Hyperglycemia induces oxidative damage in SW872 cells

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ABSTRACT

The high level of oxidative stress in obesity-linked type 2 diabetes is associated with elevated formation of advanced glycation end products (AGEs). Given that very little is known about the role of proteasome and AGE receptors (CD36 and RAGE) and ligands (HMGB1) in adipocyte response to hyperglycemia-induced oxidative stress, this study was focused on assessing the impact of either chronic or intermittent hyperglycemia on proteasome expression and activity, RAGE and CD36 expressions and HMGB1 mRNA levels in SW872 cell lines. 15% and 80% increases in ROS production and carbonyl accumulation were observed, respectively, when cells were incubated in hyperglycemic conditions compared to the control. Enhanced immunoproteasome expression was observed by Western blot attesting the inflammatory situation of SW872 cells when incubated in chronic hyperglycemia. Using fluorescent specific substrates, significant enhanced trypsin like activity of the proteasome (+60%) were observed in cells incubated in hyperglycemic conditions. Chronic hyperglycemia leads to cellular protein damages to a greater extent than intermittent hyperglycemia induced. An increase in CD36 and RAGE expressions was shown in SW872 treated in hyperglycemic conditions. Using real time quantitative PCR, enhanced HMGB1 mRNA expressions were evidenced in hyperglycemic-treated SW872 cell line. Our data clearly indicate that hyperglycemia treatments result in an increase in oxidative damage in SW872 cell lines that may affect its functionality. Oxidative stress drives the activation of inflammatory processes though RAGE ligands and receptors that can perturb insulin signalling leading to glucose intolerance and diabetes.

KEY WORDS: *Diabetes; Oxidative stress; Proteasome; Adipocytes*

INTRODUCTION

Free radicals and reactive oxygen species (ROS) can induce oxidative stress and oxidative damages associated with the progression of age-related diseases[1]. Diabetes, which is characterized by hyperglycemia and insulin resistance, can lead to dramatic complications such as cardiovascular disease, which remains the leading cause of mortality in Western countries[2]. Oxidative stress in diabetes may result from several processes, such as elevated ROS production from glucose autoxidation, glycoxidized proteins and impairment of antioxidant proteins[3].

The incidence of diabetes is increasing, with a worldwide prevalence estimated to double by 2030, primarily because of sedentary lifestyle and obesity[4]. A strong link between obesity and diabetes is now well established with more than 70

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percent of people with diabetes being overweight[5]. Adipocytes are known to express and secrete a variety of active molecules, so-called adipokines, which can regulate many biological processes such as insulin-sensitivity, appetite, immunity and reproduction[6].

Several underestimated factors at the adipocyte level could be involved in adipose tissue malfunctioning in the diabetes. The first factor to be considered is the proteasome proteolytic complex. Indeed, intracellular protein degradation is an intricately regulated process that maintains protein homeostasis and exerts quality control by degrading damaged or misfolded proteins[7]. In eukaryotic cells, the majority of intracellular proteins are degraded by the ubiquitin-proteasome system[8]. If numerous studies have identified the proteasome as a modulator of oxidative stress in a variety of settings, very little is known about its role in adipose tissue and whether hyperglycemia alters its activity in this tissue[9].

Secondly, two distinct receptors, namely receptor of advanced glycation endproduct (RAGE) and CD36, a SRB1 type receptor, were identified at the adipocyte level and could be involved in the oxidative stress-induced inflammation[10]. Nonetheless their expressions in SW872 subjected to hyperglycemia have never been investigated before.

Finally, high-mobility group protein B1 (HMGB1) constitutes an alarm, which acts as a signal danger when it binds to RAGE. Human HMGB1 is a single polypeptide chain of 215 amino acids. HMGB1 was initially identified as a nuclear factor but was later discovered to be a fundamental cytokine that mediates the response to infection and injury[11,12].

In this work, we determined whether either chronic or intermittent hyperglycemia could have deleterious effect on SW872 cell lines, a liposarcoma cell line often used as a human adipocyte cell model[13-18], particularly since the impact of hyperglycemia on SW872 cells

has never been investigated before. We report the effect of hyperglycemia on proteasome expression and activity, RAGE and CD36 expression and HMGB1 mRNA levels in SW872 treated cell lines.

MATERIALS AND METHODS
Materials
The substrates Z-Leu-Leu-Arg β-naphthylamide (postglutamyl peptidase-like activity) (C0788-10MG); N-Succinyl-Leu-Leu-Val-Tyr 7-amido-4-merhylcoumarin (chymotrypsin-like activity) (S-6510); and the substrate Boc-Leu-Ser-Thr-Arg-7- amido-4-merhylcoumarin (trypsin-like activity) and MG132 (Z-leu-leu-leu-Al; C2211-5MG) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

The antibodies used in this study were antibodies against β-tubulin (SC-58883) and LMP2 (SC-28809) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). All the horseradish peroxidase-conjugated secondary antibodies were purchased from GE Healthcare. For FACS, we used an antibody against CD36 conjugated with FITC (HM2121F, Hycult biotechnology) and a rabbit antibody against RAGE (R5278-100UG, Sigma). The anti-rabbit secondary antibody conjugated with PE (EI-1007) was purchased from Vector.

Cell culture
Human SW872 cells (ATCC, HTB-92) were used as a model for adipocytes. Cells were grown at 37°C under isobaric conditions (5% CO2, 95% air) in an incubator. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin, and 0,5 µg/ml amphotericin B were used to propagate the cells[19]. For SW872 treatments, cells were grown to confluence and then stimulated for 6 hours with glucose.

Hyperglycemic conditions
In order to mimic in *vivo* conditions of normal fasting glucose and

hyperglycemia, cells were cultured with 5 mM D-glucose or 25 mM D-glucose during 6h, respectively[19].

The intermittent exposure was used to mimic fluctuations in glucose levels: cells were incubated with 5 mM glucose during 1 h 30, with 25 mM glucose during 1 h 30, then with 5 mM glucose during 1 h 30 and finally with 25 mM glucose during 1 h 30[19].

Cell viability

Cell viability was determined by trypan blue exclusion counting. Briefly, 5 µl of a 0.4% solution of trypan blue in PBS was added to 45 µl of cell suspension for 5 min; cell count results were expressed as the percentage of blue stained cells to the total cell number. After counting in trypan blue, cells were lysed for analysis of the proteasome proteolytic activities.

MTT assay

The MTT-assay was conducted to evaluate the effect of hyperglycemia on cell proliferation[20]. The assay is based on cleavage of a yellow tetrazolium salt (MTT) by metabolically active cells, yielding a purple formazan, which can be photometrically quantified. An increase in the number of living cells results in an increase in the total metabolic activity, and thus an increase in color formation. Cells were incubated in the presence of different reagents for 6 hours, 20 µL of MTT dye (5 mg/ml) was added then to each well, followed by 4 h of incubation. After discarding the media, 150 µl of isopropanol was added to each well and plates were agitated in the dark for 30 min to solubilize the resulting dark blue formazan crystals. Plates were read using a microplate reader at a wavelength of 595 nm. A negative control well (medium only; no cells and no reagent) was used as reference to zero the absorbance.

Cell lysis

After removing the culture media, cells were washed with PBS and lysed by adding lysis buffer (25mM Tris pH=8.3, 10mM KCl, 1mM EDTA and 1% Triton X-100, 1mM) without protease inhibitor for proteasomal activity measurement. The same lysis buffer with protease inhibitor (0,5X final) was used for Western Blot analyses.

For the dot blot analysis and carbonyl ELISA, a lysis buffer containing pure DMSO plus 0.5 % TFA was used.

Determination of ROS production by DCF-DA

Six hours after treatment, cells were washed twice with PBS, and incubated with 10 µM DCF-DA at 37°C. After 30 mins, cells were washed once with PBS, and the fluorescence intensity of the oxidized form of DCF was measured in a microplate-reader at 492 nm (excitation) and 520 nm (emission). Fluorescence values were calculated after background subtraction (using identical conditions without DCF).

Proteolytic activity of the proteasome

Chymotrypsin-like, trypsin-like, and caspase-like proteasome activities were assayed using fluorogenic peptides (from Sigma) Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (LLVY-MCA at 25 µM), N-t-Boc-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin (LSTR-MCA at 40 µM) and N-Cbz-Leu-Leu-Glu-b-naphthylamide (LLE-NA at 150 µM), respectively[21]. Assays were carried out on approximately 50 µg of cell lysate in 25 mM Tris–HCl (pH 7.5) and the appropriate substrate, which were incubated together at 37°C for 0–30 mins. Aminomethylcoumarin and β-naphthylamine fluorescence was measured at excitation/emission wavelengths of 350/440 and 333/410 nm, respectively, using a fluorometric microplate reader (Fluostar - BMG France). Peptidase activities were measured in the absence or presence (20 µM) of the proteasome inhibitor, MG132 (N-Cbz-Leu-Leu-leucinal), and the difference between the two values was attributed to proteasome activity.

Western Blot analysis

Total proteins (20 μg) were separated by electrophoresis on a 15% polyacrylamide gel and then electrotransferred to an immobilon-P membrane (Millipore) and blocked with 1% BSA in PBS buffer containing 0.05% Tween-20. The blocked membrane was incubated in the same buffer containing antibody against LMP2 or antibody against β-tubulin used as loading control. The membranes were washed with PBS buffer containing 0.05% Tween-20 and then incubated with horseradish peroxidase-coupled anti-goat or anti-mouse IgG as a secondary antibody. The bands were visualized using enhanced chemiluminescence detection reagent.

Carbonyl ELISA

Quantification of protein carbonyl was determined by ELISA. The proteins were incubated with a solution containing DNPH (2,4-dinitrophenylhydrazine) for 15 minutes. The reaction with the DNPH was stopped with a basic solution. Then, coating of ELISA plate was performed for 3 hours at 37°C. Blocking was performed overnight at 4°C with PBS 1X, 0.1% tween 20 and 1% BSA. For the detection of carbonylated proteins, a rabbit anti-DNP (2,4-dinitrophenol) primary antibody and a secondary HRP-conjugated antibody were used. TMB was used as substrate for the color development. After 15 minutes incubation, the reaction was neutralized with HCl 2N and absorbance was read at 450 nm. Several protein concentrations were used and the slopes of each curve were compared.

Carbonyl Dot Blot

After cell lysis by DMSO, derivatizating solution (20mM DNPH, 0.5% TFA, 92% DMSO) was added to give a protein concentration of 300 ng/μl. Each sample was spotted (1μl) on dry immobilon PVDF membrane for protein determination. To deposit the samples, the membrane was placed on a 96-well plate and to provide a guide for spotting sample, a grid from a pipette tip rack was used. The membranes were then air-dried for 15 minutes. After air-drying, membranes were washed twice in acetic acid for 2 minutes. After removal of the second wash, 5 ml of acetic acid was added to keep the membrane wet, and water was gradually added to cover the membrane. This solution was removed and replaced with water. After 5 minutes incubation, the membrane was ready for proteins or carbonyl determination[22]. For protein determination, one membrane was stained with Ponceau red while the other membrane was blocked in PBS, 0.05% tween 20, 1% BSA buffer overnight at 4°C. Then, the membrane was incubated for 2 hours with a 1:2000 dilution of goat anti-DNP primary antibody. After incubation, the membrane was washed three times with PBS, tween 20, and then incubated with a 1:4000 anti-goat secondary antibody conjugated with HRP for 2 hours. ECL was used for the detection.

To dispose of a negative or a positive control for this test, cells were treated with different concentration of AAPH during 6h. Signal intensity was determined with ImageJ free software (*http://rsb.info.nih.gov/ij/*)[23].

Flow Cytometer analysis

Cells were cultured in 6-well plate and treated when confluent. After treatment, cells were washed once in PBS and scraped in PBS/1% BSA buffer. Then, cells were incubated with FITC-conjugated CD36 primary antibody (HM2122F, Hycult biotechnology) or an antibody against RAGE (R5278-100UG, Sigma) or with FITC-conjugated CD36 primary antibody alone or in the absence of antibody. After one-hour incubation, cells were washed once with PBS/BSA. Then, cells were incubated for 30 minutes with antibody conjugated with PE or with no antibody. PE fluorescence intensity was assayed by FACScan flow cytometer (Becton-Dickinson).

qPCR analysis

HMGB1 mRNA expression was analyzed by qPCR. Total RNA was extracted from cell lysates by using Trizol reagent (Invitrogen) and resuspended in sterile water. Concentration of mRNA was determined by reading the absorbance at 260 nm. To perform reverse transcription, a dNTP mix at 10 mM each (Promega), Random Hexamer from Biosystem (N18917) and SuperScript II RT (SS II RT) from Invitrogen (P/N 100004925) were used. Then, qPCR from cDNA using SYBR Green I reagent was then performed. HMGB1 primers (Forward: *5' ACC CAG ATG CTT CAG TCA AC 3'* and reverse: *5' GGC GAT ACT CAG AGC AGA AG 3'*) and GAPDH primers (Forward: *5' TTC ACC ACC ATG GAG AAG GC 3'*; Reverse: *5' GGC ATG GAC TGT GGT CAT GA 3'*) were used and amplification was performed using ABI PRISM® 7700 Sequence Detection System.

Statistics and data analysis

The results were expressed as mean ± SD from at least three experiments performed in triplicates. For the figures reporting RAGE, CD36 and HMGB1 mRNA and protein expression levels, one or two experiments were performed in triplicate. The significance of differences between groups was assessed using Student's t-test for unpaired samples with Prism (GraphPad Software Inc., San Diego, Calif.). Significance was defined as *p <0.05, **p <0.01*.

RESULTS

Chronic hyperglycemia condition or intermittent hyperglycemia condition did not affect cell viability

We first tested whether our hyperglycemic treatment could be considered as a pathophysiological condition with no impact on cell viability. The results obtained by trypan blue staining, showed that prolonged treatment with glucose or intermittent treatment with glucose did not affect SW872 cell line viability. H_2O_2 treatment induced a 40% decrease in cell viability

(**Figure 1A** and **B**). H_2O_2 treatment was used as a positive control. Identical results were obtained using the MTT assay (**Figure 1C**).
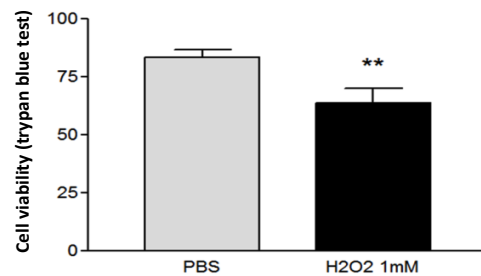

Figure 1A: Cells were treated in the presence or absence of 1mM H_2O_2. Cell viability was assessed by trypan blue counting. (n=3)
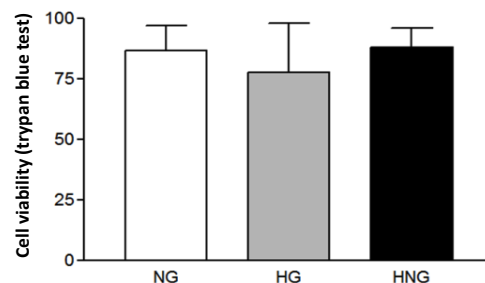

Figure 1B: Cells were treated for 6 hours in normoglycemic condition, chronic hyperglycemic condition or intermittent hyperglycemic condition. We evaluated cell viability by trypan blue counting. Conditions for glucose exposure were: NG: normoglycemic condition (5mM of glucose) for 6 hours; HG: Hyperglycemic condition (25mM of glucose) for 6 hours; HNG: Intermittent hyperglycemic condition (1H30 at 25mM; 1h30 at 5mM; 1h30 at 25mM; 1h30 at 5mM). (n=6)
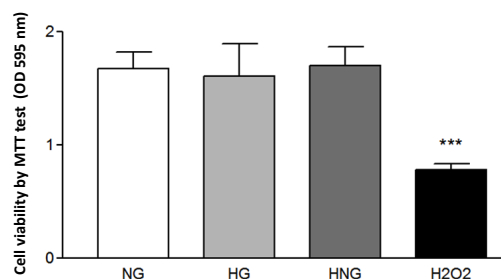

Figure 1C: MTT test. Conditions for glucose exposure were: NG: normoglycemic condition (5mM of glucose) for 6 hours; HG: Hyperglycemic condition (25mM of glucose) for 6 hours; HNG: Intermittent hyperglycemic

condition (1H30 at 25mM; 1h30 at 5mM; 1h30 at 25mM; 1h30 at 5mM). (n=6) *Results were analyzed using Student's t test for unpaired samples: ***p <0.001 (compared to the control).*

Chronic or intermittent hyperglycemia tends to enhance intracellular ROS production

To study the impact of hyperglycemia on ROS generation in adipocytes, we measured intracellular ROS levels in the treated cells (NG, HG and HNG) using the dichlorofluorescein diacetate (DCF-DA) assay. We observed that chronic hyperglycemia condition or intermittent hyperglycemia seemed to promote ROS generation after 6 hours of treatment compared to normoglycemic condition (**Figure 2**). A 16 % and 14 % increase in ROS production was observed when cells were incubated in chronic hyperglycemic or intermittent hyperglycemic conditions, respectively, compared to the control.
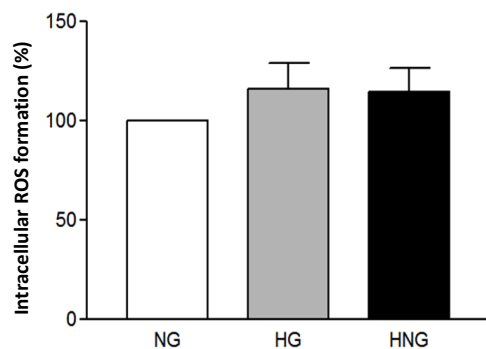

Figure 2: ROS generation was evaluated by the DCF-DA assay. DCF, the oxidation product of the reagent 2, 2'-dichlorofluorescin diacetate, was used as a marker of cellular free radical production. *NG: Normoglycemic condition; HG: Hyperglycemic condition; HNG: Intermittent Hyperglycemic condition (n=2)*

Chronic hyperglycemia condition or intermittent hyperglycemia condition increased proteasome expressions and proteasome activities

Proteasome expression was assayed by Western blot analysis (**Figure 3A**). Antibodies used to performed proteasome Western blots were specific for the LMP2 subunit. This LMP2 subunit is included to the proteasome complex in inflammatory situation and leads to the formation of the immunoproteasome[24].
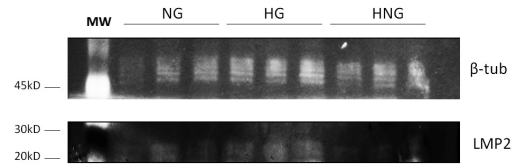

Figure 3A: Proteasome expression was assayed by Western Blot. The detection of β-tubulin and LMP2 proteasome subunit were determined using specific antibodies as described in Material and Method section. *NG: Normoglycemic condition; HG: Hyperglycemic condition; HNG: Intermittent Hyperglycemic condition*

Noteworthy, immunoproteasome expression was increased in chronic hyperglycemia condition compared to the normoglycemic condition.

Proteasome activity was assayed using fluorescent substrates. The trypsin-like activity of the proteasome was significantly increased in chronic and intermittent hyperglycemia condition compared to control cells in normoglycemic condition (**Figure 3B**).
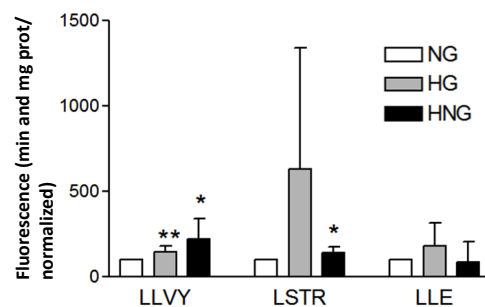

Figure 3B: Proteasome activities were measured using different fluorescent probes. We determined chymotrypsin-like activity, postglutamyl- peptidase-like activity, and trypsin-like activity using N-LLVY-MCA, N-LLE-β-napthylamine, and N-LLR-MCA substrates, respectively. Proteins samples were incubated with or without proteasome inhibitor MG132. The difference between the two fluorescence rates was attributed to the proteasome. *NG: Normoglycemic condition;*

## Chronic hyperglycemia condition promotes protein oxidation

To test if glucose concentration fluctuations may influence protein oxidation in SW872 adipocytes, the cells media was switched between media containing 5 or 25 mM glucose over a period of 6 h. In parallel, we tested the influence of a chronic hyperglycemic treatment on protein oxidation.

Dot blot analysis was used to visualize the rate of protein oxidation. Signal quantification was performed using ImageJ freeware[23]. Chronic hyperglycemia condition significantly increased protein oxidation and intermittent hyperglycemia did not affect the rate of oxidized proteins in comparison with normoglycemic condition (control; **Figure 4A**).

This result was confirmed by carbonyl ELISA showing a significant increase (80%) in carbonylated proteins chronic hyperglycemia-treated cells in comparison with the control (**Figure 4B**). Conversely, intermittent hyperglycemia did not significantly affect the rate of oxidized protein accumulation in comparison with normoglycemic condition (**Figure 4B**).
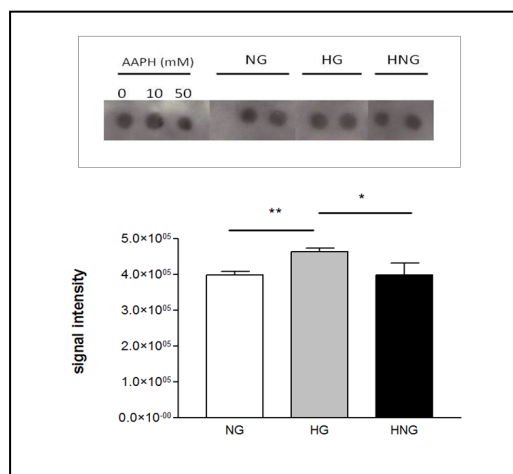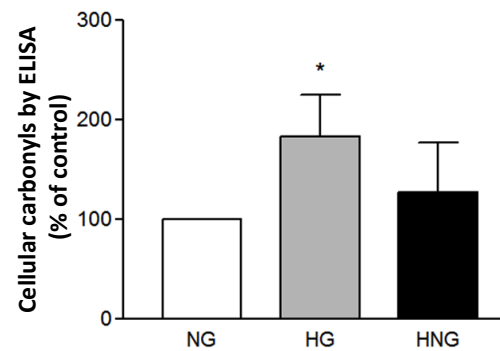

B

Figure 4: Oxidized protein accumulation in treated cells was determined by dot blot analysis (A) and ELISA carbonyl (B) *NG: Normoglycemic condition; HG: Hyperglycemic condition; HNG: Intermittent Hyperglycemic condition* **(n=3)** *Results were analyzed using Student's t test for unpaired samples: *p <0.05, **p <0.01 (compared to the control)*

CD36 and RAGE expression are enhanced in hyperglycemic condition

To determine whether CD36 and RAGE expressions were modulated by hyperglycemia, we performed a flow cytometer analysis. 80% of cells exhibited RAGE labeling in normoglycemic condition whereas CD36 labeling in normoglycemic condition was less than 15% (**Figure 5A**). In chronic hyperglycemic condition, no significant increase in RAGE and CD36 levels was observed when compared to the control (**Figure 5B, Figure 6**). In intermittent hyperglycemia, RAGE and CD36 expression were significantly increased when compared to normoglycemic condition. High glucose condition increased CD36 and RAGE levels by 1.2-, and 1.7-fold, respectively (**Figure 5C, Figure 6**). Despite their involvement in diabetes-associated cellular disorders, RAGE and CD36 expression at SW872 cell line surface have never been reported before. We showed that RAGE and CD36 expressions are enhanced in SW872 treated in hyperglycemic condition. This result indicates that RAGE and CD36 may play a role in inflammation due to diabetes at the adipocyte level.

A

5A   5B   5C
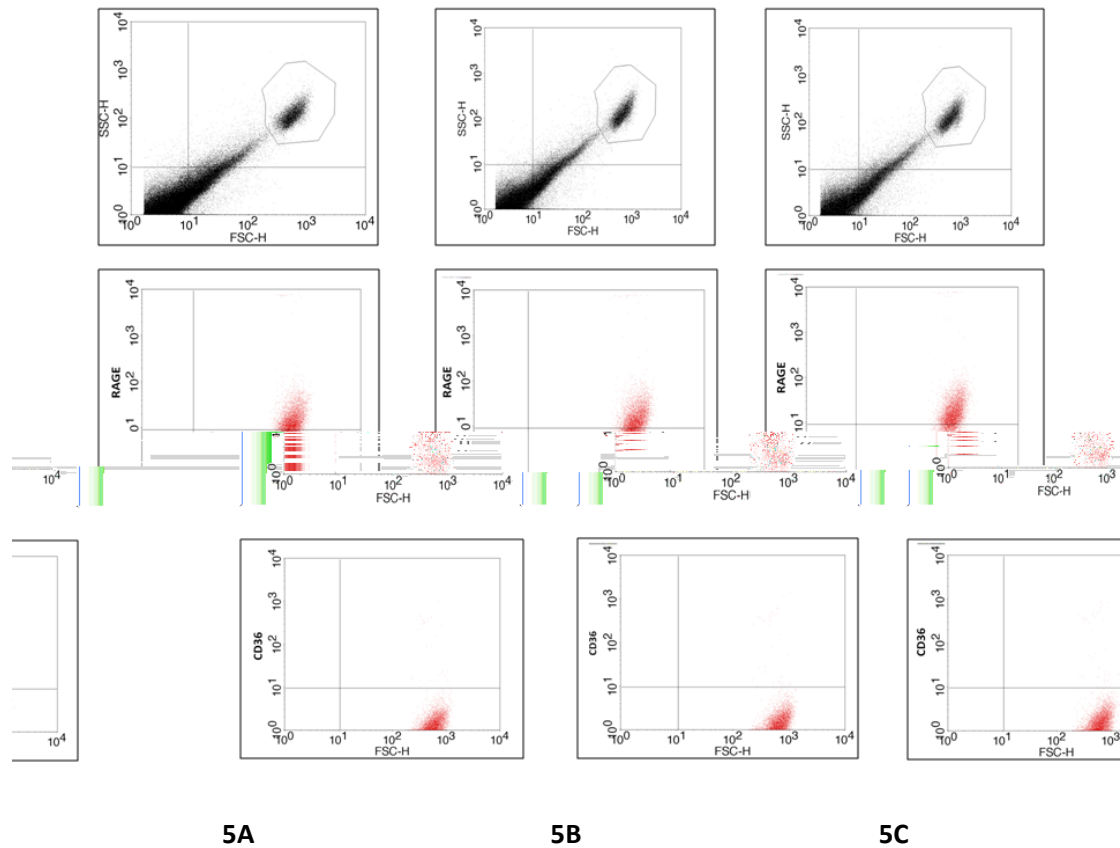
Figure 5: FACS analyses for cellular RAGE and CD36 expressions: CD36 and RAGE expression were determined using Flow cytometer analysis. For the detection of RAGE and CD36, we used FITC-conjugated CD36 primary antibody and an antibody directed against RAGE. (A) normoglycemic condition; (B) chronic hyperglycemia condition and (C) intermittent hyperglycemia condition.
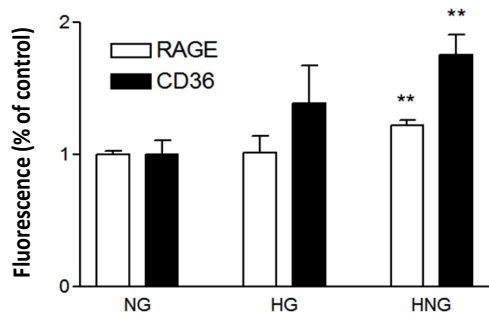

Figure 6: Expression levels of RAGE and CD36 receptors measured by FACS, were expressed relative to control (normoglycemic condition), normalized to 1. *NG: Normoglycemic condition; HG: Hyperglycemic condition; HNG: Intermittent Hyperglycemic condition*. *Results were analyzed using Student's t test for unpaired samples: **p < 0.01 (compared to the control).*

HMGB1 mRNA expression increases in hyperglycemic condition

HMGB1 constitutes one of the ligands for RAGE receptor and is implicated in RAGE-mediated inflammation pathway. To examine whether HMGB1 mRNA transcript was expressed in SW872 cells and modulated by hyperglycemia, cells were maintained either in a chronic hyperglycemia condition for 6 hours or intermittent hyperglycemia condition. Compared to normoglycemic condition, cell treatment in chronic hyperglycemic or intermittent conditions resulted in an increase in HMGB1 mRNA expression (**Figure 7**).
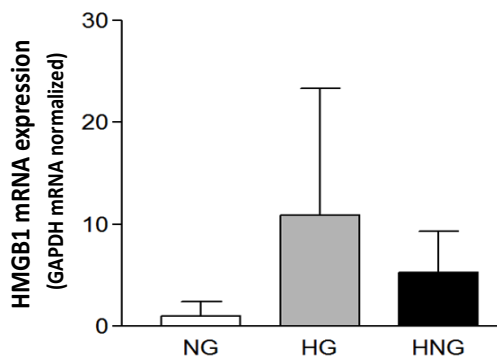
Figure 7: HMGB1 mRNA expression was determined by qPCR analysis using SYBR Green method. Amplification and detection was performed using 7700 system software. *NG: Normoglycemic condition; HG: Hyperglycemic condition; HNG: Intermittent Hyperglycemic condition (n=2)*

DISCUSSION

Our present work brings new insight on the impact of hyperglycemia on adipocytes by inducing intracellular ROS formation, carbonylated protein accumulation, impairment in proteasome activity and enhancement in RAGE, CD36 and HMGB1 expressions. All deleterious effects of hyperglycemia are observed in pathophysiological conditions where cellular viability was not affected.

The diabetic pathology is characterized by high ROS production and leads to an important level of oxidative stress. In this pathology, enhanced oxidative stress is linked to hyperglycemia via several processes, such as elevated ROS production from glucose autoxidation, glycoxidized proteins and impairment of antioxidant proteins[3]. In the long term, the accumulation of ROS can cause damages to DNA and oxidation of proteins, which then exert altered functions. These changes can lead to cellular damages. In this study, we observed that high H_2O_2 concentrations, used as positive control, did induce a significant reduction (-40%) in cell viability. However no such effect was observed in our experimental conditions suggesting that hyperglycemia treatments did not affect cell viability. Interestingly,

in the presence of pathologically relevant hyperglycemia, a non-significant increase in ROS production was noted. It can be hypothesized that a longer SW872 adipocytes exposure (in this study 6 hours) to hyperglycemia would have induced a significantly higher production of ROS in comparison to cells incubated in normoglycemic condition.

In our experimental conditions, we evidenced significant enhancements in cellular oxidized protein accumulation in adipocytes incubated in hyperglycemic conditions. Again, this result was obtained in conditions, which might be encountered in some uncontrolled pathological diabetes situations. Few studies from our group reported enhanced carbonylated proteins in adipocytes subjected to oxidative stress by AGE treatment[13,23,25]. More recently, we evidenced protective effect of citrus antioxidant on AGE-induced carbonyl accumulation in SW872 cell line[26].

Enhanced carbonylated protein accumulation was evidenced in adipose tissue biopsies from obese patients[27]. Increased adipose protein carbonylation has been associated with impaired insulin signaling in cell culture models[28,29].

Beside multiple other functions, the proteasome proteolytic system is responsible for the degradation of misfolded or oxidized proteins. In our experimental conditions, significant increase in LMP2 proteasome subunit was observed by Western blot when cells were incubated in chronic hyperglycemia. This LMP2 subunit is included to the proteasome complex in inflammatory situation and leads to the formation of the immunoproteasome[24]. Hence, SW872 cells when placed in a hyperglycemic environment exhibited an "inflammatory state". This result is in perfect agreement with recent reports from the group of Grune whereby enhanced expression of LMP2 subunit in response to oxidative stress was reported[30-32].

Though the role of the proteasome at the adipocyte level remains largely

unexplored, the very few examples present in the literature are interesting and suggest targets for drug development. Proteasome appeared to be involved in regulating lipid synthesis in adipocytes and cellular differentiation and therefore could influence the development of obesity[9,33].

In the present study, significant enhanced trypsin-like activity of the proteasome when cells were incubated in hyperglycemic conditions was shown. This enhanced proteolytic activity could be considered as a defense system triggered by the oxidative stress insult. In our experimental hyperglycemic conditions, proteasome system may be rapidly overwhelmed by oxidative stress and result in carbonylated proteins, which accumulate more importantly than in normoglycemic-treated cells. This enhanced proteasomal activity observed in hyperglycemia-treated adipocyte cells was recently measured in ependidymal adipose tissue from transgenic Db/Db diabetic mice (unpublished results).

Oxidative stress in diabetes could originate from AGEs, which constitute a heterogeneous group of well-studied biological substances[10]. Several cascades of reaction can lead to AGEs formation but the initial step is the glycation reaction known as Maillard's reaction. The glycation phenomenon corresponds to the non-enzymatic and non-oxidative covalent attachment of a glucose molecule to a protein[34]. Glycoxidation refers to the radical-mediated oxidation reaction of both free and protein-bound sugars[2]. Amadori rearrangement of the above glycated protein gives rise to advanced glycoxidation (also termed advanced glycation) end products (AGEs)[35]. Interaction of advanced glycated end products with specific receptors in particular RAGE and CD36 induces several cellular phenomena potentially relating to diabetic complications[10].

This study, for the first time, demonstrates the presence of CD36 and RAGE in SW872 cells. Furthermore, an increase in CD36 expression in SW872 treated in hyperglycemic conditions is also reported. Data revealing that the binding of AGEs could be mediated by CD36, on adipose cells are quite recent[36,37]. Since then, several studies have reported AGEs deleterious impact on adipocyte physiology[13,38-40]. To our knowledge, our present work is the first report on impact of hyperglycemia on CD36 and RAGE expression and brings new insights on adipocyte role in inflammation progression in the diabetes context.

Furthermore, hyperglycemia enhanced HMGB1 expression by SW872 cell line. HMGB1 represents a ligand for RAGE and acts as a mediator of inflammation. Recent results from our group revealed soluble HMGB1 as a novel adipokine stimulating IL-6 secretion through RAGE receptor in SW872 cell line contributing to chronic inflammation in fat tissue[41].

Our present work contains several limitations such as duration of the hyperglycemic treatment of the cells. We used 6-hour treatment and longer hyperglycemia exposure may have induced more drastic effects on cells. Also our results especially concerning CD36, RAGE and HMGB1 expressions require complementary works to gain better insight in the involvement of these receptors at the adipocyte level in hyperglycemic situations. However, our data indicate that hyperglycemic treatments enhance oxidative damage in SW872 cell lines that may affect their functionality. Oxidative stress drives the activation of inflammatory processes, though RAGE ligands and receptors that can perturb insulin signaling, leading to glucose intolerance and diabetes.

Further experiments are warranted for a better understanding of hyperglycemia-induced oxidative stress effect at the adipocyte level.

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