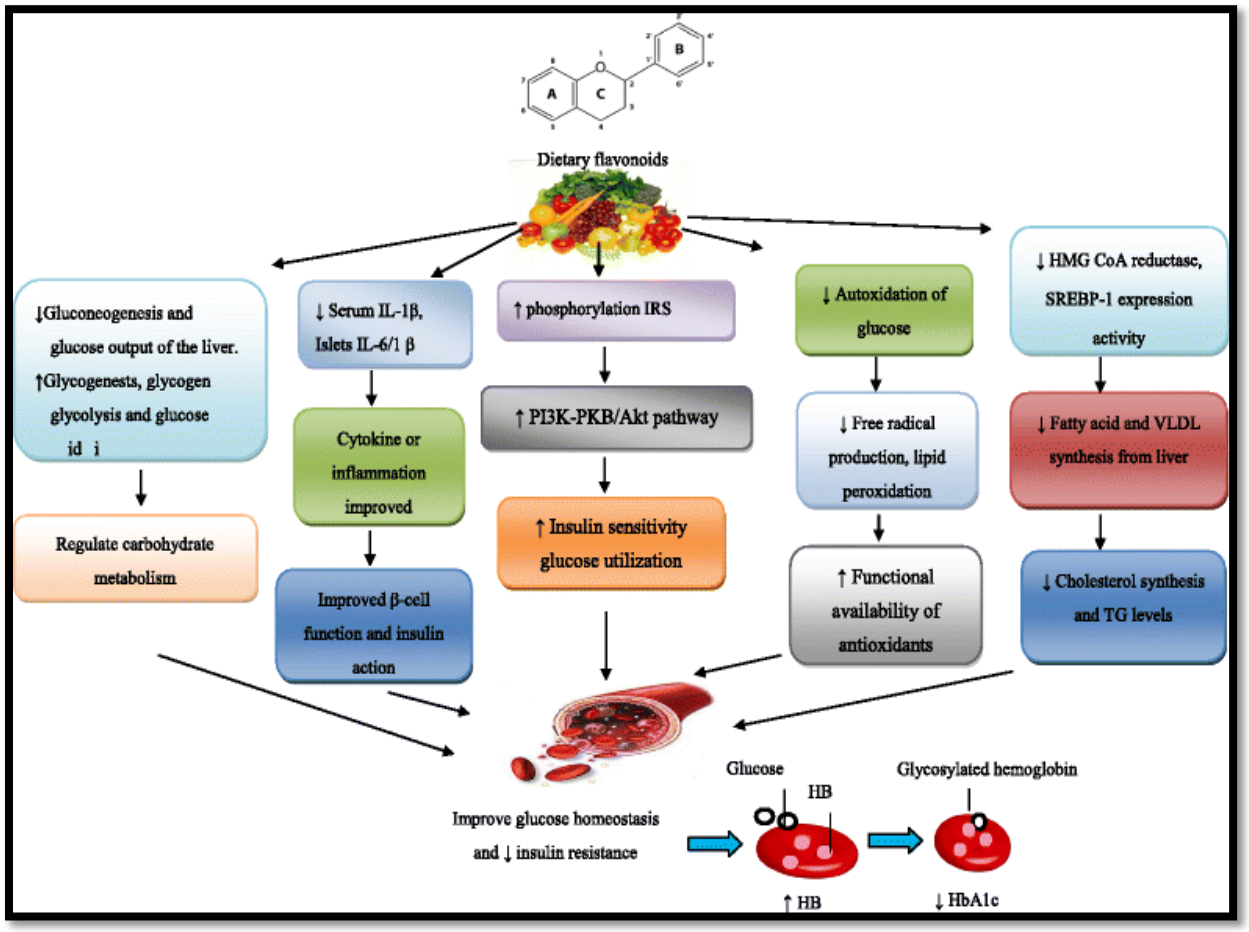


Figure 6: A flowchart of the proposed role of flavonoids on management of blood glucose in diabetes. AKT; v-akt murine thymoma viral oncogene homolog, IRS; Insulin receptor substrate, HB; hemoglobin, HbA1c; Glycated hemoglobin, HMG-CoA:3-hydroxy-3-methylglutaryl-coenzyme A, IL-1β; Interleukin-1 beta, PI3K; Phosphatidylinositol-3-kinase, SREBP-1c; Sterol regulatory element-binding protein, TG; Triglycerides, VLDL; Very low density lipoprotein, (↑ Increase, ↓ Decrease)(Vinayagam & Xu, 2015)

2.7DiosminandHesperidin

Diosmin is a derived from hesperidin by dehydrogenation (figure 7). Hesperidin is a flavone glycoside mainly found in the citrus family of fruits. Currently Diosmin is mainly indicated for the management of chronic venous insufficiency; for example in varicose veins, venous ulcers, piles, neuropathic pain and is being tested for activity against COVID-19 (Carballo-Villalobos et al., 2016; Giannini et al., 2015; Haggag et al., 2020; Toledo et al., 2017). Hesperidin is a precursor of diosmin though they are normally co-administered together in the treatment of chronic venous insufficiency(El-Shafae & El-Domiaty, 2001; Kanaze et al., 2003; "Monograph. Diosmin.," 2004).Research on rats with streptozocin induced diabetes has demonstrated that diosmin may treat diabetes (Pari & Srinivasan, 2010).

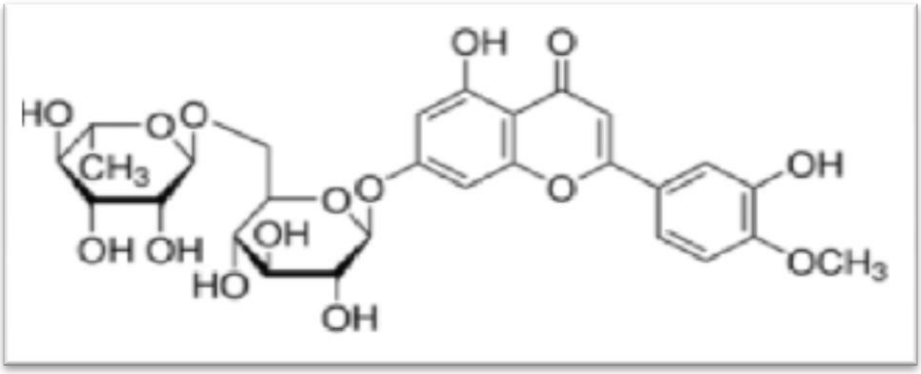


Figure 7a: Diosmin

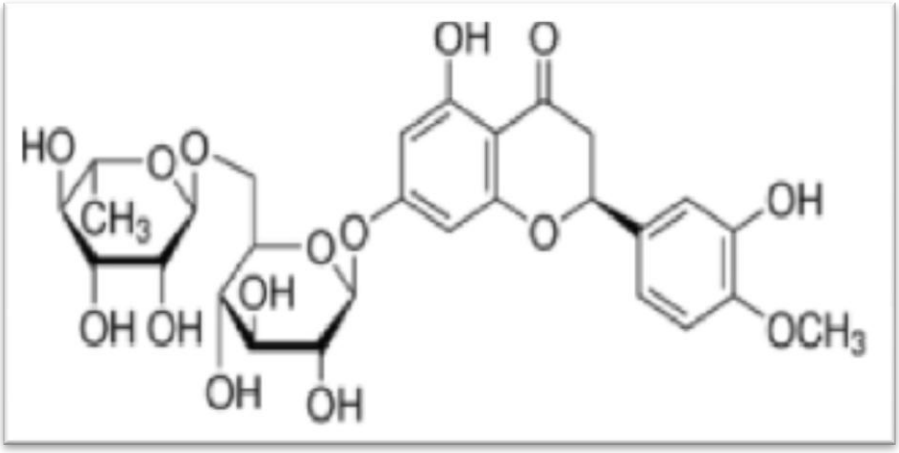


Figure 7b: Hesperidin

Figures 7 : Structures of the flavonoids Diosmin and Hesperidin (Vinayagam & Xu, 2015)

2.8Lopinavir, Atazanavir and Ritonavir

These drugs (lopinavir, atazanavir and ritonavir) are all HIV –protease inhibitors. They inhibit the enzyme HIV protease- an enzyme that is required for the cleavage of the viral glycoprotein precursors into functional -proteins in the infectious HIV(Chandwani & Shuter, 2008; Cvetkovic & Goa, 2003; Molina et al., 2010).Ritonavir, a part from inhibiting HIV-protease also inhibits the isoenzyme CYP450 3A4 which metabolizes HIV- Protease inhibitors.For this reason ritonavir is added to other HIV-protease inhibitors to protect them from these metabolizing enzymes andthus increasestheir half-lifeleading to less frequent dosing that reduces the pill burden on thepatient andincreases patient compliance.

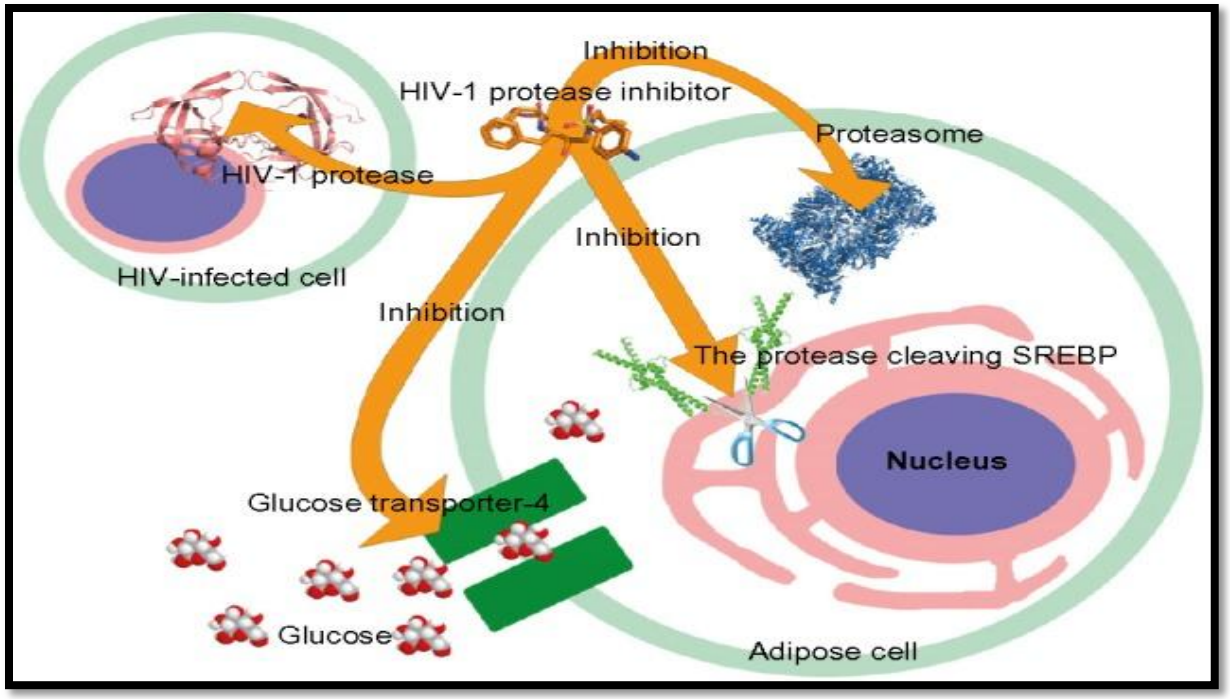


Figure8:Potential target molecules of the HIV- protease inhibitors that may lead to toxicity(X. Li et al., 2015).

Ritonavir is thus a pharmacokinetic booster. Toxicity of the HIV-PIs is due to the fact these cells inhibit other cell proteases (figure 8) (Findlay, 2007; Jamjoom, 1991; Lv et al., 2015; Molina et al., 2008; Sevrioukova & Poulos, 2010). The toxicity of HIV-PIs is due their inhibition of other cellular proteases as described displayed in figure 8.

2.9 Conceptual framework

The relationship between the HIV-PIs, flavonoids, food, blood insulin levels, blood glucose levels and blood HbA1c levels is summarized in the conceptual framework below (figure 9)

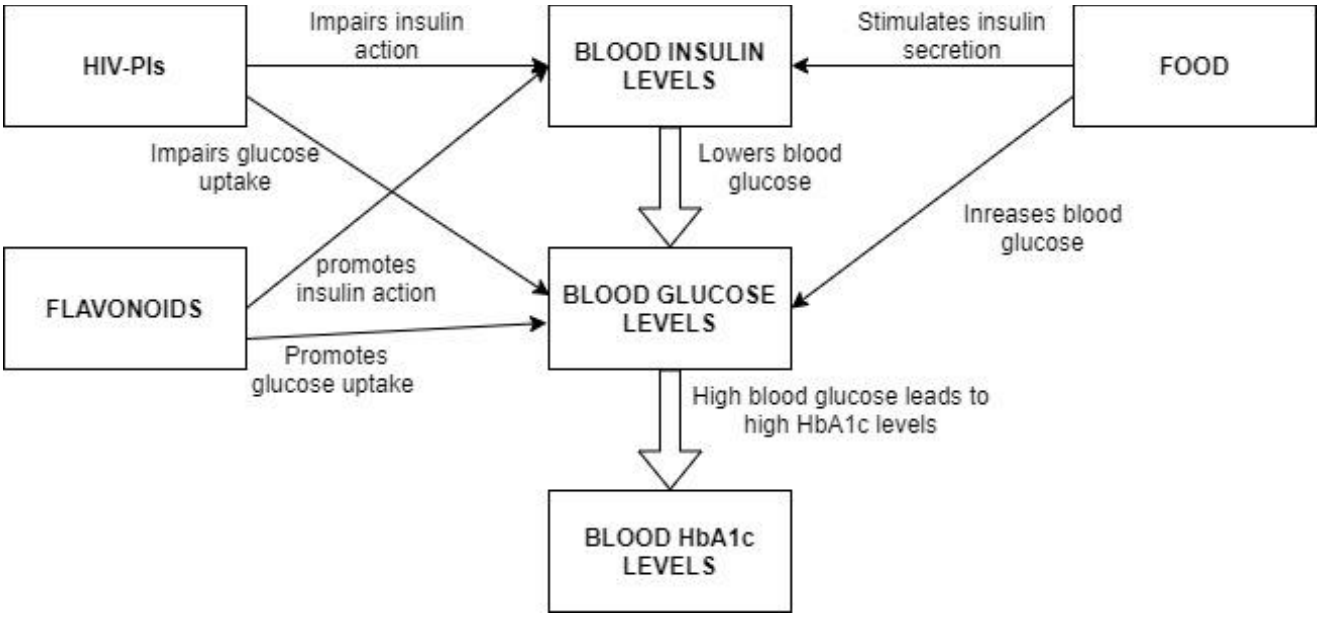


Figure 9: Conceptual Framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was conducted within the zoology Laboratories of the University of Eldoret in Uashin Gishu County, in the North rift region of Kenya. This Laboratories are well equipped and designed for animal studies.

3.2 Study design

This was a randomized controlled experimental study in which seventy twomale Wistar rats aged 3 to 4 months weighing between 170g and 250g were randomly assigned into six groups of twelve rats each. Each group was subjected to a specific treatment as described under treatments (section 3.7).

3.3 Sample size determination

For this study,the sample sizes were calculated based on blood glucose levels.Blood glucose can be classified as fasting blood glucose (FBG), random blood sugar(RBS) or postprandial blood glucose(PBG)(Jean-Marie, 2018). Fasting blood glucose (FBG) and postprandial blood glucose (PBG) in rats is 3.95±1.31 and 5.65±1.63 mmol/Lrespectively(Wang et al., 2010). Sample size was estimated using the Glenn D. Israel(Israel, 1992)statistical formula based on confidence intervals:

$$\mathbf{n= (Z^2_{1-\alpha/2*}S^2)/d^2}$$

n=sample size per group (treatment)

$\mathbf{Z_{1-\alpha/2}}$= Z-value at α-level of significance where the value of $Z_{1-\alpha/2}$,with α=0.05 is equal to1.96 (from statisticaltables).

The assumption here being that glucose concentration in blood is normally distributed in a random rat sample.

S= is the sample standard deviation of blood glucose levels which from the above figures is estimated at 1.6mmol/L(Wang et al., 2010).

d= Effect size, in this case ourd=1mmol/L level of precision in blood glucose concentration.

Therefore $\mathbf{n=(1.96^2 x\ 1.6^2)/1^2 = 9.83 = 10}$ animals per group or treatment.20% (done at the investigator's discretion)more animals were added to cater for any fatalities that may occur during the course of the study to avoid compromising the statistical power of the study.

3.4 Sampling procedure

72 male Wistarrats, aged 3-4months weighing around 170g – 200gwere randomly selected at the university of Nairobi animal house.Twelve animals were then randomly assigned into the sixdifferent treatment groups.

3.5 Treatment protocols

The 72 male Wistar rats, purchased from the university of Nairobi (Kenya) animal housewere kept for two weeks to acclimatize in their new environment at the university of Eldoret (Kenya) zoology laboratories. These labs were chosen because of their suitability for this kind study in terms of personnel, proximity and equipment. The animals were fed with commercial rat pellets (refer appendix 11 for nutrient composition of the pellets) 7g per animal three times a day: at 7 am, 1 pm and 8pm and given enough water to drinkand this was done because it is random blood sugar that was measured.Each animal was weighed (using W.C Redmon Deluxe™ digital small animal and avian scale) and given a serial number ranging from 1 to 12 for each group and

labeled appropriately using indelible ink on their backs and tails. Their baseline random blood glucose was measured using a glucometerand recorded.Any animal that was found to have a random blood glucose levels of more than 7mmol/L (this parameter value was used as a threshold for diagnosing hyperglycemia) wasretested and if confirmed wasexcluded from the procedure and replaced. The animals were then randomlyassigned into 6 groups (using simple random sampling and labelled as stated above)and placed into separate cages labelled and partitioned appropriately (based on the group treatment) to accommodate each animal individually (sections 3.6 and 3.7 below).

3.6Animals, materials and reagents

Study animals

The malewistarrats (*Rattusnorvegicusalbinus*) werethe experimental/studyanimals (animal model).

Drugs

The othermain materials for this study were:Lopinavir/Ritonavir (200/50 mg) tablets, Atazanavir/Ritonavir (300/100mg) tablets, purchased from Mylan Laboratories Ltd,India, through their Local (Kenya) distributors-Harleys Ltd, Nairobi. Diosmin/Hesperidin (450/50 mg)tablets from Servier Laboratories, France were purchased from the local (Kenya) distributors- Transwide pharmaceuticals Ltd, Nairobi. Therest of the materials are as listed under appendix 3.

Rats

72male wistarrats were randomly assigned into six treatment groups of 12 animals eachand treated as follows:

Group 1

12 rats –werefed on normal rat diet (7g three times a day)and used as the control group for the duration of the experiment (6 weeks).

Group 2

12 rats were fed on normal diet (7g three times a day) plus Lopinavir/Ritonavir 2/0.5 mg per kg body weight twelve hourly for 6 weeks.

Group 3

12 rats – Were placed on normal diet(7g three times a day) plus Atazanavir/Ritonavir 3/1 mg/kg body weight administered twelve hourly for 6 weeks.

Group 4

12 rats- were placed on normal diet (7g three times a day) plus Lopinavir/Ritonavir at a dose of 2/0.5 mg/kg plus Hesperidin/Diosmin 4.5/0.5 mg /kg body weight co-administered twelve hourly for 6 weeks.

Group 5

12 rats- were placed on normal diet (7g three times a day) plus Atazanavir/Ritonavir at a dose of 3/1mg/kg plus Hesperidin/Diosmin4.5/0.5 mg /kg body weight co-administeredtwelve hourly for 6 weeks.

Group 6

12 rats were placed on normal diet (7g three times a day) on normal diet plus Hesperidin/Diosmin 4.5/0.5 mg/kg body weight for 6 weeks.

At end of week six

At the end of the sixth week, blood was obtained by tail prick for blood glucose levels measurement and then all the remaining rats were sacrificedand 2ml blood was collected by cardiopuncture using 21 gauge needles. Half of the blood was placed into collection tubes (Vacutainers) with anticoagulant (EDTA) and the other half was placed in collection tubes without anticoagulant. The blood samples were used to analyze the blood for HbA1c levels and insulin levels. These results were recorded. Due to the small size of the animals, blood samples were collected at the beginning and the end of week six because bleeding the animals on a weekly basis to extract 2ml of blood could have killed the animals.

3.7 Detailed protocols/treatments

Treatment 1

Every day for 6 weeks group1 rats were each fed on 7g of the normal commercial rat food pellets three times a day (8am, 1pm and 8pm).On day 0 blood glucose was measured by tail prick and glucometer. On day7,day14, day21, day28, day35 and day42 each rat was taken in turn, according to its label and a drop of blood was drawn from its tail vein and blood glucose level measured using the glucometer and recorded. The animals' body weights in grams were also taken and recorded.

Treatment 2

For this group for 6 weeks each rat was fed on 7g of the rat pellets three times a day (8am, 1am and 8pm). In addition, each rat was given Lopinavir/ritonavir mixed with the food pellets at a dose of 2/0.5 mg/kg body weight twice a day (7am and 7 pm). On day0, day7, day14, day21, day28, day35 and day42 a drop of blood was taken from the tail vein and the blood glucose level

was measured using the glucometer and their weight in grams was also taken. (See appendix 4 on how to prepare a fresh lopinavir/ritonavir mixture in rat pellets)

Treatment 3

These animals were fed on 7g of the normal diet rat pellets three times a day (8am, 1am and 8 pm). Atazanavir/ritonavir was adminstered at a dose of Atazanavir/ritonavir 3mg/1 mg/kg body weight twelve hourly (7am and 7pm) mixed with the crushed food pellets. On day0, day7,day 14,day21 and day28,day35 and day42 a drop of blood was taken from the tail veins and blood glucose level measured using glucometer and body weights were taken using an electronic weighing balance and recorded on the results table (See appendix 5 on how to prepare a fresh mixture of Atazanavir/ritonavir and rat pellets)

Table 6-Treatment protocols summary

Group	Treatment
Group1	Rat food pellets
Group2	Rat food pellets+lopinavir+ritonavir
Group3	Rat food pellets+atazanavir+ritonavir
Group4	Ratfoodpellets+lopinavir+ritonavir+ hesperidin+diosmin
Group5	Ratfoodpellets+atazanavir/ritonavir +hesperidin+diosmin
Group 6	Rat food pellets +hesperidin/diosmin

Treatment 4

Each of these animalswere fedon 7g of the rat food pellets three times a day (8am,1pm and 8pm). Lopinavir/ritonavirwas administered to the animals at dose of 2/0.5 mg /kg bodyweight plusdiosmin/hesperidin at a dose of 4.5/0.5 mg/kg body weight twelve hourly (7am and 7pm).4 hours after feeding in the morning (to measure random blood sugar [RBS]), on day0, day7, day14 day21, day28, day35 and day42;a drop ofblood was taken from the tail and blood glucose was measured using a glucometer and the body weight was taken using a weighing balance. The

results were recorded in the results table (See appendix 6 on how to prepare a fresh mixture of diosmin/hesperidin in rat pellets).

Treatment 5

Each of these animalswere fed on 7g of the normal diet rat pellets three times a day (8 am, 1pm and 8pm).Atazanavir/ritonavir at a dose of Atazanavir/ritonavir 3/1mg per kg plushesperidin/diosminwas administered at a dose of 4.5/0.5mg/kg every twelve hours (7am and 7pm). On day 0, day7, day14, day21, day28, day35 and day42a drop of blood fromthe tail vein was used to measure blood glucose using a glucometer and the body weights were taken. The results were recorded in a results table. (See appendix 7 for formulation)

Treatment 6

Each of these 12 animals were fed on 7g rat pellets three times a day (8am, 1pm and 8pm). Hesperidin/Diosmin 4.5/0.5mg/kg body weight twice a day for 6 weeks. On the day 0, day7, day14, day21, day28, day 35 and day 42 a drop of blood was drawn from thetail vein and blood glucose was taken with glucometerand the body weights were taken and recorded in the results table (see Appendix 8 for formulation). This group was intended to assess the effect of the diosmin-hesperidin combination on the blood sugar of untreated (not treated with IV-PIs) animals

Procedures on day 42

On day42 the blood glucose level was measured using a drop of blood taken from the tail vein, the body weights were also measured. The animals were anaesthetized one at a time with chloroform and enough blood was collected through cardiopuncture using gauge 21 needles into

collection tubes (vacutainers) and equal amounts of 1ml were placed intocontainers with anticoagulant (EDTA)and those without anticoagulant respectivelyand stored in a fridge for assay. The insulin and HbA1c levels were measured using rat insulin ELISA test kits and HbA1c- meter respectively and the results recorded in a results table.

3.8 Inclusion and exclusion criteria

Inclusion criteria

The animals included in this study had to be males Wistar rats weighing at least 170g and 3 to 4 months old. Their random blood glucose had to be less than 7mmol/L. They also had to be free of any obvious malformation or disease.

Exclusion criteria

Any animal that was not male, less than 170g in body weight with obvious malady or malformation was excluded. Those animals with random blood glucose of 7mmol/L or more were also excluded from the study.

3.9 Data collection

Blood glucose

The random blood glucose levels were taken at the start of the study before the treatments were administered (baseline) and thereafter the blood glucose levels were measured once a week on the same day and time over the duration of the study and the results were recorded. The blood glucose levels were measured using a glucometer (On - Call plus™ by ACON laboratories Inc., 1025 Mesa Rim Road, San Diego, CA 92121, USA, 2016 model). Each animal's blood glucose levels were measured once during each session unless on measurement the glucose

levels were abnormally high or abnormally low in which case the test was repeated to confirm the results.

Blood insulin levels

Blood samples were taken from the animals from the tail vein at the start of the study in a specimen container without an anticoagulant. The specimen was allowed to clot and l00µL of the supernatantwas drawn and loaded into the ADVIA™ Centaur Insulin Assay system (Siemens™ Healthcare Diagnostics, USA) and an automated rat insulin ELISA assay was conducted by the machine. Onday 42 the animals were sacrificed and blood samples were obtained by cardiopuncture and the above insulin assay procedure was repeated until all the samples were analyzed.

Blood percentage HbA1c

At the start of the study, blood samples from the tail vein of the animals were collected into specimen tubes with an anticoagulant (EDTA) to keep the specimen uncoagulated. 100µL blood sample was taken using a pipette and placed in the sample cartridge of the MISIPA-i3™ cartridge based protein auto-analyzer (AGAPPE DIAGNOSTICS™, Switzerland GmbH). Eight minutes after loading, the results were printed and the HbA1c values in percentage were recorded. At the end of the study (Day 42) the animals were sacrificed using chloroform to induce anaesthesia and blood samples were collected by cardiopuncture into specimen tubes with anticoagulant and the HbA1c assay was repeated until all the samples were analyzed.

3.10 Data analysis

The data was summarized using the mean± SD (standard deviation) and displayed in tables and graphs to demonstrate the main trends of blood glucose (mmol/L), percentageblood HbA1c levels and bloodinsulin (µIU/mL) concentrationover the duration of the study.Analysis of the

group mean blood glucose, blood insulin levels andpercentage blood HbA1cwas done using one-way ANOVA(F-value).Normality of the data was not tested but the central limit theorem (CLT) was invoked. The equality of variances was tested using Levenne's test and was noted if significant. Differences between the group means of these measurements due to experimental treatments was assessed by computation of the Least Significant Difference (LSD)through ad hoc multiple group comparisons. The associated p-values of the differences of the group means and the effect sizes of the F-values(partial eta squared,η^2) were noted.Results were considered significant if the test statistics (F-valueand LSDs)had p –values less than or equal to the level of significancei.e. α=0.05 ($P \leq 0.05$). The effect sizeof the test statistics was interpreted using the Cohen's guidelines (J. Cohen, 1988)

3.11Ethical considerations

This study involved the use of animals - albinoWistar rats (*Rattusnorvegiccusalbinus*). Permission to use the animals as the experimental model was obtainedfrom the ethical review board on the use of laboratory animals from The University of Eastern Africa, Baraton, Kenya. Permission to conduct the study was also sought from and granted by the school of graduate studies (SGS), Maseno University(A*ppendix* 7*and* 8).

3.12 Study limitations and challenges

1. The main study limitation was the small size of the animals which made it challenging in terms of sample collection, especially blood from the live animals.

2. Due to the oily nature of the rat fur it was sometimes difficult to label the animals permanently using ink. It necessitated daily relabeling of the animals to track identity. This was not only time consuming but laborious

3. Limited financial resources posed a challenge. For this reason the blood levels of HbA1cand plasma insulinlevels had to be done only twice: at the beginning and at the end of the study. This is because these assays are quite expensive.

4. Given that the Wistar rats small animals only limited amounts of blood for laboratory assays could be drawn from the live animals at any given time without harming them. This meant that tests like insulin levels and HbA1c levels could done a limited number of times.

3.13 Disclosures

This project was wholly funded by the investigator. There are financial disclosures to be made.

CHAPTER FOUR

RESULTS

4.1 Blood glucose levels in HIV- protease inhibitor treated rats

The baseline (day 0) group mean blood glucose levels were measured before the treatments were administered with the control, LPV/RTV and ATV/RTV groups attaining mean group blood glucose levels of4.07±0.27, 4.18±0.25 and 4.09±0.37 mmol/L respectively (displayed in figure 10). These differences were not statistically significant (p=0.113, η^2=0.124).

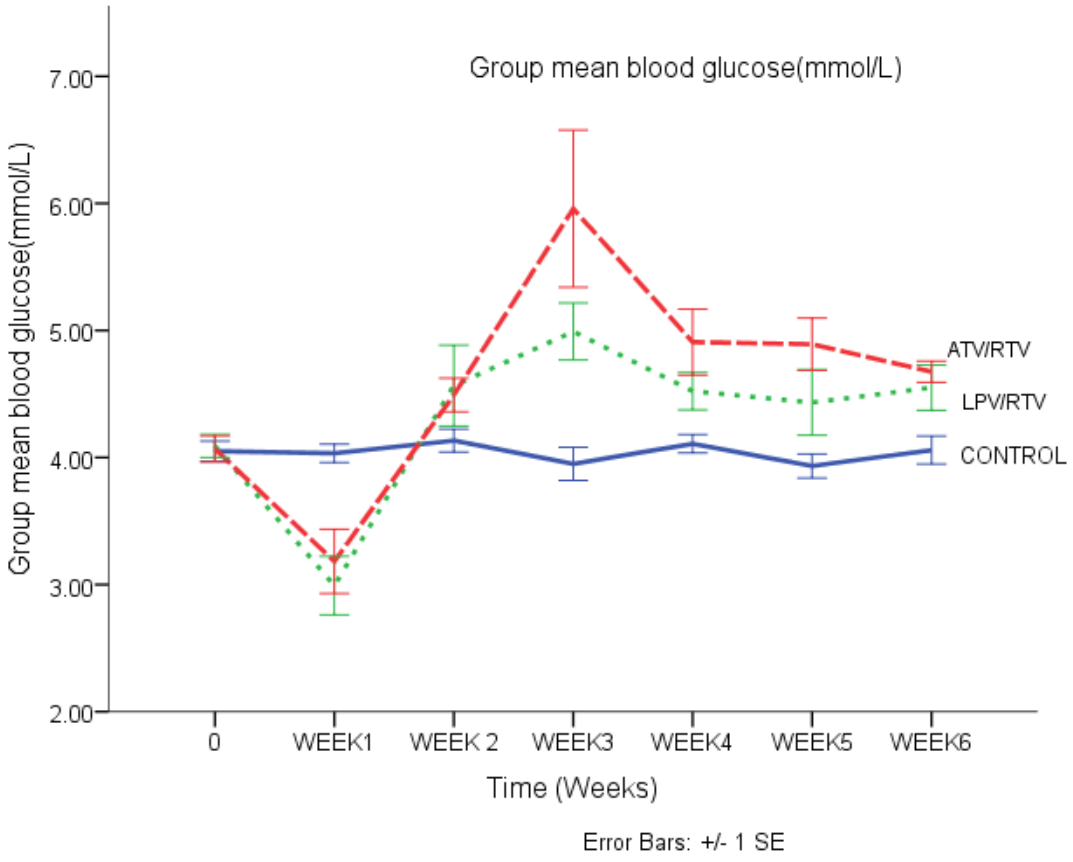


Figure 10: Mean blood glucose levels of Lopinavir /ritonavir (LPV/RTV) and Atazanavir/ritonavir (ATV/RTV) treated groups over time.

At the end of week one, with the exception of the control group (4.10±0.386 mmol/L),the other two groups showed a drastic drop in their meangroup blood glucose with the LPV/RTV treated group attainingthe lowest groupblood glucose mean (2.99±0.80 mmol/L) and the ATV/RTV treated group with 3.18±0.87mmol/L.

This drop in group mean blood glucose was demonstrated by the animals treated with HIV-protease inhibitors ($p<0.0001$, $\eta^2 =0.299$) indicating highly significant differences in the group mean blood glucose levels.During this period the effect size of the group blood glucose mean differences (partial eta squared,η^2) increased by 141% (from 0.124 at baseline to 0.299) indicating that the treatments had huge contribution(from 12.4% to 30%)towards the differences in the mean blood glucose amongst the groups. The HIV-protease inhibitor treated groups themselves did not show a significant differences in their group mean blood glucose levels: the ATV/RTV and LPV/RTV treated group had a difference of 0.192mmol/L ($p=0.450$).By the end of the second week of treatment, the group mean blood glucose levels of the on HIV- protease inhibitor treated groups had increased significantly. The LPV/RTV treated group had the highest group mean (4.78±0.65 mmol/L) followed by the ATV/RTV treated group(4.53±0.46 mmol/L) and was significant ($p=0.01$, $\eta^2=0.204$) when compared to the control (4.30±0.47 mmol/L). An ad hoc pairwise multiple comparison of the HIV-protease inhibitor treated groups based on the least significant difference(LSD) revealed that the LPV/RTV group had no significant difference in group mean blood glucose levels when compared with the ATV/RTV treated group (difference(diff.) =0.256mmol/L, $p=0.252$).

The end of the third week of treatment is the period when the groups treated with ATV/RTV and LPV/RTV attained highest group mean blood glucose levels of 5.95±2.15 mmol/L and 5.14±0.92 mmol/L respectively, compared to the control(3.88±0.42 mmol/L) indicating a highly significant

difference in the group mean blood glucose levels between thesetreatments ($p<0.0001$, $\eta^2=0.272$) and that over 27% of the group difference was due to the treatments (effect size). By the end of the fourth week the HIV-protease inhibitor treated groups still had the high group mean blood glucose levels. The ATV/RTV treated group had the highest group mean (4.91±0.90 mmol/L) followed by the LPV/RTV treated group (4.64±0.29 mmol/L) and compared to the control group (4.11+/-0.25 mmol/L). By the end of the fifth week the ATV/RTV treatedgroup still had the highest group mean blood glucose level(5.03±0.63mmol/L) followed by the LPV/RTV treated group (4.51±0.60mmol/L) and the control (4.16+/-0.38) respectively. The difference between the three groups in terms of their group mean blood glucose levels was significant ($p<0.0001$, $\eta^2=0.349$). At the end of the sixth week,the ATV/RTV treated group had the higher group mean blood glucose level (4.89±0.71mmol/L) followed by the LPV/RTV treated group (4.43±0.77 mmol/L) with the control attaining 3.94±0.32mmol/L. These group mean blood glucose levelswere significantly different ($p=0.03$,$\eta^2=0.166$) at the tail end of the study.

4.2 The blood glucose levels in rats treated with HIV- protease inhibitors (LPV/RTV OR ATV /RTV) plus flavonoids (Diosmin/Hesperidin combination)

4.2.1 The blood glucose levels of the lopinavir/ritonavirand lopinavir /ritonavir+diosmin/hesperidin treated groups

At the end of the first week of treatment there was a drastic fall in the group mean blood glucose levels (displayed in figure 11) in the LPV/RTV (2.99±0.80 mmol/L) and the LPV/RTV+DIOS/HES (3.49±0.63 mmol/L) treated animals compared to the control (4.10±0.38 mmol/L). This difference between LPV/RTV and control was significant($p<0.0001$) and the difference between the control group and the LPV/RTV+DIOS/HES was also significant

(p=0.038).The difference between the LPV/RTV and the LPV/RTV+DIOS/HES was significant too (p=0.047).At the second week of treatment the group blood glucose means of the grouptreated with LPV/RTV (4.78±0.65 mmol/L) had higher group mean blood glucose level compared to the group treated with LPV/RTV+DIOS/HES (4.32± 0.147mmol/L) and the Control group (4.30±0.47 mmol/L).

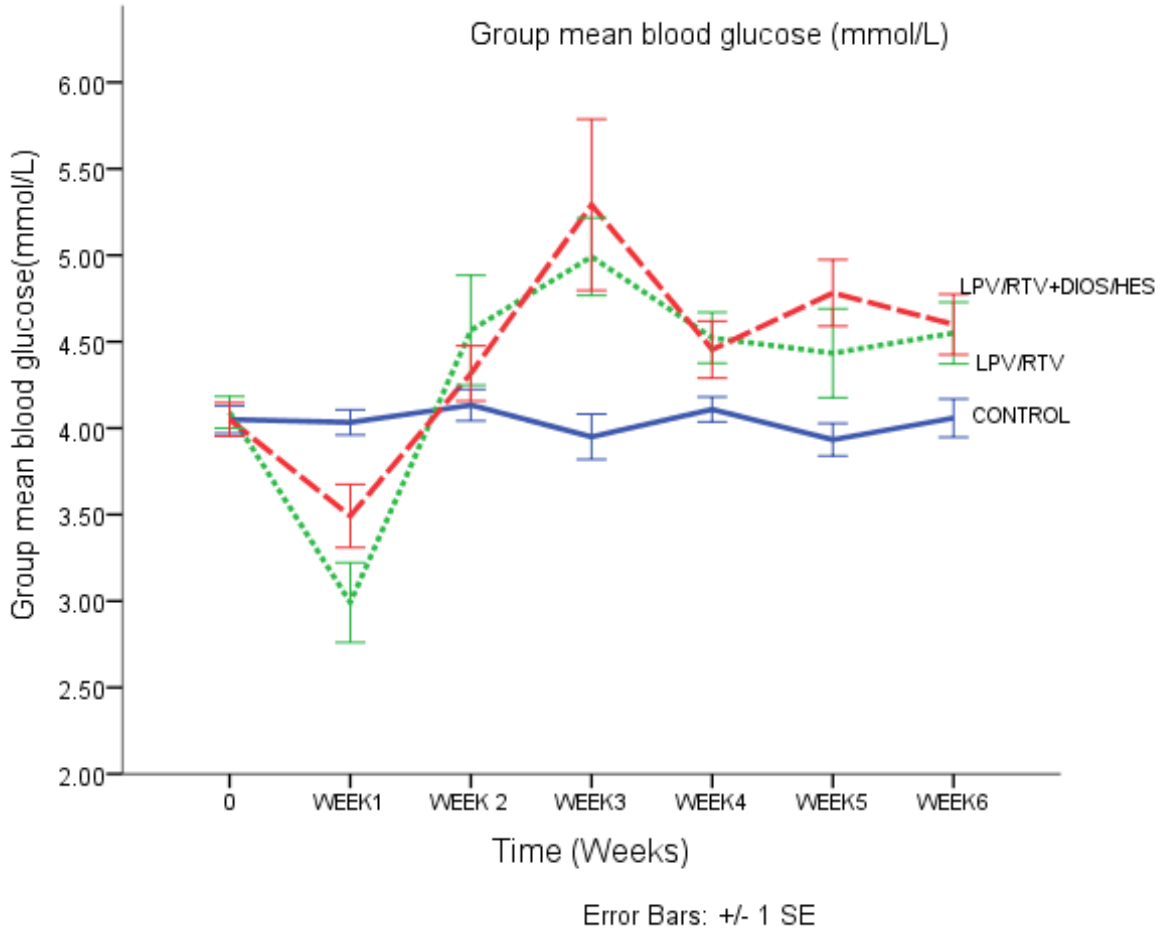


Figure 11:The group mean blood glucose of the control, the lopinavir/ritonavir(**LPV/RTV**) and the lopinavir/ritonavir+diosmin/hesperidin **(LPV/RTV+DIOS/HES**) treated groups over time.

The p-values of the differences were as follows: the control group versus LPV/RTV treated group (p=0.006), the control group versus the LPV/RTV+DIOS/HES treated group (p=0.55) and the LPV/RTV versus LPV/RTV+DIOS/HES (p=0.028). The addition of diosmin/hesperidin to

lopinavir/ritonavir did have a significant effect in terms of lowering the group mean blood glucose of the LPV/RTV+DIOS/HES to the extent that the difference was not significant when compared with the control while on the other hand the difference between the LPV/RTV and the LPV/RTV+DIOS/HES was significant (p=0.028).

By the end of the third week of treatment, the control(3.88+/-0.42 mmol/L) and the LPV/RTV (5.14+/-0.92 mmol/L) groups had significant differences in their group mean blood glucose concentrations(diff.=1.26 mmol/L, p=0.016).The control group and the LPV/RTV+DIOS/HES(5.29+/-0.1.72 mmol/L) treated groups also had a significant difference in their group mean blood glucose levels (diff.=1.41 mmol/L, p =0.007) with the LPV/RTV+DIOS/HES group attaining the highest blood glucose meanamong the three groups at this point in time (figure10). The LPV/RTV and the LPV/RTV + DIOS/HES treated groups did not show a significant difference in their group mean blood glucose levels at this point in time(0.150 mmol/L, p = 0.770)although the LPV/RTV + DIOS/HES treated group had a slightly higher blood glucose level indicating that at this point in time the administration of DIOS/HES combination did not have a significant impact on the hyperglycaemic effect of LPV/RTV treatment.

At the end of the fourth week of treatment there was no significant difference in the group mean blood glucose levels of the control (4.11±0.25 mmol/L), the LPV/RTV(4.64±029 mmol/L) and the LPV/RTV+DIOS/HES (4.46±0.55 mmol/L) treatment groups. The difference between the group mean blood glucose of the LPV/RTV group and the control was marginally significant (p=0.051). The control and the LPV/RTV+DIOS/HES groups had an insignificant difference (p=0.18)so was the group mean blood glucose between the LPV/RTV and the LPV/RTV +DIOS/HES (p=0.55). By the end of the fifth week the Control (3.94±0.77 mmol/L) and the

LPV/RTV (4.43±0.77 mmol/L) treated groups had a significant difference in the group mean blood glucose (0.492 mmol/L, p=0.043). The LPV/RTV+DIOS/HES treated group had a higher mean blood glucose than the Control group (difference=0.840 mmol/L, p<0.0001). The LPV/RTV and the LPV/RTV+DIOS/HES treated groups had no significant difference in their mean blood glucose levels (diff. =0.348 mmol/L, p=0.155). This again indicates that the DIOS/HES combination did not have an impact on the LPV/RTV induced increase in blood glucose. By the end of the sixth, the Control (4.02±0.37mmol/L) and the LPV/RTV (4.16±0.40 mmol/L) treated groups did not have a significant difference in their group mean blood glucose level (0.146mmol/L, p=0.448). The control group and the LPV/RTV+DIOS/HESgroup (3.77±0.39 mmol/L) did not show a significant difference (0.244 mmol/L, p=0.168) either, whereas the LPV/RTV and the LPV/RTV+DIOS/HES treated groups had a marginally significant difference (0.390 mmol/L, p=0.049)in their group mean blood glucose levels with the LPV/RTV group having a higher group mean blood glucose(4.16±0.40 mmol/L).

4.2.2 The blood glucose levelsin theAtazanavir/Ritonavir and Atazanavir/Ritonavir+ Diosmin/Hesperidin treated rats

At the inception of the study (day 0) there was no significant difference in the group mean blood glucose of the control (4.07±0.27mmol/L) group, the ATV/RTV9 (4.07±0.36 mmol/L) and the ATV/RTV+DIOS/HES (4.09±0.28 mmol/L) groups. This is because at day 0 (baseline) no treatment had been administered. By the end of week one however, the group mean blood glucose levels were significantly different among the groups (figure 12). The control group (4.02±0.25 mmol/L) and the ATV/RTV (3.18±0.87 mmol/L) treated group had significantly different group mean blood glucose levels (diff.=0.83mmol/L, p<0.0001).The control group had a significantlyhigher group mean blood glucose level than the ATV/RTV+DIOS/HES (3.39±0.48

mmol/L) treated group (diff.=0.625 mmol/L, p=0.014). However, the ATV/RTV and the ATV/RTV+ DIOS/HES treated groups did not show any significant difference in their group mean blood glucose levels (diff.=0.208 mmol/L, p=0.403) at this point.

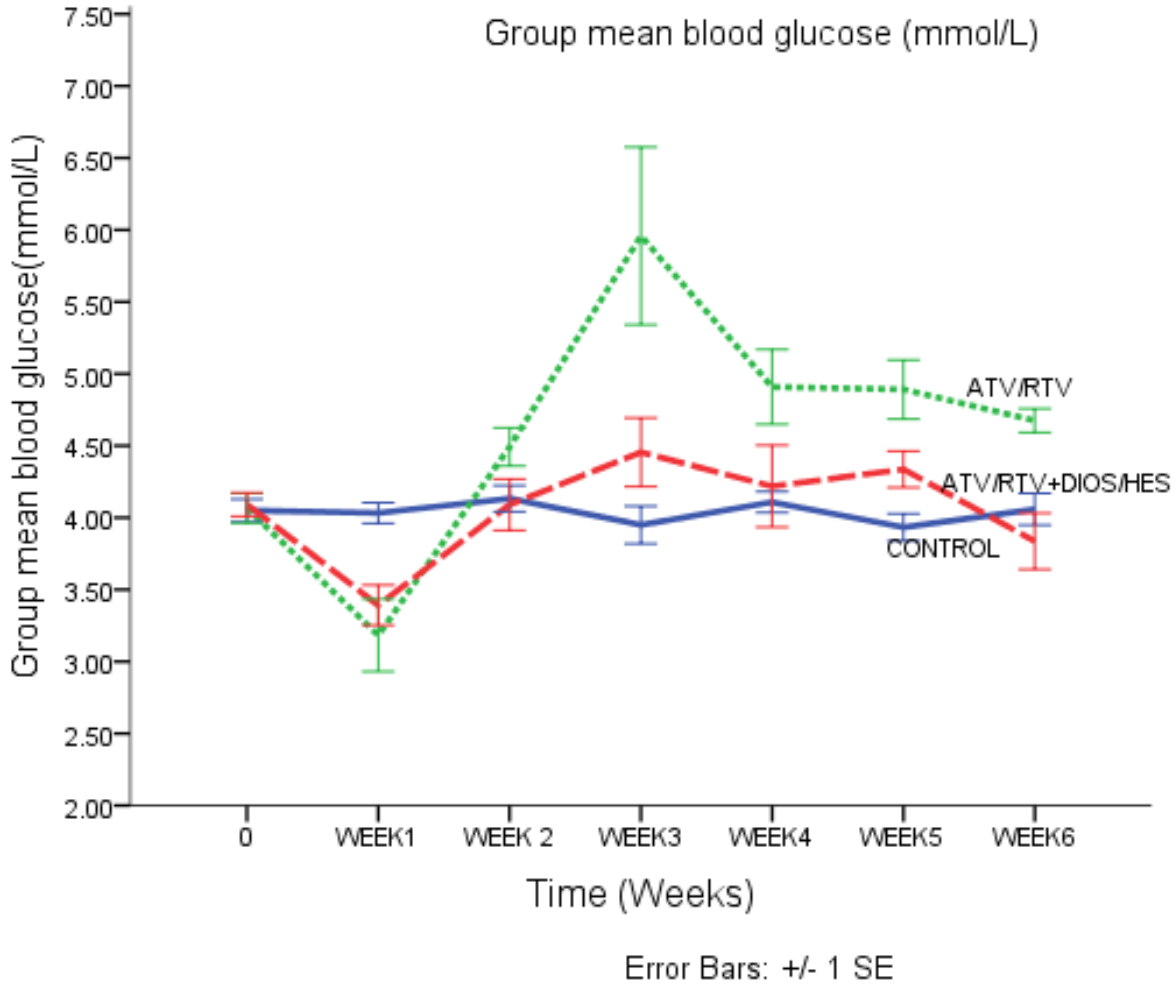


Figure 12: The group mean blood glucose of the control,Atazanavir /ritonavir (**ATV/RTV**) and Atazanavir/ritonavir+diosmin/hesperidin (**ATV/RTV+DIOS/HES**) treated groups.

At the end of the second week of treatment the ATV/RTV treated group had higher group mean blood glucose (4.51±0.45 mmol/L) than the control group (4.19±0.32 mmol/L)but the difference was not significant (diff.=0.317 mmol/L, p=0.123). At this point in time there was also no significant difference (diff.=0.101 mmol/L, p=0.665) between the group mean blood glucose

levels of the control group and the ATV/RTV+ DIOS/HES treated group (4.09±0.59 mmol/L). The ATV/RTV and ATV/RTV +DIOS/HES treated groups did not demonstrate a significant difference either(diff.=0.417 mmol/L, p= 0.054).

By the end of third week the ATV/RTV treated group showed a drastic increase in the mean blood glucose(5.95±2.15 mmol/L) compared to the control group(3.88±0.42 mmol/L)and had a highly significant difference (diff.=2.08 mmol/L,p< 0.001). There was however no significant difference in the group mean blood glucose level between the control group and the ATV/RTV+ DIOS/HES (4.45±0.79 mmol/L) treated group (diff.=0.57 mmol/L, p=0.277). There wasalso a significant difference in the group mean blood glucose levels between the ATV/RTV and the ATV/RTV + DIOS/HES treated groups (diff.=1.50 mmol/L, p= 0.006).On day 28 (end of the 4th week) the ATV/RTV treated group had a higher mean blood glucose level (4.91±0.91 mmol/L) than the control group (4.11±0.25 mmol/L) with a significant difference (diff.=0.80 mmol/L, p=0.002)confirming that this HIV-protease inhibitorcombination(ATV/RTV) increased blood glucose levels. The control group and the ATV/RTV+DIOS/HES (4.22±0.94 mmol/L) treated group did not have significantly different levels of group mean blood glucose (diff.=0.110 mm0l/L, p = 0.668) showing that the co-administration of DIOS/HES with ATV/RTV countered the hyperglycaemic effects of ATV/RTV administration to the animals. At this point in time the ATV/RTV and ATV/RTV+ DIOS/HES treated groups demonstrated a very significant difference in their blood glucose (diff.=0.69 mmol/L, p = 0.009). It is noteworthy that the addition of the flavonoids DIOS/HES to the ATV/RTV treatment regimen reduced its blood glucose elevating effect. At the end of week five, the control group (3.94+/-0.32 mmol/L) and the group treated with ATV/RTV (4.89+/-0.71 mmol/L) had a highly significant difference (diff.=0.950 mmol/L,

p<0.0001). The control group and the ATV/RTV+DIOS/HES (3.84±0.65 mmol/L) treated group did not have a significant difference in their blood glucose (diff.=0.395 mmol/L, p = 0.084).

On the other hand the ATV/RTV and ATV/RTV+DIOS/HES treated group demonstrated significantly different group mean blood glucose levels (diff.=0.56 mmol/L, p=0.019). The ATV/RTV group had a higher group mean blood glucose(4.89±0.71 mmol/L) than the ATV/RTV+DIOS/HES treated group(4.34±0.42 mmol/L).By the end of the sixth week the control group (4.02±0.37 mmol/L) and the ATV/RTV treated group (4.29±0.39 mmol/L) did not show significant difference in their blood glucose levels (diff.=0.275 mmol/L, p=0.113). The control group and the ATV/RTV+DIOS/HES treated group (3.84±0.65 mmol/L)also did not show any significant difference also at this point (diff.=0.180 mmol/L, p=0.306). However, on this same period (week six) the ATV/RTV and the ATV/RTV+DIOS/HES treated groups had a significant difference in their group mean blood glucose levels (diff.=0.455 mmol/L, p=0.011).

4.2.3Blood glucose levels of diosmin/hesperidin treated rats

The mean blood glucose levels of the diosmin/hesperidin(DIOS/HES) treated group did not show any significant differencefrom the control groupthroughout the duration of the study(as displayed in table 7). This indicated that treatment with diosmin/hesperidin did not exert any obvious effect on the blood glucose level of theanimals in the HIV-PI untreated animals. However, the mean blood glucose levels of the DIOS/HES treated group become more stable as the animals got treated with these flavonoids. The standard deviation (SD) of the group mean glucose levels of the DIOS/HES treated group declined over time, particularly from the third week (figure 13) compared to the control group whose variability of the group mean blood glucose levels (indicative of glycemic control) of the animals (as indicated by volatility of the SD) remained

relatively high throughout the duration of the study (figure 12). This shows that the intake of flavonoids may have a beneficial effect of stabilizing the blood glucose levels.

Table 7: Blood glucose levels (mmol/L) of the Control and DIOS/HES groups

Time (Weeks)	Control group(mmol/L)	Diosmin/Hesperidin Group(mmol/L)	P - value
Baseline (Day 0)	4.07±0.27 n=12	4.00±0.35 n=12	0.612
Week 1	4.02±0.25 n =12	3.91±0.32 n=12	0.663
Week 2	4.19±0.32 n=12	4.11±0.43 n = 12	0.690
Week 3	3.88±0.42 n=12	4.07±0.26 n=12	0.277
Week 4	4.11±0.25 n=12	3.97±0.24 n=12	0.572
Week 5	3.94±0.32 n=12	3.95±0.18 n=12	0.970
Week 6	4.02±0.37 n=12	3.99±0.21 n=12	0.884

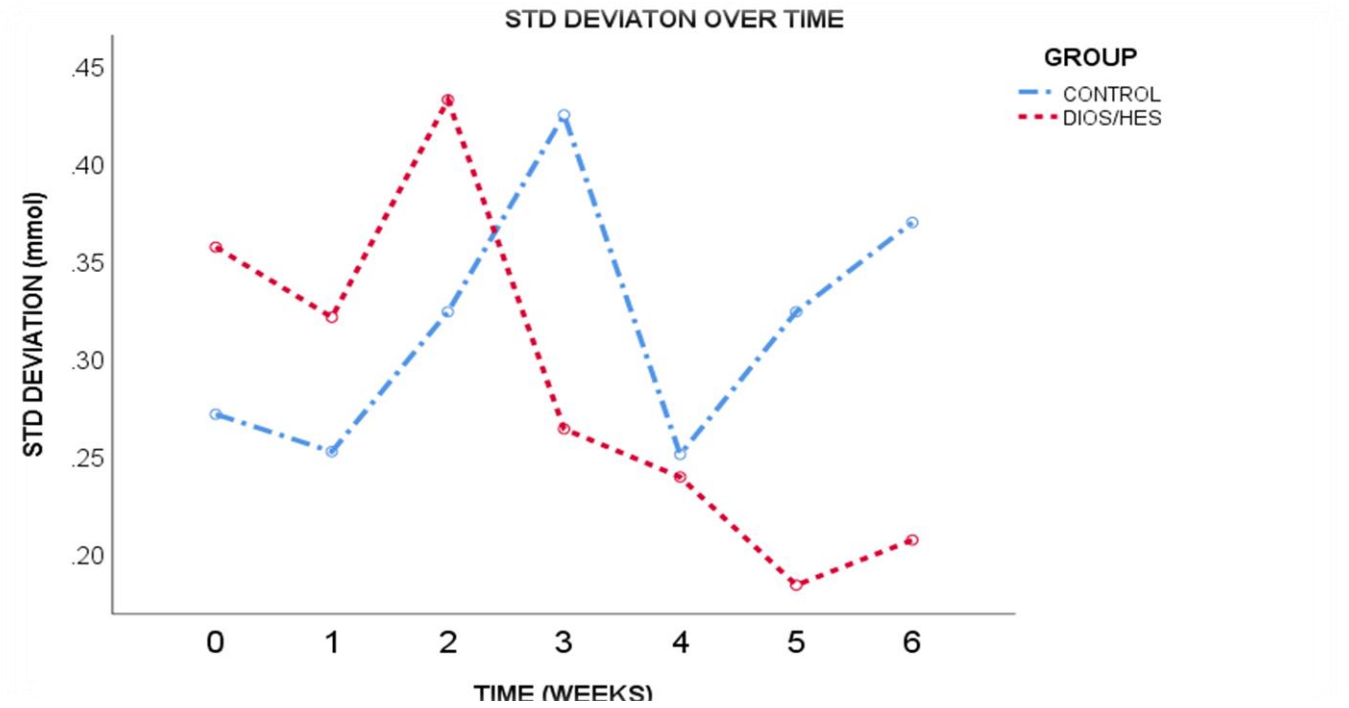


Figure 13: Variability of the group mean (standard deviation) of the of the blood glucose of the Control group and DIOS/HES treated rats.

Treatment with flavonoids over time seems to decrease the variability in the mean of the blood sugar levels thus smoothening the of blood sugar levels (glycemic control). The overall effect is that there was a decrease in the variability of the mean blood sugar in the DIOS/HES treated animals compared to the Control group. An independent t-test of the overall difference in the standard deviations of the group mean blood glucose levels between the control group and the DIOS/HES treated group however was not significant, $t_{1-\alpha/2}$ (df =12; p=0.478).

4.2.4 Theblood glucose levels of the DIOS/HES, the ATV / RTV + DIOS/HESand LPV/RTV + DIOS/HEStreated rats

For all the four groups the mean blood glucose levels were not significantly different on day zero (baseline) and at the end of the sixth week (figure 14). The control and the DIOS/HES treated groups did not exhibit any significant differences in their group mean blood glucose levels for the entire duration of the study.The control group (4.02±0.25 mmol/L) and ATV/RTV+DIOS/HES (3.39±0.48 mmol/L) had a significant difference (p=0.014) only once at the end of the first week of treatment. From the second week of treatment, thecontrol, the DIOS/HES and the ATV/RTV + DIOS/HES treated groups did not exhibitany significant difference in their group mean blood glucose levels (figure 14). The control group and the LPV/RTV+DIOS/HES (3.49±0.63 mmol/L) had a significant difference (diff.=0.53mmol/L, p=0.038) at the end of the first week of treatment. At the end of the third week, the control group (3.88±0.42 mmol/L) and the LPV/RTV+DIOS/HES (5.29±1.72 mmol/L) had a significant difference (diff.=1.41mmmol/L, p=0.007) and on week five the LPV/RTV+DIOS/HES

(4.78±0.64 mmol/L) and the control (3.94±0.32 mmol/L) had a highly significant difference (diff.=0.84mmol/L, p<0.0001). The LPV/RTV+DIOS/HES and ATV/RTV+DIOS/HES treated groups did not show any significant differences throughout the entire duration of the study.

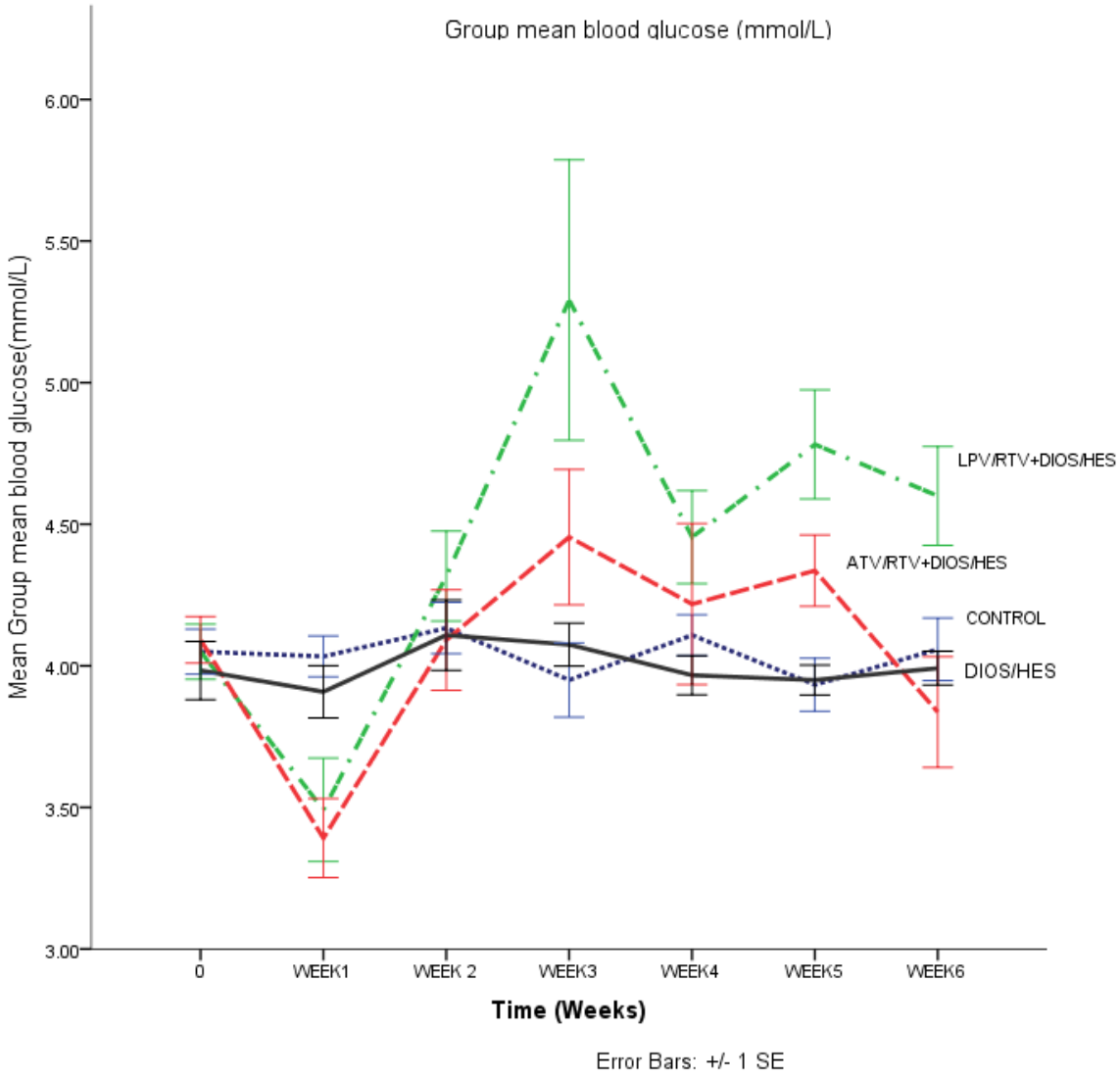


Figure 14: Group mean blood glucose levels of the control,lopinavir/ritonavir+diosmin/hesperidin (LPV/RTV + DIOS/HES), atazanavir/ritonavir+diosmin/heseridin (ATV/RTV+DIOS/HES) and the diosmin/ hesperidn(DIOS/HES) treated rats.

4.3 Plasma insulin levels before and after treatmentwith HIV-Protease inhibitors (LPV+RTV and ATV+RTV) and HIV-Protease inhibitors + diosmin/hesperidin combination and diosmin/hesperidin combination.

Results

The assays of the blood insulin levels before and after treatment shows that in both instances the group mean blood insulin levels were significantly different, from the F-values (table 8).

Table 8: Group mean insulin levels (µIU/L) before and after treatment

Treatment groups & test statistics	Blood insulin levels at baseline (day 0) (mean±SD)	Blood insulin levels on day 42(week 6) (mean±SD)
Group 1 (CONTROL)	3.42±0.33 (n=12)	17.80±2.93 (n=12)
Group 2 (LPV/RTV)	3.40±0.28 (n=12)	22.04±2.18 (n=8)
Group 3 (ATV/RTV)	3.57±0.18 (n=12)	22.08±0.64 (n=12)
Group 4 (LPV/RTV+DIOS/HES)	3.77±0.30 (n=12)	19.32±2.11 (n=11)
Group 5 (ATV/RTV+DIOS/HES)	3.73±0.14 (n=12)	20.93±0.51 (n=10)
Group 6 (DIOS/HES)	3.58±0.29 (n=12)	20.34±0.48 (n=12)
F-value(one way ANOVA)	F=3.98	F=10.2
p-value (of F-value)	P=0.003	P<0.0001
Effect size (partial η^2)	η^2=0.231	η^2 =0.455

KEY: CONTROL - Control group, LPV/RTV – Lopinavir/Ritonavir treated group, ATV/RTV – Atazanavir/aritonavir treated group, LPV/RTV+DIOS/HES – Lopinavir/ Ritonavir+ Diosmin/ Hesperidin treated group, ATV/RTV+DIOS/HES – Atazanavir/ritonavir+dios/hes treated group, DIOS/HES – Diosmin/hesperidin group, SD- standard deviation, µIU/L- micro-international units/Litre.

It was noted that at the beginning of the study the mean plasma insulin levels of the six groups were statistically significant based on one way ANOVA with $F_{1-\alpha/2}$ 5,66 =3.98 (p=0.003, η^2 =0.231). This observation was difficult to explain since the animals had not been subjected to

any treatment on day 0. At the end of the study, the difference in plasma insulin levels increased in significance with the $F_{1-\alpha/2}(5, 60) = 10.2$ with $p<0.0001$ and $\eta^2=0.455$. There was a big increase in the blood insulin levels at the end of the treatment period. The increase in insulin levels was noted in all the groups. The highest increase was observed in the HIV-PIs treated groups: LPV/RTV (from 3.40±0.33 μIU/mL to 22.04±0.2.18 μIU/mL) treated groupand the ATV/RTV (from 3.57±0.18 μIU/mL to 22.08±0.64 μIU/mL) treated group. The smallest increase was noted in control group (from 3.42±0.33 μIU/mL to 17.80±0.2.93μIU/mL). The effect size as measured by the partial eta squared (η^2) was 0.231 before and 0.455 after treatment respectively. These partial eta squared values indicate that the treatments almost doubled the effect size and hence the observed decrease in the p-value implying an increase in statistical significance of the observed difference in the mean group blood insulin levels. At day 0 (baseline) the control group had a significant difference in group mean insulin levels with the LPV/RTV + DIOS/HES group (diff. =0.348 μIU/mL, p=0.002, table 8) and the ATV/RTV + DIOS/HES group (diff. =0.314 μIU/mL, p=0.005). The LPV/RTV treated group also had significantly lower group mean blood insulin levels at baseline (3.42±0.33 μIU/mL) compared to the LPV/RTV + DIOS/HES group (3.78±0.30 μIU/L, diff. = 0.37 μIU/mL, p=0.001) and the ATV/RTV + DIOS/HES group (3.58±0.30 μIU/mL, diff. = 0.33 μIU/mL, p=0.003).At the end of the sixth week (Day 42) the control group had a significant difference in the group mean blood insulin levels compared to the LPV/RTV treated group (diff.=4.27 μIU/mL, p<0.0001), the ATV/RTV treated group (diff.=4.27μIU/mL, p<0.0001), the LPV/RTV+DIOS/HES treated group (diff.=1.56μIU/mL, p=0.037), the ATV/RTV+DIOS/HES treated group (diff.=3.16 μIU/mL, p<0.0001) and the DIOS/HES treated group (diff.=2.57 μIU/mL, p=0.001).

Significant differences in blood insulin levels were also noted between the LPV/RTV treated group and the LPV/RTV+DIOS/HES treated group (diff.=2.72 µIU/mL, p=0.001), the ATV/RTV and the LPV/RTV+DIOS/HES treated groups (diff.=2.71 µIU/mL, p<0.001), the ATV/RTV+DIOS/HES and the LPV/RTV+DIOS/HES treated groups(diff.=1.61 µIU/mL, p=0.035) , the DIOS/HES and the LPV/RTV treated group(diff.=1.70 µIU/mL, p=0.037) and the DIOS/HES and the ATV/RTV treated groups(diff.=1.69 µIU/mL, p=0.020). There was no significant difference in the blood insulin levels between the Control group and the DIOS/HES treated group (p=0.148)

4.4: HbA1c levels before and after treatmentwith HIV-Protease inhibitors (LPV/RTVand ATV/RTV) or HIV-Protease inhibitors(LPV/RTV or ATV/RTV) +flavonoids (DIOS / HES combination)

Results

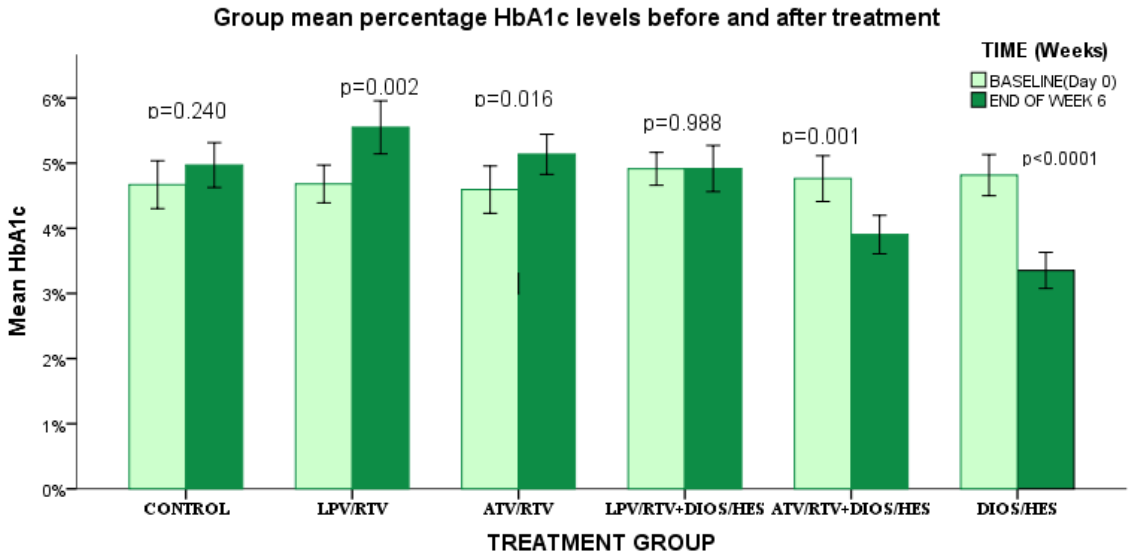


Figure 15: Group % HbA1c mean before (Day 0) and after treatment (end of week 6) with HIV-PIs and flavonoids (Error barsrepresent 95% CI).

A side by side bar graph comparison of the group % HbA1c clearly shows the main trends and group differences in the HbA1c levels before and after treatmentamong the various treatment groups (figure 15). Atthe inception of the study i.e. before treatment, the animals did not show significantly different group mean HbA1c levels among the six groups. This is demonstrated by a ONE way ANOVA done at baseline wherethe $F_{1-\alpha/2}$ (5,66) was 0.658 at α=0.05 (p=0.656, η^2=0.048).At the end of the treatment period of 6 weeks (42^{nd} day) there was a clear and significant difference among the groups in terms of their mean HbA1c percentage as shown bya one way ANOVA done at the end of week six gave a $F_{1-\alpha/2}$ (5,60) of 20.52(p<0.0001) with a partial η^2 value=0.635. A partial eta squared of 0.635 is quite a large effect size (Cohen J,1988) and demonstrates that the treatments accounted for 63.5% of the observeddifference in the group mean HbA1c levels.This study observed that the administration of Diosmin/Hesperidin combination alone produced the biggest decrease in the group mean percentage HbA1c over the duration of treatment (4.71±0.55% to 3.35±0.47%).On the other hand the groups administered with Lopinavir/Ritonavir combination(4.67±0.67% to 5.45±0.77%) and Atazanavir /Ritonavir(4.52±0.62% to 5.13±0.53%) exhibited a significant increase in the percentage HbA1c(figure14). At the end of the six weeks(day 42) the control group had a significantly higher mean HbA1c level than the ATV/RTV+DIOS/HES treated group (diff. =1.03%. p<0.0001) and the DIOS/HES treated group (diff. =1.62%, p<0.0001)

The LPV/RTV treated group also had higher group meanpercentage HbA1c levels than the DIOS/HES treated group (diff. =2.18%, p<0.0001, figure 15). The ATV/RTV treated group had higher group mean HbA1c levels than the ATV/RTV+DIOS/HES treated group (diff.=1.2%, p<0.0001) whereas the LPV/RTV+DIOS/HES treated group had higher HbA1c levels than the ATV/RTV+DIOS/HES treated group (diff.=0.96%, p<0.0001) and the DIOS/HES treated group

(diff.=1.55%, $p<0.0001$) respectively. Compared to all the other groups the DIOS/HES treated group had the lowest level of percentage blood HbA1c (3.35±0.55%,figure 14) indicating that the administration ofDIOS/HES had a significant effect of lowering the blood HbA1c levels in the experimental rats.

CHAPTER FIVE

DISCUSSION OF RESULTS

5.1Summary of findings

This study found that the two HIV-protease inhibitor combinations raised the blood glucose levels with the ATV/RTV(5.95+/-2.15 mmol/L) and LPV/RTV(5.30+/-0.92 mmol/L), compared to the control (3.88+/-0.42 mmol/L), on the third week. The ATV/RTV combination showed the highesteffect in raising blood sugar. The HIV-PI combinations ATV/RTV and LPV/RTV were used because these combinations are the most widely used HIV protease inhibitors in Kenya. Moreover ritonavir is added to ATV and LPV as a pharmacokinetic booster. The co-administration of the HIV-Protease inhibitors with the flavonoids (DIOS/HES combination) to the animals reduced the blood glucose raising effects of ATV/RTV combination but not the blood glucose raising effect of the LPV/RTV combination. The HIV-Protease inhibitors seemed to increase the magnitude of the differences in blood insulin levels as demonstrated by the increase of the grand mean from 3.58±0.29 μIU/mL at day 0 to 20.32±2.27 μIU/mL at the end of the study (Day 42) and the increase in effect size (partial eta squared) of the F values from 0.231 to 0.455 before and after treatment with the HIV-protease inhibitors respectively. However, it was observed that the increase in insulin levels was in all the groups including the control although the HIV-protease treated groups with or without the DIOS/HES combination showed a bigger margin of increase compared to the other groups.The treatmentof the animals with HIV-protease inhibitors increased the percentage HbA1c levels. The co-administration of DIOS/HES combination with the HIV-protease inhibitors reduced the HbA1c raising effects of the HIV-protease inhibitors. The reduction of HbA1c levels was more marked in the

ATV/RTV+DIOS/HES treated group comparedto the LPV/RTV+DIOS/HES treated group. The DIOS/HES treated group showed the biggest drop in HbA1c levels between Day 0 and Day 42.

5.2 Blood glucose levels before and after treatment with HIV- Protease inhibitors (LP/RTV or ATV /RTV)

The mean blood glucose of the groups treated with lopinavir/ritonavir or atazanavir/ritonavir was generally higher than that of the control and the diosmin/hesperidin treated groups.The mechanisms by which the HIV-protease Inhibitors (HIV-PIs) increase blood glucose include inhibition of insulin stimulated glucose uptake by such cells as adipocytes and skeletal muscle cells. HIV-PIs are also thought to interfere with insulin signal transduction mechanismsthat lead to the translocation offor example the GLUT-4 transporters to the cell surface to facilitate glucose uptake by cells(Jaldin-Fincati et al., 2017; Olson, 2012). Some of these HIV-PIs also induce insulin resistance. They also cause impairment in insulin secretion while stimulating endogenous glucose production (gluconeogenesis). These compounds are now known to cause oxidative stress that may result in increased β-cell apoptosis leading to decreased insulin secretion (Carr et al., 2008; Chandra et al., 2009; Dubé, 2000; Flint et al., 2009; Howard et al., 2005; Hruz et al., 2008; Hui, 2003; Lee et al., 2005; Lien & Feinglos, 2005; Murata et al., 2000; Noor et al., 2004; Sangraula et al., 2001; Schütt et al., 2004; Tsiodras et al., 2000; Viganò et al., 2009; Woerle et al., 2003b, 2003a). One of the consequences in the increase in blood glucose in patients on HIV- protease inhibitors is that it may sometimes result in overt diabetes mellitus(Barbaro, 2005; Barth et al., 2010; Carr et al., 1999; Dever et al., 2000; Reust, 2011; Salehian et al., 2005; Zeldin & Petruschke, 2004). This can result in complicating patient management because the patient may have to take more drugs to manage diabetes mellitus and this can lead to an increase in the pill (medicines) burden given that most of them are already on

ARVs (HAART).Atazanavir/ritonavir caused a higher increase in the blood glucose compared to lopinavir/ritonavir(Aberg et al., 2012; Noor et al., 2006; Stanley et al., 2009). It was also noticed that their maximum effect on blood glucose occurred at aroundthe third week of treatment after the commencement of treatment indicating that the hyperglycemic effect may be experienced after a certain period of treatment and not immediately. The results of this study are line with previous findings that some of the HIV-PIs can cause an increase in blood glucose levels in patients (Al Soub et al., 2001; De Araújo et al., 2007; Tsiodras et al., 2000)

5.3. The effects of diosmin / hesperidin combination treatment on blood glucose levels in protease inhibitor-treated rats.

Flavonoids have been demonstrated to have antidiabetic effects in rats and this may thus have contributed to their antihyperglycemic effect in this study (Chandrika et al., 2006). Mechanisms by which flavonoids like Hesperidin exertantihyperglycemic effects include upregulating PPAR-ɤ (Beekmann et al., 2015; Cortés et al., 2010; Da-Costa-Rocha et al., 2014; Gao et al., 2009; Hossain et al., 2016; Huang et al., 2010; Jung et al., 2006; Panche et al., 2016; Park et al., 2013; Röder et al., 2016; Salam et al., 2008; Tapas et al., 2008; Vinayagam & Xu, 2015). In this study the DIOS/HES combination reduced the blood glucose elevating effect of the ATV/RTV combination significantly. This was in contrast to the effect of these compounds (flavonoids)on the glucose elevating effect of the LPV/RTV combination in which they were not effective. The physiological mechanism behind the actions of these flavonoids may include the reductionof hyperglycemia and insulin resistance and can also reduction of oxidative stress on the pancreatic β-cells in patients with diabetes (Hafizur et al., 2017; Omodanisi et al., 2017; Ramkissoon et al., 2013; Testa et al., 2016). Its by these actions thatHesperidin and its derivative Diosminseem to exert their anti-diabetic actions (de Oliveira et al., 2013; Garg et al., 2001; Jung et al., 2004; C.

Li & Schluesener, 2017; Mahmoud et al., 2012; Wilmsen et al., 2005). Flavonoids by their actions in the liver where they reduce gluconeogenesis have demonstrated anti-hyperglycemic effects and this may have contributed to their action of reducing blood glucose in the HIV-protease inhibitor treated animals (Alkhalidy, Moore, Wang, Luo, McMillan, Zhen, et al., 2018; Cushnie & Lamb, 2005; Gao et al., 2009; Nijveldt et al., 2001; Williams et al., 2004). It is by these actions that flavonoids are thought to antagonize the hyperglycemic effects of HIV-PIs.

These results demonstrate that the co-administration of DIOS/HES with HIV-protease inhibitors had a bigger impact in terms of the reduction of the group mean blood glucose level elevation caused by treatment with ATV/RTV compared to the impact on the blood sugar elevation caused by treatment with LPV/RTV. The differences were even higher between the DIOS/HES combination treated and the LPV/RTV and ATV/RTV combination treated groups. During the duration of the study, the animals treated with LPV/RTV had a significant group mean blood glucose level difference with the group treated with DIOS/HES combination. The group on ATV/RTV and the group treated with DIOS/HES demonstrated significant differences in their mean blood glucose levels at the end of the first week (0.73mmol/L, $p=0.005$), at the end of the third week (1.89 mmol/L, $p<0.0001$) at the end of the fourth week (0.94 mmol/L, $p<0.0001$) and at the end of the fifth week (0.94 mmol/L, $p<0.0001$The addition of Diosmin/Hesperidin combination to the lopinavir/ritonavir and atazanavir/ritonavir treated groups had a significant effect on the blood glucose levels. The atazanavir/ritonavir+diosmin/hesperidin treated group's mean blood glucose levels were comparable tothose of the control over the entire treatment period except for the difference noted at the end of week one after the start of treatment. This is an indication that addition of the flavonoids(diosmin/hesperidin combination) had a significant group mean blood glucose stabilizing effect to the extent that it was more or less similar to that

of the control group. On the other hand thediosmin/hesperidin combination did not have a significant effect on the blood glucose levels of the lopinavir/ritonavir treated group which remained more or less elevatedWhen administered to untreated animals (those not on HIV-PIs) the diosmin/hesperidin combination did not have a significant impact on blood glucose levels.However,the flavonoids did seem to have an effect on the extent of variability (standard deviation) of the group mean. This finding suggests that the consumption of flavonoids prevent the excessive variation in the blood glucose levels (glycemic control). These findingsare in line with those of other researchers who found that flavonoids have an antihyperglycemic effect(Constantin et al., 2014; El-Marasy et al., 2014; Sahnoun et al., 2017).

5.4 Plasma insulin levels in rats before and after treatment with HIV-Protease inhibitors, HIV-Protease inhibitors+Diosmin/Hesperidin combination and Diosmin/Hesperidin combination.

Insulin is a polypeptide hormone that is required for the regulation of the metabolism of glucose, lipids and amino acids (Petersen & Shulman, 2018; Resistance, 2004). Its signal transduction mechanism involves the binding of the hormone to a tyrosine kinase receptor on the cell membrane of the target cells. In the pancreas this leads to the release of insulin by the β-cells of the islets of Langerhans and the translocation of the glucose transporters e.g. GLUT-4, to the cell membrane for example in skeletal muscle cells and adipocytes to allow inward movement of glucose into these cells resulting in lowering of blood glucose (Furtado et al., 2002, 2003; Rayner et al., 1994; Watson & Pessin, 2001).This also leadsto the entry of glucose into these cells for metabolism to produce energy or for storage as fat in the adipocytes or as glycogen in the liver and skeletal muscle (Jensen et al., 2011; Long et al., 2011).Flavonoids are known to act in a number of ways to promote the action of insulin. These include the following mechanisms:

1) Sensitizing tissues to the action of insulin i.e. antagonize insulin resistance. 2) Flavonoids reduce oxidative stress and this prevents the β-cells from premature damage and death. 3) The flavonoids counter apoptosis of the β-cells. 4) These compounds(flavonoids) are known to promote the regeneration of the β-cells(Babu et al., 2013; Cordero-Herrera et al., 2014; Hossain et al., 2016; Mohan & Nandhakumar, 2014).

The HIV-PIs used in the management of HIV infection management in the HAART have a number of side effects. These side effects include interference with glucose metabolism by promoting insulin resistance. These drugs also interfere with insulin signal transduction mechanisms (Carr, Samaras, Chisholm, et al., 1998; Lee et al., 2005; Murata et al., 2000; Santiprabhob et al., 2017). The Diosmin/Hesperidin combination significantly countered the insulin elevating effects caused by the administration of the ATV/RTV combination.The animals treated with ATV/RTV combination a lone, without the co-administration of DIOS/HES combination had a comparativelyhigher group mean blood insulin levels compared to the ATV/RTV+DIOS/HES treated group and thecontrol group.The co-administration of the flavonoid combination of Diosmin and Hesperidin(DIOS/HES)with the HIV/PIs combination of LPV/RTV did not have a significant effect on the group mean blood insulin levels which remained elevated and significantly higher compared to baseline levels. The co-administration of the DIOS/HES combination to the LPV/RTV treated group marginally reduced the group mean insulin levels compared to the group treated with LPV/RTV combination alone. However in both cases the levels were much higher than the baseline levels.The control group had the lowest group mean blood insulin levels at the end of the study (day 42).This results indicate that at the end of the study the group mean insulin levels of all the groups were higher compared to their respective baseline levels.The differences in the group mean blood insulin levels was more

pronounced at the end of the study as indicated by the decrease in the p-value and increase in effect size. The difference in insulin levels were around five to seven times higher at the end of the study compared to the levels at the beginning of the study. The group that was placed on Lopinavir/ritonavir+Diosmin/Hesperidin showed a notable lowermean insulin level compared to the group treated with LPV/RTV combination alone. The same observation was made on the ATV/RTV treated group which had a higher group mean blood insulin level compared to the ATV/RTV+DIOS/HES treated group where the ATV/RTV treated group had a higher group mean blood insulin levels. This finding demonstrated that flavonoids do counter or reduce insulin resistance leading to decrease in blood insulin levels since this compounds promote insulin action and promote insulin signaling reducing the need for high insulin levels to effect the required reduction in blood glucose. These findings seem to agree with those of other resaerachers(Alkhalidy, Moore, Wang, Luo, McMillan, Wang, et al., 2018; Alkhalidy, Moore, Wang, Luo, McMillan, Zhen, et al., 2018; Babu et al., 2013; Brahmachari, 2011; Hanhineva et al., 2010; Kahn et al., 2014; Kozłowska & Szostak-Wegierek, 2014; Russo et al., 2019). From these findings there are mixed outcomes on the effect of these treatments on blood insulin level with the control group having the lowest insulin level at the end of the study (6 weeks) period although this level(blood insulin levels) were about six times the levels at baseline (Day zero).

These results may be due to the action of flavonoids which enhance insulin secretion, promote proliferation of β-cells of the pancreas, reduce apoptosis of the β- cells, decrease insulin resistance; reduce inflammation and oxidation stress in the β-cells and promote the translocation of GLUT-4 type of glucose transporters to the cell membrane in the target tissue to enhance glucose uptake in these tissues (Cueva et al., 2017; Hanhineva et al., 2010; Koziara et al., 2019; Williamson, 2017).

5.5Effect of the administration of Diosmin-Hesperidin combination on blood HbA1c levels in HIV-protease inhibitor treated rats (*Rattusnorvegicusalbinus*)

HbA1c is glycated hemoglobin formed by the unregulated (no enzymatic involvement) reaction between hemoglobin and blood glucose. The glucose reacts with the β-chain of hemoglobin A. The levels of HbA1c in blood is dependent on the levels of blood glucose (Florkowski, 2013; Sherwani et al., 2016; Trial, 2002).Elevated levels of blood HbA1c are a reflection of chronic hyperglycemia (Rohlfing et al., 2002; Vos et al., 2012). Since the formation of HbA1c is a non-enzymatic reaction, it is mainly driven by the law of mass action (Higgins, 2012b; Ładyżyński et al., 2011; Sen et al., 2005).HbA1c is one of the advanced glycation end products (AGEs) – the products formed by chronic hyperglycemia. Elevated levels of HbA1c leads to a number of adverse effects. These includemicrovascular complications that lead to increased thrombogenicity of blood and poor wound healing incase of injuries like e.g. diabetic foot; increased levels of glucose which may result in glucotoxicity to the β-cells of the pancreas and also increased HbA1c levels which may increase the risk developing coronary heart disease and stroke.(Lind et al., 2009; Osende et al., 2001; Sherwani et al., 2016; Škrha et al., 2016). Such an increase in the risk of developing these complication in HIV infected patients can lead to the need for more medications (to control hyperglycemia and treat complications of elevated HbA1c) on top of the ones that the patients are already taking (ARVs) to prevent the progression of HIV infection to full blown AIDS. This may increase the pill (number of medicines) burden and the associated adverse effectsas the patient may require management of these complications.

In this study the treatment of the animals with HIV-Proteaseinhibitors led to an increase in the blood HbA1c levels in both the LPV/RTV and the ATV/RTV treated groups. The co-administration of the flavonoids (DIOS/HES) with HIV-Protease inhibitors LPV/RTV did not

any lead to any change in the HbA1c levels indicating that the flavonoids (DIOS/HES) did not have any significant impact on the HbA1c levels increase induced by administration of LPV/RTV HIV-PIs combination. This may to the fact that these flavonoids did not seem to counter the blood glucose elevating effects of the LPV/RTV combination. Therefore the high blood glucose may have led to the observed HbA1c levels- which did not decrease. On the other hand the co-administration of the flavonoids DIOS/HES combinationwith the HIV-Protease inhibitors ATV/RTV led to a significant reduction in the blood HbA1c levels. The group treated with the flavonoid combination of Diosmin and Hesperidin showed the largest decrease in blood HbA1c levels. The group mean HbA1c level of this group was the lowest at the end of the study. The control group showed a slight increase in the blood HbA1c levels at the end of the study though this was not statistically significant.

These findings show that these drugs (HIV-protease inhibitors) generally increased the blood glucose levels and this increased blood glucose led to the glycation of hemoglobin leading to the observed increase in the percentage HbA1c levels.This is because the levels of HbA1c are directly proportional to blood glucose levels and its generation is generally driven by the law of mass action. HbA1c levels are strongly associated with some of the endstage complications of DM (outlined above)(Farmer et al., 2007; Higgins, 2012a; Ittle, 2002; Khattab et al., 2010; Petitti et al., 2009; Sherwani et al., 2016; Stolar, 2010).This finding is consistent with those of other researchers who have demonstrated that the administration of flavonoids such diosmin and hesperidin to human and animal subjects tends to reduce the level of advanced glycated end-products(AGEs) including HbA1c in blood(Ahmad et al., 2013; Pashikanti et al., 2010).

CHAPTER SIX

CONCLUSION AND RECOMENDATIONS

6.1 Conclusion

In conclusion the present study found that:

1. The treatment of rats (*Rattusnorvegiccusalbinus*) with the HIV-PIs (ATV/RTV or LPV/RTV) increased their blood glucose levels.

2. The administration of DIOS/HES combination to rats (*Rattusnorvegicusalbinus)* treated with HIV-PIs led to a significant decrease in the group mean blood glucose levels in the ATV/RTV treated group but not in the treated LPV/RTV group.

3. The administration of HIV-PIs to the rats (*Rattusnorvegicusalbinus)* led to a larger increase in blood insulin levels compared to the control.

4. The co-administration of DIOS/HES to HIV-PIs treated rats led to a slight decrease in the insulin levels compared to the control.

5. The administration of HIV-PIs (ATV/RTV or LPV/RTV) to rats (*Rattusnorvegicusalbinus)*produced a significant increase in HbA1c levels.

6. The co-administration of the flavonoids with LPV/RTV (a HIV-PI) did not show any change in the blood HbA1c levels. On the other hand the co-administration of DIOS/HES with theATV/RTV combination(a HIV-PI combination) led to a reduction in the HbA1c levels significantly.

7. The administration of DIOS/HES flavonoid combination alone to Wistar rats not on HIV-PIs treatment did not have an effect (neither increased nor decreased) on the group mean blood glucose levels.

6.2 Recommendations

1. The flavonoids are naturally occurring polyphenolic compounds and freely available in fruits and vegetables and have virtually no adverse effects. In view of the results of these study – we recommend that HIV infected patients on HIV-PIs can be encouraged to take fruits and vegetables liberallyas this will counter the hyperglycemic effects of the HIV-PIs and reduce the likelihood of developing iatrogenic (drug induced) hyperglycemia which may lead to overt diabetes mellitus. Individual flavonoids can be isolated, purified and characterized to design dosages for specified administration to manage hyperglycemia in patients on drugs that induce hyperglycemia such as HIV-Protease inhibitors.

3. The control of hyperglycemia may lead to the reduction in HbA1c levels and this will protect these patients from the complications of chronically elevated HbA1c levels. In future these flavonoids can be isolated and purified for a titrated co-administration with these HIV-PIs and other drugs that induce hyperglycemia for example glucocorticoids used for management chronic conditionse.g.autoimmune diseases in patients who are predisposed to develop hyperglycemia.

3. We recommend larger animal models such as pigs and primates to be used for further investigationsas these animals are phylogenetically closer to humans.

REFERENCES

Abebe, M., Kinde, S., Belay, G., Gebreegziabxier, A., Challa, F., Gebeyehu, T., Nigussie, P., & Tegbaru, B. (2014). Antiretroviral treatment associated hyperglycemia and dyslipidemia among HIV infected patients at Burayu Health Center, Addis Ababa, Ethiopia: A cross-sectional comparative study. *BMC Research Notes*, *7*(1). https://doi.org/10.1186/1756-0500-7-380

Aberg, J. A., Tebas, P., Overton, E. T., Gupta, S. K., Sax, P. E., Landay, A., Falcon, R., Ryan, R., & De La Rosa, G. (2012). Metabolic effects of darunavir/ritonavir versus atazanavir/ritonavir in treatment-naive, HIV Type 1-infected subjects over 48 weeks. *AIDS Research and Human Retroviruses*, *28*(10), 1184–1195. https://doi.org/10.1089/aid.2011.0327

Achari, A. E., & Jain, S. K. (2017). Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. In *International Journal of Molecular Sciences* (Vol. 18, Issue 6). https://doi.org/10.3390/ijms18061321

Aguirre, G. A., Ita, J. R., Garza, R. G., & Castilla-Cortazar, I. (2016). Insulin-like growth factor-1 deficiency and metabolic syndrome. *Journal of Translational Medicine*. https://doi.org/10.1186/s12967-015-0762-z

Ahima, R. S., Qi, Y., Singhal, N. S., Jackson, M. B., & Scherer, P. E. (2006). Brain adipocytokine action and metabolic regulation. *Diabetes*, *55*(SUPPL. 2). https://doi.org/10.2337/db06-S018

Ahmad, S., Shahab, U., Baig, M. H., Khan, M. S., Khan, M. S., Srivastava, A. K., Saeed, M., & Moinuddin. (2013). Inhibitory Effect of Metformin and Pyridoxamine in the Formation of Early, Intermediate and Advanced Glycation End-Products. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0072128

Akiyama, S., Katsumata, S. I., Suzuki, K., Nakaya, Y., Ishimi, Y., & Uehara, M. (2009). Hypoglycemic and hypolipidemic effects of hesperidin and cyclodextrin-clathrated hesperetin in Goto-Kakizaki rats with type 2 diabetes. *Bioscience, Biotechnology and Biochemistry*, *73*(12), 2779–2782. https://doi.org/10.1271/bbb.90576

Al-Ghamdi, A. A. (2004). Role oF HbA1c in management of diabetes mellitus. *Saudi Medical Journal*, *25*(3), 342–345.

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, *9*(9). https://doi.org/10.3390/biom9090430

Al Soub, H., Al Khal, A. L., Al Alousi, F., & Fabella, A. (2001). Hyperglycemia associated with protease inhibitors in HIV infected patients in Qatar. *Qatar Medical Journal*.

Alam, M. A., Subhan, N., Rahman, M. M., Uddin, S. J., Reza, H. M., & Sarker, S. D. (2014). Effect of Citrus Flavonoids, Naringin and Naringenin, on Metabolic Syndrome and Their Mechanisms of Action. *Advances in Nutrition*, *5*(4), 404–417. https://doi.org/10.3945/an.113.005603

Alberti, K. G. M. M., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Medicine*, *15*(7), 539–553. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

Alkhalidy, H., Moore, W., Wang, A., Luo, J., McMillan, R. P., Wang, Y., Zhen, W., Hulver, M. W., & Liu, D. (2018). Kaempferol ameliorates hyperglycemia through suppressing hepatic gluconeogenesis and enhancing hepatic insulin sensitivity in diet-induced obese mice. *Journal of Nutritional Biochemistry*, *58*, 90–101. https://doi.org/10.1016/j.jnutbio.2018.04.014

Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R. P., Zhen, W., Zhou, K., & Liu, D. (2018). The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. *Molecules*, *23*(9). https://doi.org/10.3390/molecules23092338

Anuurad, E., Bremer, A., & Berglund, L. (2010). HIV protease inhibitors and obesity. In *Current Opinion in Endocrinology, Diabetes and Obesity* (Vol. 17, Issue 5, pp. 478–485). https://doi.org/10.1097/MED.0b013e32833dde87

Aronoff, S. L., Berkowitz, K., Shreiner, B., & Want, L. (2004). Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum*, *17*(3), 183–190. https://doi.org/10.2337/diaspect.17.3.183

Azaïs-Braesco, V., Sluik, D., Maillot, M., Kok, F., & Moreno, L. A. (2017). A review of total & added sugar intakes and dietary sources in Europe. In *Nutrition Journal* (Vol. 16, Issue 1). https://doi.org/10.1186/s12937-016-0225-2

Babu, P. V. A., Liu, D., & Gilbert, E. R. (2013). Recent advances in understanding the anti-diabetic actions of dietary flavonoids. In *Journal of Nutritional Biochemistry* (Vol. 24, Issue 11, pp. 1777–1789). https://doi.org/10.1016/j.jnutbio.2013.06.003

Bahadoran, Z., Mirmiran, P., & Azizi, F. (2013). Dietary polyphenols as potential nutraceuticals in management of diabetes: A review. In *Journal of Diabetes and Metabolic Disorders* (Vol. 12, Issue 1). https://doi.org/10.1186/2251-6581-12-43

Bakari, A. G., Sani-Bello, F., Shehu, M. S., Mai, A., Aliyu, I. S., & Lawal, I. I. (2007). Anti-retroviral therapy induced diabetes in a Nigerian. *African Health Sciences*, *7*(3), 133–135. https://doi.org/10.5555/afhs.2007.7.3.133

Barbaro, G. (2005). Metabolic and Cardiovascular Complications of Highly Active Antiretroviral Therapy for HIV Infection. *Current HIV Research*, *4*(1), 79–85. https://doi.org/10.2174/157016206775197664

Baril, J. G., Junod, P., LeBlanc, R., Dion, H., Therrien, R., Laplante, F., Falutz, J., Côté, P., Hébert, M. N., Lalonde, R., Lapointe, N., Lévesque, D., Pinault, L., Rouleau, D., Tremblay, C., Trottier, B., Trottier, S., Tsoukas, C., & Weiss, K. (2005). HIV-associated lipodystrophy syndrome: A review of clinical aspects. In *Canadian Journal of Infectious Diseases and Medical Microbiology* (Vol. 16, Issue 4, pp. 233–243). https://doi.org/10.1155/2005/303141

Barré-Sinoussi, F. (1996). HIV as the cause of AIDS. In *Lancet*. https://doi.org/10.1016/S0140-6736(96)09058-7

Barret, K. E., BArman, S. M., Boitano, S., & Brooks, H. L. (2012). Ganong's Review of Medical Physiology 24th Edition's Review of Medical Physiology. In *Ganong's Review of Medical Physiology 24th Edition's Review of Medical Physiology*.

Barth, R. E., van der Loeff, M. F. S., Schuurman, R., Hoepelman, A. I., & Wensing, A. M. (2010). Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. In *The Lancet Infectious Diseases* (Vol. 10, Issue 3, pp. 155–166). https://doi.org/10.1016/S1473-3099(09)70328-7

Beekmann, K., Rubió, L., De Haan, L. H. J., Actis-Goretta, L., Van Der Burg, B., Van Bladeren, P. J., & Rietjens, I. M. C. M. (2015). The effect of quercetin and kaempferol aglycones and glucuronides on peroxisome proliferator-activated receptor-gamma (PPAR-γ). *Food and Function*, *6*(4), 1098–1107. https://doi.org/10.1039/c5fo00076a

Berg, J. P. (2013). Hba1c as a diagnostic tool in diabetes mellitus. *Norsk Epidemiologi*. https://doi.org/10.5324/nje.v23i1.1596

Bergman, M. (2013). Pathophysiology of prediabetes and treatment implications for the prevention of type 2 diabetes mellitus. In *Endocrine* (Vol. 43, Issue 3, pp. 504–513). https://doi.org/10.1007/s12020-012-9830-9

Bhowmik, B., Diep, L. M., Munir, S. B., Rahman, M., Wright, E., Mahmood, S., Afsana, F., Ahmed, T., Khan, A. K. A., & Hussain, A. (2013). HbA1c as a diagnostic tool for diabetes and pre-diabetes: The Bangladesh experience. *Diabetic Medicine*, *30*(3). https://doi.org/10.1111/dme.12088

Bodasing, N., & Fox, R. (2003). HIV-associated Lipodystrophy Syndrome: Description and Pathogenesis. *Journal of Infection*, *46*(3), 149–154. https://doi.org/10.1053/jinf.2002.1102

Bonert, V. S., & Melmed, S. (2017). Growth Hormone. In *The Pituitary: Fourth Edition*. https://doi.org/10.1016/B978-0-12-804169-7.00004-0

Bonora, E., & Tuomilehto, J. (2011). The pros and cons of diagnosing diabetes with A1C. In *Diabetes Care* (Vol. 34, Issue SUPPL. 2). https://doi.org/10.2337/dc11-s216

Brahmachari, G. (2011). Bio-flavonoids with promising anti- diabetic potentials : A critical survey. *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry - Research Signpost*, *661*(2), 187–212.

British Nutrition Foundation. (2019). Macronutrients - Energy , fat , carbohydrates and protein. *Nutrition Requirements*.

Brown, T. T., Cole, S. R., Li, X., Kingsley, L. A., Palella, F. J., Riddler, S. A., Visscher, B. R., Margolick, J. B., & Dobs, A. S. (2005). Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Archives of Internal Medicine*, *165*(10), 1179–1184. https://doi.org/10.1001/archinte.165.10.1179

Bryant, N. J., Govers, R., & James, D. E. (2002). Regulated transport of the glucose transporter GLUT4. In *Nature Reviews Molecular Cell Biology* (Vol. 3, Issue 4, pp. 267–277). https://doi.org/10.1038/nrm782

Calderhead, D. M., Kitagawa, K., Tanner, L. I., Holman, G. D., & Lienhard, G. E. (1990). Insulin regulation of the two glucose transporters in 3T3-L1 adipocytes. *Journal of Biological Chemistry*, *265*(23), 13800–13808.

Calza, L., Manfredi, R., & Chiodo, F. (2004). Insulin resistance and diabetes mellitus in HIV-infected patients receiving antiretroviral therapy. In *Metabolic Syndrome and Related Disorders* (Vol. 2, Issue 4, pp. 241–250). https://doi.org/10.1089/met.2004.2.241

Campbell, J. E., & Drucker, D. J. (2013). Pharmacology, physiology, and mechanisms of incretin hormone action. In *Cell Metabolism* (Vol. 17, Issue 6, pp. 819–837). https://doi.org/10.1016/j.cmet.2013.04.008

Carayannopoulos, M. O., Chi, M. M. Y., Cui, Y., Pingsterhaus, J. M., McKnight, R. A., Mueckler, M., Devaskar, S. U., & Moley, K. H. (2000a). GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(13), 7313–7318. https://doi.org/10.1073/pnas.97.13.7313

Carayannopoulos, M. O., Chi, M. M. Y., Cui, Y., Pingsterhaus, J. M., McKnight, R. A., Mueckler, M., Devaskar, S. U., & Moley, K. H. (2000b). GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(13), 7313–7318. https://doi.org/10.1073/pnas.97.13.7313

Carballo-Villalobos, A. I., González-Trujano, M. E., Pellicer, F., & López-Muñoz, F. J. (2016). Antihyperalgesic Effect of Hesperidin Improves with Diosmin in Experimental Neuropathic Pain. *BioMed Research International*. https://doi.org/10.1155/2016/8263463

Carr, A., Ritzhaupt, A., Zhang, W., Zajdenverg, R., Workman, C., Gatell, J. M., Cahn, P., & Chaves, R. (2008). Effects of boosted tipranavir and lopinavir on body composition, insulin sensitivity and adipocytokines in antiretroviral-naive adults. *AIDS*, *22*(17), 2313–2321. https://doi.org/10.1097/QAD.0b013e328315a7a5

Carr, A., Samaras, K., Burton, S., Law, M., Freund, J., Chisholm, D. J., & Cooper, D. A. (1998). A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS*, *12*(7). https://doi.org/10.1097/00002030-199807000-00003

Carr, A., Samaras, K., Chisholm, D. J., & Cooper, D. A. (1998). Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. In *Lancet* (Vol. 351, Issue 9119, pp. 1881–1883). https://doi.org/10.1016/S0140-6736(98)03391-1

Carr, A., Samaras, K., Thorisdottir, A., Kaufmann, G. R., Chisholm, D. J., & Cooper, D. A. (1999). Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: A cohort study. *Lancet*, *353*(9170), 2093–2099. https://doi.org/10.1016/S0140-6736(98)08468-2

Chandra, S., Mondal, D., & Agrawal, K. C. (2009). HIV-1 protease inhibitor induced oxidative stress suppresses glucose stimulated insulin release: Protection with thymoquinone. *Experimental Biology and Medicine*, *234*(4), 442–452. https://doi.org/10.3181/0811-RM-317

Chandrika, U. G., Wedage, W. S., Wickramasinghe, S. M. D. N., & Fernando, W. S. (2006). Hypoglycaemic action of the flavonoid fraction of Artocarpus heterophyllus leaf. *African Journal of Traditional, Complementary and Alternative Medicines*, *3*(2), 42–50. https://doi.org/10.4314/ajtcam.v3i2.31156

Chandwani, A., & Shuter, J. (2008). Lopinavir/ritonavir in the treatment of HIV-1 infection: A review. In *Therapeutics and Clinical Risk Management* (Vol. 4, Issue 5, pp. 1023–1033). https://doi.org/10.2147/tcrm.s3285

Cheatham, B., Volchuk, A., Kahn, C. R., Wang, L., Rhodes, C. J., & Klip, A. (1996). Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(26), 15169–15173. https://doi.org/10.1073/pnas.93.26.15169

Chen, F., Xiong, H., Wang, J., Ding, X., Shu, G., & Mei, Z. (2013). Antidiabetic effect of total flavonoids from Sanguis draxonis in type 2 diabetic rats. *Journal of Ethnopharmacology*, *149*(3), 729–736. https://doi.org/10.1016/j.jep.2013.07.035

Clifton, P. M., Keogh, J. B., Woonton, B. W., Taylor, C. M., Janakievski, F., & De Silva, K. (2009). Effect of glycomacropeptides (GMP) on satiety hormones and food intake. *Australian Journal of Dairy Technology*, *64*(1), 29–31.

Cohen, A. M., Maxon, H. R., Goldsmith, R. E., Schneider, H. J., Wiot, J. F., Loudon, R. G., & Altemeier, W. A. (1977). Metastatic Pulmonary Calcification in Primary Hyperparathyroidism. *Archives of Internal Medicine*, *137*(4), 520–522. https://doi.org/10.1001/archinte.1977.03630160080016

Cohen, J. (1988). Statistical power for the social sciences. *Hillsdale, NJ: Laurence Erlbaum and Associates*.

Constantin, R. P., Constantin, R. P., Bracht, A., Yamamoto, N. S., Ishii-Iwamoto, E. L., & Constantin, J. (2014). Molecular mechanisms of citrus flavanones on hepatic gluconeogenesis. *Fitoterapia*, *92*, 148–162. https://doi.org/10.1016/j.fitote.2013.11.003

Cordero-Herrera, I., Martín, M. Á., Goya, L., & Ramos, S. (2014). Cocoa flavonoids attenuate high glucose-induced insulin signalling blockade and modulate glucose uptake and production in human HepG2 cells. *Food and Chemical Toxicology*. https://doi.org/10.1016/j.fct.2013.11.014

Cortés, S., Pulgar, H., Sanhueza, V., Aspé, E., & Fernández, K. (2010). Identificación de las proantocianidinas extraídas desde corteza de pinus radiata D. Don. *Ciencia e Investigacion Agraria*, *37*(2), 15–25. https://doi.org/10.4067/S0718-16202010000200002

Coskun, O., Kanter, M., Korkmaz, A., & Oter, S. (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. *Pharmacological Research*, *51*(2), 117–123. https://doi.org/10.1016/j.phrs.2004.06.002

Cueva, C., Gil-Sánchez, I., Ayuda-Durán, B., González-Manzano, S., González-Paramás, A. M., Santos-Buelga, C., Bartolomé, B., & Victoria Moreno-Arribas, M. (2017). An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health. In *Molecules* (Vol. 22, Issue 1). https://doi.org/10.3390/molecules22010099

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. In *International Journal of Antimicrobial Agents* (Vol. 26, Issue 5, pp. 343–356). https://doi.org/10.1016/j.ijantimicag.2005.09.002

Cvetkovic, R. S., & Goa, K. L. (2003). Lopinavir/ritonavir: A review of its use in the management of HIV infection. In *Drugs* (Vol. 63, Issue 8, pp. 769–802). https://doi.org/10.2165/00003495-200363080-00004

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). Hibiscus sabdariffa L. - A phytochemical and pharmacological review. In *Food Chemistry* (Vol. 165, pp. 424–443). https://doi.org/10.1016/j.foodchem.2014.05.002

De Araújo, P. S. R., Ximenes, R. A. D. A., Lopes, C. F. C., Duarte, J. Y., Da Silva, M. M., & Carneiro, E. M. (2007). Antiretroviral treatment for HIV infection/aids and the risk of developing hyperglycemia and hyperlipidemia. *Revista Do Instituto de Medicina Tropical de Sao Paulo*. https://doi.org/10.1590/s0036-46652007000200002

de Oliveira, D. M., Dourado, G. K. Z. S., & Cesar, T. B. (2013). Hesperidin associated with continuous and interval swimming improved biochemical and oxidative biomarkers in rats. *Journal of the International Society of Sports Nutrition*, *10*. https://doi.org/10.1186/1550-2783-10-27

DeFronzo, R. A. (2010). Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: The missing links. The Claude Bernard Lecture 2009. In *Diabetologia* (Vol. 53, Issue 7, pp. 1270–1287). https://doi.org/10.1007/s00125-010-1684-1

DeFronzo, Ralph A. (2004). Pathogenesis of type 2 diabetes mellitus. In *Medical Clinics of North America* (Vol. 88, Issue 4, pp. 787–835). https://doi.org/10.1016/j.mcna.2004.04.013

Del Prato, S. (2009). Role of glucotoxicity and lipotoxicity in the pathophysiology of Type 2 diabetes mellitus and emerging treatment strategies. In *Diabetic Medicine*. https://doi.org/10.1111/j.1464-5491.2009.02847.x

Devaskar, S. U., & Mueckler, M. M. (1992). The mammalian glucose transporters. *Pediatric Research*, *31*(1), 1–13. https://doi.org/10.1203/00006450-199201000-00001

Dever, L. L., Oruwari, P. A., Figueroa, W. E., O'Donovan, C. A., & Eng, R. H. K. (2000). Hyperglycemia associated with protease inhibitors in an urban HIV- infected minority patient population. *Annals of Pharmacotherapy*, *34*(5), 580–584. https://doi.org/10.1345/aph.19231

Di Angelantonio, E., Gao, P., Khan, H., Butterworth, A. S., Wormser, D., Kaptoge, S., Kondapally Seshasai, S. R., Thompson, A., Sarwar, N., Willeit, P., Ridker, P. M., Barr, E. L. M., Khaw, K. T., Psaty, B. M., Brenner, H., Balkau, B., Dekker, J. M., Lawlor, D. A., Daimon, M., … Harshfield, E. (2014). Glycated hemoglobin measurement and prediction of cardiovascular disease. *JAMA - Journal of the American Medical Association*, *311*(12), 1225–1233. https://doi.org/10.1001/jama.2014.1873

Diatewa, M., Badila Samba, C., Hondi Assah, T. C., & Abena, A. A. (2004). Hypoglycemic and antihyperglycemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of Cogniauxia podoleana Baillon in normal and alloxan-induced diabetic rats. *Journal of Ethnopharmacology*, *92*(2–3), 229–232. https://doi.org/10.1016/j.jep.2004.02.017

Drucker, D. J. (2006). The biology of incretin hormones. In *Cell Metabolism* (Vol. 3, Issue 3, pp. 153–165). https://doi.org/10.1016/j.cmet.2006.01.004

Dubé, M. P. (2000). Disorders of Glucose Metabolism in Patients Infected with Human Immunodeficiency Virus. *Clinical Infectious Diseases*. https://doi.org/10.1086/317491

El-Marasy, S. A., Abdallah, H. M. I., El-Shenawy, S. M., El-Khatib, A. S., El-Shabrawy, O. A., & Kenawy, S. A. (2014). Anti-depressant effect of hesperidin in diabetic rats. *Canadian Journal of Physiology and Pharmacology*, *92*(11), 945–952. https://doi.org/10.1139/cjpp-2014-0281

El-Shafae, A. M., & El-Domiaty, M. M. (2001). Improved LC methods for the determination of diosmin and/or hesperidin in plant extracts and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis*. https://doi.org/10.1016/S0731-7085(01)00476-9

Esposito, K., Chiodini, P., Capuano, A., Petrizzo, M., Improta, M. R., & Giugliano, D. (2012). Basal supplementation of insulin lispro protamine suspension versus insulin glargine and detemir for type 2 diabetes: Meta-analysis of randomized controlled trials. *Diabetes Care*, *35*(12), 2698–2705. https://doi.org/10.2337/dc12-0698

Estrada, V., Martínez-Larrad, M. T., González-Sánchez, J. L., de Villar, N. G. P., Zabena, C., Fernández, C., & Serrano-Ríos, M. (2006). Lipodystrophy and metabolic syndrome in HIV-infected patients treated with antiretroviral therapy. *Metabolism: Clinical and Experimental*, *55*(7), 940–945. https://doi.org/10.1016/j.metabol.2006.02.024

Farmer, A., Wade, A., Goyder, E., Yudkin, P., French, D., Craven, A., Holman, R., Kinmonth, A. L., & Neil, A. (2007). Impact of self monitoring of blood glucose in the management of patients with non-insulin treated diabetes: Open parallel group randomised trial. *British Medical Journal*, *335*(7611), 132–136. https://doi.org/10.1136/bmj.39247.447431.BE

Feng, Z., Hou, X., Zhu, C., Zhu, J., & Jiang, C. (2019). Epigallocatechin gallate ameliorates morphological changes of pancreatic islets in diabetic mice and downregulates blood sugar level by inhibiting the accumulation of AGE-RAGE. *Journal of Cellular Biochemistry*, *120*(5), 8510–8520. https://doi.org/10.1002/jcb.28139

Findlay, V. J. (2007). Ritonavir. In *xPharm: The Comprehensive Pharmacology Reference* (pp. 1–6). https://doi.org/10.1016/B978-008055232-3.62543-7

Finkelstein, J. L., Gala, P., Rochford, R., Glesby, M. J., & Mehta, S. (2015). HIV/AIDS and lipodystrophy: Implications for clinical management in resource-limited settings. In *Journal of the International AIDS Society*. https://doi.org/10.7448/IAS.18.1.19033

Flint, O. P., Noor, M. A., Hruz, P. W., Hylemon, P. B., Yarasheski, K., Kotler, D. P., Parker, R. A., & Bellamine, A. (2009). The role of protease inhibitors in the pathogenesis of HIV-associated lipodystrophy: Cellular mechanisms and clinical implications. In *Toxicologic Pathology* (Vol. 37, Issue 1, pp. 65–77). https://doi.org/10.1177/0192623308327119

Florkowski, C. (2013). HbA1c as a diagnostic test for diabetes mellitus - Reviewing the evidence. *Clinical Biochemist Reviews*.

Foundation, B. N. (2016a). Nutrition Requirements. In *British Nutrition Foundation*. https://doi.org/10.1300/5513_12

Foundation, B. N. (2016b). Nutrition Requirements. In *British Nutrition Foundation*. https://doi.org/10.1300/5513_12

Fukami, A., Seino, Y., Ozaki, N., Yamamoto, M., Sugiyama, C., Sakamoto-Miura, E., Himeno, T., Takagishi, Y., Tsunekawa, S., Ali, S., Drucker, D. J., Murata, Y., Seino, Y., Oiso, Y., & Hayashi, Y. (2013). Ectopic expression of GIP in pancreatic β-cells maintains enhanced insulin secretion in mice with complete absence of proglucagon-derived peptides. *Diabetes*, *62*(2), 510–518. https://doi.org/10.2337/db12-0294

Furtado, L. M., Poon, V., & Klip, A. (2003). GLUT4 activation: Thoughts on possible mechanisms. *Acta Physiologica Scandinavica*. https://doi.org/10.1046/j.1365-201X.2003.01160.x

Furtado, L. M., Somwar, R., Sweeney, G., Niu, W., & Klip, A. (2002). Activation of the glucose transporter GLUT4 by insulin. In *Biochemistry and Cell Biology*. https://doi.org/10.1139/o02-156

Gallo, R. C., & Montagnier, L. (2003). The Discovery of HIV as the Cause of AIDS. In *New England Journal of Medicine*. https://doi.org/10.1056/NEJMp038194

Gao, X., Wang, W., Wei, S., & Li, W. (2009). Review of pharmacological effects of Glycyrrhiza Radix and its bioactive compounds. In *Zhongguo Zhongyao Zazhi* (Vol. 34, Issue 21, pp. 2695–2700).

Garg, A., Garg, S., Zaneveld, L. J. D., & Singla, A. K. (2001). Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. In *Phytotherapy Research* (Vol. 15, Issue 8, pp. 655–669). https://doi.org/10.1002/ptr.1074

George, V. C., Dellaire, G., & Rupasinghe, H. P. V. (2017). Plant flavonoids in cancer chemoprevention: role in genome stability. In *Journal of Nutritional Biochemistry* (Vol. 45, pp. 1–14). https://doi.org/10.1016/j.jnutbio.2016.11.007

Gerozissis, K. (2008). Brain insulin, energy and glucose homeostasis; genes, environment and metabolic pathologies. In *European Journal of Pharmacology* (Vol. 585, Issue 1, pp. 38–49). https://doi.org/10.1016/j.ejphar.2008.01.050

Giannini, I., Amato, A., Basso, L., Tricomi, N., Marranci, M., Pecorella, G., Tafuri, S., Pennisi, D., & Altomare, D. F. (2015). Flavonoids mixture (diosmin, troxerutin, hesperidin) in the treatment of acute hemorrhoidal disease: a prospective, randomized, triple-blind, controlled trial. *Techniques in Coloproctology*. https://doi.org/10.1007/s10151-015-1302-9

Gibney, M. J., Forde, C. G., Mullally, D., & Gibney, E. R. (2017). Ultra-processed foods in human health: A critical appraisal. In *American Journal of Clinical Nutrition* (Vol. 106, Issue 3, pp. 717–724). https://doi.org/10.3945/ajcn.117.160440

Gkrania-Klotsas, E., & Klotsas, A. E. (2007). HIV and HIV treatment: Effects on fats, glucose and lipids. In *British Medical Bulletin*. https://doi.org/10.1093/bmb/ldm030

González-Scarano, F., & Martín-García, J. (2005). The neuropathogenesis of AIDS. In *Nature Reviews Immunology*. https://doi.org/10.1038/nri1527

Goodyear, PhD, L. J., & Kahn, MD, B. B. (1998). EXERCISE, GLUCOSE TRANSPORT, AND INSULIN SENSITIVITY. *Annual Review of Medicine*. https://doi.org/10.1146/annurev.med.49.1.235

Gosslau, A., Zachariah, E., Li, S., & Ho, C.-T. (2018). Effects of a flavonoid-enriched orange peel extract against type 2 diabetes in the obese ZDF rat model. *Food Science and Human Wellness, 7*(4), 244–251. https://doi.org/10.1016/j.fshw.2018.10.001

Gran, J. M., Wasmuth, L., Amundsen, E. J., Lindqvist, B. H., & Aalen, O. O. (2008). Growth rates in epidemic models: Application to a model for HIV/AIDS progression. *Statistics in Medicine*. https://doi.org/10.1002/sim.3219

Grassi, D., Desideri, G., Mai, F., Martella, L., De Feo, M., Soddu, D., Fellini, E., Veneri, M., Stamerra, C. A., & Ferri, C. (2015). Cocoa, Glucose Tolerance, and Insulin Signaling: Cardiometabolic Protection. In *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.5b00913

Gutierrez, A. D., & Balasubramanyam, A. (2012). Dysregulation of glucose metabolism in HIV patients: Epidemiology, mechanisms, and management. In *Endocrine* (Vol. 41, Issue 1, pp. 1–10). https://doi.org/10.1007/s12020-011-9565-z

Hafizur, R. M., Momin, S., & Fatima, N. (2017). Prevention of advanced glycation end-products formation in diabetic rats through beta-cell modulation by Aegle marmelos. *BMC Complementary and Alternative Medicine*, *17*(1). https://doi.org/10.1186/s12906-017-1743-y

Hage, M., Zantout, M. S., & Azar, S. T. (2011). Thyroid disorders and diabetes mellitus. In *Journal of Thyroid Research* (Vol. 2011). https://doi.org/10.4061/2011/439463

Haggag, Y. A., El-Ashmawy, N. E., & Okasha, K. M. (2020). Is hesperidin essential for prophylaxis and treatment of COVID-19 Infection? *Medical Hypotheses*. https://doi.org/10.1016/j.mehy.2020.109957

Hajiaghaalipour, F., Khalilpourfarshbafi, M., Arya, A., & Arya, A. (2015). Modulation of glucose transporter protein by dietary flavonoids in type 2 diabetes mellitus. *International Journal of Biological Sciences*, *11*(5), 508–524. https://doi.org/10.7150/ijbs.11241

Hammer, S. M., Eron, J. J., Reiss, P., Schooley, R. T., Thompson, M. A., Walmsley, S., Cahn, P., Fischl, M. A., Gatell, J. M., Hirsch, M. S., Jacobsen, D. M., Montaner, J. S. G., Richman, D. D., Yeni, P. G., & Volberding, P. A. (2008). Antiretroviral treatment of adult HIV infection: 2008 Recommendations of the international AIDS society-USA panel. In *JAMA - Journal of the American Medical Association* (Vol. 300, Issue 5, pp. 555–570). https://doi.org/10.1001/jama.300.5.555

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. In *International Journal of Molecular Sciences* (Vol. 11, Issue 4, pp. 1365–1402). https://doi.org/10.3390/ijms11041365

Higgins, T. (2012a). HbA 1c - An analyte of increasing importance. In *Clinical Biochemistry* (Vol. 45, Issues 13–14, pp. 1038–1045). https://doi.org/10.1016/j.clinbiochem.2012.06.006

Higgins, T. (2012b). HbA1c - An analyte of increasing importance. In *Clinical Biochemistry*. https://doi.org/10.1016/j.clinbiochem.2012.06.006

Holst, J. J., Vilsbøll, T., & Deacon, C. F. (2009). The incretin system and its role in type 2 diabetes mellitus. In *Molecular and Cellular Endocrinology* (Vol. 297, Issues 1–2, pp. 127–136). https://doi.org/10.1016/j.mce.2008.08.012

Hossain, M. K., Dayem, A. A., Han, J., Yin, Y., Kim, K., Saha, S. K., Yang, G. M., Choi, H. Y., & Cho, S. G. (2016). Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. In *International Journal of Molecular Sciences* (Vol. 17, Issue 4). https://doi.org/10.3390/ijms17040569

Howard, A. A., Floris-Moore, M., Arnsten, J. H., Santoro, N., Fleischer, N., Lo, Y., & Schoenbaum, E. E. (2005). Disorders of Glucose Metabolism among HIV-Infected Women. *Clinical Infectious Diseases*. https://doi.org/10.1086/429824

Hruz, P. W. (2011). Molecular mechanisms for insulin resistance in treated HIV-infection. In *Best Practice and Research: Clinical Endocrinology and Metabolism* (Vol. 25, Issue 3, pp. 459–468). https://doi.org/10.1016/j.beem.2010.10.017

Hruz, P. W., Yan, Q., Struthers, H., & Jay, P. Y. (2008). HIV protease inhibitors that block GLUT4 precipitate acute, decompensated heart failure in a mouse model of dilated cardiomyopathy. *FASEB Journal*, *22*(7), 2161–2167. https://doi.org/10.1096/fj.07-102269

Hsu, C. C., Lin, M. H., Cheng, J. T., & Wu, M. C. (2017). Diosmin, a citrus nutrient, activates imidazoline receptors to alleviate blood glucose and lipids in type 1-like diabetic rats. *Nutrients*, *9*(7). https://doi.org/10.3390/nu9070684

Hu, F. B., & Malik, V. S. (2010). Sugar-sweetened beverages and risk of obesity and type 2 diabetes: Epidemiologic evidence. *Physiology and Behavior*, *100*(1), 47–54. https://doi.org/10.1016/j.physbeh.2010.01.036

Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2010). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. In *Nutrition and Cancer* (Vol. 62, Issue 1, pp. 1–20). https://doi.org/10.1080/01635580903191585

Hui, D. Y. (2003). Effects of HIV protease inhibitor therapy on lipid metabolism. In *Progress in Lipid Research* (Vol. 42, Issue 2, pp. 81–92). https://doi.org/10.1016/S0163-7827(02)00046-2

Hussain, S. A., & Marouf, B. H. (2013). Flavonoids as alternatives in treatment of type 2 diabetes mellitus. *Academia Journal of Medicinal Plants*, *1*(2), 31–36.

Hwang, G. J., Lee, J. D., Park, C. Y., & Lim, S. K. (1996). Reversible extraskeletal uptake of bone scanning in primary hyperparathyroidism. *Journal of Nuclear Medicine*, *37*(3), 469–471.

Iglesias, P., Selgas, R., Romero, S., & Díez, J. J. (2012). Biological role, clinical significance, and therapeutic possibilities of the recently discovered metabolic hormone fibroblastic growth factor 21. In *European Journal of Endocrinology* (Vol. 167, Issue 3, pp. 301–309). https://doi.org/10.1530/EJE-12-0357

Ismail-Beigi, F. (2012). Glycemic management of type 2 diabetes mellitus. In *New England Journal of Medicine* (Vol. 366, Issue 14, pp. 1319–1327). https://doi.org/10.1056/NEJMcp1013127

Israel, G. D. (1992). Determining Sample Size 1 The Level Of Precision. *The Level Of Precision (Pp. 1–5).*, *November*, 1–5. http://sociology.soc.uoc.gr/socmedia/papageo/metaptyxiakoi/sample_size/samplesize1.pdf

Ittle, R. A. R. L. (2002). De ning the Relationship Between Plasma Glucose and HbA. *Diabetes Care*, *25*(2), 1–4. https://doi.org/10.2337/diacare.25.2.275

Jadhav, R., & Puchchakayala, G. (2012). Hypoglycemic and antidiabetic activity of flavonoids: Boswellic acid, Ellagic acid, Quercetin, Rutin on streptozotocin-nicotinamide induced type 2 diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(2), 251–256.

Jaeger, B. N., Parylak, S. L., & Gage, F. H. (2018). Mechanisms of dietary flavonoid action in neuronal function and neuroinflammation. In *Molecular Aspects of Medicine* (Vol. 61, pp. 50–62). https://doi.org/10.1016/j.mam.2017.11.003

Jaldin-Fincati, J. R., Pavarotti, M., Frendo-Cumbo, S., Bilan, P. J., & Klip, A. (2017). Update on GLUT4 Vesicle Traffic: A Cornerstone of Insulin Action. In *Trends in Endocrinology and Metabolism*. https://doi.org/10.1016/j.tem.2017.05.002

Jamjoom, G. A. (1991). Protease inhibitors as potential therapeutic agents for AIDS. In *Annals of Saudi Medicine* (Vol. 11, Issue 5, pp. 568–575). https://doi.org/10.5144/0256-4947.1991.568

Janssens, B., Van Damme, W., Raleigh, B., Gupta, J., Khem, S., Soy Ty, K., Vun, M. C., Fordd, N., & Zachariah, R. (2007). Offering integrated care for HIV/AIDS, diabetes and hypertension within chronic disease clinics in Cambodia. In *Bulletin of the World Health Organization* (Vol. 85, Issue 11, pp. 880–885). https://doi.org/10.2471/BLT.06.036574

Jean-Marie, E. (2018). Diagnosis and classification of diabetes mellitus. In *Encyclopedia of Endocrine Diseases*. https://doi.org/10.1016/B978-0-12-801238-3.65822-1

Jensen, J., Rustad, P. I., Kolnes, A. J., & Lai, Y. C. (2011). The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. In *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2011.00112

Jiang, G., & Zhang, B. B. (2003). Glucagon and regulation of glucose metabolism. In *American Journal of Physiology - Endocrinology and Metabolism* (Vol. 284, Issues 4 47-4). https://doi.org/10.1152/ajpendo.00492.2002

Jibhkate, S., Valand, A., Ansari, S., & Bharambe, B. (2014). Hyperparathyroidism complicating pregnancy: A diagnostic challenge? *Journal of Postgraduate Medicine*, *60*(3), 329–331. https://doi.org/10.4103/0022-3859.138825

Jiraungkoorskul, K., & Jiraungkoorskul, W. (2015). Sesbania grandiflora: New nutraceutical use as antidiabetic. In *International Journal of Pharmacy and Pharmaceutical Sciences* (Vol. 7, Issue 7, pp. 26–29).

Johnson, R. J., Nakagawa, T., Sanchez-Lozada, L. G., Shafiu, M., Sundaram, S., Le, M., Ishimoto, T., Sautin, Y. Y., & Lanaspa, M. A. (2013). Sugar, uric acid, and the etiology of diabetes and obesity. In *Diabetes* (Vol. 62, Issue 10, pp. 3307–3315). https://doi.org/10.2337/db12-1814

Joint United Nations Programme on HIV/AIDS. (2013). Global report: UNAIDS report on the global AIDS epidemic. In *UNAIDS*. http://files.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf

Jung, U. J., Lee, M.-K., Jeong, K.-S., & Choi, M.-S. (2004). The Hypoglycemic Effects of Hesperidin and Naringin Are Partly Mediated by Hepatic Glucose-Regulating Enzymes in C57BL/KsJ-db/db Mice. *The Journal of Nutrition*, *134*(10), 2499–2503. https://doi.org/10.1093/jn/134.10.2499

Jung, U. J., Lee, M. K., Park, Y. B., Kang, M. A., & Choi, M. S. (2006). Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. *International Journal of Biochemistry and Cell Biology*, *38*(7), 1134–1145. https://doi.org/10.1016/j.biocel.2005.12.002

Kahn, S. E., Cooper, M. E., & Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. In *The Lancet* (Vol. 383, Issue 9922, pp. 1068–1083). https://doi.org/10.1016/S0140-6736(13)62154-6

Kalra, S., & Agrawal, N. (2013). Diabetes and HIV: Current understanding and future perspectives. In *Current Diabetes Reports* (Vol. 13, Issue 3, pp. 419–427). https://doi.org/10.1007/s11892-013-0369-9

Kalra, S., Kalra, B., Agrawal, N., & Unnikrishnan, A. (2011). Understanding diabetes in patients with HIV/AIDS. In *Diabetology and Metabolic Syndrome* (Vol. 3, Issue 1). https://doi.org/10.1186/1758-5996-3-2

Kanaze, F. I., Gabrieli, C., Kokkalou, E., Georgarakis, M., & Niopas, I. (2003). Simultaneous reversed-phase high-performance liquid chromatographic method for the determination of diosmin, hesperidin and naringin in different citrus fruit juices and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis*. https://doi.org/10.1016/S0731-7085(03)00289-9

Kaur, J., Jiang, C., & Liu, G. (2019). Different strategies for detection of HbA1c emphasizing on biosensors and point-of-care analyzers. *Biosensors and Bioelectronics*, *123*, 85–100. https://doi.org/10.1016/j.bios.2018.06.018

Kazafeos, K. (2011). Incretin effect: GLP-1, GIP, DPP4. *Diabetes Research and Clinical Practice*, *93*(SUPPL. 1). https://doi.org/10.1016/S0168-8227(11)70011-0

Kent, B. D., McNicholas, W. T., & Ryan, S. (2015). Insulin resistance, glucose intolerance and diabetes mellitus in obstructive sleep apnoea. In *Journal of Thoracic Disease* (Vol. 7, Issue 8, pp. 1343–1357). https://doi.org/10.3978/j.issn.2072-1439.2015.08.11

Keogh, J. B., Woonton, B. W., Taylor, C. M., Janakievski, F., Desilva, K., & Clifton, P. M. (2010). Effect of glycomacropeptide fractions on cholecystokinin and food intake. *British Journal of Nutrition*, *104*(2), 286–290. https://doi.org/10.1017/S0007114510000280

Khattab, M., Khader, Y. S., Al-Khawaldeh, A., & Ajlouni, K. (2010). Factors associated with poor glycemic control among patients with Type 2 diabetes. *Journal of Diabetes and Its Complications*, *24*(2), 84–89. https://doi.org/10.1016/j.jdiacomp.2008.12.008

Kirschbaum, C., Bono, E. G., Rohleder, N., Gessner, C., Pirke, K. M., Salvador, A., & Hellhammer, D. H. (1997). Effects of Fasting and Glucose Load on Free Cortisol Responses to Stress and Nicotine 1. *The Journal of Clinical Endocrinology & Metabolism*, *82*(4), 1101–1105. https://doi.org/10.1210/jcem.82.4.3882

Kleinridders, A., Ferris, H. A., Cai, W., & Kahn, C. R. (2014). Insulin action in brain regulates systemic metabolism and brain function. *Diabetes*, *63*(7), 2232–2243. https://doi.org/10.2337/db14-0568

Koster, J. C., Remedi, M. S., Qiu, H., Nichols, C. G., & Hruz, P. W. (2003). HIV protease inhibitors acutely impair glucose-stimulated insulin release. *Diabetes*, *52*(7), 1695–1700. https://doi.org/10.2337/diabetes.52.7.1695

Koutkia, P., & Grinspoon, S. (2004). HIV–Associated Lipodystrophy: Pathogenesis, Prognosis, Treatment, and Controversies. *Annual Review of Medicine*, *55*(1), 303–317. https://doi.org/10.1146/annurev.med.55.091902.104412

Koziara, Baranowska, Bartoszek, & Namieśnik. (2019). Comparison of Redox Properties of Flavonoid Aglycones and Corresponding Glycosides and Their Mixtures in the Cellular Model. *Proceedings*, *11*(1), 25. https://doi.org/10.3390/proceedings2019011025

Kozka, I. J., Clark, A. E., & Holman, G. D. (1991). Chronic treatment with insulin selectively down-regulates cell-surface GLUT4 glucose transporters in 3T3-L1 adipocytes. *Journal of Biological Chemistry*, *266*(18), 11726–11731.

Kozłowska, A., & Szostak-Wegierek, D. (2014). Flavonoids--food sources and health benefits. In *Roczniki Pa??stwowego Zak??adu Higieny* (Vol. 65, Issue 2, pp. 79–85).

Ładyżyński, P., Wójcicki, J. M., Bąk, M. I., Sabalińska, S., Kawiak, J., Foltyński, P., Krzymień, J., & Karnafel, W. (2011). Hemoglobin glycation rate constant in non-diabetic individuals. *Annals of Biomedical Engineering*. https://doi.org/10.1007/s10439-011-0366-6

Lavle, N., Shukla, P., & Panchal, A. (2016). Role of Flavonoids and Saponins in the Treatment of Diabetes Mellitus. *Journal of Pharmaceutical Science Bioscientific Research*, *6*(64), 535–541. http://www.jpsbr.org/volume_6/JPSBR_Vol_6_Issue_1_htm_files/JPSBR16RS4020.pdf

Lee, G. A., Rao, M. N., & Grunfeld, C. (2005). The effects of HIV protease inhibitors on carbohydrate and lipid metabolism. In *Current HIV/AIDS reports* (Vol. 2, Issue 1, pp. 39–50). https://doi.org/10.1007/s11904-996-0008-z

Li, C., & Schluesener, H. (2017). Health-promoting effects of the citrus flavanone hesperidin. *Critical Reviews in Food Science and Nutrition*, *57*(3), 613–631. https://doi.org/10.1080/10408398.2014.906382

Li, J., Bai, L., Li, X., He, L., Zheng, Y., Lu, H., Li, J., Zhong, L., Tong, R., Jiang, Z., & Shi, J. (2019). Antidiabetic potential of flavonoids from traditional Chinese medicine: A review. In *American Journal of Chinese Medicine* (Vol. 47, Issue 5, pp. 933–957). https://doi.org/10.1142/S0192415X19500496

Li, X., Xue, Y., Cheng, H., Lin, Y., Zhou, L., Ning, Z., Wang, X., Yu, X., Zhang, W., Shen, F., Zheng, X., Gai, J., Li, X., Kang, L., Nyambi, P., Wang, Y., Zhuang, M., Pan, Q., Zhuang, X., & Zhong, P. (2015). HIV-1 genetic diversity and its impact on baseline CD4+T cells and viral loads among recently infected men who have sex with men in Shanghai, China. *PLoS ONE*, *10*(6). https://doi.org/10.1371/journal.pone.0129559

Lien, L. F., & Feinglos, M. N. (2005). Protease inhibitor-induced diabetic complications: Incidence, management and prevention. In *Drug Safety* (Vol. 28, Issue 3, pp. 209–226). https://doi.org/10.2165/00002018-200528030-00003

Lind, M., Odén, A., Fahlén, M., & Eliasson, B. (2009). The true value of HbA1c as a predictor of diabetic complications: Simulations of HbA1c variables. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0004412

Long, Y. C., Cheng, Z., Copps, K. D., & White, M. F. (2011). Insulin Receptor Substrates Irs1 and Irs2 Coordinate Skeletal Muscle Growth and Metabolism via the Akt and AMPK Pathways. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.00983-10

Lowe, N. M. (2007). Introduction to Human Nutrition. *Maternal & Child Nutrition*, *3*(1), 69–69. https://doi.org/10.1111/j.1740-8709.2007.00073.x

Lukačínová, A., Mojžiš, J., Beňačka, R., Keller, J., Maguth, T., Kurila, P., Vaško, L., Rácz, O., & Ništiar, F. (2008). Preventive effects of flavonoids on alloxan-induced diabetes mellitus in rats. *Acta Veterinaria Brno*, *77*(2), 175–182. https://doi.org/10.2754/avb200877020175

Lukačínová, A., Mojžiš, J., Beňačka, R., Rácz, O., & Ništiar, F. (2008). Structure-activity relationships of preventive effects of flavonoids in alloxan-induced diabetes mellitus in rats. *Journal of Animal and Feed Sciences*. https://doi.org/10.22358/jafs/66635/2008

Lv, Z., Chu, Y., & Wang, Y. (2015). HIV protease inhibitors: A review of molecular selectivity and toxicity. *HIV/AIDS - Research and Palliative Care*, *7*, 95–104. https://doi.org/10.2147/HIV.S79956

Maemondo, M., Inoue, A., Kobayashi, K., Sugawara, S., Oizumi, S., Isobe, H., Gemma, A., Harada, M., Yoshizawa, H., Kinoshita, I., Fujita, Y., Okinaga, S., Hirano, H., Yoshimori, K., Harada, T., Ogura, T., Ando, M., Miyazawa, H., Tanaka, T., … Nukiwa, T. (2010). Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *New England Journal of Medicine*, *362*(25), 2380–2388. https://doi.org/10.1056/NEJMoa0909530

Mahajan, R. D., & Mishra, B. (2011). Using Glycated Hemoglobin HbA1c for diagnosis of Diabetes mellitus : An Indian perspective . *International Journal of Biological & Medical Research*, *2*(2), 508–512.

Mahmoud, A. M., Ahmed, O. M., Ashour, M. B., & Abdel-Moneim, A. (2015). In vivo and in vitro antidiabetic effects of citrus flavonoids; a study on the mechanism of action. *International Journal of Diabetes in Developing Countries*, *35*(3), 250–263. https://doi.org/10.1007/s13410-014-0268-x

Mahmoud, A. M., Ashour, M. B., Abdel-Moneim, A., & Ahmed, O. M. (2012). Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats. *Journal of Diabetes and Its Complications*, *26*(6), 483–490. https://doi.org/10.1016/j.jdiacomp.2012.06.001

Marella, S. (2017). Flavonoids-The Most Potent Poly-phenols as Antidiabetic Agents: An Overview. *Modern Approaches in Drug Designing*, *1*(3). https://doi.org/10.31031/madd.2017.01.000513

Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C., & Cañizo-Gómez, F. J. del. (2016). Update on the treatment of type 2 diabetes mellitus. *World Journal of Diabetes*, *7*(17), 354. https://doi.org/10.4239/wjd.v7.i17.354

Miranda Pedroso, T. F. de, Bonamigo, T. R., da Silva, J., Vasconcelos, P., Félix, J. M., Cardoso, C. A. L., Souza, R. I. C., dos Santos, A. C., Volobuff, C. R. F., Formagio, A. S. N., & Trichez, V. D. K. (2019). Chemical constituents of Cochlospermum regium (Schrank) Pilg. root and its antioxidant, antidiabetic, antiglycation, and anticholinesterase effects in Wistar rats. *Biomedicine and Pharmacotherapy*, *111*, 1383–1392. https://doi.org/10.1016/j.biopha.2019.01.005

MOH. (2014). Guidelines on use of antiretroviral drugs for treating and preventing HIV infection. Rapid Advice. In *National AIDS and STI Control Program, Ministry of Health Kenya* (Issue June). http://healthservices.uonbi.ac.ke/node/1963

Mohan, S., & Nandhakumar, L. (2014). Role of various flavonoids: Hypotheses on novel approach to treat diabetes. *Journal of Medical Hypotheses and Ideas*. https://doi.org/10.1016/j.jmhi.2013.06.001

Molina, J. M., Andrade-Villanueva, J., Echevarria, J., Chetchotisakd, P., Corral, J., David, N., Moyle, G., Mancini, M., Percival, L., Yang, R., Thiry, A., & McGrath, D. (2008). Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. *The Lancet*, *372*(9639), 646–655. https://doi.org/10.1016/S0140-6736(08)61081-8

Molina, J. M., Andrade-Villanueva, J., Echevarria, J., Chetchotisakd, P., Corral, J., David, N., Moyle, G., Mancini, M., Percival, L., Yang, R., Wirtz, V., Lataillade, M., Absalon, J., & McGrath, D. (2010). Once-daily atazanavir/ritonavir compared with twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 96-week efficacy and safety results of the CASTLE study. *Journal of Acquired Immune Deficiency Syndromes*, *53*(3), 323–332. https://doi.org/10.1097/QAI.0b013e3181c990bf

Monograph. Diosmin. (2004). In *Alternative medicine review : a journal of clinical therapeutic* (Vol. 9, Issue 3, pp. 308–311).

Moran, C. A., Neale Weitzmann, M., & Ofotokun, I. (2016). The protease inhibitors and HIV-associated bone loss. In *Current Opinion in HIV and AIDS* (Vol. 11, Issue 3, pp. 333–342). https://doi.org/10.1097/COH.0000000000000260

Murata, H., Hruz, P. W., & Mueckler, M. (2000). The mechanism of insulin resistance caused by HIV protease inhibitor therapy. *Journal of Biological Chemistry*, *275*(27), 20251–20254. https://doi.org/10.1074/jbc.C000228200

Murata, H., Hruz, P. W., & Mueckler, M. (2002). Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations. *AIDS*, *16*(6), 859–863. https://doi.org/10.1097/00002030-200204120-00005

Nacc/Nascop. (2012). The Kenya AIDS epidemic UPDATE 2012. *The Kenya AIDS Epidemic Update 2012*, 1–62. https://doi.org/13.12.2013

NACC. (2014). Kenya AIDS Strategic Framework 2014/2015 - 2018/2019. *National AIDS Control Council*, 1–84. http://www.nacc.or.ke/attachments/article/181/KNASP III.pdf

Nathan, D. M., Kuenen, J., Borg, R., Zheng, H., Schoenfeld, D., & Heine, R. J. (2008). Translating the A1C assay into estimated average glucose values. *Diabetes Care*, *31*(8), 1473–1478. https://doi.org/10.2337/dc08-0545

Neely, R. D. G., Rooney, D. P., Bell, P. M., Bell, N. P., Sheridan, B., Atkinson, A. B., & Trimble, E. R. (1992). Influence of growth hormone on glucose-glucose 6-phosphate cycle and insulin action in normal humans. *American Journal of Physiology - Endocrinology and Metabolism*, *263*(5 26-5). https://doi.org/10.1152/ajpendo.1992.263.5.e980

Nichols, J. D., Bailey, L. L., Jr., O. A. F., Talancy, N. W., Grant, E. H. C., Gilbert, A. T., Annand, E. M., Husband, T. P., & Hines, J. E. (2008). Importance of regional species pools and functional traits in colonization processes: predicting re-colonization after large-scale destruction of ecosystems. *Journal of Applied Ecology*, *45*(NOVEMBER), 1321–1329. https://doi.org/10.1111/j.1365-2664.2007.0

Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E. C., Boelens, P. G., Van Norren, K., & Van Leeuwen, P. A. M. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. In *American Journal of Clinical Nutrition* (Vol. 74, Issue 4, pp. 418–425). https://doi.org/10.1093/ajcn/74.4.418

Noor, M. A., Flint, O. P., Maa, J. F., & Parker, R. A. (2006). Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity: Demonstrable differences in vitro and clinically. *AIDS*, *20*(14), 1813–1821. https://doi.org/10.1097/01.aids.0000244200.11006.55

Noor, M. A., Parker, R. A., O'Mara, E., Grasela, D. M., Currie, A., Hodder, S. L., Fiedorek, F. T., & Haas, D. W. (2004). The effects of HIV protease inhibitors atazanavir and lopinavir/ritonavir on insulin sensitivity in HIV-seronegative healthy adults. *AIDS*. https://doi.org/10.1097/00002030-200411050-00005

Oladele, S. ., Ayo, J. ., & Adaudi, A. . (2010). Medicinal and physiological properties of flavonoids, coumarin derivatives and anthraqoinones of plant origin. *West African Journal of Pharmacology and Drug Research*, *11*(1). https://doi.org/10.4314/wajpdr.v11i1.53394

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: A review of current trends. In *Oman Medical Journal* (Vol. 27, Issue 4, pp. 269–273). https://doi.org/10.5001/omj.2012.68

Olson, A. L. (2012). Regulation of GLUT4 and Insulin-Dependent Glucose Flux. *ISRN Molecular Biology*. https://doi.org/10.5402/2012/856987

Omodanisi, E. I., Aboua, G. Y., & Oguntibeju, O. O. (2017). Therapeutic potentials and pharmacological properties of moringa oleifera lam in the treatment of diabetes mellitus and related complications. In *Tropical Journal of Pharmaceutical Research* (Vol. 16, Issue 7, pp. 1737–1746). https://doi.org/10.4314/tjpr.v16i7.35

Osende, J. I., Badimon, J. J., Fuster, V., Herson, P., Rabito, P., Vidhun, R., Zaman, A., Rodriguez, O. J., Lev, E. I., Rauch, U., Heflt, G., Fallon, J. T., & Crandall, J. P. (2001). Blood thrombogenicity in type 2 diabetes mellitus patients is associated with glycemic control. *Journal of the American College of Cardiology*. https://doi.org/10.1016/S0735-1097(01)01555-8

Ozougwu, O. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology*, *4*(4), 46–57. https://doi.org/10.5897/jpap2013.0001

Palella, F. J., Delaney, K. M., Moorman, A. C., Loveless, M. O., Fuhrer, J., Satten, G. A., Aschman, D. J., & Holmberg, S. D. (1998). Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *New England Journal of Medicine*, *338*(13), 853–860. https://doi.org/10.1056/NEJM199803263381301

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. In *Journal of Nutritional Science* (Vol. 5). https://doi.org/10.1017/jns.2016.41

Pari, L., & Srinivasan, S. (2010). Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. *Biomedicine and Pharmacotherapy*, *64*(7), 477–481. https://doi.org/10.1016/j.biopha.2010.02.001

Park, H. J., Jung, U. J., Cho, S. J., Jung, H. K., Shim, S., & Choi, M. S. (2013). Citrus unshiu peel extract ameliorates hyperglycemia and hepatic steatosis by altering inflammation and hepatic glucose- and lipid-regulating enzymes in db/db mice. *Journal of Nutritional Biochemistry*, *24*(2), 419–427. https://doi.org/10.1016/j.jnutbio.2011.12.009

Pashikanti, S., de Alba, D. R., Boissonneault, G. A., & Cervantes-Laurean, D. (2010). Rutin metabolites: Novel inhibitors of nonoxidative advanced glycation end products. *Free Radical Biology and Medicine*. https://doi.org/10.1016/j.freeradbiomed.2009.11.019

Paterson, D. L., Swindells, S., Mohr, J., Brester, M., Vergis, E. N., Squier, C., Wagener, M. M., Singh, N., & Hudson, B. (2000). Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Annals of Internal Medicine*, *133*(1), 21–30. https://doi.org/10.7326/0003-4819-133-1-200007040-00004

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. In *Physiological Reviews*. https://doi.org/10.1152/physrev.00063.2017

Petitti, D. B., Klingensmith, G. J., Bell, R. A., Andrews, J. S., Dabelea, D., Imperatore, G., Marcovina, S., Pihoker, C., Standiford, D., Waitzfelder, B., & Mayer-Davis, E. (2009). Glycemic Control in Youth with Diabetes: The SEARCH for Diabetes in Youth Study. *Journal of Pediatrics*, *155*(5). https://doi.org/10.1016/j.jpeds.2009.05.025

Petoumenos, K., & Worm, S. W. (2011). HIV infection, aging and cardiovascular disease: Epidemiology and prevention. *Sexual Health*, *8*(4), 465–473. https://doi.org/10.1071/SH11020

Piatti, P. M., Monti, L. D., Caumo, A., Conti, M., Magni, F., Galli-Kienle, M., Fochesato, E., Pizzini, A., Baldi, L., Valsecchi, G., & Pontiroli, A. E. (1999). Mediation of the hepatic effects of growth hormone by its lipolytic activity. *Journal of Clinical Endocrinology and Metabolism*, *84*(5), 1658–1663. https://doi.org/10.1210/jcem.84.5.5685

Pokorná, J., Machala, L., Řezáčová, P., & Konvalinka, J. (2009). Current and novel inhibitors of HIV protease. In *Viruses* (Vol. 1, Issue 3, pp. 1209–1239). https://doi.org/10.3390/v1031209

Qaid, M. M., & Abdelrahman, M. M. (2016). Role of insulin and other related hormones in energy metabolism���A review. *Cogent Food & Agriculture*. https://doi.org/10.1080/23311932.2016.1267691

Ramkissoon, J. S., Mahomoodally, M. F., Ahmed, N., & Subratty, A. H. (2013). Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs. *Asian Pacific Journal of Tropical Medicine*, *6*(7), 561–569. https://doi.org/10.1016/S1995-7645(13)60097-8

Ramnanan, C. J., Edgerton, D. S., Kraft, G., & Cherrington, A. D. (2011). Physiologic action of glucagon on liver glucose metabolism. In *Diabetes, Obesity and Metabolism* (Vol. 13, Issue SUPPL. 1, pp. 118–125). https://doi.org/10.1111/j.1463-1326.2011.01454.x

Ravishankar, D., Rajora, A. K., Greco, F., & Osborn, H. M. I. (2013). Flavonoids as prospective compounds for anti-cancer therapy. In *International Journal of Biochemistry and Cell Biology* (Vol. 45, Issue 12, pp. 2821–2831). https://doi.org/10.1016/j.biocel.2013.10.004Review

Rayner, D. V., Thomas, M. E. A., & Trayhurn, P. (1994). Glucose transporters (GLUTs 1-4) and their mRNAs in regions of the rat brain: Insulin-sensitive transporter expression in the cerebellum. *Canadian Journal of Physiology and Pharmacology*. https://doi.org/10.1139/y94-069

Resistance, I. (2004). Insulin Resistance and ᴺʟ -Cell Function. *Diabetes*.

Reust, C. E. (2011). Common adverse effects of antiretroviral therapy for HIV disease. *American Family Physician*, *83*(12), 1443–1451.

Rizza, R. A., Mandarino, L. J., & Gerich, J. E. (1982). Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. *Diabetes*, *31*(8), 663–669. https://doi.org/10.2337/diab.31.8.663

Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. In *Experimental & molecular medicine* (Vol. 48, p. e219). https://doi.org/10.1038/emm.2016.6

Rohlfing, C. L., Wiedmeyer, H. M., Little, R. R., England, J. D., Tennill, A., & Goldstein, D. E. (2002). Defining the relationship between plasma glucose and HbA1c: Analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. *Diabetes Care*. https://doi.org/10.2337/diacare.25.2.275

Rudich, A., Vanounou, S., Riesenberg, K., Porat, M., Tirosh, A., Harman-Boehm, I., Greenberg, A. S., Schlaeffer, F., & Bashan, N. (2001). The HIV protease inhibitor nelfinavir induces insulin resistance and increases basal lipolysis in 3T3-L1 adipocytes. *Diabetes*, *50*(6), 1425–1431. https://doi.org/10.2337/diabetes.50.6.1425

Rufinatscha, K., Ress, C., Folie, S., Haas, S., Salzmann, K., Moser, P., Dobner, J., Weiss, G., Iruzubieta, P., Arias-Loste, M. T., Crespo, J., Tilg, H., & Kaser, S. (2018). Metabolic effects of reduced growth hormone action in fatty liver disease. *Hepatology International*, *12*(5), 474–481. https://doi.org/10.1007/s12072-018-9893-7

Ruiz, E., Rodriguez, P., Valero, T., Ávila, J. M., Aranceta-Bartrina, J., Gil, Á., González-Gross, M., Ortega, R. M., Serra-Majem, L., & Varela-Moreiras, G. (2017). Dietary intake of individual (Free and intrinsic) sugars and food sources in the Spanish population: Findings from the ANIBES study. *Nutrients*, *9*(3). https://doi.org/10.3390/nu9030275

Ruiz, E., & Varela-Moreiras, G. (2017). Adequacy of the dietary intake of total and added sugars in the Spanish diet to the recommendations: ANIBES study. *Nutricion Hospitalaria*, *34*, 45–52. https://doi.org/10.20960/nh.1571

Russo, B., Picconi, F., Malandrucco, I., & Frontoni, S. (2019). Flavonoids and insulin-resistance: from molecular evidences to clinical trials. In *International Journal of Molecular Sciences* (Vol. 20, Issue 9). https://doi.org/10.3390/ijms20092061

Sahnoun, M., Trabelsi, S., & Bejar, S. (2017). Citrus flavonoids collectively dominate the α-amylase and α-glucosidase inhibitions. *Biologia (Poland)*, *72*(7), 764–773. https://doi.org/10.1515/biolog-2017-0091

Saini, V. (2010). Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. *World Journal of Diabetes*, *1*(3), 68. https://doi.org/10.4239/wjd.v1.i3.68

Salam, N. K., Huang, T. H. W., Kota, B. P., Kim, M. S., Li, Y., & Hibbs, D. E. (2008). Novel PPAR-gamma agonists identified from a natural product library: A virtual screening, induced-fit docking and biological assay study. *Chemical Biology and Drug Design*, *71*(1), 57–70. https://doi.org/10.1111/j.1747-0285.2007.00606.x

Salehian, B., Bilas, J., Bazargan, M., & Abbasian, M. (2005). Prevalence and incidence of diabetes in HIV-infected minority patients on protease inhibitors. *Journal of the National Medical Association*, *97*(8), 1088–1092.

Salib, J. Y., Michael, H. N., & Eskande, E. F. (2013). Anti-diabetic properties of flavonoid compounds isolated from Hyphaene thebaica epicarp on alloxan induced diabetic rats. *Pharmacognosy Research*, *5*(1), 22–29. https://doi.org/10.4103/0974-8490.105644

Sangraula, H., Sharma, K. K., & Dwivedi, S. (2001). Antiretroviral protease inhibitor therapy leads to hyperglycaemia, hyperlipidaemia and lipodystrophy. *National Medical Journal of India*, *14*(6), 347–348.

Santiprabhob, J., Tanchaweng, S., Maturapat, S., Maleesatharn, A., Lermankul, W., Sricharoenchai, S., Wittawatmongkol, O., Lapphra, K., Phongsamart, W., & Chokephaibulkit, K. (2017). Metabolic Disorders in HIV-Infected Adolescents Receiving Protease Inhibitors. *BioMed Research International*. https://doi.org/10.1155/2017/7481597

Sayed, A., Alyafei, F., De Sanctis, V., Soliman, A., & Elgamal, M. (2018). Translating the HbA1c assay into estimated average glucose values in children and adolescents with type 1 diabetes mellitus. *Acta Biomedica*, *89*, 22–26. https://doi.org/10.23750/abm.v89iS4.7357

Schütt, M., Zhou, J., Meier, M., & Klein, H. H. (2004). Long-term effects of HIV-1 protease inhibitors on insulin secretion and insulin signaling in INS-1 beta cells. *Journal of Endocrinology*, *183*(3), 445–454. https://doi.org/10.1677/joe.1.05620

Sellamuthu, P. S., Arulselvan, P., Muniappan, B. P., Fakurazi, S., & Kandasamy, M. (2013). Mangiferin from salacia chinensis prevents oxidative stress and protects pancreatic β-cells in streptozotocin-induced diabetic rats. *Journal of Medicinal Food*, *16*(8), 719–727. https://doi.org/10.1089/jmf.2012.2480

Sen, S., Kar, M., Roy, A., & Chakraborti, A. S. (2005). Effect of nonenzymatic glycation on functional and structural properties of hemoglobin. *Biophysical Chemistry*. https://doi.org/10.1016/j.bpc.2004.05.005

Sevrioukova, I. F., & Poulos, T. L. (2010). Structure and mechanism of the complex between cytochrome P4503A4 and ritonavir. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(43), 18422–18427. https://doi.org/10.1073/pnas.1010693107

Sharp, P. M., & Hahn, B. H. (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harbor Perspectives in Medicine*. https://doi.org/10.1101/cshperspect.a006841

Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A., & Sakharkar, M. K. (2016). Significance of HbA1c test in diagnosis and prognosis of diabetic patients. In *Biomarker Insights* (Vol. 11, pp. 95–104). https://doi.org/10.4137/Bmi.s38440

Shrayyef, M. Z., & Gerich, J. E. (2010). Normal Glucose Homeostasis. In *Principles of Diabetes Mellitus* (pp. 19–35). https://doi.org/10.1007/978-0-387-09841-8_2

Sim, E. H. A., Yang, I. A., Wood-Baker, R., Bowman, R. V., & Fong, K. M. (2018). Gefitinib for advanced non-small cell lung cancer. In *Cochrane Database of Systematic Reviews* (Vol. 2018, Issue 1). https://doi.org/10.1002/14651858.CD006847.pub2

Singh, R., Kaur, N., Kishore, L., & Kumar Gupta, G. (2013). Management of diabetic complications: A chemical constituents based approach. In *Journal of Ethnopharmacology* (Vol. 150, Issue 1, pp. 51–70). https://doi.org/10.1016/j.jep.2013.08.051

Škrha, J., Šoupal, J., Škrha, J., & Prázný, M. (2016). Glucose variability, HbA1c and microvascular complications. In *Reviews in Endocrine and Metabolic Disorders*. https://doi.org/10.1007/s11154-016-9347-2

Sreelatha, S., & Inbavalli, R. (2012). Antioxidant, Antihyperglycemic, and Antihyperlipidemic Effects of Coriandrum sativum Leaf and Stem in Alloxan-Induced Diabetic Rats. *Journal of Food Science*, *77*(7). https://doi.org/10.1111/j.1750-3841.2012.02755.x

Stanley, T. L., Joy, T., Hadigan, C. M., Liebau, J. G., Makimura, H., Chen, C. Y., Thomas, B. J., Weise, S. B., Robbins, G. K., & Grinspoon, S. K. (2009). Effects of switching from lopinavir/ritonavir to atazanavir/ritonavir on muscle glucose uptake and visceral fat in HIV-infected patients. *AIDS*, *23*(11), 1349–1357. https://doi.org/10.1097/QAD.0b013e32832ba904

Stolar, M. (2010). Glycemic Control and Complications in Type 2 Diabetes Mellitus. *American Journal of Medicine*, *123*(3 SUPPL.). https://doi.org/10.1016/j.amjmed.2009.12.004

Stumvoll, M., Goldstein, B. J., & Van Haeften, T. W. (2005). Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet*, *365*(9467), 1333–1346. https://doi.org/10.1016/S0140-6736(05)61032-X

Sun, H. ming, Chen, F., Yin, H. lin, Xu, X. yong, Liu, H. bing, & Zhao, B. lei. (2017). Rapid development of metastatic pulmonary calcifications in primary hyperparathyroidism: A case report and literature review. *Diagnostic Pathology*, *12*(1). https://doi.org/10.1186/s13000-017-0628-1

Taborsky, G. J. (2010). The physiology of glucagon. *Journal of Diabetes Science and Technology*, *4*(6), 1338–1344. https://doi.org/10.1177/193229681000400607

Tapas, A., Sakarkar, D., & Kakde, R. (2008). Flavonoids as Nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research*, *7*(3). https://doi.org/10.4314/tjpr.v7i3.14693

Tebas, P. (2008). Insulin resistance and diabetes mellitus associated with antiretroviral use in HIV-infected patients: pathogenesis, prevention, and treatment options. In *Journal of acquired immune deficiency syndromes (1999): Vol. 49 Suppl 2*. https://doi.org/10.1097/QAI.0b013e31818651e6

Testa, R., Bonfigli, A. R., Genovese, S., De Nigris, V., & Ceriello, A. (2016). The possible role of flavonoids in the prevention of diabetic complications. In *Nutrients* (Vol. 8, Issue 5). https://doi.org/10.3390/nu8050310

Toledo, R. R., Santos, M. E. R. de C., & Schnaider, T. B. (2017). Effect of Pycnogenol on the Healing of Venous Ulcers. *Annals of Vascular Surgery*. https://doi.org/10.1016/j.avsg.2016.04.014

Trial, C. (2002). Defining the Relationship Between Plasma Glucose and HbA 1c. *Diabetes Care*.

Tsiodras, S., Mantzoros, C., Hammer, S., & Samore, M. (2000). Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: A 5-year cohort study. *Archives of Internal Medicine*, *160*(13), 2050–2056. https://doi.org/10.1001/archinte.160.13.2050

Vancampfort, D., Holt, R. I. G., Stubbs, B., De Hert, M., Samaras, K., & Mitchell, A. J. (2016). Type 2 Diabetes Mellitus. In *Life-Threatening Effects of Antipsychotic Drugs* (pp. 255–272). https://doi.org/10.1016/B978-0-12-803376-0.00012-5

Vélez, E. J., Perelló, M., Azizi, S., Moya, A., Lutfi, E., Pérez-Sánchez, J., Calduch-Giner, J. A., Navarro, I., Blasco, J., Fernández-Borràs, J., Capilla, E., & Gutiérrez, J. (2018). Recombinant bovine growth hormone (rBGH) enhances somatic growth by regulating the GH-IGF axis in fingerlings of gilthead sea bream (Sparus aurata). *General and Comparative Endocrinology*, *257*, 192–202. https://doi.org/10.1016/j.ygcen.2017.06.019

Vessal, M., Hemmati, M., & Vasei, M. (2003). Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *135*(3), 357–364. https://doi.org/10.1016/S1532-0456(03)00140-6

Viganò, A., Brambilla, P., Pattarino, G., Stucchi, S., Fasan, S., Raimondi, C., Cerini, C., Giacomet, V., Zuccotti, G. V., & Bedogni, G. (2009). Long-term evaluation of glucose homeostasis in a cohort of HAART-treated HIV-infected children: A longitudinal, observational cohort study. *Clinical Drug Investigation*, *29*(2), 101–109. https://doi.org/10.2165/0044011-200929020-00004

Vinayagam, R., & Xu, B. (2015). Antidiabetic properties of dietary flavonoids: A cellular mechanism review. In *Nutrition and Metabolism* (Vol. 12, Issue 1). https://doi.org/10.1186/s12986-015-0057-7

Vos, M. J., Lenters-Westra, E., & Bilo, H. J. G. (2012). [HbA1c]. *Nederlands Tijdschrift Voor Geneeskunde*.

Wang, Z., Yang, Y., Xiang, X., Zhu, Y., Men, J., & He, M. (2010). [Estimation of the normal range of blood glucose in rats]. *Wei Sheng Yan Jiu = Journal of Hygiene Research*, *39*(2).

Watson, R. T., & Pessin, J. E. (2001). Intracellular organization of insulin signaling and GLUT4 translocation. In *Recent Progress in Hormone Research*. https://doi.org/10.1210/rp.56.1.175

Weaver, C. M., Dwyer, J., Fulgoni, V. L., King, J. C., Leveille, G. A., MacDonald, R. S., Ordovas, J., & Schnakenberg, D. (2014). Processed foods: Contributions to nutrition. *American Journal of Clinical Nutrition*, *99*(6), 1525–1542. https://doi.org/10.3945/ajcn.114.089284

Weiss, R. A. (1993). How does HIV cause AIDS? *Science*. https://doi.org/10.1126/science.8493571

Wensing, A. M. J., van Maarseveen, N. M., & Nijhuis, M. (2010). Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance. In *Antiviral Research* (Vol. 85, Issue 1, pp. 59–74). https://doi.org/10.1016/j.antiviral.2009.10.003

Weykamp, C., John, W. G., & Mosca, A. (2009). A review of the challenge in measuring hemoglobin A1c. *Journal of Diabetes Science and Technology*, *3*(3), 439–445. https://doi.org/10.1177/193229680900300306

Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). Flavonoids: Antioxidants or signalling molecules? In *Free Radical Biology and Medicine* (Vol. 36, Issue 7, pp. 838–849). https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Williamson, G. (2017). The role of polyphenols in modern nutrition. In *Nutrition Bulletin* (Vol. 42, Issue 3, pp. 226–235). https://doi.org/10.1111/nbu.12278

Wilmsen, P. K., Spada, D. S., & Salvador, M. (2005). Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. *Journal of Agricultural and Food Chemistry*, *53*(12), 4757–4761. https://doi.org/10.1021/jf0502000

Woerle, H. J., Mariuz, P. R., Meyer, C., Reichman, R. C., Popa, E. M., Dostou, J. M., Welle, S. L., & Gerich, J. E. (2003a). Mechanisms for the deterioration in glucose tolerance associated with HIV protease inhibitor regimens. *Diabetes*, *52*(4), 918–925. https://doi.org/10.2337/diabetes.52.4.918

Woerle, H. J., Mariuz, P. R., Meyer, C., Reichman, R. C., Popa, E. M., Dostou, J. M., Welle, S. L., & Gerich, J. E. (2003b). Mechanisms for the deterioration in glucose tolerance associated with HIV protease inhibitor regimens. *Diabetes*, *52*(4), 918–925. https://doi.org/10.2337/diabetes.52.4.918

Wong, V. W., Chong, S., Mediratta, S., & Jalaludin, B. (2017). Measuring glycated haemoglobin in women with gestational diabetes mellitus: How useful is it? *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *57*(3), 260–265. https://doi.org/10.1111/ajo.12511

Wood, I. S., & Trayhurn, P. (2003). Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *British Journal of Nutrition*. https://doi.org/10.1079/bjn2002763

Yamagata, K. (2018). Metabolic Syndrome: Preventive Effects of Dietary Flavonoids. In *Studies in Natural Products Chemistry*. https://doi.org/10.1016/B978-0-444-64181-6.00001-2

Yu, J., Bi, X., Yu, B., & Chen, D. (2016). Isoflavones: Anti-inflammatory benefit and possible caveats. In *Nutrients* (Vol. 8, Issue 6). https://doi.org/10.3390/nu8060361

Zeldin, R. K., & Petruschke, R. A. (2004). Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients. *Journal of Antimicrobial Chemotherapy*, *53*(1), 4–9. https://doi.org/10.1093/jac/dkh029

Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. In *Nature Reviews Endocrinology* (Vol. 14, Issue 2, pp. 88–98). https://doi.org/10.1038/nrendo.2017.151

Zhuang, J., Jetzt, A. E., Sun, G., Yu, H., Klarmann, G., Ron, Y., Preston, B. D., & Dougherty, J. P. (2002). Human Immunodeficiency Virus Type 1 Recombination: Rate, Fidelity, and Putative Hot Spots. *Journal of Virology*, *76*(22), 11273–11282. https://doi.org/10.1128/jvi.76.22.11273-11282.2002

Zick, Y. (2003). Role of Ser/Thr kinases in the uncoupling of insulin signaling. *International Journal of Obesity*, *27*, S56–S60. https://doi.org/10.1038/sj.ijo.0802503

Zick, Yehiel. (2001). Insulin resistance: A phosphorylation-based uncoupling of insulin signaling. In *Trends in Cell Biology* (Vol. 11, Issue 11, pp. 437–441). https://doi.org/10.1016/S0962-8924(01)02129-8

Zolopa, A. R., Andersen, J., Komarow, L., Sanne, I., Sanchez, A., Hogg, E., Suckow, C., & Powderly, W. (2009). Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: A multicenter randomized strategy trial. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0005575

APPENDICES

Appendix 1:Animals, materials and reagents

1. Seventy two maleWistar rats with average weights of 200 ±50g.

3. Daflon™ tablets- 5*30 tablets

4.2cc syringes – 200 pieces

5. Pestle and mortar

6. Measuring cylinder (500ml)

7. 500ml beakers- 4

8. Glucometer machine (On call PLUS™) -2

9. Glucometer strips-1000 strips

10. Rat housings/cages-60

11. Drinking water bottles 500ml - 12

12. Commercial rats feed or pellets – 100KG

13. Rat labels-enough

14.1ml microfine insulin syringes and needles-12

15. Clean drinking water for the rats – 1000 Litres

16. Lopinavir 200mg/Ritonavir 50mg-2*30, s

17. Atazanavir 300mg/Ritonavir 100mg- 2*30s

18. W.C Redmon Deluxe™ digital small animal and aviary weighing scale.

19. Rat pellets serving dishes(metallic-stainless steel) – 250 ml

Appendix 2: Preparation of lopinavir/ritonavir 2/0.5mg per 1 gram of feed

One tablet of lopinavir200/50mg was ground and mixed with 99.75g of dry rat pellets. This gave a mixture containing 2/0.5 mg per gram of the mixture W/W. Each rat was apportioned a mixture of the food at rate equivalent to 2/0.5mg of the drug combination per kg body weight twice a day. This dosage is based on the dose administered to human. To ensure that all the drug was consumed by the animals, each animal was given the drug- food mixture before feeding first thing in the morning (7 am) before feeding and in the evening (7pm) before feeding

Appendix 3: Preparation of atazanavir/ritonavir with 3/1 mg per gram of feed

One tablet of Atazanavir/Ritonavir 300/100mg was crushed and ground and mixed with 99.6g of rat pellets to produce a mixture containing 3/1 mg per gram of the mixture W/W. Each rat was apportioned a mixture of the food at a rate equivalent to 3/1 mg per kg body weight twice a day.This dosage is based on the dose administered to human. To ensure that all the drug was consumed by the animals, each animal was given the drug- food mixture before feeding first thing in the morning (7 am) before feeding and in the evening (7pm) before feeding

Appendix 4: Preparation of lopinavir/ritonavir 2/0.5mg combined with diosmin/hesperidin 4.5/0.5 mg per 1 gram of feed

One tablet of lopinavir/ritonavir 200/50mg and one tablet of diosmin/hesperidin 450/50mg were ground and mixed with 92.5 g of dry rat pellets. This gave a mixture containing lopinavir/ritonavir2/0.5mg plus diosmin/hesperidin 4.5/0.5mg per gram of the mixture W/W. Each rat was apportioned a mixture of the food at rate equivalent to lopinavir/ritonavir 2/0.5mg with diosmin/hesperidin 4.5/0.5 mg per kg body weight twice a day.This dosage is based on the dose administered to human. To ensure that all the drug was consumed by the animals, each animal was given the drug- food mixture before feeding first thing in the morning (7 am) before feeding and in the evening (7pm) before feeding

Appendix5: Preparation of atazanavir/ritonavir 3/1mg combined with diosmin/hesperidin 4.5/0.5 mg per 1 gram of feed

One tablet of lopinavir/ritonavir 300/100mg and one tablet of diosmin/hesperidin 450/50mg were ground and mixed with 91.0g of dry rat pellets. This gave a mixture containing atazanavir/ritonavir 3/1 mg plus diosmin/hesperidin 4.5/0.5mg per gram of the mixture W/W. Each rat was apportioned a mixture of the food at rate equivalent to atazanavir/ritonavir 3/1mg with diosmin/hesperidin 4.5/0.5 mg per kg body weight twice a day.This dosage is based on the dose administered to human. To ensure that all the drug was consumed by the animals, each animal was given the drug- food mixture before feeding first thing in the morning (7 am) before feeding and in the evening (7pm) before feeding

Appendix 6: Preparation of diosmin/hesperidin 4.5/0.5mg per gram of feed

One tablet of Daflon 500(diosmin 450mg/hesperidin 50mg) wascrushed, ground and mixed with 99.5g of dry rat pellets to produce a mixture containing 4.5/0.5 mg per a gram of the mixture W/W. Each rat was given the mixture at a dose equivalent to diosmin/hesperidin 4.5/0.5 mg per kg body weight twice a day. This dosage is based on the dose administered to human. To ensure that all the drug was consumed by the animals, each animal was given the drug- food mixture before feeding first thing in the morning (7 am) before feeding and in the evening (7pm) before feeding

Appendix 7: Ethical clearance

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B92272019 July 22, 2019

TO: Nahashon Gichana Akunga
Department of Medical Physiology
Maseno University

Dear Nahashon,

RE: EFFECT OF DIOSMIN-HEPSPERIDIN COMBINATION ON HIV-PROTEASE INHIBITOR INDUCED GLUCOSE INTOLERANCE IN *Rattus norvegicus*

This is to inform you that the Research Ethics Committee (REC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number UEAB/REC/09/07/2019. The approval period is 22 July, 2019-21st July, 2020.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Jackie K Obey

University of Eastern Africa, Baraton
22 JUL 2019
Research Ethics Committee

PROF. JACKIE K. OBEY, PHD
CHAIRPERSON, RESEARCH ETHICS COMMITTEE

A SEVENTH-DAY ADVENTIST INSTITUTION OF H IGHER LEARNING
CHARTERED 1991

Appendix8:Permission to conduct research by the SGS

MASENO UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MSC/SM/00053/015

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 18th January, 2019

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR NAHASHON AKUNGA — MSC/SM/00053/2015

The above named is registered in the Master of Science in Medical Physiology Programme in the School of Medicine, Maseno University. This is to confirm that his research proposal titled "Effect of Diosmin-Hesperidin Combination on HIV-Protease Inhibitor Induced Glucose Intolerance in *Rattus norvegicus*" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

Prof. J.O. Agutu
DEAN, SCHOOL OF GRADUATE STUDIES

18 JAN

Maseno University *ISO 9001:2008 Certified*

Appendix 9: Effect of the treatment groups on animal survival

The treatments not only had an effect on blood glucose levels but also had an impact on the survival of the animals (*Figure* 34). High mortality was noted among members of the groups treated with lopinavir/ritonavir (4animals), followed by the atazanavir/ ritonavir+ diosmin/ hesperidin (2 animals) treated group and the lopinavir/ritonavir+diosmin/hesperidin (1 animal)treated group respectively.

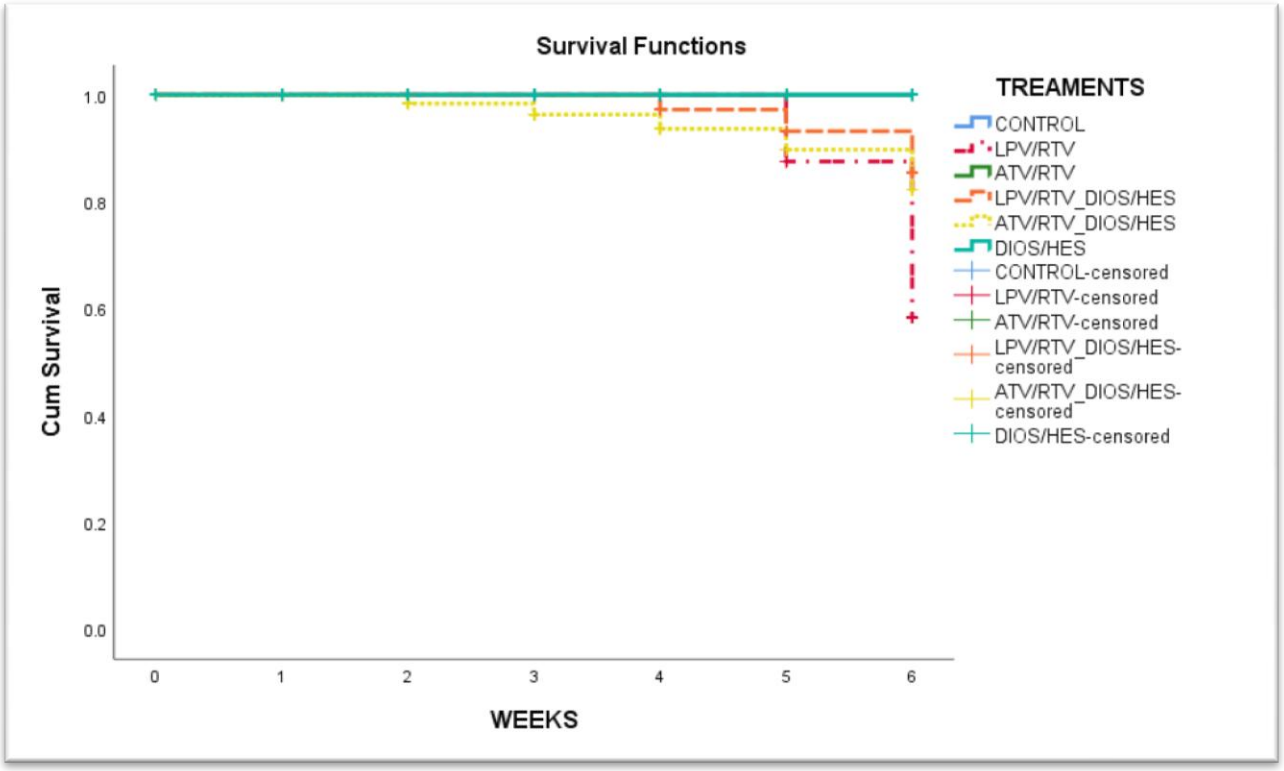


Figure 16: Kaplan – Meyer [KM] plots of the cumulative survival probability of the various treatment groups over time.

The log-rank test calculated as χ^2 with 5 degrees of freedom was 18.2 (p=0.003) at α=0.05 confirms that survival between the groups was significantly different. The LPV/RTV had the highest mortality followed by the ATV/RTV+DIOS/HES and the LPV/RTV+DIOS/HES treated group respectively. No fatalities were recorded in the control, the DIOS/HES and ATV/RTV treatedgroups (*figure* 34).

Appendix 10: Impact of treatments on group mean body weight

Apart from the initial decline in the mean weight observed at the end of the first week of treatment in all the groups apart from the control, the mean group body weight remained relatively constant with small increases observed among the groups during the rest of the treatment period.

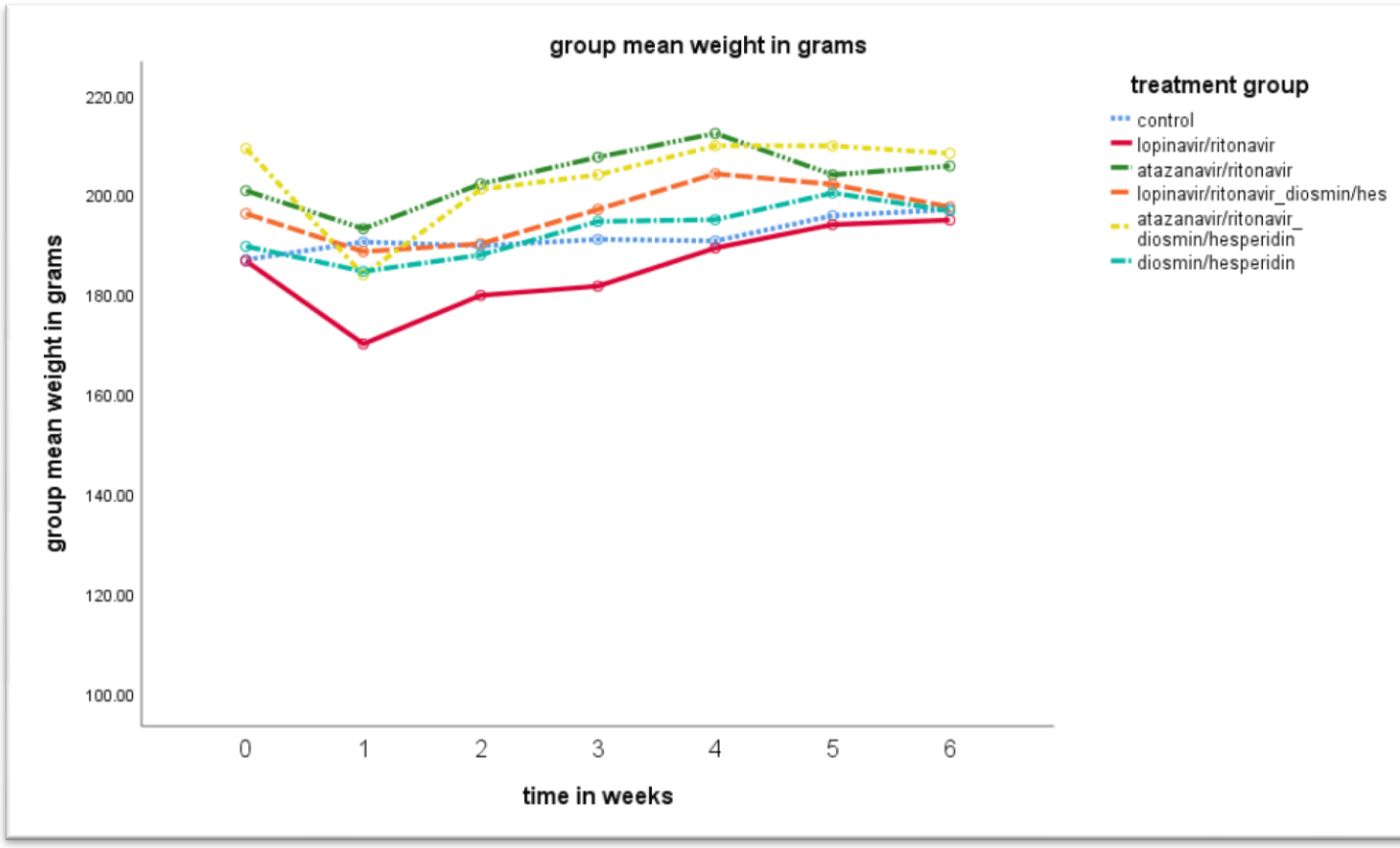


Figure 17: Trends of the group mean weight over the duration of the six weeks

The values calculated by one way ANOVA at the beginning and during the rest of the treatment period till the end were all statistically insignificant. At baseline (day 0) $F_{1-\alpha/2}(5,66)$ was 1.26(p=0.292),at the end of week one $F_{1-\alpha/2}(5,66)$ was 1.13(p=0.355), at the end of week two F_{1-}

$_{\alpha/2}(5,65\alpha)$ was 1.24(p=0.302),at the end of week three $F_{1-\alpha/2}(5,65)$ was 1.506 (p=0.200),at the end of week four $F_{1-\alpha}(5,61)$ was 0.169 (p=0.151), at the end of week five $F_{1-\alpha/2}(5,61)$ was 0.658(p=0.657) and finally at the end of the sixth week $F_{1-\alpha/2}(5,60)$ was 0.504(p=0.772). It is noteworthy that none of the F-values was significant indicating that there were no significant differences in the group mean body weight among the various treatment groups throughout the duration of the study irrespective of treatment.

Appendix11: Composition and ingredients of commercial rat pellets (food)

A. Composition (nutrients)

1. Crude protein - 23%
2. Crude fat – 3%
3. Crude carbohydrates – 39%
4. Acid insoluble ash – 8%
5. Calcium - 1-2.5%
6. Phosphorus - 0.9%
7. Sodium – 0.5%
8. Moisture -12%

B. Main ingredients (raw materials)

1. Corn (crushed)
2. Soybean pulp
3. Sunflower seed meal
4. Meat and bone meal
4. Marble dust
5. Vitamins
6. Minerals