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# The effects of integrase-transfer inhibitors on pancreatic beta-cell (INS-1) function

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BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**THE EFFECTS OF INTEGRASE-TRANSFER INHIBITORS ON PANCREATIC  
BETA-CELL (INS-1) FUNCTION**

By

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M.S., Shahid Beheshti University of Medical Sciences, 2014

Submitted in partial fulfillment of the  
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**THE EFFECTS OF INTEGRASE-TRANSFER INHIBITORS ON PANCREATIC  
BETA-CELL (INS-1) FUNCTION**

**FATEMEH HOOSHMAND**

**ABSTRACT**

**Objective:** Evidence links initiation of integrase strand-transfer inhibitors (INSTIs) to excess weight gain in people living with HIV, potentially through changes in  $\beta$ -cell function. Thus, our goal was to investigate potential mechanisms that contribute to alterations in pancreatic  $\beta$ -cells insulin secretion by following exposure to two commonly used integrase inhibitors, Dolutegravir (DTG) and Elvitegravir (ELV).

**Methods:** Insulin content and secretion were measured from clonal pancreatic  $\beta$ -cells (INS-1) cultured in medium containing 4 mM and 11 mM glucose and were exposed to either dimethyl sulfoxide (DMSO) as a control, DTG, or ELV at physiological concentrations 72 hours. Insulin was measured by fluorescence using an HTRF insulin kit (Perkin Elmer). Changes in mitochondrial function were determined using a Seahorse mitochondrial stress test. Intracellular  $\text{Ca}^{2+}$  was measured in fura-2 loaded INS-1 cells as the ratio of fluorescence at 340 nm to 380 nm using a Hitachi 2000 spectrofluorometer. The data were presented as mean  $\pm$  standard deviation (SD). Statistical analysis was conducted using Student's t-test or analysis of variance (ANOVA).

**Results:** Dolutegravir treatment significantly reduced insulin secretion in cells cultured at 11G, even when normalizing for insulin content. In the 11G condition, Dolutegravir administration reduced oxygen consumption rate (OCR) compared to ELV and control,

but no such difference was observed in the 4G condition. Consistent with reduced oxygen consumption, when INS-1 cells were cultured at 11 mM glucose with Dolutegravir, the glucose-stimulated increase in intracellular  $\text{Ca}^{2+}$  was observed to be reduced by 80% compared to the control group.

**Conclusion:** Our study reveals that exposure to DTG leads to impaired insulin secretion, less oxygen consumption and reduced calcium influx in  $\beta$ -cells under high glucose conditions. These findings provide important insights into the potential effects of Dolutegravir (DTG) on  $\beta$ -cell function and glucose regulation regarding the weight gain observed in people living with HIV (PLWH) who started or switched to INSTI treatment. Further investigations are warranted to elucidate the underlying mechanisms responsible for the observed effects of DTG on insulin synthesis, storage, and calcium signaling pathways. Understanding these mechanisms could potentially lead to the development of strategies to mitigate the detrimental impacts of DTG on  $\beta$ -cell function and optimize therapeutic approaches in patients receiving DTG therapy.

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## LIST OF ABBREVIATIONS

| <b>Abbreviations</b> | <b>Definition</b>                                     |
|----------------------|---|
| ACS                  | Acyl-CoA syntheses                                    |
| AANCC                | Age-Associated Non-communicable Comorbidities         |
| AIDS                 | Acquired immunodeficiency syndrome                    |
| ART                  | Antiretroviral Therapy ART                            |
| ATP                  | Adenosine Triphosphate                                |
| AZT                  | Azidothymidine  |
| BAT                  | Brown adipose tissue                                  |
| BIC                  | Bictegravir   |
| BME                  | $\beta$ -mercaptoethanol                              |
| cART                 | combination Antiretroviral Therapy                    |
| CNS                  | Central Nervous System                                |
| CPT-1                | Carnitine Palmitoyl Transferase 1                     |
| DTG                  | Dolutegravir  |
| EDTA                 | Ethylenediaminetetraacetic Acid                       |
| ELV                  | Elvitegravir  |
| ETC                  | Electron Transport Chain                              |
| FADH <sub>2</sub>    | Flavin Adenine Dinucleotide                           |
| FFCP                 | Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone |
| GK                   | Glucose-6-Phosphate by Glucokinase                    |
| GLUT-2               | Glucose Transporter-type                              |
| GSIS                 | Glucose-Stimulated Insulin Secretion                  |
| HEPES                | 4-(2-HydroxylEthyl)-1 Piperazine Ethane Sulfate       |
| HI                   | Hyperinsulinemia                                      |
| HIV                  | human immunodeficiency virus                          |

|         |  |
|---------|--|
| HL      | Hyperlipidemia                                     |
| HOMA-IR | Homeostatic Model Assessment of Insulin Resistance |
| IR      | Insulin Resistance                                 |
| INSTI   | Integrase Strand Transfer Inhibitors               |
| MC4R    | Melanocortin 4Receptor                             |
| mtROS   | mitochondrial Reactive Oxygen Species              |
| NADH    | Nicotinamide Adenine Dinucleotide                  |
| NNRTI   | Nonnucleoside Reverse Transcriptase Inhibitor      |
| NRTIs   | Transcriptase Inhibitors                           |
| OCR     | Oxygen Consumption Rate                            |
| OxPhos  | Oxidative Phosphorylation                          |
| PBS     | Phosphate-Buffered Saline                          |
| PEP     | Phosphoenolpyruvate                                |
| PFK     | Phosphofructokinase                                |
| PK      | Pyruvate kinase                                    |
| PKC     | Protein kinase C                                   |
| PLWH    | People living with HIV/AIDS                        |
| POMC    | Hypothalamic Proopiomelanocortin                   |
| RAL     | Raltegravir  |
| RPM     | Roswell Park Memorial Institute                    |
| TRIS    | Tris hydroxymethyl Aminomethane                    |

## **INTRODUCTION**

### **Acquired immunodeficiency syndrome**

The human immunodeficiency virus (HIV) is a leading cause of the acquired immunodeficiency syndrome (AIDS), which is an infectious disease first detected in non-human primates in Africa (Sharp, 2011). HIV is a viral infection that targets the human immune system, mainly through destroying large quantities of CD4-T lymphocytes, thereby compromising the human body's immune system (Mogadam, 2020; Ceulemans, 2019)

### **Mortality and Morbidity of HIV/AIDS**

According to a CDC report, the first official reporting of what will be known as AIDS goes back to the early 1800s, when five young men, who identified as homosexual, received treatment for biopsy-confirmed *Pneumocystis carinii* pneumonia (a serious infection caused by the fungus *Pneumocystis jirovecii* that weakens the immune system) (Sokulska, 2015) in Los Angeles, California (CDC, 1981). The number of people newly infected with HIV increased over the years, and in 1992 AIDS became the number one cause of death for 64 US cities (Selik, 1993). From 1992 to 1995, globally 3.7 million more people developed AIDS with a highest prevalence rate in Sub-Saharan Africa (Mann, 1992). During the initial period of the AIDS pandemic, significant effort was made by global health communities for scientific understanding of HIV and its prevention and treatment. In March 1987, the U.S. Food and Drug Administration

granted approval to Azidothymidine (AZT), making it the first drug authorized for the treatment of AIDS. Further the availability of new classes of drugs has marked a key achievement in the history of HIV disease, introduced in 1995-96, known as combinational antiretroviral therapy lead the HIV infection into a manageable chronic condition (Palella Jr, 1998).

### **Antiretroviral therapy**

The antiretroviral drugs include non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors, and integrase inhibitors. Antiretroviral drugs are categorized based on the specific stage of the viral life cycle they target. Additionally, their classification may also consider their chemical structure, which influences their distinct mechanisms of action (Vella, 2012).

As of 2021, the global population of people with HIV was estimated to be 38.8 million. Sub-Saharan Africa is particularly affected by HIV, with a significant number of infections occurring in this region (51% of new infections). By the end of December 2021, approximately 28.7 million individuals had access to antiretroviral therapy. Interestingly, by using the combination antiretroviral therapy (cART) the AIDS-related morbidity and mortality greatly reduced (Palella, 1998; Wada, 2013). However, individuals infected with HIV have a shorter life expectancy than non-HIV population (Hogg et al. 2018; Wada et al. 2013). When people living with HIV on cART treatment aged, they increasingly experience more Age-Associated Noncommunicable Comorbidities (AANCC) compared with HIV-uninfected patients (Schouten, 2014 6;

Hasse, 2011). Of note, a greater proportion of causes of death in cART-treated patients are non-AIDS comorbidities (Weber, 2013; Collaboration, 2010; Wada, 2013). In observational studies, metabolic syndrome (MS), including risk factors which are associated with increasing risk of cardiovascular diseases (CVD) (Sarfo, 2021) (Muller, 2019) and type 2 diabetes (Galli, 2012), has been increasingly reported among HIV-infected patients (Jantarapakde, 2014).

### **Potential mechanism**

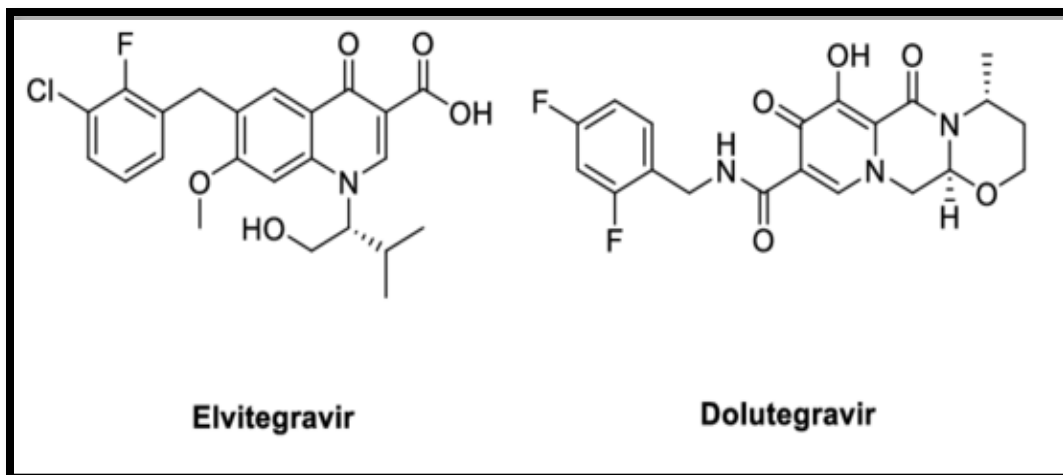
The underlying mechanisms that cause this greater cardiometabolic risk in people living with HIV/AIDS (PLWH) are still under investigation. Prevalent hypotheses include toxicity by exposure to the Antiretroviral Therapy (ART), immune system dysfunction, and inflammation related to the infection (Deeks, 2009; Wada, 2013; Deeks, 2009; Deeks, 2013; Deeks, 2011; Armah, 2012; Deeks, 2011).

### **Early ART toxicity**

Combinatorial ART over an extended period in PLWH may cause cumulative toxicity, leading to comorbidities in older patients including cardiovascular disease, and diabetes (Triant, 2008; Lucas, 2008; Tien, 2007; Group, 2008; Morse, 2015). Further, long term early generation ART exposure has been associated with increased risk of dyslipidemia (Ang, 2021) (Nduka, 2015; Fiseha, 2021), hypertension (Nduka, 2016; Xu, 2017) and Fasting Plasma Glucose (FPG) (Phuphuakrat, 2020)

## **Newer forms of ART and weight gain**

In 2018, multiple guidelines have recommended the use of Integrase strand transfer inhibitors (INSTI) for the AIDS treatment. (Saag, 2018; WHO, 2018), including Dolutegravir (DTG), Elvitegravir (ELV), Raltegravir (RAL), and Bictegravir (BIC). Integrase inhibitors interact with the integrase, an HIV enzyme responsible for incorporating genetic material of virus into human DNA. By doing so, it inhibits the binding of integrase to retroviral DNA, consequently preventing the strand transfer process required for HIV replication. Even though integrase inhibitors with more tolerability and efficacy compared to older regimens (Iwamoto, 2008; Mondy, 2019), studies have found that patients treated with integrase inhibitors have more weight gain than people who use conventional antiviral therapy (discussed below). Dolutegravir (DTG) and Elvitegravir (ELV), are two commonly used integrase inhibitors, and this weight gain is seen more significantly in people using Dolutegravir (DTG).



**Figure 1 Chemical structure of Elvitegravir and Dolutegravir**

### **Integrase strand transfer inhibitors and weight gain**

Weight gain is observed in both treatment naïve individuals and patients switching to INSTI containing regimens. A study showed that in treatment naïve individuals, use of INSTI-based regimens represented a significant contributing factor for weight gain during nearly one year, with 6.0 kg weight gain for Dolutegravir, and 2.6 kg and 0.5 kg for NNRTIs and Elvitegravir respectively (Bourgi et al. 2020). In another study for a period of 96 weeks, the most weight gain of 3.24 kg reported for patients was taking integrase inhibitors. Whereas similar weight gain observed between people who was treated with the protease inhibitor (PI) group, or nonnucleoside reverse transcriptase inhibitor (NNRTI), 1.72 kg and 1.93 kg respectively (Sax, 2020). An observational cohort study of 563 participants compared persons taking non-nucleoside reverse transcriptase inhibitor with those who received an INSTI. Those receiving an INSTI

showed greater weight gain around 2.7 kg at 96 weeks (Wu, 2021). Patients exposed to DTG had a higher weight of 1.78 kg from beginning to 12 months follow-up in compare those who remained on efavirenz, indicating that integrase inhibitors, especially *Dolutegravir*, was associated with more weight gain than older NRTIs (Brennan, 2023). Weight gain following by use of ART among people living with HIV could lead to the increasing incident of obesity and subsequently enhances the risk of metabolic syndrome and cardiovascular diseases (Kumar, 2018; Koethe, 2016; Chang, 2022). Therefore, it is important to examine the underlying mechanisms causing this observed weight-gain following integrase inhibitors treatment.

### **Hypotheses for observed weight gain in people taking integrase inhibitors.**

Imbalance between energy intake and expenditure is the main cause of weight gain, but the specific mechanism by which weight gain results from integrase inhibitors exposures is unknown. It remains unclear whether weight gain associated with treatment with integrase inhibitors affects appetite, and/or energy expenditure. There are several hypotheses proposed to explain the mechanism of ART-associated weight gain among PLWH including controlling appetites by effects of DTG on Melanocortin 4Receptor (MC4R). Another mechanism which explained is affecting the adipose tissue dysfunction. We will later explain how it could influence on energy balance leading to gain weight. Recently, clinical studies have seen increasing in the insulin resistance following integrase inhibitors, which may lead to hyperinsulinemia and promotion of lipogenic pathways (Ngono Ayissi, 2022).

### **Appetite regulation**

Some evidence indicates that integrase inhibitors may affect hypothalamic regulation of hunger. This is through the hypothalamic system, proopiomelanocortin (POMC)/melanocortin 4 receptor (MC4R) (POMC/MC4R), which is the most influential mechanism control food intake and metabolic energy balance. It is proposed that integrase inhibitors alter the appetite response by the direct impact on the melanocortin system (Domingo, 2020).

### **Fat tissue dysfunction**

Brown adipose tissue (BAT) is a thermogenic organ which has a fundamental role in regulating cardiovascular health and diseases. It promotes glucose and lipid metabolism leading to enhancing energy expenditure (Chen, 2021). Studies have implicated adipose tissue dysfunction because of INSTI exposure, and an underlying factor in INSTI-associated weight gain.

One study that examined subcutaneous and visceral adipose tissue from ART treated patients with HIV showed that INSTI-treatment was associated with greater lipid accumulation in adipocytes by enhancing both adipogenic and lipogenic pathways in adipocytes, as well as increasing oxidative stress, mitochondrial dysfunction, and decreasing serum adiponectin. Reductions in adiponectin could cause insulin resistance followed by weight gain due to its roles in regulation of glucose and lipid metabolism (Gorwood, 2020). In mouse studies, the integrase inhibitors suppressed adipocytes differentiation, and in vivo, inhibition in cellular oxygen consumption and energy

expenditure observed after administration of DTG promoting a positive energy balance leading to weight gain (Jung, 2021)

## **Insulin**

Insulin is a critical regulator of energy metabolism (Qaid, 2016), which is a potential driver of weight gain. Hyperinsulinemia (HI) including basal or fasting insulin elevation causes increased nutrient consumption and Hyperlipidemia (HL) leading to Insulin Resistance (IR). IR is a condition in which tissues become resistant to insulin's effect. In this case cells develop reduced sensitivity to insulin's impact on glucose transport while still responding to its lipogenic effects. This highlights the hypothesis that HI is a leading cause of gaining weight and obesity (Williams, 2016). Some studies have indicated insulin resistance following exposure to integrase inhibitors. In these studies an increase in the mean Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) showed in integrase inhibitors treated patients (Gupta, 2013) (Dirajlal-Fargo, 2016). In a cohort study in which people living with HIV who were on an Efavirenz were changed to integrase inhibitor (Raltegravir (RAL) or Stribild (Elvitegravir+Cobistat)), patients that switched to one containing Elvitegravir showed a 20% increase in HOMA-IR after 8 weeks, mostly due to increases in fasting insulin (R. Taylor Pickering, 2022).

Insulin secretion is the first step in body response to elevated glucose which is associated with weight gain. A causal role in the pathogenesis of obesity has been proposed for hyperinsulinemia, insulin resistance, and insulin secretion inhibition (Schwartz, 1995).

However, the specific mechanisms by insulin secretion through which integrase inhibitors may cause weight gain to have not been fully explained.

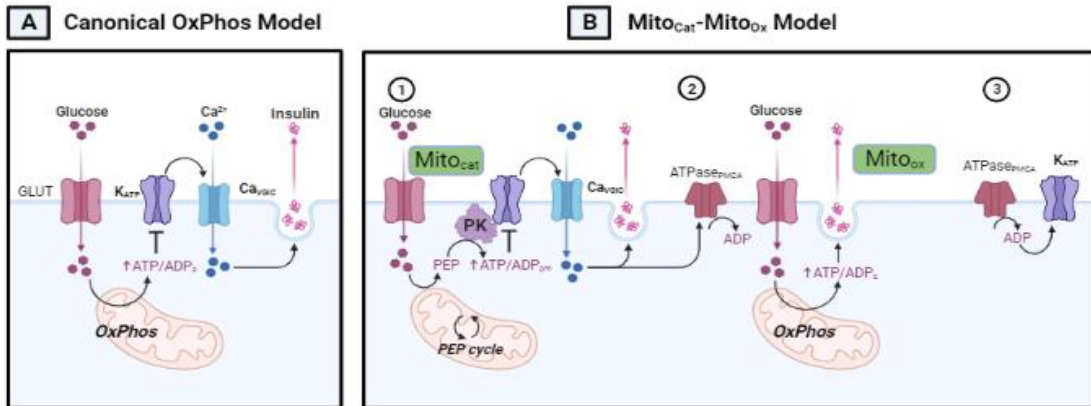
### **Insulin secretion and $\beta$ -cell function**

Pancreatic  $\beta$ -cells synthesize and secrete insulin for regulating the blood glucose level. Studies started in 1960 led to the discoveries of the three key factors explaining for the insulin stimulus-secretion in  $\beta$ -cell. First, glucose metabolization in  $\beta$ -cell is necessary for insulin secretion (Grotsky, 1963; Coore, 1964). Second, glucose showed no increase in insulin secretion in the absence of extracellular  $\text{Ca}^{2+}$ , leading to the conclusion that  $\text{Ca}^{2+}$  effects insulin secretion (Grotsky, 1966; Hales, 1968). Third, the pancreatic  $\beta$ -cell is electrically excitable demonstrated by the action potentials recording in glucose-stimulated  $\beta$ -cells (Dean, 1968). These three important elements established the concept that insulin secretion controlled by triggering and amplifying signals in  $\beta$ -cells.

### **The triggering and amplifying pathway of Glucose-induced Insulin secretion**

The triggering pathway involves the following sequence of events. Glucose enters the  $\beta$ -cell by the Glucose Transporter-type (GLUT-2) (Matschinsky, 1986). By the effect of the Glucokinase (GK), glucose phosphorylate to Glucose-6-Phosphate. Pyruvate, which is a product of glycolysis, is further oxidized in the mitochondria to produce Adenosine Triphosphate (ATP). Glycoltic oscillations are driven by Phosphofructokinase (PFK). PFK is activated by high AMP and inhibited by high citrate and ATP (low AMP). This enzyme regulation results in oscillatory flux through glycolysis resulting in oscillations

in the ATP/ADP ratio. These oscillations are amplified by fructose 1, 6 bisphosphate, the product of the reaction, which activates PFK. Oscillations in the ATP/ADP ratio led to subsequent oscillations in the activity of the ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channel. This leads to the depolarization of cell membrane, allowing  $Ca^{2+}$  entry into the cell through the voltage-operated calcium channels (VOCC). Finally, the rise in intracellular  $Ca^{2+}$  levels lead to exocytosis of insulin secretory granules (Deeney, 2000) Adenosine triphosphate (ATP) is produced by the oxidative phosphorylation process. According to the "canonical" model, mitochondrial oxidative phosphorylation (OxPhos) leads to an increase in the ATP/ADP ratio. This leads to closure of the ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channel, and subsequent  $Ca^{2+}$  influx and release of insulin from the pancreatic  $\beta$ -cells.  $Mito_{Cat}$ - $Mito_{Ox}$ , is another model of glucose signaling in the  $\beta$ -cell which was suggested in the recent review by Merrins et al (Merrins, 2022). They propose that during the electrically silent triggering phase ( $Mito_{Cat}$ ), the ATP that closes the channel is generated through the Pyruvate kinase (PK) reaction, utilizing Phosphoenolpyruvate (PEP) as a substrate within the mitochondria. In this model after the initial closure of the  $K_{ATP}$  channel by PK and the PEP cycle, the increase of the cytosolic ATP/ADP through the electrically active secretory phase ( $Mito_{Ox}$ ) phase OxPhos-dependent, may help to keep the  $K_{ATP}$  channel closed (Fig. 2)



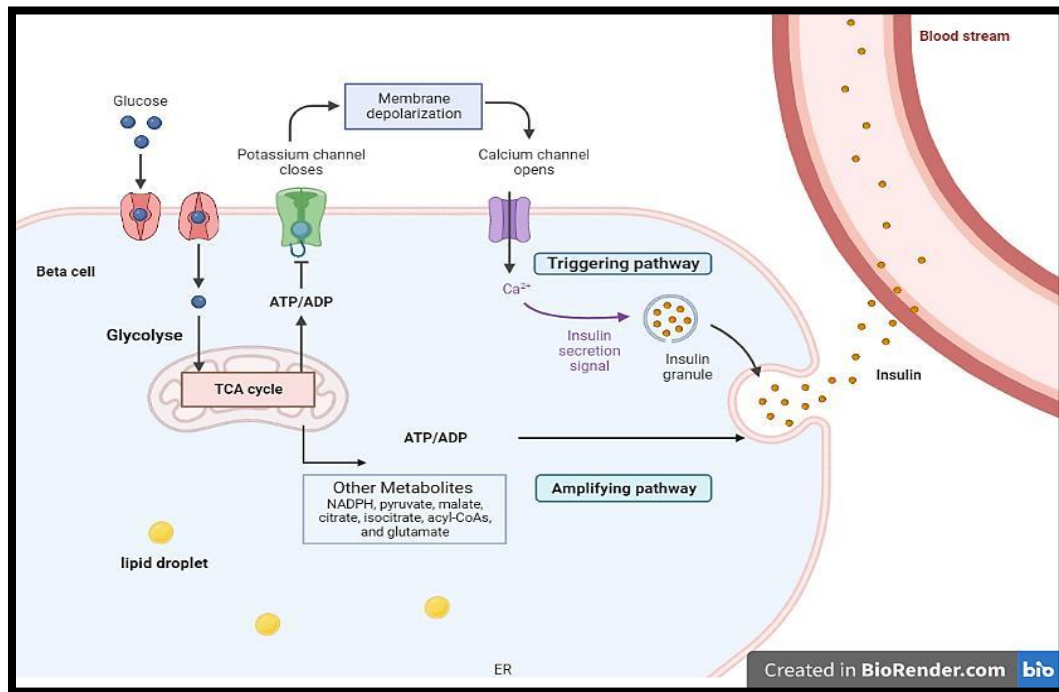
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**Figure 2 Oxidative Phosphorylation and ATP/ADP Regulation in Glucose-Stimulated Insulin Secretion.** A) Oxidative phosphorylation (OxPhos) increases ATP/ADP to close ligand gated K<sup>+</sup> channels (K<sub>ATP</sub>), open voltage gated Ca<sup>2+</sup> channels (Ca<sub>vac</sub>), and trigger Glucose S

### **Amplification pathway**

LC-CoA has been suggested as a potential modulator of insulin secretion, as suggested by the work of Prentki, Corkey, and their colleagues (Corkey, 1989; Prentki, 1992). Increased LC-CoA in the  $\beta$ -cell can result from metabolism of glucose (Liang, 1991) and FFA (Frazee, 1985). In fasting, LC-CoA produced from fatty acids by Acyl-CoA synthetase (ACS) and via Carnitine Palmitoyl Transferase 1 (CPT-1) enters the mitochondria to be oxidized (Berne, 1975). In contrast, after a carbohydrate-containing meal, synthesis of malonyl-CoA inhibits transport of long chain acyl-CoA into the mitochondria by blocking CPT-1 (Prentki, 2002).

The potential stimulating effects of Long-chain acyl-CoA (LC-CoA) on insulin release (Deeney, 2000), could be explained by its impact on regulating enzymes like Protein kinase C (PKC) (Majumdar, 1991) (Yaney, 2000).

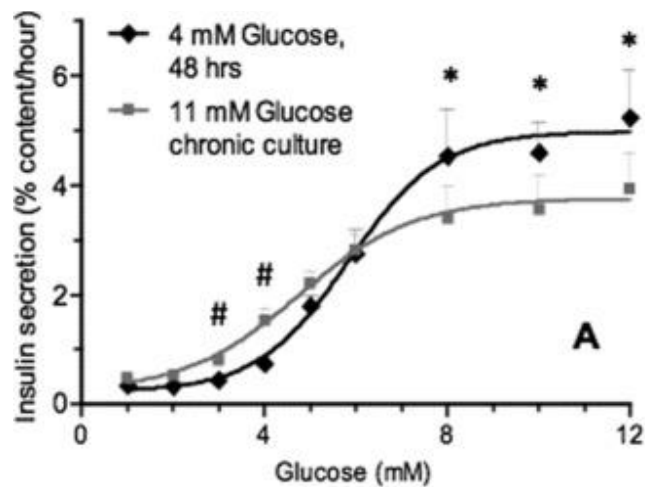


**Figure 3 The triggering and amplifying pathway of Glucose-induced Insulin secretion (Lazo-de-la-Vega-Monroy, 2011)**

### **Chronic Exposure to Excess Nutrients Left-Shifts Glucose-Stimulated Insulin Secretion**

Elevating insulin in plasma, under normal glucose conditions, is Hyperinsulinemia (HI). Erion et al, showed that chronic exposure to the excess nutrients in  $\beta$ -cell resulting in HI, characterized by a shift in the concentration dependency of glucose-stimulated insulin secretion. The results concluded from the study by culturing INS-1 cells in a 4 mM and 11 mM glucose as a physiological and high glucose concentration with or without exposure to oleate. Culture in excess nutrients exhibited the left-shifted concentration dependence of GSIS with reduced insulin content and increased lipid stores (Figure 4).

The observed shift in the concentration of insulin secretion was not attributed to increased glucose influx into the  $\beta$ -cell. Instead, it is suggested that a change in the sensitivity of the exocytotic machinery to  $\text{Ca}^{2+}$  played a role in this left shift (Erion, 2015).

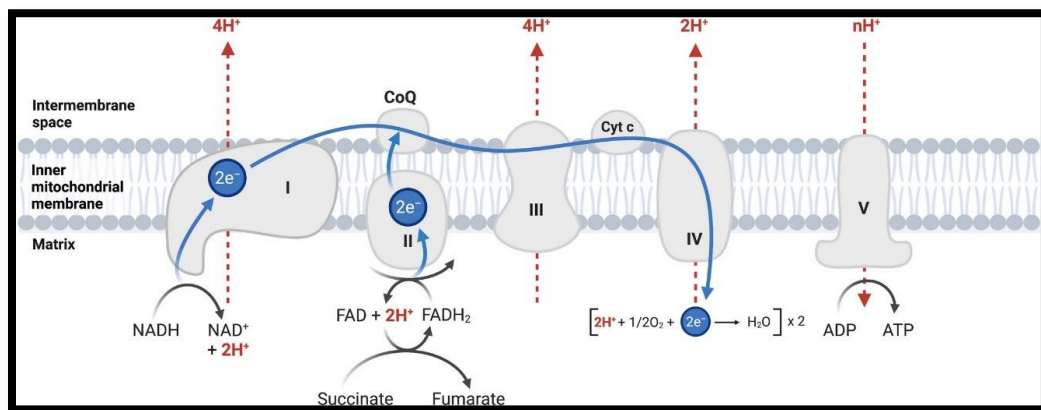


**Figure 4** INS-1 left-shifts in insulin secretion in cells cultured in excess nutrients. A, change condition from 11G to 4 mM glucose for 48 h. Data are represent as the mean and Error bars show S.E. (\*,  $p < 0.05$ ) (Erion, 2015).

### Mitochondrial Metabolic Cycles in GSIS

In pancreatic  $\beta$ -cells, mitochondria has a significant role in the process of GSIS by connecting glucose metabolism to insulin secretion (Kennedy, 1998 ).The initiation of this process is by entering of glucose through GLUT2 within  $\beta$ -cell plasma membrane and then phosphorylation by glucokinase, leading to glycolysis (Kennedy, 1998 ). The

products of glycolysis include pyruvate, ATP, and NADH. The regeneration of NAD<sup>+</sup> from NADH is essential for the cell to continue to generate any ATP (Kennedy, 1998). In most tissues, ensuring NADH oxidation to NAD<sup>+</sup> is by conversion of pyruvate to lactate. While in  $\beta$ -cells, the conversion of NADH to NAD<sup>+</sup> is facilitated by mitochondrial NADH shuttles. In mitochondria reducing equivalents including NADH and FADH<sub>2</sub> are transferred to the Electron Transport Chain (ETC), leading to generation of ATP (Fig. 5). The redox reactions of the four protein complexes of electron transport chain, causing an electrochemical gradient that leads to the creation of ATP by oxidative phosphorylation (Maechler, 2013). ATP is then leading to insulin exocytosis by transferring to the cytosol and raising the ATP/ADP ratio.



**Figure 5 The mitochondrial electron transport chain.** Electrons are fed into the ETC by NADH at complex I and FADH<sub>2</sub> from succinate oxidation at complex II. Electrons are then transferred to complex III by CoQ, through Cyt c to complex IV, where it is used to reduce oxygen into water. The movement of electrons, across the mitochondrial membrane, establishes an electrochemical gradient that powers ATP synthesis at complex V. (Yin, 2021) (Modified)

**Aim of the study**

The aim of this study was to investigate the potential mechanisms that contribute to insulin secretion by pancreatic  $\beta$ -cells following exposure to two commonly used integrase strand-transfer inhibitors (INSTIs), Dolutegravir (DTG) and Elvitegravir (ELV), in people living with HIV. Specifically, we sought to examine the effects of these INSTIs on insulin secretion, mitochondrial function, and intracellular calcium levels in INS-1 cells, a well-established pancreatic  $\beta$ -cell model.

## METHODS

### INS-1 Cell Culture

The experiments were performed using clonal pancreatic INS-1 832/13 cells (Hohmeier et al., 2000). These cells were grown in either 4 mM or 11 mM glucose in Roswell Park Memorial Institute (RPMI 1640) media containing 10 mM 4-(2-Hydroxyethyl)-1 Piperazine Ethane Sulfate (HEPES), 2 mM glutamine, 1 mM pyruvate, 50 IU/mL penicillin, 50 µg/mL streptomycin, 10% Fetal Bovine Serum (FBS), and 50 µM β-mercaptoethanol (BME). Cells were passaged once a week and the Cells from passage 65 to 80 were used. Cells that were to be used in experiments were plated into 48-well plates (Corning, NY) to total approximately 240,000 cells per well on the day of the secretion experiment. This cell number was based on the doubling time, which was 33 hours in 11 mM glucose media, and 56 hours in 4 mM glucose media. Cells were typically grown one to two days before chronic exposure (three days) to either ELV or DTG, while control cells were administered only DMSO ELV and DTG were diluted 1000-fold in glucose RPMI medium (1G RPMI) with final concentrations of (6 mM) and (7.4 mM) respectively INS-1 Cells were administered with ELV or DTG stock for 72 hours. Cells were kept under normal culture conditions 4G and 11G. Cells administered DMSO without integrase inhibitors served as a control condition. Afterward, cells were ready for Glucose-Stimulated Insulin Secretion (GSIS) experiments.

### **Insulin secretion and insulin content**

Following integrase inhibitors administration, cells were examined to ensure successful cell growth and non-contamination. Growth conditions (4G and 11G) were removed, and cells were incubated for 2 hours with 1 mM glucose RPMI medium (1G RPMI). After 2-hrs 1G RPMI was removed, and then cells were pre-incubated for 30 minutes with KRB containing 119 mM NaCl, 4.6 mM KCl, 5 mM NaHCO<sub>3</sub>, 2 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 0.15 mM NaHPO<sub>4</sub>, 0.4 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM HEPES, 1 mM glucose, and 0.05% fatty acid free bovine serum albumin (BSA) (Sigma, St. Louis, MO), pH 7.4 (1G KRB) at 37°C. Next cells were placed on ice for 5 minutes. 1G KRB was then removed and replaced with test solutions. Test solutions for each condition consisted of either 1 mM or 12 mM glucose made up in KRB. Cells were then incubated for 2 hrs. in the water bath and then cooled on ice for 5 minutes. Finally, samples were transferred to a 96-well plate where they were diluted 1:1 with 1% BSA. At least 3 wells were selected for each condition in 4G and 11G, were incubated with 400 µL/well trypsin for 5 minutes until cells became rounded, and finally all trypsin zed cells were mixed and transferred to the Eppendorf tubes, where 10 uL samples were counted by hemocytometer. After the cells were counted, Eppendorf tubes were centrifuged for 3 minutes, trypsin was aspirated, and cells were kept in 150µl of 1% BSA and 150µl of insulin extraction buffer Phosphate-Buffered Saline (PBS) solution containing 0.1% Triton X-100 (Sigma, St. Louis, MO) and 25 mM NaOH. All samples including the 96-well plate for the Insulin Assay were frozen at -20° C.

### **Cell counting**

The cell detachment process involved the utilization of trypsin followed by cell counting using a hemocytometer.

### **Insulin Assay**

All samples were diluted with the 0.5% BSA. Assays were diluted until they reached an optimal insulin level (as determined by the standard insulin curve). 2  $\mu$ L of each sample were then individually transferred to a 1536-well plate and centrifuged for 15 seconds. 2  $\mu$ L of HTRF insulin antibody was then added to each sample and centrifuged. The assay plate was securely sealed and placed in a humidified chamber for overnight incubation. On the day of measurement, the plates underwent an additional centrifugation step for 15 seconds, and the fluorescence was subsequently measured using a Tecan Infinite M 1000 PRO multimode plate reader. The obtained results were exported to Microsoft Excel for further analysis.

### **Calcium Activity Measurement**

Cytosolic-free  $\text{Ca}^{2+}$  in pancreatic  $\beta$ -cells was measured in DMSO (control) and DTG conditions. Cells were plated 4 days before measurements in two T25 flasks at a seeding density of approximately 2-3 million cells in 11 mM glucose RPMI media. After one day DMSO was added to one flask and DTG added to the other (1 to 1000 dilutions). On the day of the experiments 11 G media was removed and replaced by 1G RPMI without serum and incubated for 1 hr. After 1 hour cells were trypsinized, centrifuged and resuspended in 1G KRB with 2  $\mu$ M fura-2 AM (Invitrogen, Carlsbad, CA) for 30 min at

37°C. After 30 min fura-2 AM solution was removed, and cells were resuspended in 1G KRB without fura-2 for 10 min. At the end, cells were centrifuged for 3 minutes the media removed and cells resuspended in 300  $\mu$ L fresh 1G KRB and stored on ice. Fura 2 fluorescence was measured using a Hitachi F-2000 spectrophotometer. Measurements of fura-2 within a frequency of 340-380 nm were obtained across 1800 seconds. Basal measurements were recorded for 5 minutes in two different traces for control and DTG and were followed by a perfusion of higher glucose 1 mM for 15 minutes. During the last five minutes, to ensure normal cellular response, a calibration process was conducted using Ethylenediaminetetraacetic Acid (EDTA), Tris hydroxymethyl Aminomethane (TRIS), and Triton X-100. Each of these substances was perfused individually, with 16.5 $\mu$ L of each being used. Fluorescence was measured every 20 seconds at two separate excitation wavelengths (340 nm and 380 nm) and emission at 510 nm was collected from each wavelength. The data of the ratio F1/F2 was then exported to excel for analysis.

### **Mitochondrial Respiration Measurement**

Cells were plated in Seahorse XFe96 cell culture microplates 3 days prior to the experiments. The final density of cells was approximately 50,000 cells/well for cells cultured at either 4 mM or 11 mM glucose. 2 days before doing experiment, cells were treated with DMSO as control, DTG and ELV at final concentrations of 7.4  $\mu$ M and 6  $\mu$ M respectively. The night before the experiment the Agilent Seahorse XFe96 Sensor Cartridges was hydrated by placing the sensor cartridge into the utility plate containing 200  $\mu$ L of deionized water overnight in a CO<sub>2</sub> free incubator. The cartridge was then

placed in XF Seahorse Calibrant for at least 2 hours the next day. On the day of the experiment, growth condition media, 4 mM or 11 mM glucose, were first removed from the cells and replaced with 1G RPMI (or XF media). The Seahorse plate was then incubated for 1 hour at 37°C without CO<sub>2</sub>. After the 1-hour incubation period, 1G RPMI was removed and replaced with 1X KRB for 30 minutes. Immediately before the experiment, the buffer was changed to fresh 1X KRB.

Just before the experiment started the sensor cartridge was loaded with test solutions and calibrants. Glucose in Port A, and the calibrants oligomycin in Port B, Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) Port C, and antimycin in Port D. Test solutions and calibrants were injected as 10X solutions, which after dilution reached final concentrations of 11 mM glucose, 2 μM oligomycin, 3 μM FCCP and 3 μM antimycin. The sensor and cell plate were then loaded into the XFe96 Seahorse machine to measure oxygen consumption rate (OCR).

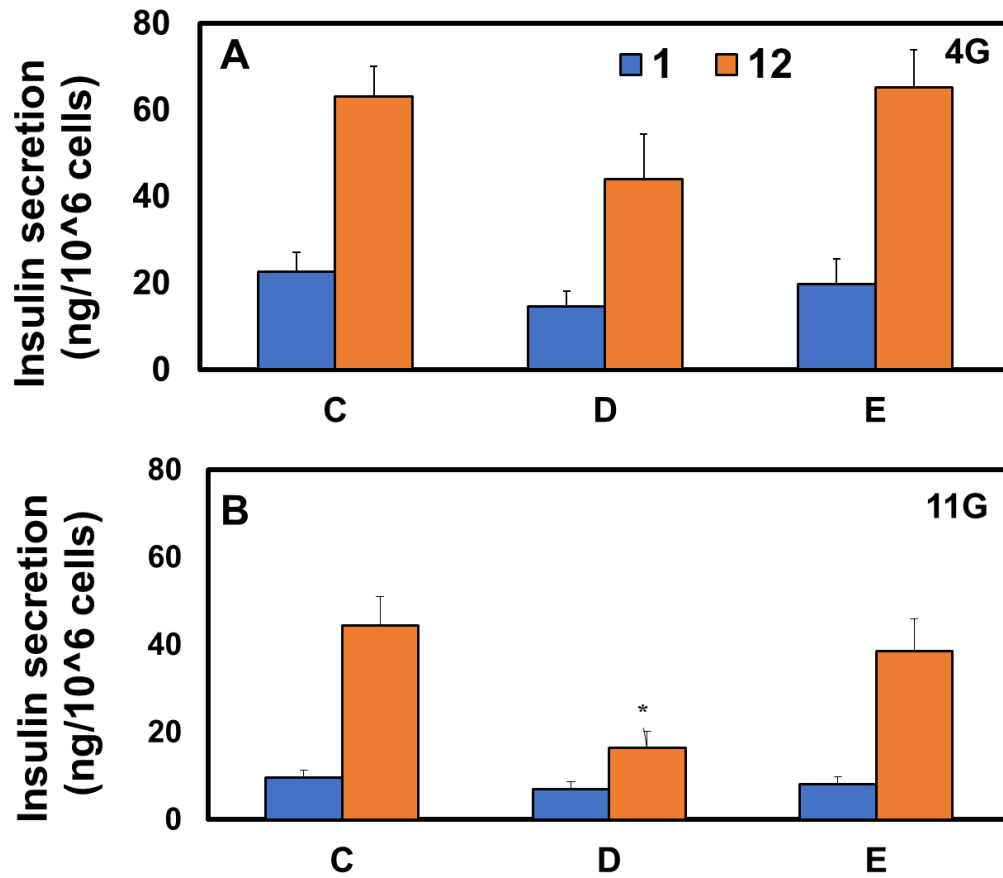
## RESULTS

### **Effects of Dolutegravir and Elvitegravir on GSIS from INS-1 Cells Cultured at 4 mM and 11mM Glucose.**

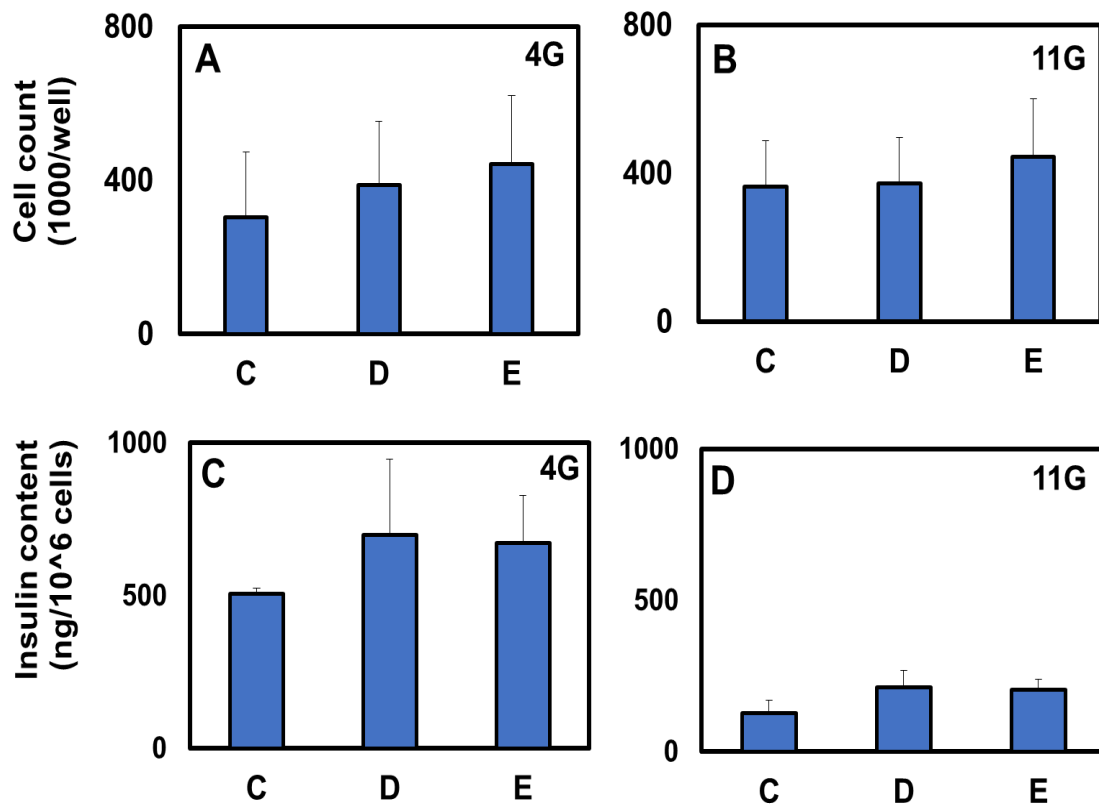
Insulin secretion was measured from INS-1 cells cultured in 4 mM and 11 mM glucose with and without, Dolutegravir (7.4  $\mu$ M) and Elvitegravir (6  $\mu$ M) for 3 days (Figures 6). There was no effect of either compound on basal (1 mM G) insulin release (blue bars) in cells cultured at low or high glucose. In contrast, Dolutegravir inhibited GSIS (12mM G) (orange bars) in cells cultured at high glucose. Inhibition in cells cultured at 4 mM glucose was not significant although there was a trend toward reduced secretion by Dolutegravir under these conditions (Fig 6 A). Dolutegravir inhibited GSIS from cells cultured at 11 mM glucose by 80% ( $P=0.018$ ) (Fig. 6 B). Elvitegravir was without effect in cells cultured at either low or high glucose.

### **Effects of Dolutegravir and Elvitegravir on cell proliferation and Insulin Content from INS-1 Cells cultured at low and high glucose.**

Since both cell number and insulin content can result in differences in GSIS we measured the effect of Dolutegravir and Elvitegravir on both parameters. Figure 7 shows the effect of 3 days exposure to Dolutegravir and Elvitegravir on INS-1 cell proliferation. Neither compound was shown to have significant effect on proliferation of cells cultured at low 4 mM glucose (Fig 7A) or high 11 mM glucose (7B). Insulin content cultured under these same conditions was also not significantly affected (Fig. 7 C and D). Note that insulin content is significantly reduced in cells cultured at high glucose.



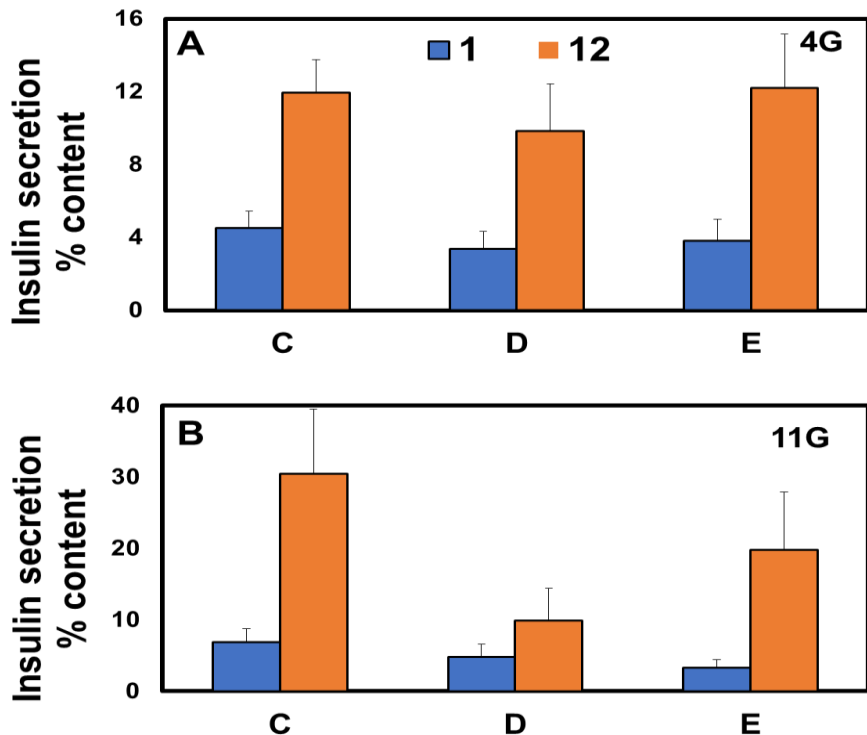
**Figure 6 Dolutegravir significantly reduced insulin secretion in INS-1 Cells Cultured at 11mM Glucose.** A) Dolutegravir and Elvitegravir on GSIS from INS-1 Cells Cultured at 4 mM Glucose. B). 11 mM Glucose. Blue:1 (mM) Glucose, Red 12 (mM) Glucose, (\* p<.05, the t-tst is used to represent a comparative statistically significant result, n=4). Error bars represent  $\pm$  SE. (C: Control, D: Dolutegravir, E: Elvitegravir)



**Figure 7 Effects of Dolutegravir and Elvitegravir on cell proliferation and Insulin Content from INS-1 Cells cultured at low and high glucose A) Cell counts in different condition at 4G and B) at 11G, C) Effects of Dolutegravir and Elvitegravir on Insulin Content from INS-1 Cells Cultured at 4 mM Glucose and D) at 11 mM Glucose. C: Control, D: Dolutegravir, E: Elvitegravir)**

**Effects of Dolutegravir and Elvitegravir on Insulin Secretion as a % of content from INS-1 Cells Cultured at 4 mM and 11 mM Glucose.**

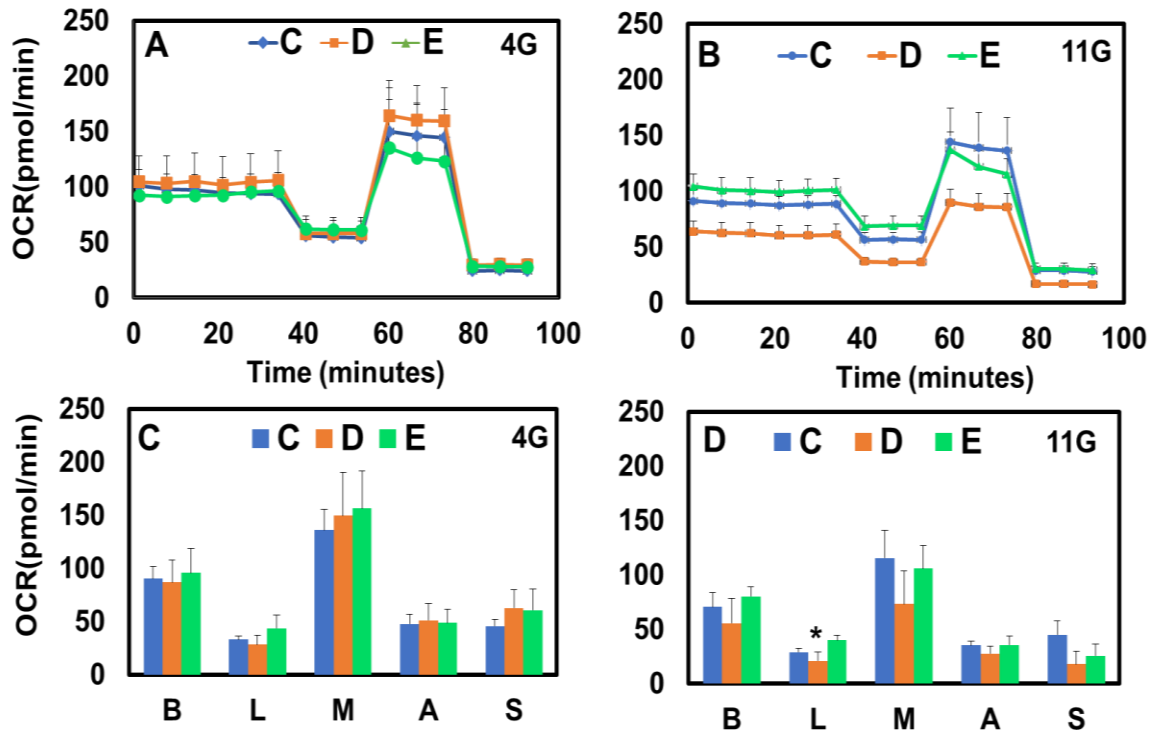
GSIS from cells cultured in both 4 and 11 mM glucose (Figure 6) was normalized to insulin content (figure 7 B) and the results shown in figure 8. Insulin release as a percentage of content was higher in cells cultured in 11 mM glucose compared to cells cultured in 4 mM glucose (Figure 8). This relative inverse in the measured levels of insulin secretion is due to the much higher insulin content in cells cultured at low glucose. Correcting for insulin content did not alter the effects of Dolutegravir and Elvitegravir on GSIS from cells cultured at 4 and 11 mM glucose (Figure 8 A and B).



**Figure 8 Dolutegravir reduced Insulin Secretion in 11G after normalizing for percent of content.** A) Effects of Dolutegravir and Elvitegravir on Insulin secretion for percent of Content from INS-1 Cells Cultured at 4 mM Glucose. B) 11 mM Glucose, Blue:1 (mM) Glucose, Red: 12 (mM) Glucose, (\*  $p < .05$ , the t-tst is used to represent a comparative statistically significant result,  $n=3$ ). (C: Control, D: Dolutegravir, E: Elvitegravir)

### **Effects of Dolutegravir and Elvitegravir on Mitochondrial Respiration**

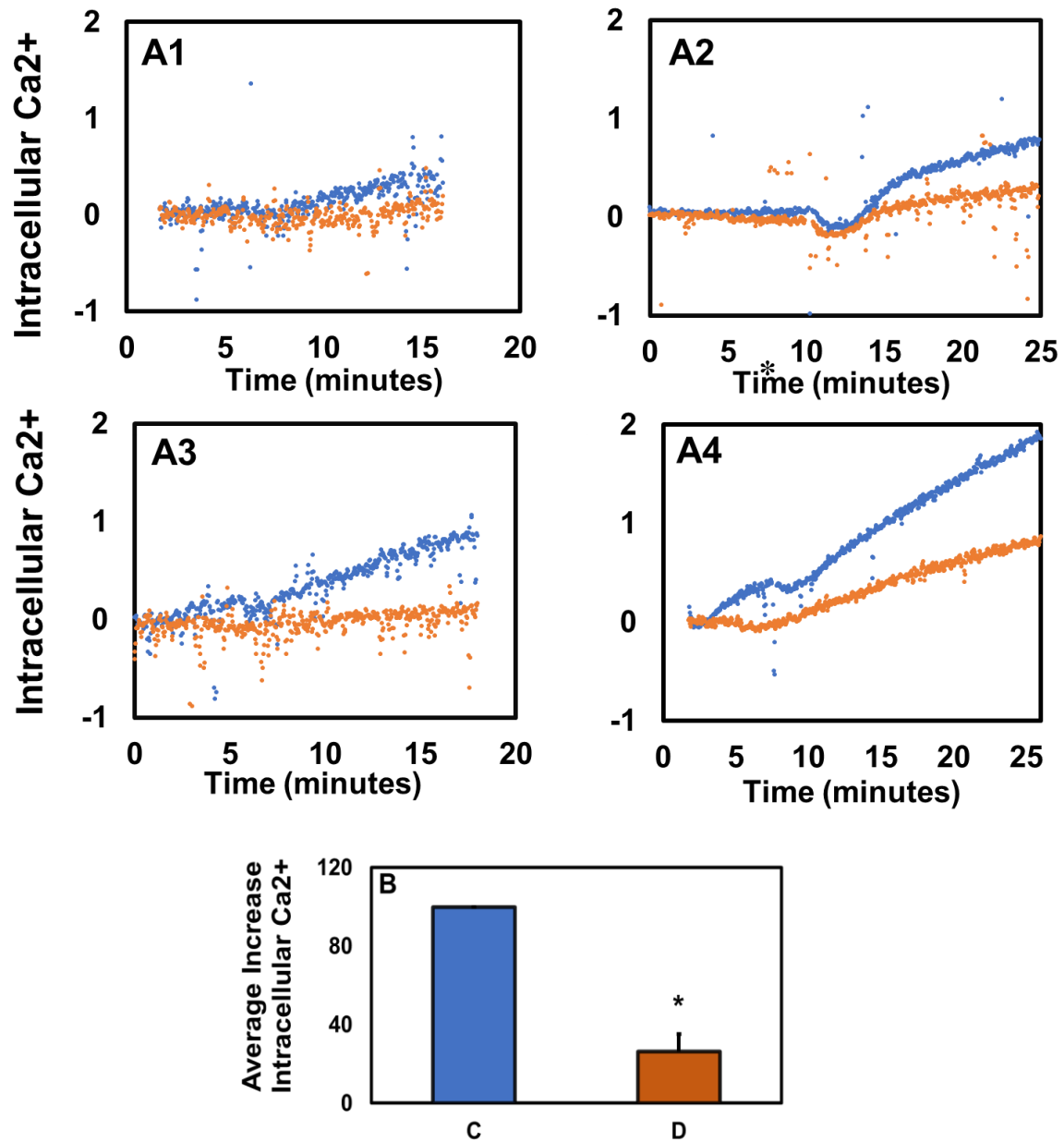
Oxygen consumption rates were measured from INS-1 cells cultured in 4 mM and 11 mM glucose with and without, Dolutegravir (7.4  $\mu$ M) and Elvitegravir (6  $\mu$ M) for 3 days. Oxygen consumption rates showed no difference between control, Dolutegravir or Elvitegravir in cells cultured at 4 mM glucose (Fig. 9 A). In contrast Dolutegravir in cells cultured at 11 mM glucose reduced all oxygen consumption rates compared to the control and Elvitegravir (Fig. 9 B). Calculated differences for derived mitochondrial parameters (Fig. 9 C and D) exhibited the same trends, with a significant difference of protein leak between DTG and ELV at cells cultured at 11 mM glucose ( $P=0.04$ ) (Fig. 9 B)



**Figure 9 Dolutegravir inhibits Oxygen consumption rate (OCR) at Basal in INS-1 cells cultured in 11 mM glucose.** A) Oxygen consumption rate (OCR) of INS-1 cells cultured in 4 mM and B) at 11 mM glucose after 3 days exposure to control, Dolutegravir, Elvitegravir.

### **Effects of Dolutegravir on Average Intracellular $\text{Ca}^{2+}$**

INS-1 cells were incubated for 3 days in 11 mM glucose with either DMSO as control or Dolutegravir. In four separate experiments there were no effects of Dolutegravir on basal calcium influx (1 mM G) (orange graph). However, Dolutegravir showed less increase in calcium influx at 12mM G in comparison to the control group (blue graph) (Fig. 10 A 1-4). Dolutegravir reduced the average percent increase of calcium influx in cells stimulated with 12mM G (orange bar) vs control (blue bar) by 80% ( $p = .00016$ ) (Fig. 10 B).



**Figure 10 Dolutegravir showed reduction in calcium influx after adding 1M glucose VS. Control.** A1-4) Effects of Dolutegravir on Average Intracellular Ca<sup>2+</sup> Activity, Ca<sup>2+</sup> Activity in 4 experiments, B) Average Intracellular Ca<sup>2+</sup> Activity. (\* p<.05, the t-tst is used to represent a comparative statistically significant result, n=4).

## DISCUSSION

In this study we utilized INS-1 cells to examine potential mechanisms that could contribute to weight gain and changes in insulin homeostasis in people living with HIV following exposure to integrase inhibitors.

We have demonstrated here, for the first time, that Dolutegravir but not Elvitegravir reduces glucose-stimulated insulin secretion (GSIS) in  $\beta$ -cells. Insulin plays a vital role in blood glucose level regulation and facilitating the storage of excess energy. However, when there is a disruption in normal insulin homeostasis or function, excess weight gain can occur. The decreased insulin secretion observed in DTG-treated  $\beta$ -cells under high glucose conditions (11G) suggests that DTG may interfere with the mechanisms involved in insulin release. This result of our study is consistent with the finding that insulin secretion inhibition contributes to weight gain (Schwartz, 1995). Several mechanisms could explain this relationship.

Insufficient insulin secretion can hinder the uptake of glucose by cells, leading to elevated blood glucose levels. In response, the body may produce more insulin to compensate. However, if insulin secretion remains inadequate, cells may not receive the necessary nutrients, leading to increased hunger and subsequent overeating, which can contribute to weight gain (Torbatinejad, 2009). Furthermore, in response to reduced insulin secretion, the body may undergo metabolic adaptations that favor weight gain. This can include a decrease in energy expenditure, known as reduced thermogenesis. This was shown in a study examining the potential impact of HIV integrase inhibitors,

specifically dolutegravir and Bictegravir, on the reduction of adipose tissue (Ngono Ayissi, 2022).

We sought to understand the broader effects of DTG on cellular metabolism leading to reduced insulin secretion. The cellular metabolic oxygen consumption rate (OCR) refers to the amount of oxygen consumed by cells during various metabolic processes, such as oxidative phosphorylation and mitochondrial respiration. OCR serves as a vital indicator of cellular energetics and overall metabolic activity. Changes in OCR can reflect alterations in cellular function, including shifts in energy production pathways, cellular stress response, and ATP production. Changes in OCR affect insulin production and secretion in pancreatic  $\beta$ -cells. Increased OCR reflects higher metabolic activity, indicating that the  $\beta$ -cells are utilizing more glucose to generate ATP. This rise in ATP levels triggers the release of insulin vesicles, leading to increased insulin secretion (Henquin, 2009). Additionally, Changes in OCR can influence the generation of Reactive Oxygen Species (ROS) within cells (Yin, 2021). ROS are natural byproducts of oxidative metabolism and can act as signaling molecules. Moderate levels of ROS are necessary for proper insulin secretion, as by enhancement of  $\text{Ca}^{2+}$  flux they contribute to the coupling mechanism between glucose metabolism and insulin release. However, excessive ROS production, often associated with dysfunctional OCR, can lead to  $\beta$ -cell damage and impaired insulin secretion (Pi, 2007).

Our findings showed a significant reduction in OCR at 11G cells exposed to DTG, suggesting an effect of the drug on ATP production and insulin secretion. A study employing a mouse model compared impacts of various Antiretrovirals (ARVs) on

mitochondrial function and adipocyte differentiation and found alterations in OCR in adipocytes following exposure to DTG (Jung, 2021).

These changes in OCR could be attributed to multiple factors, including mitochondrial dysfunction, oxidative stress, or indirect effects resulting from the inhibition of viral replication. Some studies have proposed that DTG might affect mitochondrial activity (Chui, 2020). This effect could be explained by DTG interfering with mitochondrial DNA replication or by altering oxidative stress. Certain antiretroviral drugs, including DTG, have been associated with mitochondrial health, as evidenced by an increase in the mitochondrial Reactive Oxygen Species (mtROS) (Ajaykumar, 2023)

Moreover, we assessed the effects of DTG on calcium influx, a critical step in the regulation of insulin secretion. Our results indicate that compared to control cells, after the introduction of 12 mM glucose, DTG-treated  $\beta$ -cells exhibit a significantly lower increase in calcium influx. This reduction in calcium influx amounted to 80 %, highlighting the potential inhibitory effect of DTG on the calcium signaling pathway involved in GSIS. This is consistent with the current understanding of the underlying mechanisms regulating GSIS by  $\text{Ca}^{2+}$  entry into pancreatic  $\beta$ -cells through voltage-dependent  $\text{Ca}^{2+}$  channels. The elevated  $\text{Ca}^{2+}$  levels induce the fusion of insulin-containing secretory granules with the plasma membrane, leading to the exocytosis of insulin into the bloodstream (Deeney, 2000).

Another proposed mechanism underlying weight gain in patients treated with integrase inhibitors involves the interaction of DTG with MC4R receptors. Preclinical studies have suggested that DTG may bind to MC4R and modify its signaling, potentially leading to

increased food intake and weight gain. Some scholars have suggested that the difference in weight gain observed with integrase inhibitors may be related to their effect on adipocyte activity (Macallan, 1995). Another hypothesis suggests that integrase inhibitors might affect the gut microbiota in HIV/AIDS patients (Moure, 2016).

In summary, this study investigated the effects of Dolutegravir (DTG) and Elvitegravir on glucose-stimulated insulin secretion (GSIS) in  $\beta$ -cells using INS-1 cells. The study found that DTG reduced insulin secretion in  $\beta$ -cells under high glucose conditions, suggesting a disruption in insulin release mechanisms. Changes in cellular metabolic oxygen consumption rate (OCR) and calcium influx were also observed in DTG-exposed cells, indicating potential effects on ATP production and the calcium signaling pathway involved in GSIS.

Our study had several Strengths. The study presents novel findings by demonstrating, for the first time, that DTG reduces GSIS in  $\beta$ -cells, while ELV does not. Furthermore, the study integrates multiple mechanisms to explain the observed reduction in insulin secretion. It considers changes in glucose metabolism, mitochondrial function (OCR), and calcium influx, providing a comprehensive understanding of the potential pathways affected by DTG. The limitation of our study includes the lack of in vivo data. The study solely focuses on in vitro experiments using cell culture. Secondly, limited clinical relevance. The study primarily focuses on cellular mechanisms and does not directly investigate the clinical implications or long-term effects of DTG on weight gain and insulin homeostasis in people living with HIV. While the study establishes a link between DTG and reduced insulin secretion, it does not directly measure or correlate this effect

with weight gain. The proposed mechanisms linking insulin secretion to weight gain remain hypothetical and require further investigation to establish a clear causal relationship. Future studies focusing on elucidating the molecular pathways involved in these differential effects may shed light on the specific targets and mechanisms by which DTG modulates  $\beta$ -cell function.

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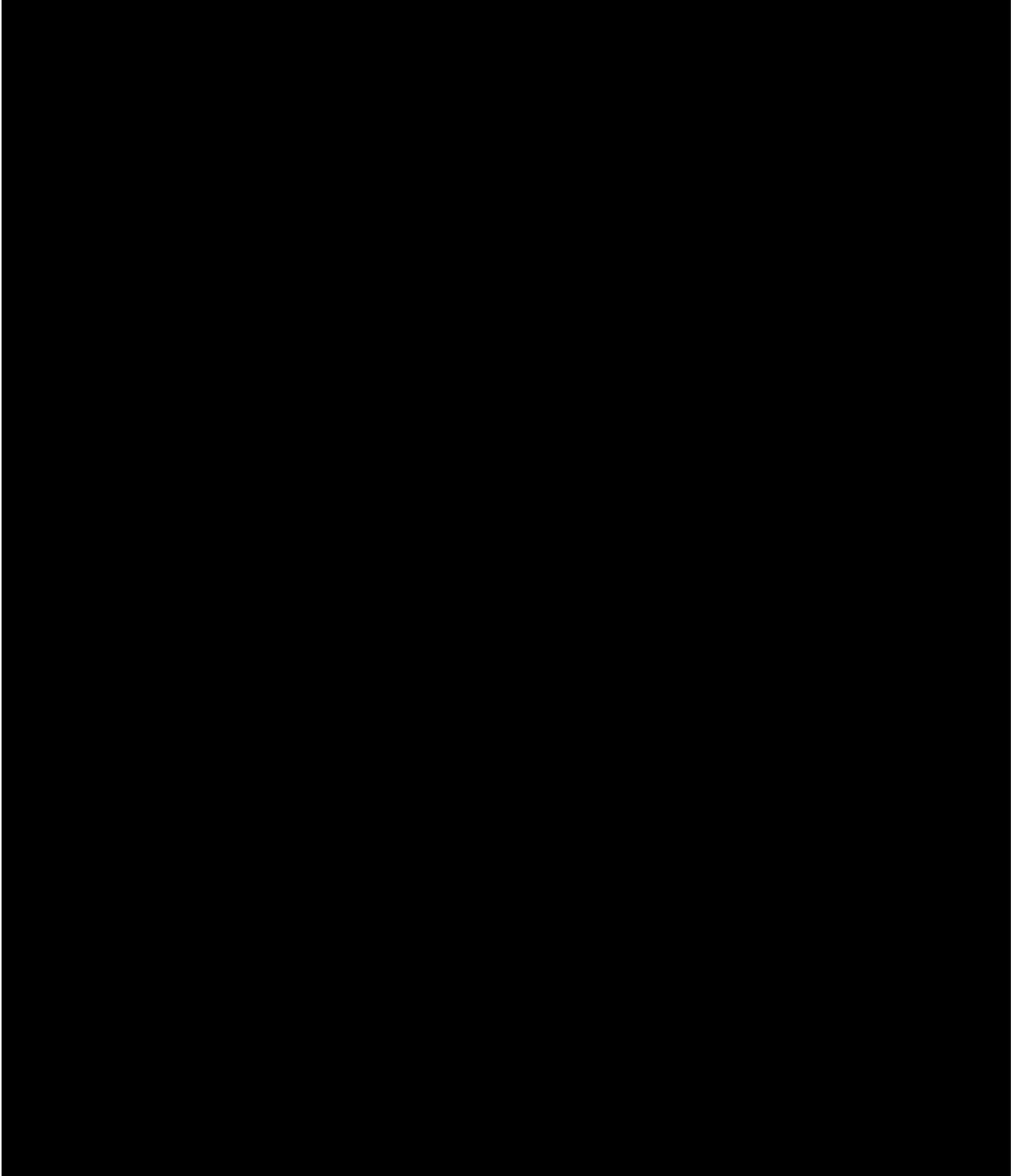
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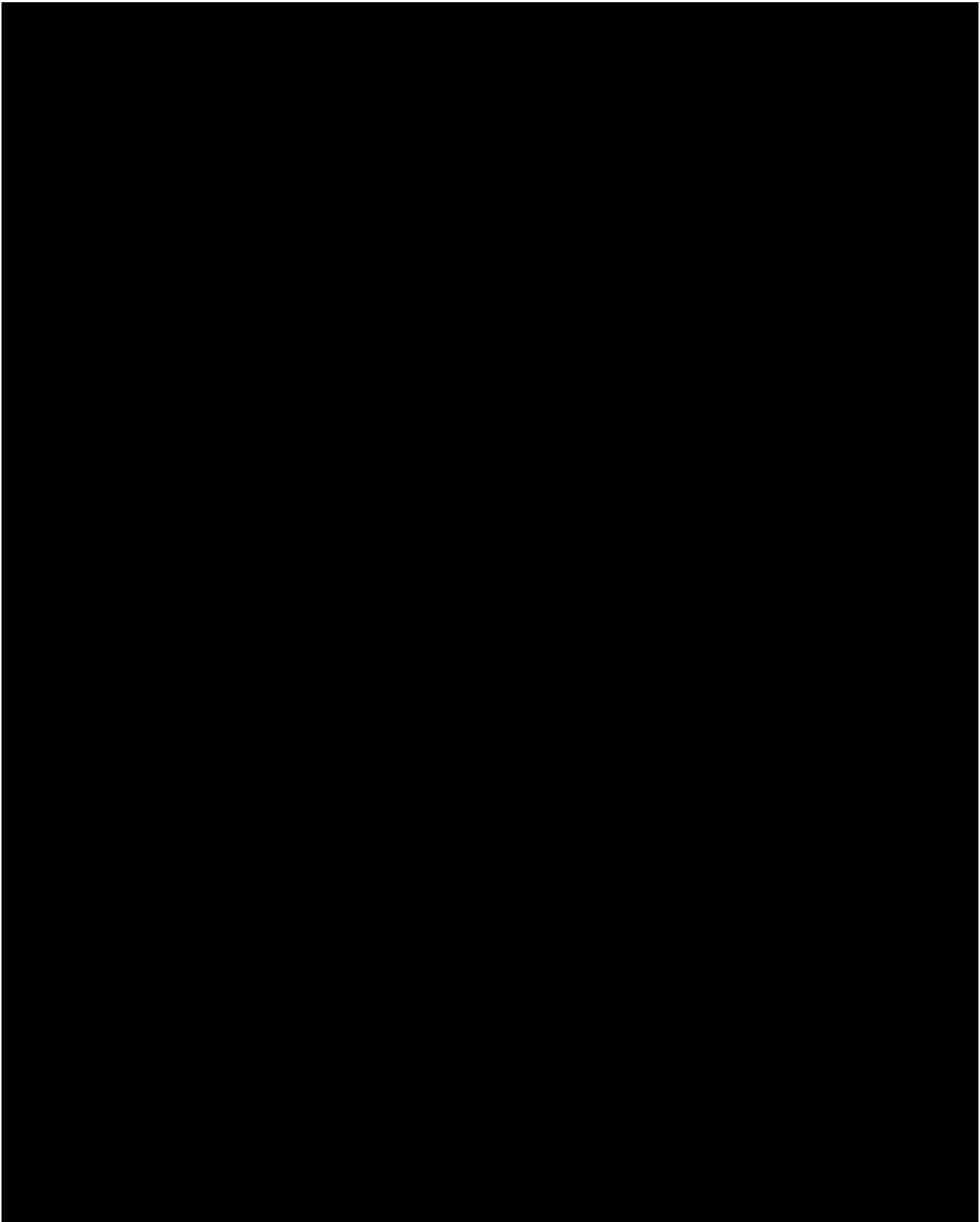
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**CURRICULUM VITAE**





**Mar. 2016** **Journal:** Golaleh Asghari, Emad Yuzbashian, Parvin Mirmiran, **Fatemeh Hooshmand**, Reza Najafi and Fereidoun Azizi. **Dietary Approaches to Stop Hypertension (DASH) Dietary Pattern Is Associated with Reduced Incidence of Metabolic Syndrome in Children and Adolescents.**  
<http://dx.doi.org/10.1016/j.jpeds.2016.03.077>

**Certificates:**

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- Biosafety Level 1 & 2 Training, Boston University, Oct 2021
- Chemical Safety Training, Boston University, Oct 2021
- Laboratory Safety Training, Boston University, Oct 2021

**VOLUNTEERING**

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- 2011-2014** **Iranian nutrition society; board of student and alumina group**
- Planned and implemented workshop program for students, staff and researchers
  - Facilitated introduction of speakers and discussion during workshop sessions
- July-2018** **Abstract reviewer for American Society of Nutrition meeting**
- rated abstract submissions objectively according to defined criteria
  - educate nutrition facts to public

**COMPUTER SKILLS**

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- Expert in Statistical programs: SPSS
- Expert in Nutritional programs: N3, N4
- Expert in Reference manager Endnote
- Expert in Microsoft Office
- Expert in Illustrator

**LANGUAGE SKILLS**

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- Persian Mother Tongue
- English: **Ielts: 6.5**(10/24/2020 ), **GRE:295**(06/09/2020)
- Arabic Familiar