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Iron Ascorbic Acid-Mediated Oxidative Stress Leads to Abnormal Insulin Secretion in Pancreatic Beta Cells

Ntimbane T[1], Spahis S[1,2], Rabasa-Lhoret R[2,3], Mailhot G[1,2], Berthiaume Y[3,4] and Levy E[1,2]*

[1]Research Centre, CHU Sainte-Justine

[2]Department of Nutrition, Université de Montréal, Montreal, Quebec, Canada

[3]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM)

[4]Institut de Recherches Cliniques de Montréal, Quebec, Canada

***Corresponding author:** Emile Levy, GI-Nutrition Unit, CHU Sainte-Justine, 3175 Côte Ste-Catherine, Montreal, Quebec, Canada, H3T 1C5, Tel: (514) 345-7783; E-mail: emile.levy@recherche-ste-Justine.ca

Research Article

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Abstract

Background: Oxidative stress (OxS) is involved in organ damage during the pathogenesis of diabetes, but the relationship between OxS and the deterioration of β-cell function remains unclear. Due to their low expression of many antioxidant enzymes, β-cells may be susceptible to OxS in response to the increased production of reactive oxygen species (ROS) associated with the hyperglycemic condition.

Objectives: In the current study, we investigated the relationship between chemical-induced ROS generation and glucose-stimulated insulin secretion under OxS conditions.

Methods: Murine pancreatic βTC-tet cells were treated with the pro-oxidant iron-ascorbate (0.2 mM/2 mM) complex in the presence or absence of the powerful antioxidant Trolox (1mM).

Results: Chronic exposure of the pancreatic β-cell to the pro-oxidant system iron/ascorbate markedly lowered endogenous antioxidant enzyme defence (catalase and superoxide dismutase) while increasing ROS and lipid peroxidation as noted by measurement of the fluorescent probe CM-H_2DCFDA, malondialdehyde and 4-hydroxy-2-nonenal-protein adducts. Concomitantly, glucose-induced insulin secretion was altered and potential mechanisms for insulin output blunting include activation of the inflammatory transcription factor NF-κB, depressed ATP/ADP ratio and reduced arachidonic acid levels. The antioxidant and scavenger compound Trolox prevented the harmful effect of OxS and restored the insulin secretion profile.

Conclusions: Taken together, these findings indicate that OxS may disturb insulin secretion via alterations of various intracellular pathways, a phenomenon that may be prevented by antioxidants.

Keywords: Oxidative Stress; Pancreatic Cells; Insulin Secretion; Diabetes

Abbreviations: CAT-Catalase; Fe/Asc-Iron/ascorbate; GPx-Glutathione peroxidase; GSH-Glutathione; GSIS-Glucose-stimulated insulin secretion; H_2O_2-Hydrogen peroxide; MDA-Malondialdehyde; OxS-Oxidative stress; ROS-Reactive oxygen species; SOD-Superoxide dismutase.

Introduction

Oxidative stress (OxS), which results from an increased reactive oxygen species (ROS) production that can overwhelm cell antioxidant defence mechanisms [1,2], has been described in the pathogenesis of various diseases, including type I and II diabetes [3,4]. OxS is implicated in β-cell dysfunction or death caused by inflammatory processes in type I diabetes, whereas it is associated with the impairment of β-cell function in type II diabetes [5,6]. The mechanisms by which OxS affects β-cell function remain unclear but several OxS by-products are found increased in diabetic than control β-cells in association with impaired glucose-stimulated insulin secretion (GSIS) [7], suggesting that OxS might play a role in the onset of diabetes. The GSIS process in β-cells is a stepwise mechanism that relies on three major parameters such as glucose metabolism, ATP production and calcium mobilization [8]. Glucose metabolism being the pivotal event controlling insulin secretion, this mechanism is also the most one generating ROS trough mitochondrial activation that leads to ATP synthesis [9-13]. Although the endogenous ROS production is involved in normal β-cell signalling [14]and glucose responsiveness [15], many evidences suggest that increased ROS levels in hyperglycemic conditions can alter GSIS in β-cells by indirectly interfering with the metabolic steps involved in ATP production and calcium homeostasis [16, 17]. Moreover, it has been proposed that extra and intracellular ROS production can directly affect insulin secretion in β-cells by damaging their plasma membrane components (lipids and proteins) [18] and then altering the fluidity needed for the exocytosis process of insulin granules. Numerous studies have also demonstrated that ROS might activate intracellular signalling pathways that lead to cell dysfunction [19-21].

Pancreatic β-cells are also known to express low levels of major ROS-scavenging enzymes and the weakness of their intracellular antioxidant defence system renders them particularly sensitive to OxS [22-24], especially in conditions of hyperglycemia-induced ROS production [25]. Seeing that OxS can arise when the cellular endogenous antioxidant network fails to neutralize ROS production or when the cell is unable to provide a sufficient compensatory response to restore antioxidant levels, β-cells have develop a fine tune on ROS production to avoid any overwhelm that might later lead to cell dysfunction [19,24] can be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and then to oxygen and water mainly by catalase (CAT) and glutathione peroxidase (GPx) [13,26] which are the main antioxidant enzymes that maintain proper oxidation/reduction (redox) homeostasis and prevent irreversible oxidative damage in β-cells [19]. Moreover, thiol-containing molecules such as reduced glutathione (GSH) are essential to prevent ROS effects by scavenging or reducing them to not reactive substances [27,28] in order to preserve the redox state and to fully maintain the insulin secretion process in β-cells [29].

Despite their noxious effects on β-cell function, clues of the beneficial role of ROS in the GSIS process have been highlighted in various studies [30,31]. Data from *in vitro* studies have demonstrated that glucose-induced ROS production in β-cells was an obligatory stimulus for GSIS and that the insulin secretion was highly correlated with ROS levels whereas it was dependently blunted by antioxidants [31]. Moreover, evidences from diabetic animal models suggest that increased levels of antioxidant enzymes (metallothionein and CAT) can accelerate spontaneous diabetes [32]. Although the endogenous ROS production seems a well-controlled mechanism, this raises the problem to what extent the β-cells are able to thwart an enhanced ROS production as encountered during diabetic conditions.

While facing an excessive or a sustained ROS production, as it occurs during OxS condition, β-cells can activate either the redox-sensitive or the apoptotic pathway regulated by the transcription factor NF-κB then resulting in an increased inflammatory response associated with more ROS production [2,6]. Since both *in vitro* [33,34] as well as *in vivo* studies [35,36] have shown the ability of exogenous antioxidants to inhibit NF-κB activation, the β-cell function and survival rely on their use in case of defective endogenous antioxidant network.

In this study, we first aimed to establish a well-controlled system of exogenous OxS using the reliable murine pancreatic βTC-tet cell line. Second, we aimed to examine the activity status of noticeable cellular antioxidant enzymes and to ascertain the role of a short and long standing OxS in the GSIS mechanisms.

Materials and Methods

Chemicals

Culture media and penicillin-streptomycin were obtained from GIBCO-BRL (Grand Island, NY, USA) whereas horse serum was purchased from Invitrogen Life Technologies (Burlington, ON, Canada) and fetal bovine serum from Flow, (McLean, VA, USA). All chemicals including iron/ascorbate (Fe/Asc), Trolox, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1-methyl-2-vinyl-pyridium trifluoromethane sulfonate, metaphosphoric acid, 5,5'-dithiobis-(2-nitrobenzoic acid, 3-isobutyl-1-methylxanthine, oxidized GSH and thiobarbituric acid were obtained from Sigma (St-Louis, MO, USA). The

fluorescent probe 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester was from Molecular Probes (Eugene, OR, USA). Phosphoric acid was from Fisher Scientific (Nepean, ON, Canada). Antibodies directed against NF-κB were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) whereas those against I-κB from Cell Signaling Technology (Beverly, MA, USA). All aqueous solutions were prepared with water-purified milli-Q-system (Millipore, Montreal, QC, Canada).

Cell Culture

βTC-tet murine pancreatic β-cells were kindly provided by Dr PS. Haddad (Department of pharmacology, University of Montreal, Montreal, Canada). Cells were cultured at 37°C in a humidified 5%:95% CO_2:air and in high-glucose (4.5 g/l) Dulbecco's modified eagle medium (DMEM), supplemented with 15% horse serum, 2.5% foetal bovine serum and 1% penicillin-streptomycin until they reached 85% of confluence.

Cell Treatment

Once cell cultures reached 85% of confluence, the medium was changed to serum-free DMEM supplemented with 1% penicillin-streptomycin. βTC-tet cells were incubated with the mixture Fe/Asc (0.2 mM/2 mM) for 2 or 18h, with or without the antioxidant Trolox (1 mM), which was added to the medium 1h prior the incubation with Fe/Asc.

MTT Reduction Assay

The βTC-tet cell metabolism and viability were examined with the MTT colorimetric assay as previously described [37]. The absorbance measured at 535 nm is directly proportional to viable cell number. Viability was calculated as the percentage of the absorbance of treated relatively to control untreated cultures.

Determination of ROS, Lipid Peroxidation Markers and Redox Factor in βTC-tet Cells

ROS quantification: Levels of intracellular ROS were measured by confocal microscopy using the fluorescent probe CM-H_2DCFDA as previously described [30]. After treatment with Fe/Asc in the presence or absence of the antioxidant Trolox, βTC-tet cells were loaded with CM-H_2DCFDA (5 μM) for 30 min at 37°C, followed by three washing with PBS. Fluorescence images were obtained at excitation and emission wavelengths of 485 and 535 nm, respectively with a LSM 510 laser scanning confocal microscope (Carl Zeiss, Thornwood, NY, USA). Individual cells from randomly selected microscopic fields were outlined using the Image J software (http://rsb.info.nih.gov/ij) and the fluorescence intensity determined.

Malondialdehyde (MDA) determination: The amount of free MDA generated during incubation with Fe/Asc and/or Trolox was determined by HPLC as described previously [37]. Proteins were first precipitated with a 0.44 M phosphoric acid solution. The protein free supernatants were then reacted with an equivalent volume of 0.5% (wt/vol) thiobarbituric acid solution (TBA) at 95°C for 1h. After cooling to room temperature, the pink chromogene [(TBA) 2-MDA] was extracted with 1-butanol and dried down with nitrogen at 37°C. The dry extract was resuspended in KH_2PO_4/methanol (70:30; pH 7.0) before MDA determination by HPLC with fluorescence detection.

4-hydroxy-2-nonenal (HNE)-protein adducts determination: Quantification of HNE bound to proteins (HNE-proteins) in cell homogenates was assessed as previously described [38] with slight modifications. Analysis was performed using a Hewlett Packard 6890 Series GC System version A.02.14 (Hewlett Packard, Paolo Alto, CA, USA) equipped with HP-5 capillary column (50m x 0.2mm x 0.33μm) coupled to a Mass Spectrometer (Agilent Technologies Mass Selective Detector 5973 Network). Only the fragmented ions m/z 257, 258 and 268 were measured while the MS source and quadrupole were set at 300°C-176°C respectively.

Measurement of GSH-to-GSSG ratio: The ratio of reduced glutathione to glutathione disulfide (GSH/GSSG) was determined after exposure to Fe/Asc in the presence or absence of Trolox using the Bioxytech GSH/GSSG-412 kit (OxisResearch, Portland, OR, USA). βTC-tet cells were washed twice with ice-cold PBS and harvested in 5% meta-phosphoric acid followed by a centrifugation at 6500 x g, 4°C for 5 min. The resulting supernatants were divided into two aliquots: one for the measurement of total glutathione (GSH+GSSG) (immediately stored at -80°C), and the other for the GSSG assessment (stored at -80°C after being mixed with thiol scavenging 1-methyl-2-vinyl-pyridium trifluoromethane sulfonate). After addition of the chromogen DTNB, absorbance was recorded at 412 nm to measure total GSH concentration whereas GSSG levels were determined using a calibration curve.

Antioxidant Enzyme Activities

The activity of the antioxidant enzymes: Mn-SOD, CAT and GPx were measured in βTC-tet cell homogenates after treatment with Fe/Asc either pre-incubated or not with Trolox. Briefly, the specific substrate for each enzyme was added to the cell homogenates and the rate of its disappearance was then measured by spectrophotometry. Cytosolic and

mitochondrial SOD activities were determined as described by McCord *et al*. [39]. The assay for the CAT activity was adapted from the protocol reported by Jiang *et al*. [40] with measurement of xylenol orange oxidation at 560 nm in the presence of ferrous ions upon addition of H_2O_2 (10 mM) to cell homogenates. For the GPx activity, cell homogenates were added to a PBS buffer containing 10 mM GSH, 0.1 U of GSH reductase and 2 mM NADPH with H_2O_2 (1.5%) to initiate the reaction. Absorbance was then monitored at 340 nm for 5 min.

Insulin Secretion Assay

All experiments were performed in static incubation. Briefly, βTC-tet cells were seeded in 12-well plates at a density of 2.5 x 10^5 cells/well. Upon reaching 60-85% confluence, cells were exposed to Fe/Asc +/- Trolox in the culture medium and then pre-incubated at 37°C for 1h in a Krebs-Ringer buffer (10 mM HEPES, 25 mM $NaHCO_3$, 2 mM NaH_2PO_4, 118 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1mM $MgSO_4$, 0.1% fatty acid free BSA, pH 7.4) containing no glucose. To assess insulin secretion, the buffer was removed and replaced by a Krebs buffer containing 0.5 mM IBMX and either 4, 6, 10, 16 mM glucose (for insulin stimulation), or 2 mM glucose plus 50 mM K^+ (non-fuel secretagogue) (buffer adjusted to 50 mM KCl and 73 mM NaCl). After a 2h-incubation, media were collected, centrifuged for 3 min at 4°C, 3,000 x g to remove cell debris and stored at -80°C until further analysis. Insulin release into the medium was determined by radioimmunoassay (RIA) as described below. Cellular insulin content was measured in cells exposed to 4 (basal secretion) and 16 mM glucose for 2h. Briefly, intracellular insulin was extracted overnight at 4°C in 0.2 mM HCl-75% ethanol. These samples were briefly sonicated and centrifuged at 30,000 x g for 5 min before measurement of insulin by RIA in the supernatant (# RI-13 K, Linco Research, St-Charles, MO, USA). Measurements were performed on samples diluted between 100 and 300 times according to the manufacturer's instructions. All insulin concentrations are expressed per mg of proteins.

Measurement of ATP Levels

Cellular ATP contents were measured using an ATP/ADP bioluminescent assay kit (BioAssay Systems). Briefly, βTC-tet cells were seeded onto 96-well microplates, incubated with Fe/Asc in the presence or absence of Trolox and then lysed to release ATP and ADP. The first phase of the assay consists in the luciferase-catalyzed reaction of cellular ATP and D-luciferin, which produced a luminescent signal. Then, ADP was converted into ATP through an enzyme reaction and the newly formed ATP reacts with D-luciferin. The second light intensity represented the total ADP and ATP content; ADP levels were obtained by subtraction. The calculated ATP/ADP ratio was normalized to the total protein content of the wells.

Fatty Acid Analysis

Cellular fatty acids were assayed by a method established in plasma samples and commonly used in our laboratory [41][41]. Briefly, cell homogenates were subjected to direct trans-esterification and then injected into a gas chromatograph using the Varian 8400 GC Auto Sampler system equipped with a (90m x 0.32mm) WCOT-fused silica capillary column VF-23ms coated with 0.25µm film thickness (Varian Inc. Canada).

Western Blot Analysis

Cells were homogenized and adequately prepared for Western blotting as described previously [37]. Nitrocellulose membranes were incubated in 1% defatted milk containing primary antibodies directed against NF-κB (Santa Cruz Biotechnology, Santa Cruz, CA) and I-κB (Cell Signaling Technology, Beverly, MA). The relative amount of primary antibody was detected with species-specific horseradish peroxidase-conjugated secondary antibody in 0.5% milk. NF-κB and I-κB were quantitated using an HP Scanjet scanner equipped with a transparency adapter and the UN-SCAN-IT gel 6.1 software.

Statistical Analysis

Data are expressed as means ± SEM. To assess the differences between means, Student *t* test or one-way Anova followed by Tukey's post hoc tests were performed. A P value < 0.05 was considered as significant.

Results

Effect of Iron/Ascorbate on βTC-tet Cell Viability

Cell viability was assessed by the MTT assay following incubation with Fe/Asc and +/- Trolox in serum-free medium. As shown in Figure 1, the exposure of βTC-tet cells to Fe/Asc and Fe/Asc + Trolox after 2h (data not shown) and 18h did not significantly affect cell viability. Similar data were observed when viability was evaluated with the Trypan blue exclusion test (data not shown).

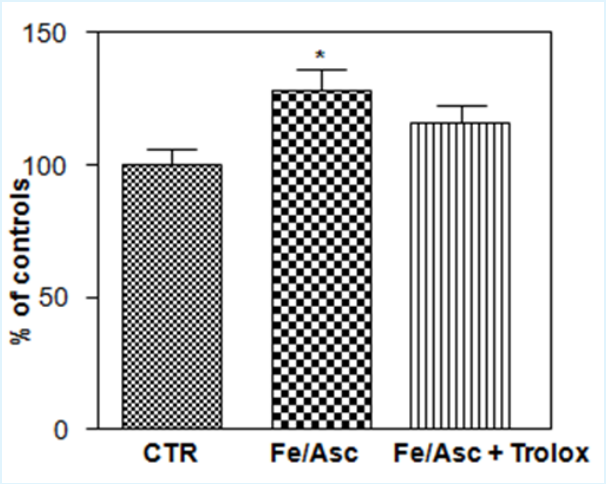

Figure 1: Cell viability upon iron/ascorbate exposure.

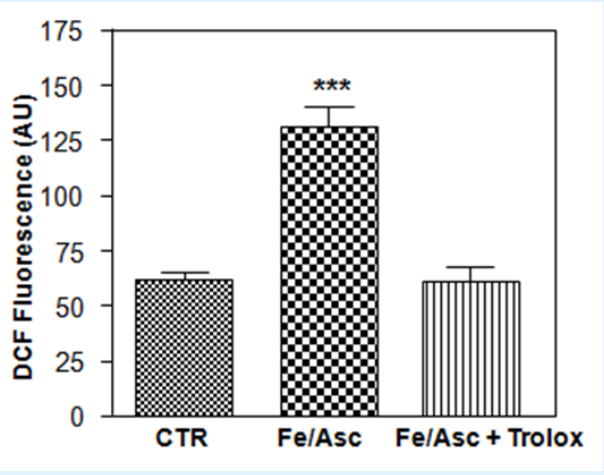

Figure 2: ROS production in βTC-tet cells.

βTC-tet cells were incubated with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1mM Trolox for 18h. Non-treated cells served as controls (CTR). After a 2h-incubation in the MTT solution, the MTT product Formazan was colorimetrically measured at 535 nm. Data represent means ± SEM for 3 independent experiments performed in triplicate on different cell preparations. *P<0.05 vs. CTR.

Iron/Aascorbate-Induced ROS Production in βTC-tet Cells

Intracellular ROS levels were measured using the fluorescent probe CM-H2DCFDA, which is metabolized into cells and reacts with ROS, then producing the fluorescent component 2', 7'-dichlorofluorescein. Fluorescence intensity in βTC-tet cells upon exposure to Fe/Asc +/- Trolox was quantitated after 2h (data not shown) and 18h incubation. Our pro-oxidant mixture elicited a significant increase in ROS levels (mostly after 18h) whereas the addition of Trolox neutralized this production as exemplified by the decrease in fluorescence intensity to levels comparable to controls (**Figure 2**).

After 18h-treatments with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1mM Trolox, cells were incubated in the presence of the fluorescent probe CM-H2DCFDA for 45 min at 37°C. Untreated cells served as controls (CTR). Fluorescence intensity was quantitated and expressed as arbitrary unit. Results represent the means ± SEM of 25 to 41 cells selected randomly in 6 to 7 microscopic fields. ***P<0.0001 vs. CTR.

Time Course of Lipid pPeroxidation in βTC-tet Cells

Experiments were subsequently carried out to examine the effects of Fe/Asc on lipid peroxidation following time incubations. Lipid peroxidation markers as determined by the intracellular MDA (Figures 3A, 3B) and HNE-proteins (Figures 3C, 3D) levels showed a significant increase following Fe/Asc administration at both times of exposure. Indeed, MDA levels were increased about 77 and 94% upon 2 and 18h incubation with Fe/Asc whereas similar increase was also observed for HNE-protein levels (79 and 87%). The combination of Trolox and Fe/Asc normalized the production of both MDA and HNE-protein levels in comparison with control values.

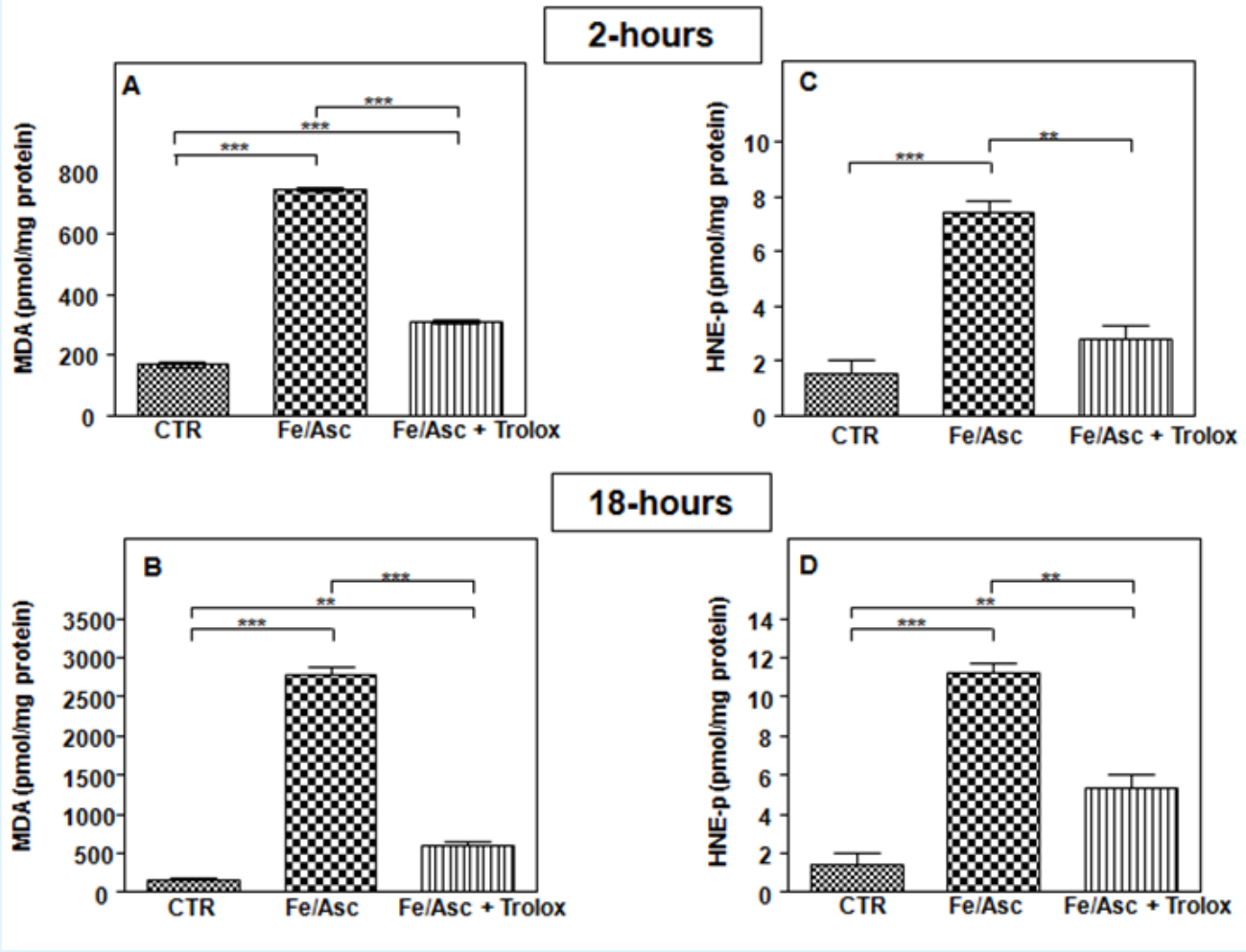

Figure 3: Lipid peroxidation markers in βTC-tet cells.

After a 2h (A, C) or a 18h (B, D) treatment with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1 mM Trolox, cells were collected and assessed for malondialdehyde (MDA) and 4-hydroxynonenal bound to proteins (HNE-proteins) cell contents as described in the MATERIALS AND METHODS section. Untreated cells served as controls (CTR). Data represent means ± SEM of 3 separate experiments. ***P*<0.001; ****P*<0.0001.

Modulation of Endogenous Antioxidant Defences and Redox Factor in Btc-Tet Cells by Iron/Ascorbate

To determine whether the oxidative effects of Fe/Asc were associated with consequences on the cell antioxidant system, we measured changes in some of its major components. Alterations in the endogenous antioxidant defence occur in βTC-tet cells upon Fe/Asc administration only after 18h incubation as evidenced by the modifications of GSH levels and enzymatic activities of Mn-SOD and CAT. As illustrated in (Figure 4), we observed a significant increase in the GSH/GSSG ratio in response to Fe/Asc along with a significant decrease in both activities of Mn-SOD and CAT. The co-administration of Trolox restored CAT activity to control levels but failed to restore the Mn-SOD activity back to control values, whereas the GSH/GSSG ratio remained high. No significant changes were noticed in cytosolic SOD and GPx activities, suggesting that these enzymes were less affected (data not shown).

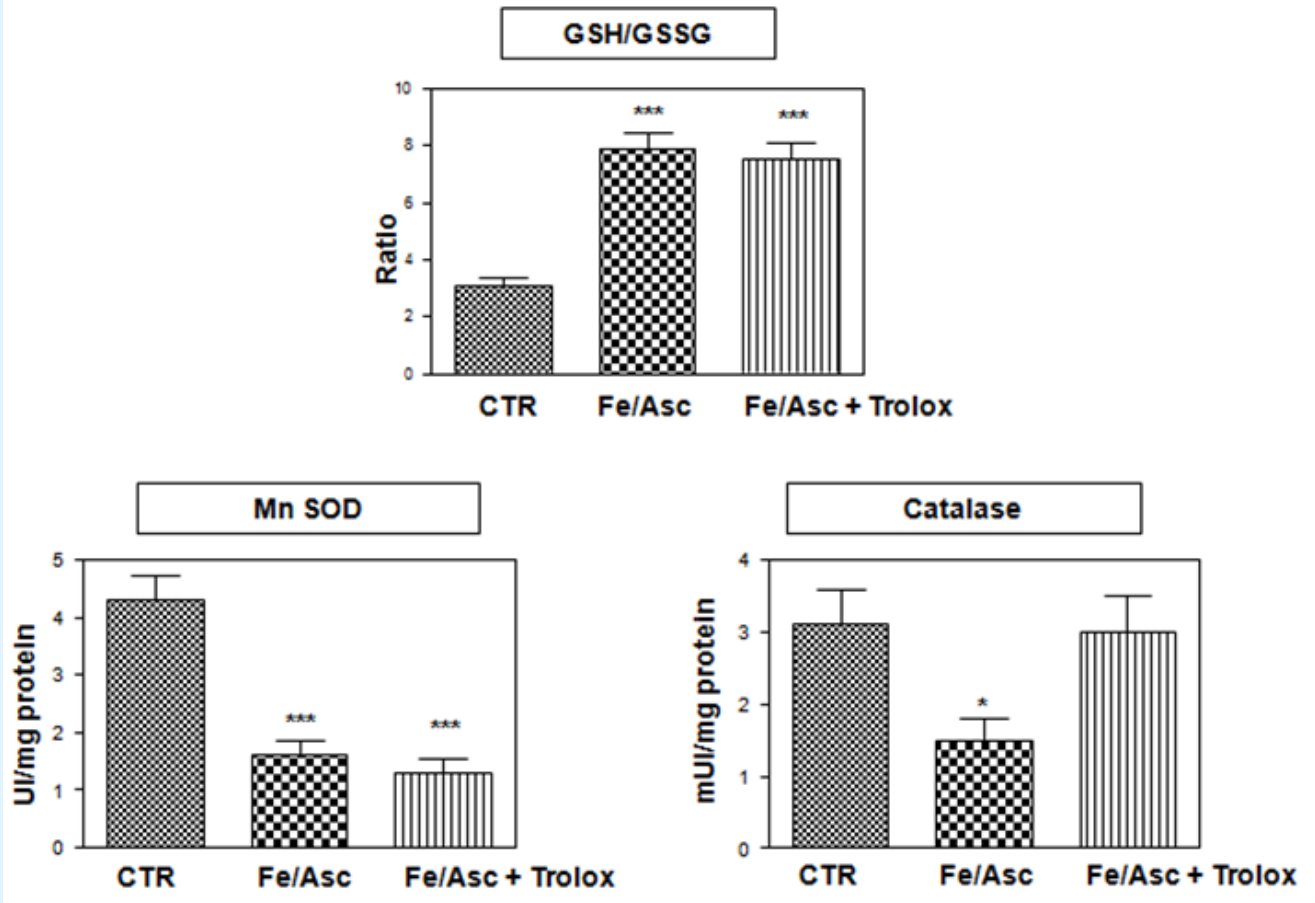

Figure 4: Endogenous antioxidant status in βTC-tet cells.

The GSH-to-GSSG ratio (**A**), the activity of mitochondrial superoxide dismutase (Mn-SOD) (**B**) and Catalase (**C** were measured following a 18h-incubation with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1 mM Trolox as described in the MATERIALS AND METHODS section. Untreated cells served as controls (CTR). Data represented means ± SEM of 3 separate experiments. **P*<0.05, ****P*<0.0001 vs. CTR;

Impact of Iron/Ascorbate on Insulin Secretion in Btc-Tet Cells

As βTC-tet cells represent an established experimental model for testing insulin release in response to increasing glucose concentrations, we first assessed glucose-induced insulin secretion following a 2h-incubation in the presence or absence of Fe/Asc. In control cells, insulin secretion was dose-dependently increased when glucose concentration raised from physiological fasting (4 mM) to pathophysiological (20 mM) levels (Figure 5A). Although addition of Fe/Asc resulted in a significant increase in insulin secretion at 4 and 6 mM glucose, further augmentation in glucose concentrations had little effect. Longer exposure with Fe/Asc (18h) did not raise insulin secretion in response to low concentrations of glucose (4-6 mM) but markedly blunted the response to higher concentrations of glucose (Figure 5B).

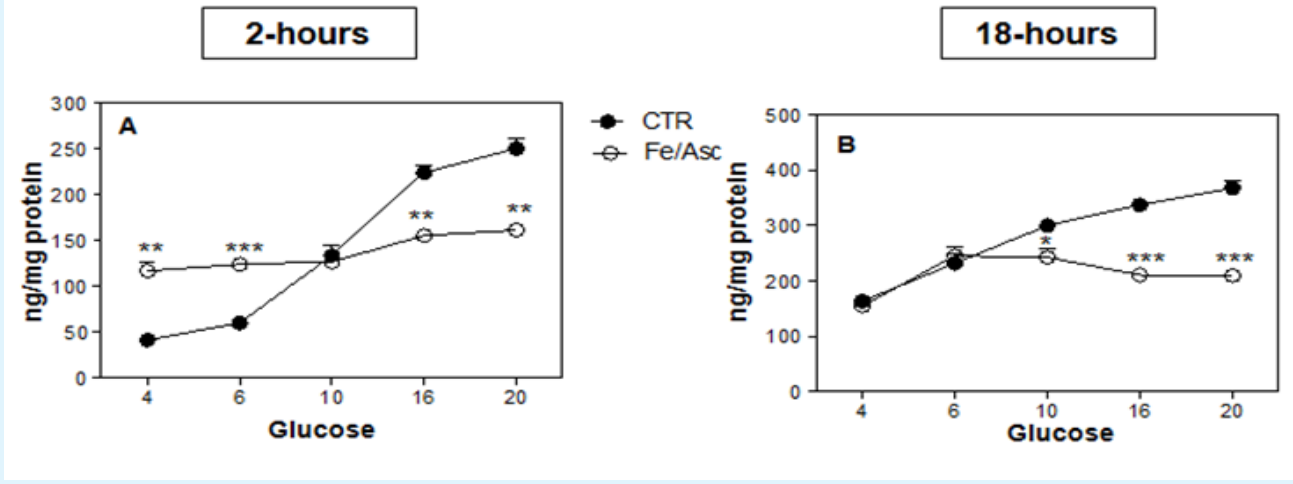

Figure 5: Glucose-stimulated insulin secretion in βTC-tet cells.

After a 2 (**A**) or 18h (**B**)-incubation with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM), insulin secretion in response to 4, 6, 10, 16 or 20 mM glucose was measured as described in the materials and methods section. Control cells (CTR) represent untreated cells. Results presented are means ± SEM of triplicate coming from 3 separate experiments. **P*<0.05, ***P*<0.001, ****P*<0.0001 vs. CTR using unpaired Student *t* tests.

We then focused on insulin secretion in response to 4 and 16 mM of glucose, two concentrations that lead to different responses in relation with insulin secretion following exposure to Fe/Asc. The use of Trolox in combination with Fe/Asc completely normalized insulin secretion, regardless of the time of incubation (Figures 6A, 6B). These data were further confirmed with measurements of the intracellular insulin contents. When insulin secretion was expressed as the percentage of total insulin release, short-term Fe/Asc treatment still significantly increased insulin secretion following the administration of 4 mM glucose (Figure 6C). The percentages of total insulin output upon stimulation with 16 mM glucose remained invariable in comparison with the stimulation by 4 mM glucose at both periods of exposure (Figures 6C, 6D).

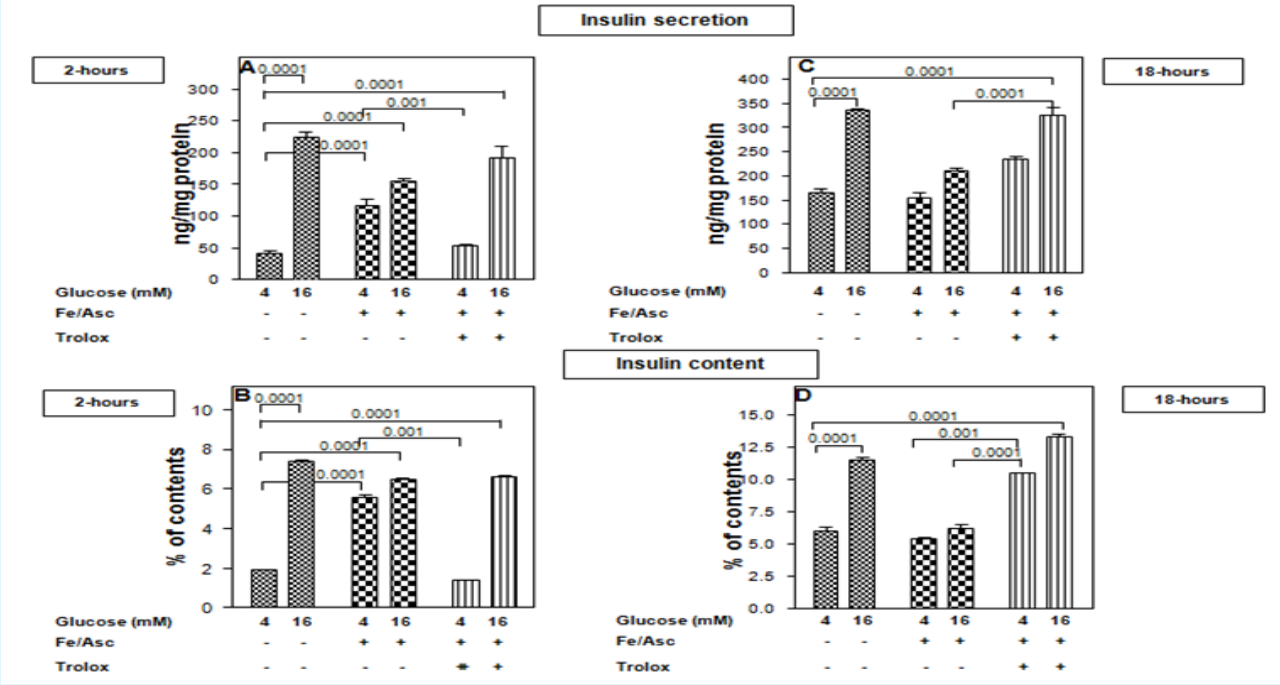

Figure 6: Effect of iron/ascorbate on glucose-stimulated insulin secretion and intracellular insulin content.

βTC-tet cells were incubated in various conditions without any agent at 4 and 16 mM glucose followed by the addition of iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) or 1 mM Trolox. The insulin secretion profile after 2h (A) or 18h (B) incubation is illustrated for 3 independent experiments, n = 6 per group. Intracellular insulin contents were measured in the same conditions after cell incubations for 2 (C) or 18h (D). Intracellular insulin levels were determined according to the MATERIALS AND METHODS section and levels of secreted insulin were analysed in relation with the intracellular insulin contents. Results are means ± SEM from 3 separate experiments. P<0.001, P<0.0001.

Impact of Iron/Ascorbate on K-Stimulated Insulin Secretion and ATP/ADP Ratio

The effects of Fe/Asc treatment on insulin release through mechanisms independent from glucose stimulation were also evaluated. Depolarizing concentration of potassium was used to trigger insulin secretion. Insulin secretion was similar between control and Fe/Asc-treated cells after the addition of 50 mM KCl (data not shown), suggesting that iron/ascorbate affects mainly intracellular events linked to glucose metabolism and involved in insulin secretion. A rise in the ATP/ADP ratio is a key cellular event that precedes glucose-stimulated insulin secretion. We therefore evaluated changes in ADP and ATP concentrations following the administration of Fe/Asc and found that ATP/ADP ratio was significantly decreased after 18h-incubation with Fe/Asc (Figure 7). These results show that the ATP/ADP ratio is more sensitive to long standing OxS, and can at least partly explain the negative effects of Fe/Asc on the glucose-stimulated insulin secretion process.

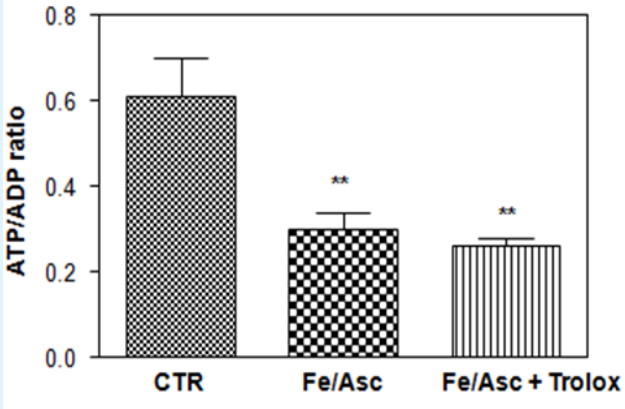

Figure 7: ATP-to-ADP ratio in βTC-tet cells.

Intracellular levels of ATP and ATP were assessed in βTC-tet cells as described in MATERIALS AND METHODS. Cells were incubated for 18h with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1 mM Trolox. Untreated cells served as controls (CTR). Results are means ± SEM, n = 6 wells per group. $^{**}P< 0.001$ vs. CTR.

Iron/Ascorbate and Fatty Acid Composition in Btc-Tet Cells

As lipid peroxidation by-products were modulated by the treatment with Fe/Asc, we verified whether these modulatory effects were associated with changes in cellular fatty acid composition. Overall, polyunsaturated fatty acid levels showed a decreasing trend in βTC-tet cells exposed to Fe/Asc, but only concentrations of arachidonic acid (20:4n-6) were significantly reduced (mostly after 18h) when compared to those in controls and cells treated with Fe/Asc and Trolox (Figure 8). Furthermore, the ratio of eicosatrienoic acid to arachidonic acid [20:3(n-9)/20:4(n-6)], an index of essential fatty acid deficiency, was not modified by Fe/Asc-mediated oxidative effects (data not shown).

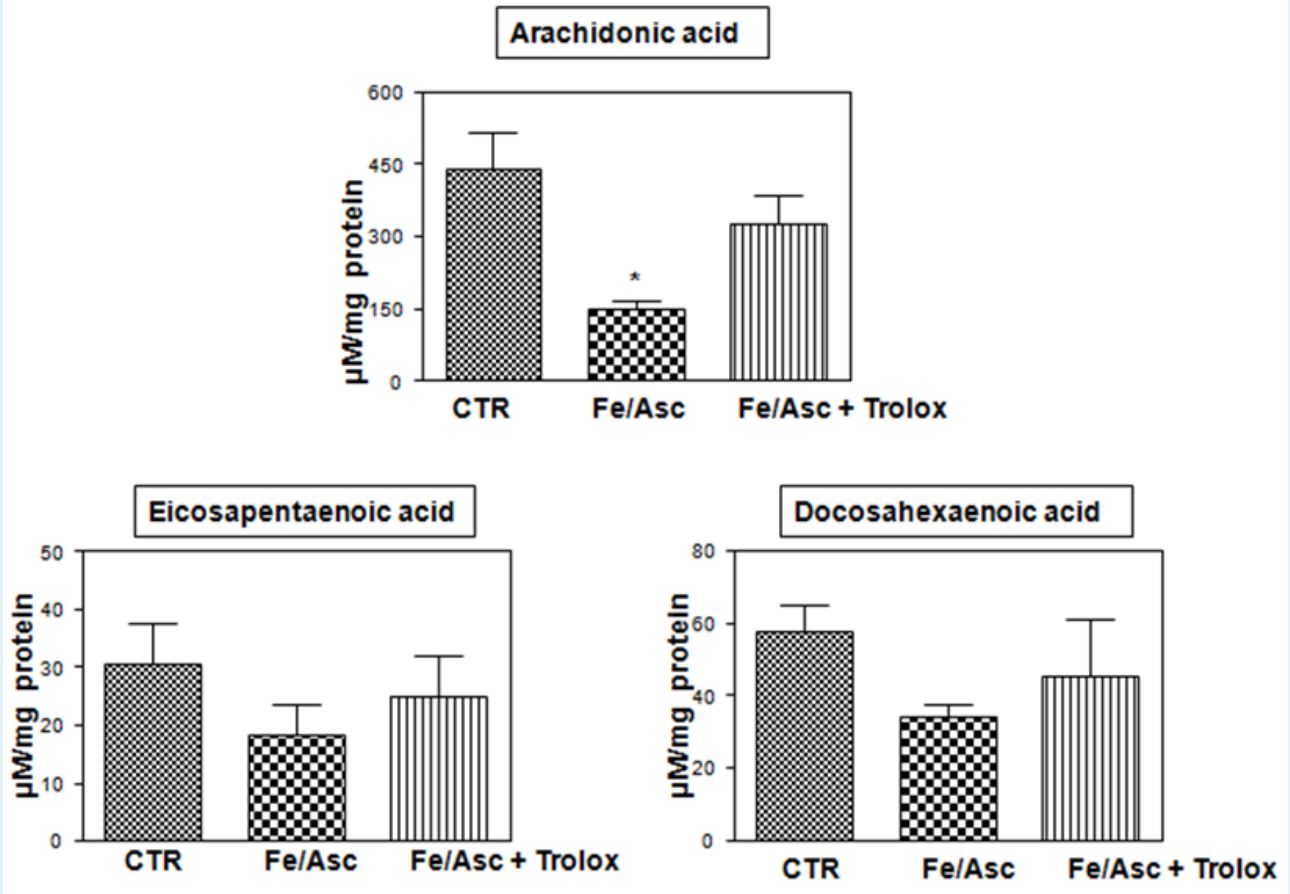

Figure 8: Arachidonate Fatty acid levels in βTC-tet cells.

After a 18h-treatments with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1 mM Trolox, cells were collected and fatty acids assayed by gas liquid chromatography after direct trans-esterification. Untreated cells served as controls (CTR). Results are means ± SEM of 3 separate experiments and expressed as µM/mg of cellular total protein content. **P*<0.05 *vs.* CTR.

Iron/Ascorbate-Induced NF-Kb Activation Following ROS Production

In order to verify whether Fe/Asc could modulate inflammation pathway, expression of the inhibitory protein I-κBα and transcription factor NF-κBα was analysed. Following the incubation with Fe/Asc for 18h, the expression of the 37-kDa protein I-κBα was decreased in βTC-tet cell homogenates (**Figure 9)**, which could account for NF-κB rise in the cytosol. Degradation of I-κBα and increased release of NF-κB were both inhibited by the presence of Trolox, showing that the pro-oxidant mixture Fe/Asc, can activate this transcription factor. Therefore, long-term incubation of βTC-tet cells with Fe/Asc activates NF-κB, which could ultimately lead to the transcription of genes involved in inflammation protein synthesis.

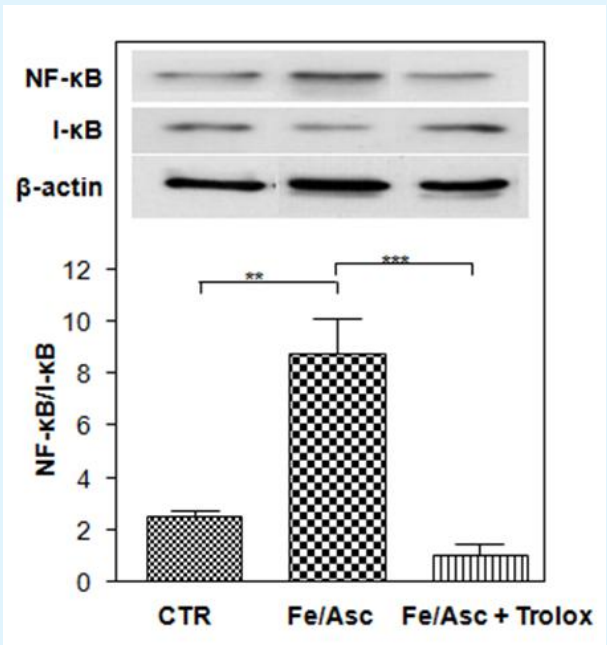

Figure 9: I-κB degradation and NF-κB activation in βTC-tet cells.

After 18h-treatments with iron/ascorbate (Fe/Asc, 0.2 mM/2 mM) and 1 mM Trolox, cells were collected and lysed in protein extraction buffer. Untreated cells served as controls (CTR). Fifty µg of protein were applied on a SDS-PAGE and immunoblotted as described in materials and methods. The ratio NF-κB to I-κB was calculated. Results represent means ± SEM of 3 separate experiments. $^{**}P<0.001$ vs. CTR; $^{***}P<0.0001$ vs. Fe/Asc + Trolox.

Discussion

Most studies have evidenced the role of OxS in the rise of diabetic complications [42] whereas others have demonstrated the involvement of OxS in β-cell dysfunction during the early phases of diabetes [5,6]. A feature of pancreatic β-cells is their low expression and activity of the major enzymes involved in antioxidant defence mechanisms, which render them susceptible to the harmful effect of OxS [43]. However, the relationship between increased OxS and the impaired glucose metabolism surrounding the appearance of diabetes in patients is not fully established and still remains a matter of hypotheses.

The first step consisted in examining whether pancreatic β-cells are sensitive to OxS. We used the Fe/Asc mixture as the pro-oxidant system since this iron-catalyzed model of OxS is known to generate hydroxyl radicals ($HO^{\bullet}$) from hydrogen peroxide (H_2O_2) in presence of ferrous ions [44]. Our previous studies in intestinal epithelial cells describe the Fe/Asc oxygen radical-generating system as a powerful tool for the production of highly reactive ROS involved in lipid peroxidation in inflammatory bowel diseases [45] and iron is a known catalyst for the oxidation of biological molecules, such as lipids, proteins and DNA [46]. In the iron-catalyzed lipid oxidation process, ROS (mostly $HO^{\bullet}$) react with polyunsaturated lipids to initiate chain reactions that lead to the formation of lipid hydroperoxides. The latter may undergo breakdown by Fe (II), leading to the formation of other radicals and the propagation of lipid oxidation process via the Fenton-like reaction. In the past, our laboratory and other groups reported that Fe/Asc-mediated lipid peroxidation altered the composition and properties of the bilayer lipid environment, affected the functions of sterol regulatory enzymes and integral membrane proteins of the endoplasmic reticulum, and disturbed cholesterol homeostasis [45,47], whereas ascorbic acid can amplify iron-oxidative potential by promoting metal ion-induced lipid peroxidation [48-50]. The data presented here clearly indicate that the Fe/Asc system efficiently elicited marked lipid peroxidation in βTC-tet cells in a time-dependant manner, probably via the oxidation of membrane polyunsaturated fatty acids, yielding a loss of cellular arachidonic acid. It is possible that this long fatty acid is more sensitive to lipid peroxidation, thereby producing increased concentrations of MDA and HNE-protein products, as suggested previously [51]. Accordingly, our findings were capable to document not only the reaction of the fluorescent probe (CM-H2DCFDA) with oxygen radicals and peroxides keeping the same spectral properties [30], but also the intracellular accumulation of MDA and HNE-proteins. Moreover, the administration of the antioxidant and lipid peroxide scavenger Trolox together with Fe/Asc almost normalized lipid peroxidation evaluated by analysis of MDA and HNE-proteins. Overall, the use of the pro-oxidant Fe/asc system was not only efficient in inducing high levels of lipid peroxidation in βTC-tet cells, but also in informing as to the high susceptibility of these pancreatic cells to OxS.

Endogenous antioxidant enzymes such as SOD and CAT are essential to limit the production of oxidative molecules in OxS conditions [52]. It is now well established that β-cells contain low levels of SOD, which helps remove oxygen radicals and CAT involved in the elimination of peroxides [53]. Our results also showed a decrease in CAT and Mn-SOD activities upon long-term incubation with Fe/Asc. The addition of Trolox prevented the drop in CAT activity but showed no significant effect on SOD activity. As a vitamin E analog, the water-soluble antioxidant Trolox displayed the ability to enter into cell and reduce intracellular ROS accumulation when co-administrated with Fe/Asc. This is consistent with our results since Trolox normalized the ROS-induced fluorescence and CAT activity after Fe/Asc incubation. As to the failure of Trolox to restore SOD activity upon incubation with our pro-oxidant system, our results are coherent and convergent with those of another study in which the polyethylene glycol-conjugated superoxide dismutase scavenger failed to rescue cells exposed to the pro-oxidant mixture of $CuSO_4$, phenanthroline, and ascorbic acid [54]. Since the lipid peroxidation chain breaker Trolox is a powerful scavenger that protects against both $HO^{\bullet}$ and H_2O_2 noxious effects in cells and that CAT levels are extremely lower in β-cells (only 1% of the expression levels in the liver) [55], it is not surprising, in our study, that CAT activity normalized with Trolox co-administrated with Fe/Asc. Meanwhile, SOD scavenging activity is exclusive to the conversion of superoxide anion (O_2^{-}) into H_2O_2. At least few mechanisms can explain why Trolox failed to normalize mitochondrial SOD activity in the present work: differential ability of the antioxidant scavenger to cross organelle membranes and nonspecific activity of Trolox toward O_2^{-}. With our pro-oxidant model, H_2O_2 which does equilibrate across cell and organelle membranes, can be converted into O_2^{-} by reverse dismutation reaction or into $HO^{\bullet}$ by the Fenton reaction. Therefore, mitochondrial SOD activity may not be able to handle the overwhelming increases in O_2^{-} due to either Trolox nonspecific activity or its inability to cross the mitochondria membranes.

Among the mechanisms of defence against ROS toxicity in β-cells, GSH is the most abundant ROS-scavenging molecule and the key element in the maintenance of cellular redox homeostasis and in reducing oxidative damage [53-56]. GSH is converted by oxidation to GSSG upon its reaction with ROS produced elsewhere in cell compartments. Thus, the balance between GSH and GSSG is an important indicator of OxS [57]. Changes in the GSH system, especially a decreased GSH-to-GSSG ratio, were expected in our ROS-generating model with Fe/Asc. However, these changes occurred with rather an increase in GSH levels and a significant increase in the GSH/GSSG ratio. Our results are consistent with previous studies where β-cells exposure to OxS increased GSH levels in order to prevent intracellular ROS accumulation [30]. Nguyen et al., have also demonstrated an enhanced expression of enzymes involved in GSH synthesis and recycling in condition of OxS in β-cells [58]. Another study has demonstrated that a long-term exposure to HNE-proteins dramatically increased the total cellular antioxidant potential in β-cells. Increased GSH levels as occurring in the current study might represent a self protection mechanism of βTC-tet cells as described by Lacraz et al., like being an adaptive up-regulation of antioxidant defences in β-cells against chronic OxS [59]. One can speculate that GSH being the most abundant and powerful ROS-scavenger in β-cells its endogenous up-regulation is required to counteract the Fe/Asc-induced OxS.

Alterations in insulin secretion mechanism in β-cells have been reported to be detrimental to glucose control. Disturbances related to glucose-stimulated insulin secretion from β-cells exposed to acute ROS production have been reported in many studies by different exposure times being less than 2h [30,60,61]. In our study, we investigated the influence of OxS on insulin secretion mechanisms during short- and long-term (2 and 18h, respectively) periods of cell exposition to OxS. The glucose-stimulated insulin secretion process relies on 3 parameters: 1) glucose metabolism; 2) ATP production; and 3) calcium mobilization [62,63]. Upon glucose metabolism, increased cytosolic ATP/ADP ratio will lead to closure of K_{ATP} channels [64,65], opening of voltage-dependent calcium channels and insulin exocytosis [62]. We were able to show increased insulin levels when βTC-tet cells were challenged with basal glucose concentrations (4-6 mM) whereas higher glucose concentrations (10-20 mM) failed to raise insulin levels during short-term ROS production. The discrepancies observed in this insulin secretion profile occurred without any significant changes regarding the ATP/ADP ratio (data not shown). However, our observations are in agreement with other studies showing that increased ROS (exogenous or endogenous) generation can induce insulin release at basal glucose concentrations. Decreased insulin levels found in the presence of increased glucose concentrations (10-20 mM) upon 2h incubation with Fe/Asc might be explained, at least, by other mechanisms different to those involved in ATP synthesis for insulin secretion including an altered ion transport during short-term ROS production.

After 18-h incubation with Fe/Asc, the insulin response to higher concentrations of glucose was markedly blunted. Normalization of the insulin secretion profile upon Trolox addition clearly point out the involvement of ROS. This observation was further confirmed by the decreased ATP/ADP ratio upon Fe/Asc treatment whereas insulin secretion was unchanged in control and Fe/Asc-treated cells after the addition of 50 mM. KCl (data not shown). To rule out a putative damaging effect on membrane integrity due to ROS-induced lipid peroxidation, we calculated the index for membrane fluidity and we did not detect any significant differences between control and Fe/Asc-treated cells. Moreover, data relative to the proportion of insulin content demonstrate that the insulin secretion process in βTC-tet cells was altered by treatment with Fe/Asc. The present report on these findings point out an oxidative insult occurring under long-term ROS production that can further stimulate mitochondrial ROS production (ROS-induced ROS release) [66,67] leading to ATP depletion to an extent that cause β-cell dysfunction. Moreover, it has been demonstrated that increasing exogenous ROS can impair ATP production, thus opening K_{ATP} channels, limiting calcium influx and therefore glucose-induced insulin secretion [68].

OxS has been associated to inflammatory gene expression in both type 1 and 2 diabetes. Consequently, we examined whether Fe/Asc-mediated lipid peroxidation and the inflammation pathways could modulate the control of insulin secretion in βTC-tet cells. Since NF-κB is a master stress-sensitive transcription factor involved in inflammatory response, its activation by various stimuli including OxS will lead to the degradation of its inhibitory protein I-κB with the ensuing translocation of NF-κB to the nucleus [69]. NF-κB activation due to an increased production of intracellular ROS is responsible for the activation of stress-sensitive signalling pathways involved in both altered insulin secretion and action [2]. An increase in NF-κB activation is followed by an increase in the release of pro-inflammatory cytokines, which can lead to β-cells dysfunction through mechanisms involving ROS production. In our study, treatment of βTC-tet cells with Fe/Asc resulted in the reduction of cytosolic IκB with concomitant increase in NF-κB levels in the nucleus. Decreased insulin levels upon long-term exposure with Fe/Asc followed this translocation. The presence of the antioxidant Trolox prevented the reduction of cytosolic IκB, thereby confirming that NF-κB is subject to redox regulation and that it is involved in the control of insulin secretion in βTC-tet cells. These observations are in agreement with other studies

showing that antioxidant treatment can inhibit NF-κB activation and ameliorate insulin secretion [2]. For further information about the role of inflammation in the control of insulin secretion, we then focused on arachidonic acid levels since this polyunsaturated fatty acid is both involved on glucose-stimulated insulin secretion mechanisms and NF-κB activation. Indeed, it is well established that insulin secretion from pancreatic β-cells is stimulated by arachidonic acid [70] and decreased levels of arachidonic acid can suppress insulin secretion [71]. Furthermore, arachidonic acid and its metabolites (enzymatic and non enzymatic) are known to participate in pro-inflammatory processes through NF-κB activation [70]. Long term exposure of βTC-tet cells to Fe/Asc showed a significantly decrease in arachidonic acid levels whereas the addition of Trolox prevented the drop. Thus, arachidonic acid might affect insulin secretion mechanisms through altered exocytosis of insulin granule and activation of inflammatory pathways.

Data from the current study can be analyzed in the context of some events involved in oxidative-induced altered insulin secretion in diabetic patients and the effect of iron overload on pancreatic function. For the first time we demonstrated that the pro-oxidant mixture Fe/Asc can affect insulin secretion in βTC-tet cells in different manners depending on the exposure time. We can then propose that results obtained after 2h of incubation with Fe/Asc could simulate an acute OxS condition with an altered insulin secretion process in β-cells; a situation that may occur during the onset of diabetes. The function of β-cells is more worsened with the longer incubation with Fe/Asc for 18h showing that prolonged OxS induced an inadequate β-cell response and insulin secretion to glycemic changes. The data obtained with Trolox suggest that antioxidant treatment of glucose intolerance could be beneficial to restore β-cell function and delay the appearance of diabetes. Finally, our results on inflammation suggest that these factors can contribute to the installation of chronic OxS events and more β-cell dysfunction during the development of diabetes. However, we have to keep in mind that *in vitro* findings should be interpreted with cautions.

In summary, our data confirm the harmful effects of OxS in β-cell function with some discrepancy between acute (2h) and long-term (18h) effects. The poor antioxidant equipment of β-cells renders them more vulnerable to OxS events, even those occurring at distant sites. The Fe/Asc pro-oxidant model we used in this study is a good system for *in vitro* studies since it responds to the antioxidant Trolox.

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References

1. Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82(1): 47-95.

2. Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes 52(1): 1-8.

3. Chen H, Li X, Epstein PN (2005) MnSOD and catalase transgenes demonstrate that protection of islets from oxidative stress does not alter cytokine toxicity. Diabetes 54(5): 1437-1446.

4. Kehrer JP (1993) Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 23(1): 21-48.

5. Donath MY, Storling J, Maedler K, Mandrup-Poulsen T (2003) Inflammatory mediators and islet beta-cell failure: a link between type 1 and type 2 diabetes. J Mol Med (Berl) 81(8): 455-470.

6. Mandrup-Poulsen T (2003) Apoptotic signal transduction pathways in diabetes. Biochem Pharmacol 66(8): 1433-1440.

7. Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, et al. (2005) Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes 54(3): 727-735.

8. Henquin JC (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49(11): 1751-1760.

9. Deeney JT, Prentki M, Corkey BE (2000) Metabolic control of beta-cell function. Semin Cell Dev Biol 11(4): 267-275.

10. Patterson GH, Knobel SM, Arkhammar P, Thastrup O, Piston DW (2000) Separation of the glucose-stimulated cytoplasmic and mitochondrial NAD(P)H responses in pancreatic islet beta cells. Proc Natl Acad Sci 97(10): 5203-5207.

11. Skulachev VP (1996) Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. Q Rev Biophys 29(2): 169-202.

12. Kadenbach B (2003) Intrinsic and extrinsic uncoupling of oxidative phosphorylation. Biochim Biophys Acta 1604(2): 77-94.

13. Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552(Pt 2): 335-344.

14. Antonucci S, Tagliavini A, Pedersen MG (2015) Reactive oxygen and nitrogen species disturb Ca(2+) oscillations in insulin-secreting MIN6 beta-cells. Islets 7(4): e1107255.

15. Schultz J, Waterstradt R, Kantowski T, Rickmann A, Reinhardt F, et al. (2016) Precise expression of Fis1 is important for glucose responsiveness of beta cells. J Endocrinol 230(1): 81-91.

16. Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51(2): 216-226.

17. Archer SL, Wu XC, Thebaud B, Moudgil R, Hashimoto K, et al. (2004) O_2 sensing in the human ductus arteriosus: redox-sensitive K_+ channels are regulated by mitochondria-derived hydrogen peroxide. Biol Chem 385(3-4): 205-216.

18. Fu J, Cui Q, Yang B, Hou Y, Wang H, et al. (2017) The impairment of glucose-stimulated insulin secretion in pancreatic beta-cells caused by prolonged glucotoxicity and lipotoxicity is associated with elevated adaptive antioxidant response. Food Chem Toxicol 100: 161-167.

19. Hoarau E, Chandra V, Rustin P, Scharfmann R, Duvillie B (2014) Pro-oxidant/antioxidant balance controls pancreatic beta-cell differentiation through the ERK1/2 pathway. Cell Death Dis 5: e1487.

20. Shang SW, Yang JL, Huang F, Liu K, Liu BL (2014) Modified Si-Miao-San ameliorates pancreatic B cell dysfunction by inhibition of reactive oxygen species-associated inflammation through AMP-kinase activation. Chin J Nat Med 12(5): 351-360.

21. Prause M, Christensen DP, Billestrup N, Mandrup-Poulsen T (2014) JNK1 protects against glucolipotoxicity-mediated beta-cell apoptosis. PLoS One 9(1): e87067.

22. Gerber PA, Rutter GA (2016) The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus. Antioxid Redox Signal.

23. Bo J, Xie S, Guo Y, Zhang C, Guan Y, et al. (2016) Methylglyoxal Impairs Insulin Secretion of Pancreatic beta-Cells through Increased Production of ROS and Mitochondrial Dysfunction Mediated by Upregulation of UCP2 and MAPKs. J Diabetes Res 2016: 2029854.

24. Fu J, Zheng H, Wang H, Yang B, Zhao R, et al. (2015) Protective Role of Nuclear Factor E2-Related Factor 2 against Acute Oxidative Stress-Induced Pancreatic beta -Cell Damage. Oxid Med Cell Longev 2015: 639191.

25. Robertson RP, Harmon J, Tran PO, Poitout V (2004) Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 53(S1): S119-S124.

26. Rhee SG, Yang KS, Kang SW, Woo HA, Chang TS (2005) Controlled elimination of intracellular H(2)O(2): regulation of peroxiredoxin, catalase, and glutathione peroxidase via post-translational modification. Antioxid Redox Signal 7(5-6): 619-626.

27. Lenzen S, Freytag S, Panten U (1988) Inhibition of glucokinase by alloxan through interaction with SH groups in the sugar-binding site of the enzyme. Mol Pharmacol 34(3): 395-400.

28. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, et al. (1999) Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells. Diabetologia 42(12): 1417-1424.

29. Ivarsson R, Quintens R, Dejonghe S, Tsukamoto K, in 't Veld P, et al. (2005) Redox control of exocytosis: regulatory role of NADPH, thioredoxin, and glutaredoxin. Diabetes 54(7): 2132-2142.

30. Pi J, Bai Y, Zhang Q, Wong V, Floering LM, et al. (2007) Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes 56(7): 1783-1791.

31. Leloup C, Tourrel-Cuzin C, Magnan C, Karaca M, Castel J, et al. (2009) Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. Diabetes 58(3): 673-681.

32. Li X, Chen H, Epstein PN (2006) Metallothionein and catalase sensitize to diabetes in nonobese diabetic mice: reactive oxygen species may have a protective role in pancreatic beta-cells. Diabetes 55(6): 1592-1604.

33. Suzuki YJ, Packer L (1993) Inhibition of NF-kappa B activation by vitamin E derivatives. Biochem Biophys Res Commun 193(1): 277-283.

34. Harakeh S, Jariwalla RJ (1997) NF-kappa B-independent suppression of HIV expression by ascorbic acid. AIDS Res Hum Retroviruses 13(3): 235-239.

35. Ho E, Chen G, Bray TM (1999) Supplementation of N-acetylcysteine inhibits NFkappaB activation and

protects against alloxan-induced diabetes in CD-1 mice. FASEB J 13(13): 1845-1854.

36. Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW (1996) In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J Immunol 157(4): 1630-1637.

37. Ntimbane T, Mailhot G, Spahis S, Rabasa-Lhoret R, Kleme ML, et al. (2016) CFTR silencing in pancreatic beta-cells reveals a functional impact on glucose-stimulated insulin secretion and oxidative stress response. Am J Physiol Endocrinol Metab 310(3): E200-E212.

38. Ntimbane T, Krishnamoorthy P, Huot C, Legault L, Jacob SV, et al. (2008) Oxidative stress and cystic fibrosis-related diabetes: a pilot study in children. J Cyst Fibros 7(5): 373-384.

39. McCord JM, Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 244(22): 6049-6055.

40. Jiang ZY, Woollard AC, Wolff SP (1991) Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids 26(10): 853-856.

41. Spahis S, Alvarez F, Dubois J, Ahmed N, Peretti N, et al. (2015) Plasma fatty acid composition in French-Canadian children with non-alcoholic fatty liver disease: Effect of n-3 PUFA supplementation. Prostaglandins Leukot Essent Fatty Acids 99: 25-34.

42. Brown WV (2008) Microvascular complications of diabetes mellitus: renal protection accompanies cardiovascular protection. Am J Cardiol 102(12A): 10L-13L.

43. Gurgul E, Lortz S, Tiedge M, Jorns A, Lenzen S (2004) Mitochondrial catalase over expression protects insulin-producing cells against toxicity of reactive oxygen species and proinflammatory cytokines. Diabetes 53(9): 2271-2280.

44. Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12(10): 1161-1208.

45. Taha R, Seidman E, Mailhot G, Boudreau F, Gendron FP, et al. (2010) Oxidative stress and mitochondrial functions in the intestinal Caco-2/15 cell line. PLoS One 5(7): e11817.

46. Welch KD, Davis TZ, Van Eden ME, Aust SD (2002) Deleterious iron-mediated oxidation of biomolecules. Free Radic Biol Med 32(7): 577-583.

47. Marcil V, Lavoie JC, Emonnot L, Seidman E, Levy E (2011) Analysis of the effects of iron and vitamin C co-supplementation on oxidative damage, antioxidant response and inflammation in THP-1 macrophages. Clin Biochem 44(10-11): 873-883.

48. Bachowski GJ, Thomas JP, Girotti AW (1988) Ascorbate-enhanced lipid peroxidation in photooxidized cell membranes: cholesterol product analysis as a probe of reaction mechanism. Lipids 23(6): 580-586.

49. Brasitus TA, Davidson NO, Schachter D (1985) Variations in dietary triacylglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membranes. Biochim Biophys Acta 812(2): 460-472.

50. Jourd'Heuil D, Vaananen P, Meddings JB (1993) Lipid peroxidation of the brush-border membrane: membrane physical properties and glucose transport. Am J Physiol 264(6 Pt 1): G1009-G1015.

51. Precourt LP, Seidman E, Delvin E, Amre D, Deslandres C, et al. (2009) Comparative expression analysis reveals differences in the regulation of intestinal paraoxonase family members. Int J Biochem Cell Biol 41(7): 1628-1637.

52. Gier B, Krippeit-Drews P, Sheiko T, Aguilar-Bryan L, Bryan J, et al. (2009) Suppression of KATP channel activity protects murine pancreatic beta cells against oxidative stress. J Clin Invest 119(11): 3246-3256.

53. Itoh K, Tong KI, Yamamoto M (2004) Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med 36(10): 1208-1213.

54. Schlieve CR, Lieven CJ, Levin LA (2006) Biochemical activity of reactive oxygen species scavengers do not predict retinal ganglion cell survival. Invest Ophthalmol Vis Sci 47(9): 3878-3886.

55. Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46(11): 1733-1742.

56. Jones DP (2002) Redox potential of GSH/GSSG couple: assay and biological significance. Methods Enzymol 348: 93-112.

57. Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30(11): 1191-1212.

58. Nguyen T, Sherratt PJ, Pickett CB (2003) Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. Annu Rev Pharmacol Toxicol 43: 233-260.

59. Lacraz G, Figeac F, Movassat J, Kassis N, Coulaud J, et al. (2009) Diabetic beta-cells can achieve self-protection against oxidative stress through an adaptive up-regulation of their antioxidant defenses. PLoS One 4(8): e6500.

60. Janjic D, Wollheim CB (1992) Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay. Diabetologia 35(5): 482-485.

61. Courtois F, Suc I, Garofalo C, Ledoux M, Seidman E, et al. (2000) Iron-ascorbate alters the efficiency of Caco-2 cells to assemble and secrete lipoproteins. Am J Physiol Gastrointest Liver Physiol 279(1): G12-G19.

62. Wollheim CB, Maechler P (2002) Beta-cell mitochondria and insulin secretion: messenger role of nucleotides and metabolites. Diabetes 51(S1): S37-S42.

63. Maechler P, Wollheim CB (2001) Mitochondrial function in normal and diabetic beta-cells. Nature 414(6865): 807-812.

64. Ronner P, Naumann CM, Friel E (2001) Effects of glucose and amino acids on free ADP in betaHC9 insulin-secreting cells. Diabetes 50(2): 291-300.

65. Tsuboi T, Lippiat JD, Ashcroft FM, Rutter GA (2004) ATP-dependent interaction of the cytosolic domains of the inwardly rectifying K+ channel Kir6.2 revealed by fluorescence resonance energy transfer. Proc Natl Acad Sci U S A 101(1): 76-81.

66. Brady NR, Elmore SP, van Beek JJ, Krab K, Courtoy PJ, et al. (2004) Coordinated behavior of mitochondria in both space and time: a reactive oxygen species-activated wave of mitochondrial depolarization. Biophys J 87(3): 2022-2034.

67. Zorov DB, Juhaszova M, Sollott SJ (2006) Mitochondrial ROS-induced ROS release: an update and review. Biochim Biophys Acta 1757(5-6): 509-517.

68. Drews G, Krippeit-Drews P, Dufer M (2010) Oxidative stress and beta-cell dysfunction. Pflugers Arch 460(4): 703-718.

69. Baeuerle PA, Henkel T (1994) Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 12: 141-179.

70. Lee SH, Na SI, Heo JS, Kim MH, Kim YH, et al. (2009) Arachidonic acid release by H_2O_2 mediated proliferation of mouse embryonic stem cells: involvement of Ca_{2+}/PKC and MAPKs-induced EGFR transactivation. J Cell Biochem 106(5):787-797.

71. Ramanadham S, Gross RW, Han X, Turk J (1993) Inhibition of arachidonate release by secretagogue-stimulated pancreatic islets suppresses both insulin secretion and the rise in beta-cell cytosolic calcium ion concentration. Biochemistry 32(1): 337-346.