The Glycolytic Pathway is the Predominate Path for Glucose Utilization in Human Pancreatic Beta Cells (1.1B4)

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Abstract

The oxidative metabolism of energy substrates has a paramount role in the stimulus secretion pathway of insulin. However, the role of glycolytic pathway in pancreatic beta cells is not very well understood. To address this, we have investigated and compared the functional effects of two mitochondrial substrates (glucose and α-ketoisocaproate) between the human (1.1B4) and murine (MIN6) pancreatic beta cell lines. MTS assay was conducted as an indicator of the metabolic activity of both cell lines. Polarographic detection of (ΔO_2) and lactate were used to measure the oxygen consumption rate and anaerobic glycolysis respectively. The mitochondrial redox state was monitored via RH123 distribution and NAD(P)H autofluorescence. The metabolic assays showed glucose stimulated MTS reduction in MIN6 cells in a time and concentration dependent manner and nor in 1.1B4. Both sub strates failed to affect OCR, NADPH and increased lactate production in 1.1B4 cells. However, they stimulated OCR, increased NADPH, increased lactate output but was less extent and hyperpolarized the mitochondria in MIN6 cells. The above results showed that 1.1B4 cells are mainly depending on the glycolytic pathway different from MIN6 cells which rely on mitochondrial respiration. In conclusion, 1.1B4 cell line represents a new model to study the bioenergetics profile because it depends on the anaerobic glycolysis rather than aerobic respiration of the other models such as MIN6 and islets.

Keywords: Oxidative metabolism, glycolysis, mitochondria, insulin secretion, pancreatic β-cell

Introduction

Glycolysis is the process by which one mole of glucose breaks down to produce two moles of pyruvate and to provide energy as ATP. Glucose

enters beta cells via two types of glucose transporters such as glut-2 and glut -1by facilitated diffusion process. It is phosphorylated to glucose -6-phosphate by glucokinase enzyme (GK). This enzyme has high affinity to glucose (Km of = 5 mM) which explains the concentration dependence of the beta cell response to glucose in the physiological range (Meglasson and Matschinsky, 1984, 1986; Matschinsky *et al.*, 1993; Aguilar-Bryan and Bryan, 1999). In the lack of oxygen, pyruvate produces lactate by anaerobic glycolysis (Barrett *et al.*, 2012). However, in cancer cells and some cell lines lactate can be produced in the presence of sufficient amount of oxygen according to Warburg effect (WARBURG, 1956). In the presence of oxygen pyruvate enters the mitochondria and metabolizes in tricarboxylic acid cycle (TCA) to generate 38 molecules of adenosine triphosphate (ATP).

The mitochondrion consists of matrix surrounded by two membranes inner and outer with intermembrane space in between them. TCA cycle happens in the matrix while oxidative phosphorylation occurs in the inner membrane. In the oxidative phosphorylation process, electrons are transferred from electron donors (NADH and $FADH_2$) produced from TCA cycle to electron acceptors (oxygen) in redox reactions. The redox reactions achieved by several steps called electron transport chain via five protein complexes (I-V). The process commences when the electron donors are donate two electrons during the reacting with NADH dehydrogenase enzyme, which is also called complex I. As a result of passing of those electrons through complex I, four protons (H^+) will be transported from matrix to intermembrane space. Moreover, coenzyme Q10 or ubiquinone (Q) will be reduced to ubiquinol (QH_2) (Hirst, 2010). Second step in electron transport chain is the effect of complex-II which is also known as succinate dehydrogenase enzyme. It oxidizes succinate to fumarate and transports the electrons via reduction of flavin adenine dinucleotide (FAD) which leads to reduce ubiquinone as well. The oxidation of succinate in complex II produces less energy than that of NADH in complex I. Therefore, complex II does not transport (H^+) throughout the membrane and it is considered the only complex that transport electrons without pumping (H^+) to the intermembrane space. Complex III (cytochrome c reductase) consists of two protein subunits or dimer. In each subunit, it oxidises the ubiquinone to ubiquinol, releasing two (H^+) to the intermembrane space and the electrons are passed through the cytochrome units of the complex (Chaban *et al.*, 2014).

The final enzyme of the mitochondrial electron transport chain is cytochrome c oxidase or complex IV which receives electrons from complex III. Two water molecules will be generated from the reaction of the elections with the final acceptor (oxygen). During this process, four (H^+) are pumped to the intermembrane space (Calhoun *et al.*, 1994; Chaban *et al.*, 2014). As each complex moves electrons along the chain, protons are pumped out of the

matrix into the intermembrane space. The proton gradient generated is used to drive ATP synthesis by Complex V (F_1 F_0 ATP synthase), which phosphorylates ADP to ATP (Kim *et al.,* 2008). This proton gradient may commonly refer to as the mitochondrial membrane potential (~150-180mV) which is significantly higher than the plasma membrane potential; (Valdez *et al.,* 2006) vs.(~60-90mV (Wright, 2004).

The widely accepted key process of glucose stimulated insulin secretion is ATP-sensitive K^+ channels (K^+-$_{ATP}$) dependent. The increase of ATP/ADP ratio causes closure of the K^+-$_{ATP}$ sensitive channels is leading to a depolarization of cell membrane and opening the voltage gated calcium channel (Aguilar-Bryan and Bryan, 1999). This will increase the cytosolic Ca^{2+} levels which is directly promoting exocytosis of insulin.

The aim of this study is to compare metabolic profiles of two different pancreatic beta cell lines, (1.1B4) human derived cell line vs murine (MIN6). The rational is to find out which cell line is more relevant for the study of the pancreatic beta cells bioenergetics.

Methodology

Cell lines

Two cell lines had been used in this study. Firstly, 1.1B4 (ECACC-87092802) a cell line designed by the electrofusion of a primary culture of human pancreatic islets with PANC-1, a human pancreatic ductal carcinoma cell line (Lieber *et al.*, 1975; McCluskey *et al.*, 2011). 1.1B4 cells are considered to be a good model for secreting pure insulin (McCluskey *et al.*, 2011). Cells maintained with RPMI 1640 which supplemented by 10% (v/v) foetal calf serum (FCS) in addition to + 1% (w/v) Penicillin 1000 unit/ml and streptomycin 0.1mg/ml. Passage numbers 32-40 were used.

The MIN6 cell line is a transgenic mouse insulinoma cells established by Miyazaki in 1990. We have chosen Min6 because it is homogenous and response to glucose with the physiological range in addition to it expresses glucose transporter and glucose which make Min6 ideal for this study (Ishihara et al., 1993). Although MIN6 have been reported to contain and secrete other pancreatic endocrine hormones, these cells still remain primarily b-cell in function (Nakashima et al., 2009). Cell were cultured in RPMI 1640, supplemented by 10 %(v/v) foetal calf serum (FCS). Passages numbers 19-40 were used.

MTS Assay

The water-soluble salt MTS (3-[4, 5dimethylthiazol-2-yl] -5-[3-carboxymethoxy-phenyl] -2-[4-sulfophenyl] -2H-tetrazolium) assay depends on reduction of the MTS substrate to produce coloured formazan. This assay does not need to solubilize the cells by using acidified isopropanol, which may

disrupt the cells integrity. MTS receives electrons via an intermediate electron acceptor Phenazine methosulfate (PMS) which amplifies (fluorescence signal at 490 nM (Cory *et al.*, 1991; Janjic and Wollheim, 1992; Segu *et al.*, 1998). The MTS assay was performed according to the manufacturer's instructions (Celltiter 96 aqueous nonradioactive cell- proliferation assay, Promega, Madison, WI). The absorbance was measured at 490 nm by plate reader. Readings were corrected for the background by subtracting the blank (MTS/PMS regent in Hanks without the cells) from the readings. Three experiments have been performed with triplicate wells in each experiment.

Oxygen Consumption Rate (OCR)

Mitochondrial respiratory rate of 1.1B4 and MIN6 cell suspensions were measured polarographically using Clark Oxygen. The partial pressure of O_2 (PO_2) was measured at a polarographic voltage of 0.6 V with electrodes previously calibrated at 100% air saturation (vigorous gassing with air, 0.25mM) and 0% (addition of $Na_2S_2O_4$) (Daunt *et al.*, 2006). The background of Oxygen consumption by electrode was determined by the addition of 6 mM sodium azide to block oxidative respiration at cytochrome C.

Lactate production

The rate of lactate production of both1.1B4 and MIN6 was measured polarographically with sarissa lactate enzyme electrodes (Sarissa Biomedical Ltd Coventry, UK) as previously mentioned by Brown *et al.*, 2012. 4 mL of cell suspension were loaded in to a chamber, where the potential of the lactate electrode was set at 500 mV relative to Ag/AgCl reference electrode. Electrical current will be generated due to the oxidation of lactate by the lactate oxidase enzyme on the surface of a carbon fiber electrode. This current is a stoichiometric measure of lactate concentration. The lactate output rate was calculated according to the following equation:

ESubstrate (nmol 107 cell-1 min-1) = (substrate)*60/ (cells count)/calibration value

Mitochondrial membrane potential

Rhodamine123 (Rh123) was used to monitor the mitochondrial membrane potential, $\Delta\Psi m$, with methods similar to those described previously with some modifications (Duchen et al., 1993). The dye was excited at 480 nm and the emitted fluorescence monitored at 530 nm. Cells were plated on 22 mm cover-slips and incubated with 10 µg/ml rh123 for 10 min at room temprature. Images were captured at a frequency of 1Hz with a Photonics ISIS CCD camera, DT3155 frame grabber (Data translation, UK) and Imaging workbench software (IW5.2 INDEC BioSystems, Santa Clara, CA, USA). Only single and clusters of cells that responded to glucose were chosen for

further analysis using Origin software with bespoke scripts written in Labtalk (OriginLab Corporation, MA USA). Images were background corrected and the average fluorescence intensity for each field of cells calculated.

NAD(P)H measurements

NAD(P)H experiments were carried out using epifluorescence microscope as previously described (e.g. Duchen, 1992a,b; Duchen and Biscoe, 1992a,b). NAD(P)H produced from glucose and/or amino acid metabolism in the mitochondria . Cells were excited by light at 350 nm and measured with a bandpass fliter combination between 400 and 500 nm. To normalize the NAD(P)H response experiments, 10 mM glucose was used to reach a maximal response and this was set as a 100%.

Dose-response relationships glucose and KIC:

The dose-response relationships were quantified by best fits of the data with the following equation using prism via best fit non-linear regression:

$$Y = \frac{Y_{MAX} - Y_{MIN}}{1 + (EC_{50}/G)^{h}} + Y_{MIN}$$

Where G is the concentration of substrates, EC_{50} is the concentration that produces the half-maximal response, Y is the response magnitude, Y_{MAX} is the maximal response, Y_{MIN} is the minimal response, and h is an index of slope. Fits were performed in PRISM 6 (GraphPad Software Inc., San Deigo, CA).

Statistical analysis

Data are expressed as mean ± S.E.M. Parametric statistical analysis was used when the data are normally distributed (D' Agostino Pearson ominubus normality test). All statistical analysis had been done Prism (6.0, GraphPad software, San Diego California, USA). EC_{50} and h values are quoted with 95% confidence intervals. Statistical significant was accepted at $P < 0.05$.

Results

MTS

In 1.1B4 cells, there was a slight increase in MTS reduction after adding of 1mM glucose, which saturated at 10mM. The EC_{50} of glucose stimulated MTS reduction was 1.6 mM (0.4 to 6 95% CI) within an h (slope of index) of 1 (Fig. 1). In MIN6 cells, the basal MTS signal was lesser than in 1.1B4 cells. There was a progressive increase in MTS reduction with

increasing glucose concentration at 0.01mM glucose, which saturated at 10mM as well. The EC_{50} of glucose stimulated MTS reduction in MIN6 was 1.8 µM (2 to 30 95% CI) and h of 1 (Fig. 1)

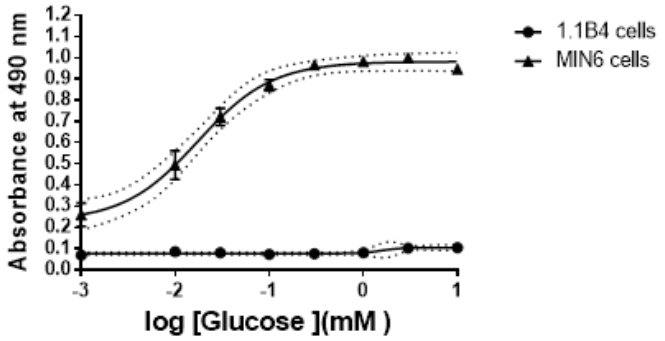

Fig. 1: **Glucose concentration-response relationship for the reduction of MTS in 1.1B4 and MIN6 cells**. Solid lines are best fits of equation 1 with the parameters given in text. Dots line are confidence interval Cells were incubated for 60 min. Data are mean ± SEM. N=6.

Oxygen consumption rate

In this study, we were conducted the OCR experiments as an indicator to assess the mitochondrial activity of 1.1B4 and MIN6 cell lines. In the absence of added substrate, 1.1.B4 cells possessed a linear basal respiratory rate at -27 nmoles 10^7 $cells^{-1}$ min^{-1} (Fig. 2A). Adding 10 mM glucose inhibited the OCR by ~24 ± 4.6% (P< 0.001, Wilcoxon matched-pairs signed rank test)(Fig. 2A). The basal OCR of MIN6 cell was -23 nmoles 10^7 $cells^{-1}$ min^{-1}, and within 1 min of adding 10 mM glucose the OCR increased by ~25 ± 4.5% (P< 0.001, Wilcoxon matched-pairs signed rank test) (Fig. 2B).

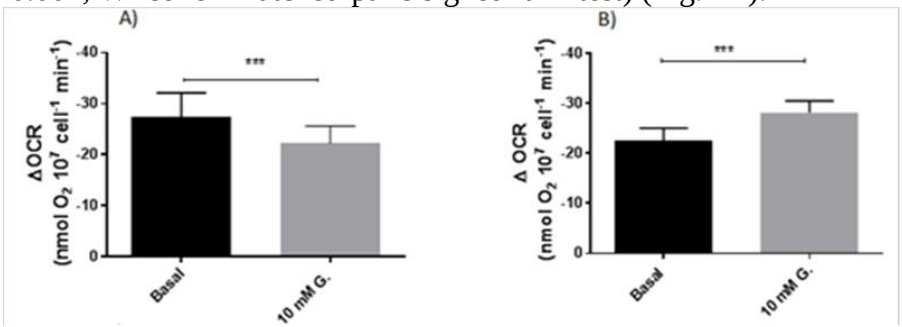

Fig. 2: The effect of glucose 10mM on Oxygen consumption rate (OCR) in 1.1B4 (A) MIN6 cells (B). Glucose inhibits oxygen consumption 1.1B4 (A) and stimulated it in MIN6 cells (B) compared to those measured in the absence of exogenous substrate (basal). Data are mean ± SEM. Statistical significance determined by paired T test. N=15.

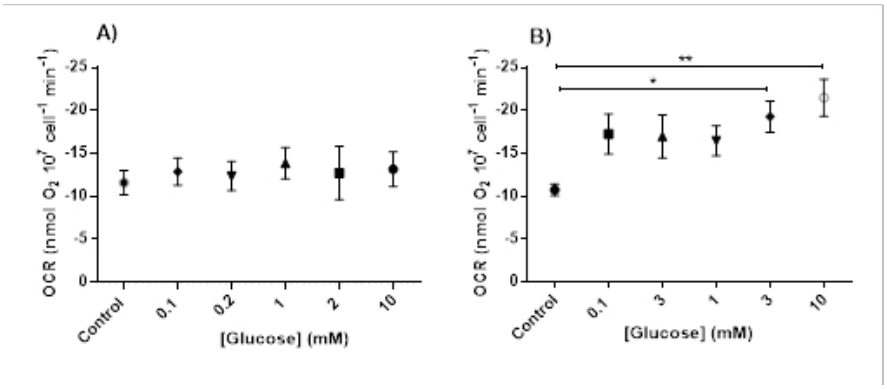

Fig. 3: Relationship between glucose concentration and O_2 consumption in 1.1B4 cells (A) and MIN6 cells (B). Data are mean ± SEM. N=18.

Different concentrations of glucose had been failed to change the OCR in comparison to the control (H_2O) in 1.1B4 cells (as shown in figure 3A). While in MIN6 cells, glucose Stimulated OCR in a concentration-dependent manner which started from 3 mM in comparison with vehicle control (H_2O) ($P< 0.05$, Dunnett's multiple comparisons test). Further increased in OCR had been achieved by 10 mM glucose stimulated OCR ($P< 0.01$, Dunnett's multiple comparisons test) (Fig 3B). Since glucose did not affect the OCR in 1.1B4 cells and it did in MIN6, this suggests that there are differences in the mitochondrial function between 1.1B4 and MIN6. Thus, we used α-ketoisocaproate (a fuel that feeds directly into the mitochondrial tricarboxylic acid cycle (Duchen *et al.*, 1993; Gao *et al.*, 2003; Daunt *et al.*, 2006). We found that α-ketoisocaproate failed to stimulate OCR compared to the Vehicle control (H_2O) in 1.1B4 cells (Fig. 4.A). However, in MIN6 cells, α-ketoisocaproate increased OCR in a concentration-dependent manner in comparison with Control (H_2O). ($P<0.01$, Dunnett's multiple comparisons test)(Fig. 4B).

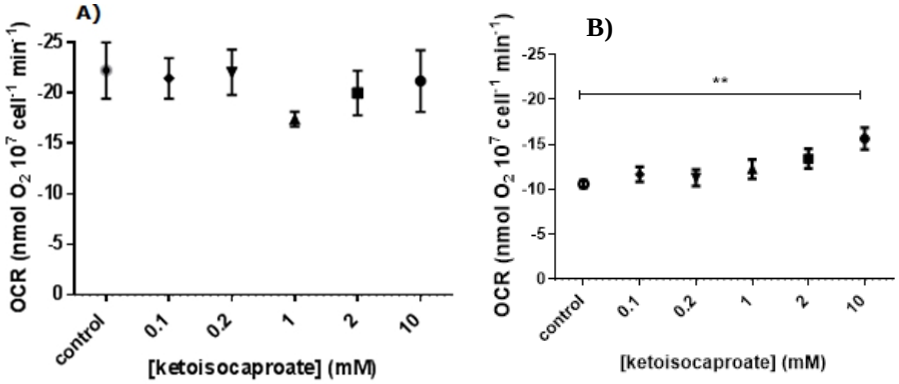

Fig. 4: Relationship between α-ketoisocaproate concentration and O_2 consumption in1.1B4 cells (A) and MIN6 cells (B). Data are mean ± SEM. N=8.

Lactate production

In order to compare the rate of glycolysis of 1.1B4 and MIN6 cells, we measured the lactate production as an indicator of conversion of pyruvate in to lactate. In the absence of exogenous metabolic substrate, 1.1B4 cells possessed a linear basal lactate production at 2 nmoles 10^7 cells^{-1} min^{-1} (Fig. 5A). Adding 10 mM glucose significantly increased lactate production by ~93 ± 9.5% (P< 0.001, Wilcoxon matched-pairs signed rank test) compared to the basal (Fig. 5A). The basal rate of lactate production of MIN6 cells was at -10 ± 2.4 nmoles 10^7 cells^{-1} min^{-1}. Within 1 min of adding 10 mM glucose, lactate production significantly increased by ~25 ± 4.5% (P< 0.001, Wilcoxon matched-pairs signed rank test) (Fig. 5B).

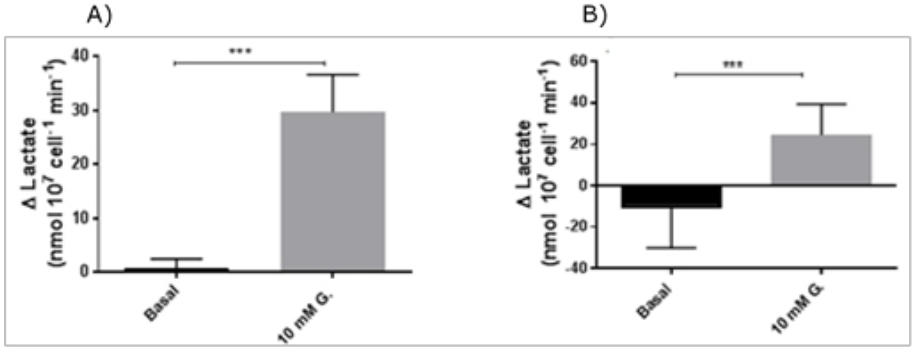

Fig. 5: The effect of glucose 10mM on lactate production rate in 1.1B4 (A) MIN6 cells (B). 10mM glucose (G.) significantly increased lactate output in both 1.1B4 (A) and MIN6 (B). Data are mean ± SEM. Statistical significance determined by paired T test. N=15.

The effect of glucose and α-ketoisocaproate (KIC) on the mitochondrial membrane potential

In addition to OCR, we investigated the mitochondrial redox by monitoring mitochondrial membrane potential Δψm using Rh123 fluorescence in MIN6 cells. In the absence of exogenous metabolic substrate, Δψm was static in both glucose and KIC experiments (Fig. 6B&D). After 1 min of adding 10 mM glucose, it produced significant decrease in Rh123 fluorescence ~28±1.25% (P< 0.0001, paired t test) (Fig. 6A). Similar result had obtained by adding 10 mM KIC which decreased the Rh123 fluorescence by ~20 ±1.25% (P< 0.0001, paired t test) as shown in (Fig. C&D). Addition of 1μM FCCP, a mitochondrial uncoupler which collapses of ΔΨm (Duchen *et al.*, 1993; Daunt *et al.*, 2006) as shown in (Fig. 6B&D).

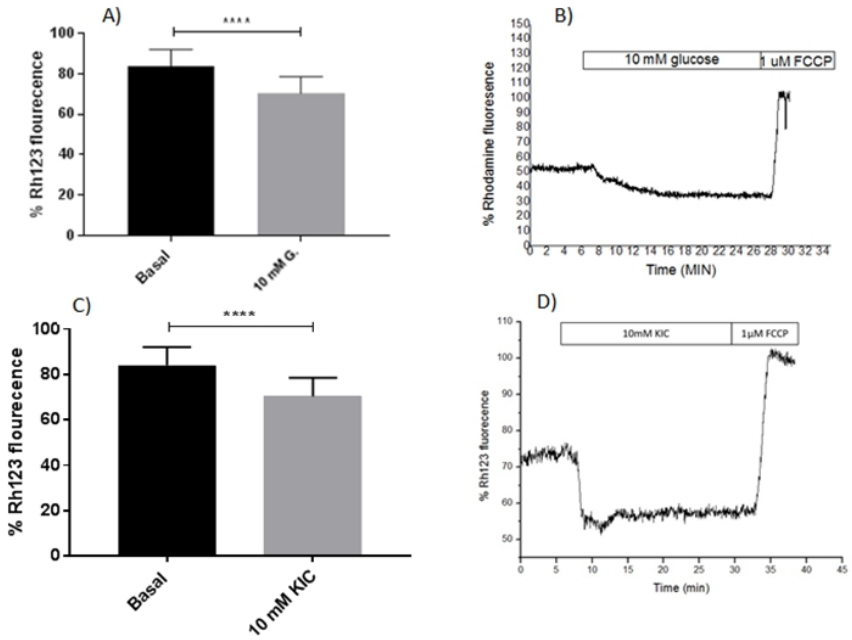

Fig. 6: Rh123 fluorescence as a measure of the mitochondrial membrane potential, ΔΨmit. Effects of 10mM glucose (G.)(A) and KIC (C) as indicated on ΔΨmit, in MIN6 cells. E,D, ΔΨmit is represented by the change in Rh-123 fluorescence relative to that measured in the absence of the sugar(100%). Records are representative of at least 3 different experiments for each condition and are formed from the average of at least 7 ROIs. Data are the means SD. Data is from at least 5 independent preparations in each case, where n is the total number of ROI measured for each condition. `

Changes in 1.1B4 NAD(P)H autofluorescence in comparison to MIN6 cells in response to 10 mM glucose and α-ketoisocaproate (KIC).

The increase in the substrate level leads to the increment in the NAD(P)H and subsequently tricarboxylic acid cycle (TCA)(Duchen et al., 1993). The aim of this experiment is to support that the glucose stimulated OCR in MIN6 cells and it did not in 1.1B4 cells which may be due to abnormal mitochondrial oxidation. We therefore, measured autofluorescence under conditions that evoke fluorescence of NAD (P) H. Fig. 7A&B showed that the effect of 10 mM glucose and on the NAD (P) H signal of a single 1.1B4. Treating of 1.1B4 cells to glucose had no effect on the NAD(P)H levels compared to the basal (Fig. 7A&B). Subsequent addition of 1 μM rotenone (complex I inhibitor of electron transport chain) failed to affect NAD(P)H signals in the presence of glucose (Fig. 7A&B). However, exposure of MIN6 cells to 10 mM glucose increased NADPH levels by ~10 ± 0.9% ($P < 0.01$, Sidak's multiple comparisons test) after a delay of one minute compared to the basal (Fig. 7C&D). 1 μM rotenone further elevated NAD(P)H signals by ~15 ± 1.9% ($P < 0.001$, Sidak's multiple comparisons test) in response to glucose (Fig. 7C&D). However, the same challeng of 10 mM KIC mitochondrial

substrates (Duchen et al., 1993; Gao et al., 2003; Daunt et al., 2006) did not affect NAD(P)H levels compared to the basal in 1.1B4 cells (Fig. 7E&F). Also, addition of 1 μM rotenone (complex I inhibitor of electron transport chain) failed to affect NAD(P)H signals in the presence of KIC (Fig.7E&F). while in MIN6 cells ,10 mM KIC caused as increase in the NAD(P)H signals by ~5 ± 0.5% (P< 0.01, Sidak's multiple comparisons test)(Fig.7G&H). After one minute delay of 1 μM rotenone further increased NADPH levels by 15 ± 1.9% (P< 0.0001, Sidak's multiple comparisons test) in response to KIC (Fig. 7G&H).

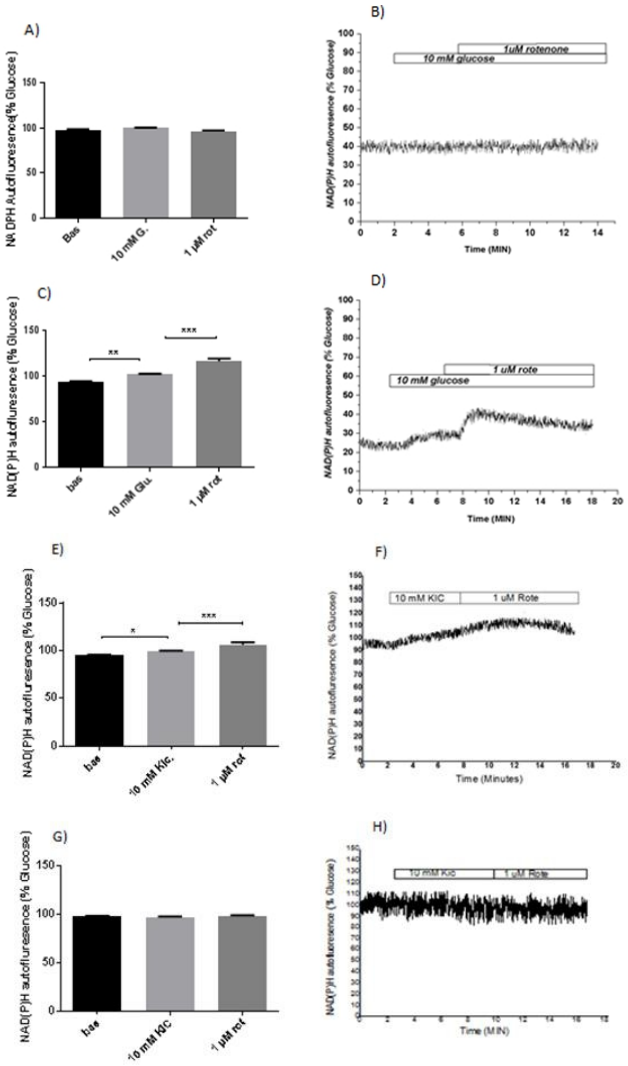

Fig. 7: Effect of 10 mM glucose (A,B) and KIC (G,H) on the NAD(P)H levels in the 1.1B4. 10 mM glucose (C,D) and KIC (E,F) on the NAD(P)H levels MIN6 cells. N= 101-130 cells collected from 4-16 experiments where the minimum number of cells is 7 in each

Discussion

MTS

Since oxidative respiration of *β*-cells are generally linked with the uptake and metabolism of glucose and other fuels, therefore assessments of the metabolic activity of 1.1B4 and MIN6 cells are important. As a result, during static incubations of these cells with glucose may increase the signal derived via the reduction of MTS in a time- and dose-dependent manner. The key rate-limiting step for glucose metabolism is its phosphorylation to glucose-6-phosphate, where the subsequent stimulus-secretion cascade leads to insulin release in pancreatic β-cells (Matschinsky *et al.*, 1993; Sakura *et al.*, 1998). Low affinity glucokinase is predominantly responsible for phosphorylation in native beta cells during the stimulus-secretion pathway, possess EC_{50} values for glucose around 5–10 mM (Sakura *et al.*, 1998; Martín *et al.*, 1999). The micromollar EC_{50s} values found for glucose phosphorylation within the stimulus-secretion pathway suggest that high-affinity hexokinase activity (Km ~300 μM) predominate in MIN6 (Ishihara *et al.*, 1993) over that of glucokinase in 1.1B4 cells (McCluskey *et al.*, 2011). In this study we found that the EC_{50} for glucose-stimulated MTS reduction of 1.1B4 was 1.6 mM compared to 1.8μM of MIN6. Although, these cell lines produce the hexokinase to metabolize glucose (Ishihara *et al.*, 1993), the variation in EC_{50} of MTS reduction could be due to high levels of glucokinase in 1.1B4 (McCluskey *et al.*, 2011) in comparison to MIN6 which mainly depends on hexokinase I (Ishihara *et al.*, 1993). The same principle would be suggested to explain the differences between highly content glucokinase mouse islet EC_{50} = 8 mM (Panten and Klein, 1982) in one hand and murine cell line MIN6 on the other hand. Since both of 1.1B4 and beta cells from pancreatic islet depend on glucokinase activity, therefore 1.1B4 had a comparable EC_{50} of MTS reduction with human pancreatic islets (EC_{50} =12 mM) found by Panten and Klein (1982) and Soria *et al.* (2010).

Oxygen consumption rate

Since we interested in the bioenergetics of the pancreatic beta cell; we then investigated aerobic respiration of 1.1B4 cells and MIN6 cells. In 1.1 B4 and MIN6 cells, basal O_2 consumption showed a linear pattern which varied between experiments. Figure 2A showed that basal OCR at 27- nmoles 10^7 $cells^{-1}$ min^{-1} of 1.1B4 cells. However, the basal OCR at 23 nmoles 10^7 $cells^{-1}$ min^{-1} of MIN6 cells. Earlier studies confirm that basal levels of Oxygen consumption rate were roughly 20 nmol 10^7 $cells^{-1}$ min^{-1} in MIN6 cell lines (Daunt et al., 2006). Difference in the basal respiration may be due to the presence of fatty acids in the cells suspensions, which metabolised by mitochondria and increase reducing equivalents leading to an increase oxidative respiration (Lopaschuk et al., 2010). The 1-fold inhibition in the rate

of (ΔO2) produced by 10 mM glucose. This suggests the adding of glucose was either metabolized solely by glycolysis and apparently not by the TCA or the cells have marginal oxidative capacity (Warburg 1956). This idea may support these findings by supplementary data stated that glucose utilization (glycolysis) occurs at a much higher rate than glucose oxidation in 1.1B4 cells (McCluskey et al., 2011) and in human islets (Doliba et al., 2012). This may lead to inhibit of oxidative respiration by glycolytic products Crabtree effect (Diaz-Ruiz et al., 2011). The 2-fold stimulation in the rate of oxygen consumption produced by 10 mM glucose MIN6 cells; an observation is comparable with previous findings, which recorded that 20 mM glucose caused an 80% increase, approximately 10 nmol O_2 10^7 $cell^{-1}$ Min^{-1} in MIN6 cells O_2 consumption (Daunt et al., 2006), 5–10 nmol O_2 10^7 $cell^{-1}$ Min^{-1} in rat islets (Hutton and Malaisse, 1980) and 40 nmol O_2 10^7 $cell^{-1}$ min^{-1} in mouse islets (Daunt et al., 2006). The above data provide strong evidence that the principle fate of glucose in MIN6 cells, which are enzymatically similar to β-cells, is mitochondrial oxidation.

Effect of glucose and α-ketoisocaproate on the OCR

The present study showed that glucose and α-ketoisocaproate failed to affect OCR compared to the control (H_2O) in 1.1B4 cells. This suggests that glucose completely metabolized by glycolysis not by TCA cycle (WARBURG, 1956). Another factor of consideration is that the problem may associate with the cell culture which results from disruption of cell-to-cell interaction. This may influence cellular functions in the organism by still-unknown mechanisms. Additionally, genetic influence of the cells might add additional complications and can eventually modify some native functions and responses of the cell, which is possibly in most pancreatic beta cell lines have defective secretory characteristics and are unable to respond to glucose in the physiological range (Skelin *et al.*, 2010). Another reason why 1.1b4 cells had a poor response to glucose is the decrease with passage number (McCluskey *et al.*, 2011). These findings consistent with poor mitochondrial function of 1.1B4 cells. However, in MIN6 cells glucose and α-ketoisocaproate stimulated OCR; an idea supported here in MIN6 by the ability of this substrate to stimulate oxidative respiration (Hutton and Malaisse, 1980; Panten and Klein, 1982; Daunt *et al.*, 2006).

Lactate production

Further to OCR, we estimated lactate production in both 1.1B4 and MIN6 cells. Figure 2B showed basal lactate production at 3 nmoles 10^7 $cells^{-1}$ min^{-1} of 1.1B4 cells. 1.1B4 cells accompanied by 9-fold stimulation in the lactate production 1.1B4 cells which leads to an increase lactate production (WARBURG, 1956; Sener and Malaisse, 1976). No previous data had been

reported for lactate output in human pancreatic beta cells. However, a study by (MacDonald and Fahien, (1990) stated that glycolytically, rather than mitochondrially, generated ATP might be crucial for closure of K^+-$_{ATP}$ channels, which in turn may regulate insulin secretion in rat β cells. Conversely, it has been shown that high mitochondrial glycerol phosphate dehydrogenase activity with low lactate dehydrogenase (LDH) activity may allow glycolytic pyruvate to enter into mitochondrial oxidation in rat *β*-cells (Sek-ine *et al.*, 1994). In terms of lactate output in MIN6 cells, glucose stimulated lactate production compared to the basal. This can be accounted for by the increase in $[NADH]_c/[NAD^+]_c$. These results consistent with that published by (Sener and Malaisse, 1976) stated that high rates of glucose-induced lactate output from rat isolated islets.

Effect of glucose and KIC on mitochondrial membrane potential (Δψm)

Since glucose and KIC stimulated oxidative respiration, we found that they produced an drop in Rh123 fluorescence compared to the basal; an effect consistent with the polarization of Δψm that occurs with stimulation of mitochondrial electron transport on oxidative respiration of sugar (Duchen *et al.*, 1993) and KIC which is a fuel that feeds directly into the mitochondrial tricarboxylic acid cycle (Duchen *et al.*, 1993; Gao *et al.*, 2003; Daunt *et al.*, 2006). Addition of FCCP evoked a rapid increase in fluorescence; an effect consistent with rapid and total collapse of Δψm caused by the mitochondrial protonphore (Duchen *et al.*, 1993). In other experiments, after energization of Δψm with 10 mM glucose, the addition of rotenone produced an immediate increase in Rh123 fluorescence. These results suggested that MIN6 cells depend on mitochondrial respiration. However, 1.1B4 cells mitochondrial membrane potential, it was difficult to measure because the cells did not take the 123RH for unknown reason.

NAD(P)H autofluorescence

The measurement of the endogenous fluorescent compound NAD(P)H serves as an indicator of glucose-induced changes of the beta cell energy metabolism (Panten *et al.*, 1973; Duchen *et al.*, 1993; Martens *et al.*, 2005; Ghaly *et al.*, 2014). As NAD(P)H autofluoresence is resulting mainly from the mitochondria (Pralong *et al.*, 1990; Patterson *et al.*, 2000; Luciani *et al.*, 2006; De Marchi *et al.*, 2014; Ghaly *et al.*, 2014). Therefore, an increase in the fluorescence signals an increase in the reduced state of the pyridine nucleotide NAD(P)H. Thus, Increased activity of the tricarboxylic acid cycle in response to increased substrate supply leads to an increase in the NAD(P)H (Duchen *et al.*, 1993), in 1.1B4 cells, the results denote that both 10 mM glucose and KIC which is considered to be a mitochondrial fuel (Duchen *et al.*, 1993; Gao *et al.*, 2003; Daunt *et al.*, 2006) had no effect on NAD(P)H

levels. This suggests that the glucose was metabolized solely by glycolysis, and not by the TCA (Diaz-Ruiz et al., 2011). Moreover, 1 μM rotenone (complex I inhibitor), which increased the NAD(P)H signal to the point where autofluoresence is likely close to maximal (De Marchi et al., 2014) did not have any effect on the NAD(P)H levels. These effects are consistent with the poor mitochondrial respiration of this cell type. However, the result found that glucose and KIC increase NAD(P)H in the MIN6 cell lines; an effect consistent with stimulation of mitochondrial electron transport on the oxidative respiration by two substrates (Pralong *et al.*, 1990; Patterson *et al.*, 2000; Luciani *et al.*, 2006; De Marchi *et al.*, 2014; Ghaly *et al.*, 2014).

Conclusion

Our results suggest that the glycolytic pathway is the predominate pathway for glucose utilization in the 1.1B4 cell and the fate of glucose in MIN6 cells, which are enzymatically similar to *β*-cells, is mitochondrial oxidation. Therefore, 1.1B4 cells are not good model to study the bioenergetics of pancreatic beta cells.

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