Biochemical and Biophysical Research Communications 473 (2016) 154–160

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Enhanced oxidative stress in adipose tissue from diabetic mice, possible contribution of glycated albumin

Florence Boyer [a, b], Nicolas Diotel [a, b], Dorothée Girard [a, b], Philippe Rondeau [a, b], M. Faadiel Essop [c], Emmanuel Bourdon [a, b, *]

[a] Inserm, UMR 1188 Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), plateforme CYROI, Sainte-Clotilde F-97490, France
[b] Université de La Réunion, UMR 1188, Sainte-Clotilde F-97490, France
[c] Cardio-Metabolic Research Group (CMRG), Department of Physiological Sciences, Stellenbosch University, Merriman Avenue, Stellenbosch 7600, South Africa

ARTICLE INFO

Article history:
Received 13 March 2016
Accepted 16 March 2016
Available online 22 March 2016

Keywords:
Oxidative stress
Hyperglycemia
Diabetes
Proteasome
Glycated albumin

ABSTRACT

Although enhanced oxidative stress and proteotoxicity constitute major contributors to the pathogenesis of multiple diseases, there is limited understanding of its role in adipose tissue. Here, we aimed at evaluating oxidative stress biomarkers in adipocytes from diabetic/obese db/db mice. The current study revealed that reactive oxygen species production was upregulated in adipocytes, together with lipid peroxidation 4-hydroxynonenal accumulation, and altered proteolytic and antioxidant activities. In parallel, acute exposure of 3T3L1 adipocyte cell lines to glycated albumin (known to be enhanced with diabetes) also elicited intracellular free radical formation. Our data provide novel insights into redox and proteolytic homeostasis in adipocytes.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Oxidative stress is defined as “an imbalance between oxidants and anti-oxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage” [1]. While reactive oxygen species (ROS) can act as signaling molecules, excessive amounts can induce lipid, DNA and protein oxidative damage thereby altering structure and function [1]. Under such circumstances antioxidant defences and/or repair processes can fail resulting in an imbalance in favor of greater intracellular ROS availability. However, under such conditions proteolytic mechanisms including the ubiquitin proteasome system (UPS) are activated to remove damaged, oxidized proteins [2].

Oxidative stress is robustly implicated in disease onset and increasing evidence implicates it in the development of insulin resistance [3,4]. Diabetes is a major health problem that is usually associated with obesity, together with hyperglycemia and advanced glycation endproducts (AGEs) formation. The concept of redox imbalance (in fat tissues) as an instigator of adipocyte dysfunction with obesity is a recent phenomenon [5]. For example, oxidative stress impaired systemic insulin sensitivity and played a causative role in the development of insulin resistance in adipose tissues [4,6]. Here the role of the proteasome remains poorly understood and recent evidence established an important function in controlling redox homeostasis and in the degradation of oxidized proteins [7]. Moreover, despite increased evidences of enhanced AGEs formation in the diabetes/obesity context, AGE involvement in adipocyte pathophysiology onset remains poorly understood [8]. As little is known regarding alterations in oxidative stress and protein homeostasis in adipose tissues, this study investigated redox homeostasis in adipocytes from the leptin receptor-deficient db/db transgenic mouse strain. To gain additional mechanistic insights, we also established an *in vitro* experimental protocol to assess the deleterious effects of hyperglycemia and albumin-AGE on 3T3L1 cells by monitoring intracellular free radical formation.

2. Materials and methods

2.1. Animals

C57BLKs/J+/Lepr^{db} mice (db/+) were obtained from Charles

Abbreviations: AGE, advanced glycation end products; DHE, dihydroethidium; GPX, glutathione peroxidase; 4HNE, 4-hydroxynonenal; PBS, saline phosphate buffer; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide disumutase.

* Corresponding author. DéTROI – Université de la Réunion, CYROI 2, rue Maxime Rivière, BP 80 005 – 97491 Sainte Clotilde Cedex La Réunion , France.
E-mail address: Emmanuel.bourdon@univ-reunion.fr (E. Bourdon).

http://dx.doi.org/10.1016/j.bbrc.2016.03.068
0006-291X/© 2016 Elsevier Inc. All rights reserved.

River Laboratories (Wilmington MA). Male heterozygous db/+ and homozygous C57BLKs/J/Lepr^{db}/Lepr^{db} (db/db) mice (n = 10 per group) were housed under constant temperature (21°±2 °C), humidity (50% ± 5%) and a 12-h light–dark cycle for a period of 12 weeks. Food and water were available *ad libitum* and all experimental procedures were conducted in compliance with animal protocols approved by the Animal Ethics Committee of Cyclotron Réunion Océan Indien (CYROI) (Project #01268.01).

2.2. Tissue collection and biochemical analyses

Twelve-week old mice were fasted overnight, weighed, anesthetized and euthanized before fasting blood glucose levels measurement using the OneTouch® Ultra Blood Glucose Monitoring System (Lifescan). Blood was collected by cardiac puncture into EDTA tubes (BD Vacutainer®) and total proteins, cholesterol, albumin, fructosamine and triglycerides levels were determined using a clinical Biochemistry automated Cobas C501 analyzer (Roche Diagnostics). Epididymal adipose tissues were excised, snap frozen and stored at −80 °C prior to analysis. Previously collected and stored epididymal adipose tissues were homogenized with a TissueLyser II (Qiagen) in a phosphate buffer (KH_2PO_4 (100 mM), DTT (1 mM), and EDTA (2 mM), pH 7.4). After centrifugation (5000 g/min for 10 min), the supernatant was used for protein quantification, carbonyl and enzymatic assays.

2.3. Immunohistochemistry and oxidative stress staining

For immunohistochemistry, adipose tissues from db/+ and db/db mice were collected and fixed in 4% paraformaldehyde in PBS. Paraffin sections (7 μm) were prepared using a microtome (Thermo Scientific, Shandon™ Finesse™ ME+) and thereafter deparaffinized and rehydrated. Antigen retrieval was performed in sodium citrate buffer (pH 6; 0.01 M) and sections were incubated overnight at room temperature with the 4-HNE antibody (1/100; Ref# ab46545; Abcam, Cambridge MA) in PBS-Triton containing 1% BSA. The next day sections were washed in PBS-Triton and incubated for 1 h 30 with secondary antibody (Alexa Fluor goat anti-rabbit 594; 1:200; Invitrogen Molecular Probes, Eugene OR). Sections were finally counterstained with DAPI and slides mounted with Vectashield anti-fading medium (H-1000, Vector Laboratories, Burlingame CA).

For ROS imaging, unfixed adipose tissues were sampled and frozen at −80 °C before being embedded in OCT (Tissue Tek, Sakura, Torrance, CA) and cut at 10 μm thickness using a conventional cryotome (Thermo Scientific, Shandon™ Cryotome FE, France). Slides were defrosted for 1 h at room temperature in a humidified chamber and subsequently incubated with 2 μM DHE (dissolved in PBS; Sigma–Aldrich, Ref: D7008, France) in a light-protected humidified chamber at 37 °C for 30 min. Slides were subsequently washed twice in PBS and mounted with Vectashield anti-fading medium (Vector Laboratories, Burlingame CA).

2.4. Microscopy

Prepared slides were viewed with a bright field/epifluorescence microscope Nikon Eclipse 80i equipped with a Hamamatsu ORCA-ER digital camera (Hamamatsu Photonics, Japan). For 4 HNE immunohistochemistry and ROS staining, pictures were acquired using the Nikon software with similar exposure times between db/+ and db/db sections. Micrographs were obtained in tiff format and adjusted for light and contrast before being assembled on plates in Adobe Photoshop CS4 (extended version 11.0).

2.5. Thiol number determination

Thiol groups were measured according to Ellman's assay using 5,5′-dithiobis, 2-nitrobenzoic acid (DTNB) [9]. A standard curve was generated for each assay and here we used 10–100 nmol of L-cysteine (Sigma–Aldrich). Briefly, 100 μL of tissue samples (in 0.1 M Tris–HCl, pH 8.0, EDTA 5 mM) were incubated with three volumes of 0.5 mM DTNB and the free thiol concentration was calculated from absorbance readings (412 nm) with the help of a standard curve. Results are expressed in nmol of free –SH groups per μg protein.

2.6. Enzymatic activity determination

SOD activity was assayed by monitoring the rate of acetylated cytochrome c reduction by superoxide radicals generated by the xanthine/xanthine oxidase system. Measurements were performed in a reagent buffer (xanthine oxidase, xanthine (0.5 mM), cytochrome c (0.2 mM), KH2PO4 (50 mM), EDTA (2 mM), pH 7.8) at 25 °C. The specific Manganese-SOD (Mn-SOD) activities were determined in the same condition after incubation of samples with NaCN (1 mM) which inhibits Cu/Zn-SOD activities. Assays were monitored by spectrophotometry at 560 nm. SOD activities were calculated using a calibration standard curve of SOD (up to 6 unit/mg). Total Mn-SOD and resulting Cu/Zn-SOD activities were expressed as international catalytic units per mg of proteins.

Glutathione peroxidase (GPX) activity was determined with cumene hydroperoxide as substrate [10]. The rate of glutathione oxidized by cumene hydroperoxide (6.5 mM) was evaluated by measuring NADPH (0.12 mM in Tris buffer) absorbance at 340 nm. Here the reaction buffer is composed of NaCN (10 mM), reduced glutathione (0.25 mM) and glutathione reductase (1 U/ml) in Tris buffer (50 mM, pH 8). GPX activity was expressed as international units per gram of proteins.

The catalase activity assay was performed on 40 μg of protein lysate in 25 mM Tris–HCl (pH 7.5). Blanks were measured at 240 nm just before adding 80 μL of H_2O_2 (10 mM final) to start the reaction. Catalase activity was determined by measuring the absorbance at 240 nm and calculated using a standard calibration curve constructed by increasing catalase amounts (between 12.5 and 125 units/ml). Catalase activity was expressed as international catalytic units per mg of proteins.

Proteasome activity measurements, i.e. chymotrypsin-like, trypsin-like, and caspase-like activities of the proteasome were assayed using fluorogenic peptides (Sigma–Aldrich, St Louis): Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (LLVYMCA at 25 mM), N-t-Boc-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin (LSTR-MCA at 40 mM) and N-Cbz-Leu-Leu-Glu-bnaphthylamide (LLE-NA at 150 mM), respectively, as described previously [11].

2.7. 3T3 L1 cell line

3T3L1 cells were cultured in completed DMEM medium 10% FBS, penicillin (100 U/ml), streptomycin (100 U/ml) and L-glutamine (2 mM) and grown in a 5% CO_2 incubator at 37 °C. Prior to treatments, 3T3 L1 cells were cultured in DMEM containing 1% FBS in 96-well plates (10 000 cells/well) for 24 h to reach about 75% confluency. At this stage cells were treated for 1 h under simulated normoglycemic conditions (5 mM glucose) in the absence (NG) or presence of 80 μM of native albumin (HSA_{G0}), glycated albumin (HSA_{MGO}) versus simulated hyperglycemic conditions (25 mM glucose; HG).

Download English Version:

https://daneshyari.com/en/article/1927871

Download Persian Version:

https://daneshyari.com/article/1927871

Daneshyari.com