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# Oxidative stress in Cohen diabetic rat model by high-sucrose, low-copper diet: inducing pancreatic damage and diabetes

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**Abstract**

Increased oxidative stress contributes to the development and progression of both types of diabetes mellitus (DM) and its complications. In the Cohen diabetic (CD) rats, a known genetic model of nutritionally induced type 2 DM, a high-sucrose, low-copper diet (HSD) induces within 4 weeks DM in the sensitive (CDs) rats but not in the resistant (CDr) rats. To assess the possible involvement of oxidative stress in the induction of DM, we studied the effect of HSD on the tissue levels of antioxidants and the extent of oxidative injuries in these animals in comparison with the regular outbred strain of nondiabetic Sabra rats. The specific aims were to investigate, at the onset of HSD-induced DM, (1) the extent of oxidative injury, as reflected by levels of malondialdehyde and protein carbonyl groups; (2) the overall antioxidant capacities to cope with increased oxidative stress; and (3) the modification of oxidative damage biomarkers in various tissues of CDr, CDs, and Sabra rats. Female CDs, CDr, and Sabra rats were fed regular diet or HSD for 4 to 5 weeks; and several parameters of oxidative injuries and antioxidant levels were determined. Changes in the levels of nonenzymatic low–molecular weight antioxidants (LMWAs) were measured by cyclic voltammetry and oxygen radical absorbance capacity. The activities of the antioxidant enzymes superoxide dismutase and catalase were measured. Oxidative damage was evaluated by measuring lipid peroxidation and protein oxidation. (1) In all animals fed HSD, the levels of LMWAs were decreased in most organs, although not plasma. (2) A significant difference was consistently found in antioxidant enzymes' activities in the pancreas of HSD-fed CDs rats, but not in other tissues. (3) The activities of superoxide dismutase and catalase and the levels of malondialdehyde and protein carbonyl group increased, whereas the levels of LMWAs decreased, in the pancreas of HSD-fed CDs rats. In the CD rats that develop DM when fed HSD, the pancreas showed susceptibility to oxidative stress–induced injuries. Thus, enhanced oxidative stress seems to play a role in the pathogenesis of DM in this strain.


## 1. Introduction

Over the recent years, researchers have focused their attention on the pathologic role of reactive oxygen species (ROS) in a variety of diseases, among which the most important are atherosclerosis, cancer, and diabetes mellitus (DM) [1]. Increased oxidative stress contributes to development and progression of DM and its complications [2,3]. It is suggested that persistent hyperglycemia in DM enhances the production of ROS from glucose autooxidation [4] and protein glycation [5], which lead to tissue damage. The tissue level of antioxidants critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of DM-borne complications [2,6].

Living organisms have [7] developed a complex antioxidant network to counteract oxidative stress that is detrimental to human life. Intracellular antioxidant defense mechanisms are attained mainly by enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase. They are supported by a large variety of nonenzymatic low–molecular weight antioxidants (LMWAs) such as glutathione (GSH),

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vitamin C or E, α-tocopherol, β-carotene, bilirubin, and flavonoids. The failure of redox homeostasis might render proteins, DNA, and lipid membranes more susceptible to oxidative stress.

The Cohen diabetic (CD) rat is a unique genetic model of inducible type 2 DM that consists of sensitive (CDs) and resistant (CDr), 2 genetically derived contrasting strains. The CDs rats develop type 2 DM when fed high-sucrose (72%), low-copper diet (HSD) for 4 weeks, whereas the CDr rats maintain normoglycemia even when fed HSD. We demonstrated previously using in vivo [7] and in vitro [8] models an inability of the CDs rat embryos (11.5 days old) to increase the activity of antioxidant enzyme in response to hyperglycemia-induced oxidative stress compared with CDr rat embryos. The studies of both in vivo and in vitro models using CD rat embryos indicated genetically determined reduced antioxidant defense efficacy. Recent studies have shown that the addition of SOD or vitamin C and E to diabetic culture medium reduced the incidence of embryonic anomalies [9,10].

Most studies of this CD rat model concerning the relationship between diabetic condition and oxidative stress performed using their embryos were focused on the pathogenesis of the diabetic embryopathy. In the present study, we investigated the changes in several biomarkers of oxidative damage in various tissues of adult female rats of this model. We examined 3 oxidative damage biomarkers to evaluate redox homeostasis in tissue level: (*a*) overall nonenzymatic LMWAs, (*b*) antioxidant enzymes (SOD and CAT), and (*c*) markers of direct oxidative injury (lipid peroxidation and protein oxidation).

The relatively large number of different LMWAs in biological samples makes it difficult to measure each antioxidant separately. The possible interaction among different antioxidants in vivo could also make the measurement of any individual antioxidant less representative of the overall antioxidant status. Therefore, several methods have been adopted to determine the total antioxidant capacities (TAC) of LMWAs for various biological samples. Methods for assaying TAC are generally classified as inhibition and noninhibition assays. Benzie and Strain [11] have recently defined all these inhibition methods as an *indirect* test of TAC, whereas the noninhibition methods were defined as a *direct* test. We have selected cyclic voltammetry (CV) as a *direct* test of TAC and oxygen radical absorbance capacity (ORAC) as an *indirect* one [11] and compared the results of both assays.

We hypothesized, based on our studies as well as of others, that HSD-fed hyperglycemic CDs rats exhibit increased oxidative injury and impaired antioxidant capacities, and that the main imbalance between oxidative stress and antioxidant defense is in the pancreas. We therefore investigated (1) lipid peroxidation, protein oxidation, and antioxidant capacity in hyperglycemic diabetic CDs rats as compared with nondiabetic CDr and Sabra rats; (2) differences in oxidative damage biomarkers in several tissues among these animals strains; and (3) modulation of oxidative damage biomarkers induced by different diets, that is, HSD vs RD.

## 2. Materials and methods

### 2.1. Animal maintenance and feeding

Four- to five-month–old Sabra, CDr, and CDs female rats were fed regular diet (RD) or HSD, and distilled water ad libitum. Postprandial blood glucose level (BGL) was examined after being fed HSD for 4.5 weeks in all strains of rats tested. Overt type 2 DM in the CDs rats after 4 weeks on HSD was confirmed if postprandial BGL was greater than 200 mg/dL. The RD consisted of a mixture of ground whole wheat, ground alfalfa, bran, skimmed milk powder, and salts, resulting in 21% protein, 60% carbohydrates, 5% fat, and 0.45% NaCl content (Koffolk, Tel Aviv, Israel). The HSD consisted of 18% casein, 72% sucrose, 4.5% butter, 0.5% corn oil, 5% salt no. II USP, water, and fat-soluble vitamins. This diabetogenic diet was copper poor (1.2 ppm), a requirement for development of full diabetic phenotype in CDs [12]. There were 6 groups of animals, 6 rats in each group, as follows: Sabra fed RD and HSD, CDr fed RD and HSD, and CDs fed RD and HSD. Of these, only the CDs on HSD developed diabetes.

### 2.2. Protocol for 2-hour postprandial BGL test

Animals were fasted overnight and fed in the morning. On the following day, they fasted overnight and fed in the morning again. Two hours after they were fed in the morning, animals were placed in individual cages; and blood was obtained from the tip of the tail of the fully awake, unanesthetized animal. Blood glucose level was determined using a glucose reagent strip and a standard automated glucometer (Elite; Bayer, Basel, Switzerland).

### 2.3. Protein measurements

Protein content was measured in the crude homogenate of each organ according to Bradford [13] using bovine serum albumin as a standard.

### 2.4. Detection of antioxidant enzyme activities

For tissue collection and assay, brain, lung, liver, heart, pancreas, spleen, kidney, and plasma were excised and immediately frozen to −80°C.

#### 2.4.1. SOD activity

The SOD activity in tissue homogenates was determined as described by McCord and Fridovich [14]. Xanthine oxidase 0.215 U/L (Sigma, St Louis, MO) added to a solution containing hypoxanthine (1.2 mmol/L) produces ROS. Cytochrome *c*, 48 μmol/L (Sigma), was used as a detector. $O_2^-$ reduces the cytochrome *c*, and the reduced cytochrome *c* is measured spectrophotometrically (Uvicon 933; Kontron, Zurich, Switzerland) at 550 nm. The SOD present in the samples decreases the amount of $O_2^-$, hence

decreasing the amount of reduced cytochrome *c* detected by the spectrophotometer. Results are expressed as units per milligram protein.

#### 2.4.2. CAT-like activity

The method was described by Thurman et al [15]. Aliquots of 15 to 20 $\mu$L of the homogenate were added to the reaction mixture containing $H_2O_2$ (0.15 mmol/L). After 10 minutes of incubation at room temperature, the reaction was stopped by adding of 200 $\mu$L trichloroacetic acid 30%; and the residual $H_2O_2$ was determined according to the procedure of Thurman et al. In brief, it measures the red complex that is formed by $H_2O_2$, ferrous ammonium sulfate (Sigma), and 25% thiocyanate (Sigma). The concentration of the complex, which is directly related to the concentration of $H_2O_2$ in the tested solution, is read by a spectrophotometer at 480 nm. Results are expressed as units per milligram protein.

### 2.5. Lipid peroxidation assay

Lipid peroxidation was assayed by measuring the content of malondialdehyde (MDA), the final product of lipid peroxidation, using thiobarbituric acid reactive substances assay (TBARS). Twenty microliters of trichloroacetic acid 60% was added to 100 $\mu$L of sample homogenate in 1.5-mL tube. After vigorous mixing, the tube was centrifuged for 10 minutes at 14 000*g*. Supernatant samples (100 $\mu$L) were placed into clean 96-well plates, and 40 $\mu$L of TBARS reagent (1.3%, dissolved in NaOH 0.3%) was mixed with the samples in the wells. The plate was wrapped with Saran (Ziploc, Indianapolis, IN), incubated for 20 minutes in 90°C bath, and then cooled on ice. The samples were analyzed in the plate at 532 nm.

### 2.6. Protein carbonyl assay

Protein carbonyls were measured spectrophotometrically using the method of Reznick and Packer [16]. Carbonyl groups react with 2,4-dinitrophenylhydrazine to generate chromophoric dinitrophenylhydrazone. After the 2,4-dinitrophenylhydrazine reaction, proteins were precipitated with an equal volume of 20% trichloroacetic acid and washed 3 times with 4 mL ethanol-ethyl acetate mixture (1:1). The samples' pellets were mechanically disrupted in the washing solution by small spatula and repelleted by centrifugation at 6000*g* for 5 minutes. Finally, the precipitates were dissolved in 6 mol/L guanidine-HCl solution; and the absorbance was measured at 380 nm using molar extinction coefficient of dinitrophenylhydrazone, $\epsilon$ = 22 000 mol/L $cm^{-1}$.

### 2.7. CV analysis

Cyclic voltammetry can be used for evaluation of the overall reducing capacity of LMWA in biological fluids or tissue homogenates. Plasma and tissue homogenates show 2 anodic currents that represent 2 classes of reducing equivalents. The first class consists of ascorbate and urate (identified by high-performance liquid chromatography–electrocehemical detection); and the second might include histidine, nicotinamide adenine dinucleotide hydrogen (NADH), melatonin, carnosine, and L-tryptophan [17]. A BAS model CV-1B cyclic voltammeter (West Lafayette, IN) was used to evaluate the reducing capacity of the plasma and tissue samples. The CV tracings were recorded at a range of −0.3 to 1.3 V and a rate of 100 mV/s vs an Ag/AgCl reference electrode. The working electrode was a glassy carbon disk (BAS MF-2012) of 3.2-mm diameter. A platinum wire served as a counterelectrode. The working electrode was polished before each measurement using a polishing kit (BAS-PK-1). The CV voltammograms were analyzed to determine peak potential and anodic current. The peak potential was measured at the half increase of the anodic wave. This potential was typical of the tested tissue and represented the ability of the tissue's reducing equivalents, composing the anodic wave, to donate electrons to working electrode. The anodic current correlated with the concentration of the reducing equivalents.

### 2.8. ORAC assay

The use of peroxyl radicals as oxidants and a protein as oxidizable substrate in the ORAC assay indicates the oxidant-removing capacity of LMWAs [18]. Fluorescein solution (150 $\mu$L, 7.5 nmol/L) and diluted tissue homogenate (40 $\mu$L) were placed in the well of the microplate. All the reaction mixtures were prepared in duplicates. The mixtures were preincubated for 15 minutes at 37°C. 2, 2′-Azobis(2-amidinopropane) dihydrochloride solution (100 $\mu$L, 320 mmol/L) was added using a multichannel pipette. The microplate was immediately placed in the reader, and the fluorescence was recorded every 2 minutes for 80 minutes in fluorescence microplate reader (FLUOstar Galaxy, Offenburg, Germany). Fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used. Phosphate buffer was used as a blank, and Trolox (25 $\mu$mol/L; Sigma-Aldrich, Rehovot, Israel) was used as a standard. The final results (ORAC value) were expressed using Trolox equivalent.

$$\text{ORAC value (in micromoles per liter)} = 25k\left(S_{\text{sample}} - S_{\text{blank}}\right)/\left(S_{\text{trolox}} - S_{\text{blank}}\right),$$

where $k$ is the sample dilution factor and $S$ is the area under the fluorescence decay curve, which is calculated as follows:

$$S = (0.5 + f_2/f_0 + f_4/f_0 + f_6/f_0 + \ldots + f_{78}/f_0 + f_{80}/f_0)*2,$$

where $f_0$ is the initial fluorescence at 0 minute and $f_i$ is the fluorescence measurement at time $i$.

### 2.9. Statistical analysis

Statistical analysis was carried out using Student $t$ test (2-tailed). Data were compared between groups. Significance was set at $P < .05$. We used SPSS (Chicago, IL) and

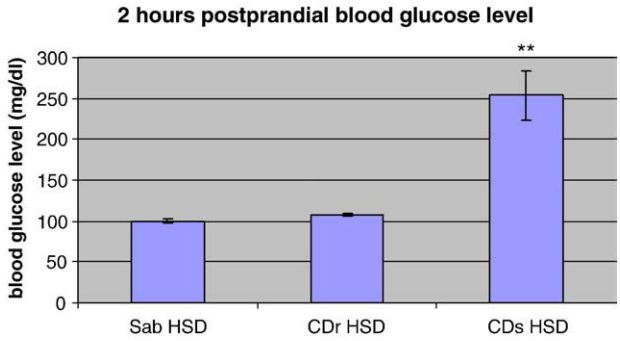


Fig. 1. Two-hour postprandial BGL of Sabra, CDr, and CDs female rats. Each female rat was fed HSD for 4.5 weeks. Overt type 2 DM was confirmed if postprandial BGL was greater than 200 mg/dL. Data are presented as means ± SEM. Unit is units per milligram protein. Statistical significance was set up at $P < .05$ or less (**$P < .01$). Significance vs Sab HSD and CDr HSD.

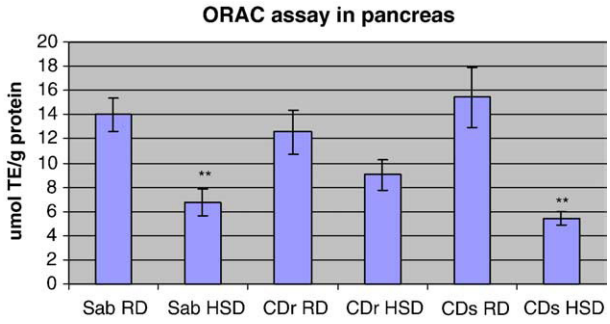


Fig. 2. The ORAC assay in pancreas in Sabra, CDr, and CDs female rats fed RD and HSD. Data are presented as means ± SEM. Statistical significance was set up at $P < .05$ or less (**$P < .01$). Significance vs RD in the same strain.

SigmaStat (Systat Software, San Jose, CA) statistical software for Windows.

## 3. Results

### 3.1. Two-hour postprandial BGL

Blood glucose levels were defined as *nondiabetic* when the maximal levels were less than 200 mg/dL. The Sabra and CDr rats showed normal range of blood glucose, whereas CDs rats showed significant hyperglycemia (Fig. 1).

### 3.2. LMWA assay

The ORAC assay results (Table 1) showed that HSD caused significant decrease in the levels of LMWAs in most organs of the 3 strains but not in the plasma. The pancreas of hyperglycemic CDs rats was most affected, showing a considerable exhaustion of LMWAs compared with normoglycemic CDs rats (5.4 ± 0.6 vs 15.4 ± 2.5, respectively, Fig. 2).

The results of CV assay showed 2 separate peaks, which represented 2 classes of reducing equivalents reflecting LMWAs. Although there were only few changes in the Sabra and CDr rats between the animals fed RD or HSD (results not shown), we found a statistically significant decrease ($P < .05$) of the LMWAs in peak 1 in the lung and in peak 2 in the liver, kidney, and pancreas of the HSD-fed hyperglycemic CDs rats compared with RD-fed normoglycemic CDs. The results of the CV in the pancreas of all animals are shown in Fig. 3. A significant decrease was found only in the CDs fed HSD, whereas the decrease in the Sabra was not statistically significant.

### 3.3. Antioxidant enzymes' assay—SOD and CAT

#### 3.3.1. Superoxide dismutase

There were no changes in the activity of SOD in the different organs of the rats except for a 3-fold increase in the activity of SOD in the pancreas of the CDs rats fed HSD compared with the CDs fed RD (2.3 ± 0.3 vs 7.3 ± 1.7, $P < .01$, Fig. 4).

A similar finding was observed in the CAT-like enzyme activity. There was a 4-fold increase in CAT activity in the pancreas of CDs fed HSD compared with the CDs fed RD (4.9 ± 1.6 vs 20.7 ± 1.8, $P < .01$, Fig. 5). It should be mentioned that an increase ($P < .05$) was also found in the lungs of the CDs rats fed HSD and in the heart of the CDr fed HSD (results not shown).

### 3.4. Oxidative injuries' assay

Malondialdehyde, a product of lipid peroxidation, measured by TBARS showed a 2-fold increase in the pancreas

Table 1
Results of ORAC assay in the different tissues of Sabra, CDr, and CDs female rats fed RD and HSD

| | Brain | Lung | Liver | Heart | Pancreas | Spleen | Kidney | Plasma |
|---|---|---|---|---|---|---|---|---|
| Sab RD | 16.4 ± 1.9 | 18.4 ± 1.2 | 12.9 ± 1.2 | 12.7 ± 1.6 | 13.9 ± 1.4 | 15.4 ± 1.4 | 18.0 ± 0.7 | 163.5 ± 3.5 |
| Sab HSD | 13.5 ± 0.8 | 9.9 ± 0.8** | 8.2 ± 0.3** | 8.6 ± 0.4 | 6.7 ± 1.1** | 8.8 ± 0.3** | 9.6 ± 0.7** | 135.9 ± 13.2 |
| CDr RD | 16.0 ± 1.4 | 15.9 ± 1.8 | 13.3 ± 2.1 | 12.1 ± 1.6 | 12.6 ± 1.8 | 14.4 ± 2.4 | 17.5 ± 2.3 | 157.2 ± 2.0 |
| CDr HSD | 12.4 ± 0.6* | 12.5 ± 1.0* | 8.0 ± 0.6 | 10.3 ± 1.3 | 9.0 ± 1.2 | 7.8 ± 0.2* | 11.1 ± 0.8* | 151.7 ± 7.3 |
| CDs RD | 16.7 ± 1.3 | 14.7 ± 1.6 | 13.1 ± 1.4 | 15.3 ± 1.8 | 15.4 ± 2.5 | 13.8 ± 1.3 | 17.6 ± 2.4 | 156.9 ± 2.1 |
| CDs HSD | 9.6 ± 0.8** | 8.7 ± 0.8* | 8.9 ± 0.7* | 7.1 ± 0.3** | 5.4 ± 0.6** | 7.4 ± 0.7** | 10.4 ± 1.0* | 152.9 ± 2.3 |

The use of peroxyl radical as oxidant and a protein as oxidizable substrate in the ORAC assay indicates the oxidant-removing power of LMWAs. Data are presented as means ± SEM. Unit is micromoles per gram protein. Statistical significance was set at $P < .05$ or less (*$P < .05$, **$P < .01$). Significance vs RD in the same strain and same tissue.

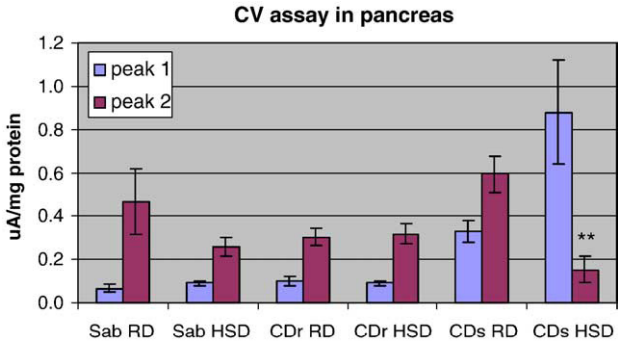


Fig. 3. The CV assay in pancreas in Sabra, CDr, and CDs female rats fed RD and HSD. Data are presented as means ± SEM. Statistical significance was set up at $P < .05$ or less ($^{**}P < .01$). Significance vs RD in the same strain and same peak.

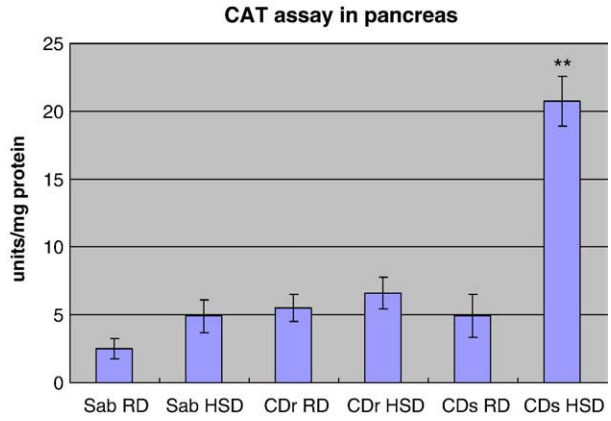


Fig. 5. The CAT assay in pancreas in Sabra, CDr, and CDs female rats fed RD and HSD. Data are presented as means ± SEM. Statistical significance was set up at $P < .05$ or less ($^{**}P < .01$). Significance vs RD in the same strain.

of HSD-fed hyperglycemic CDs rats compared with CDs rats fed RD (1923 ± 320 vs 904 ± 224, $P < .05$, Fig. 6). Decreased lipid peroxidation was observed in the spleen of HSD-fed Sabra rats and in the brain of HSD-fed CDr and CDs rats, whereas it was increased in the liver of CDr rats fed HSD in comparison with the RD-fed rats of the same strains (results not shown).

Protein carbonyl assay, carried out only for samples of liver and pancreas tissues, showed a significant increase in the pancreas of HSD-fed hyperglycemic CDs rats compared with RD-fed CDs rats (Fig. 7). No changes were found in the liver of all strains tested (Table 2).

### *3.5. Redox homeostasis change in the pancreas of hyperglycemic CDs HSD*

Redox homeostasis in the pancreas of hyperglycemic CDs rats showed significant changes in several biomarkers for measuring antioxidant status, exhibiting decreased LMWA levels and increased oxidative injuries and antioxidant enzyme activities (Figs. 2-7).

The manifestations, compared with RD-fed normoglycemic CDs rats, were as follows: decreased ORAC (Fig. 2), decreased peak 2 determined by CV (Fig. 3), increased SOD (Fig. 4) and CAT activity (Fig. 5), increased MDA level (Fig. 6), and increased levels of protein carbonyl groups (Fig. 7).

## 4. Discussion

The results of this study showed 3 significant changes in the redox status in the CD rat model. (1) When fed HSD, there was a decrease in LMWA levels in most organs of all 3 strains of rats, but no changes in the plasma. (2) There was no constant modification in antioxidant enzymes' activity except for increased activity in the pancreas of HSD-fed CDs rats. (3) There was an increase in the activity of SOD/CAT and a decrease in the level of LMWAs in the pancreas of HSD-fed CDs rats compared with RD-fed CDs rats. (4) Increased oxidative damage in the pancreas of the CDs diabetic animals was demonstrated by increased levels of MDA and protein carbonyl groups, exhibiting the role of enhanced oxidative stress in the induction of diabetes in CD rats.

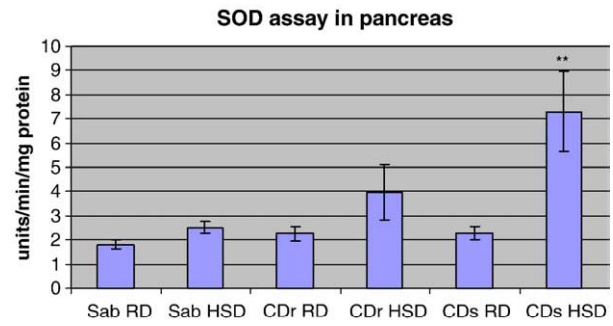


Fig. 4. The SOD assay in pancreas in Sabra, CDr, and CDs female rats fed RD and HSD. Data are presented as means ± SEM. Statistical significance was set up at $P < .05$ or less ($^{**}P < .01$). Significance vs RD in the same strain.

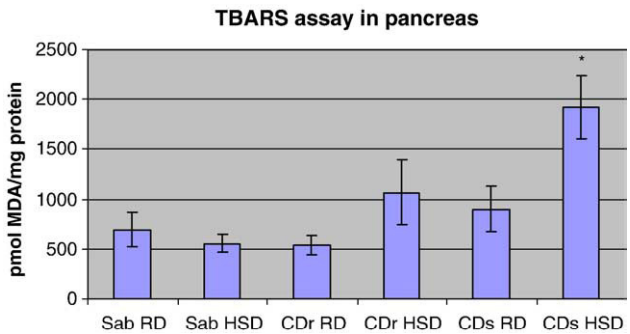


Fig. 6. The TBARS assay in pancreas in Sabra, CDr, and CDs female rats fed RD and HSD. Data are presented as means ± SEM. Statistical significance was set up at $P < .05$ or less ($^{*}P < .05$). Significance vs RD in the same strain.

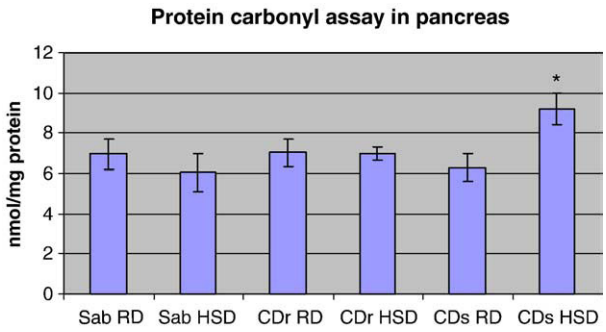


Fig. 7. Protein carbonyl assay in pancreas in Sabra, CDr, and CDs female rats fed RD and HSD. Data are presented as means ± SEM. Statistical significance was set up at $P < .05$ or less ($^*P < .05$). Significance vs RD in the same strain.

### 4.1. Changes in antioxidant mechanism

Dietary change by HSD feeding in all 3 strains caused a decrease in LMWAs, indicating that the decrease was not related to hyperglycemia because HSD-fed Sabra and CDr maintained normoglycemia. The HSD-induced dietary stress can apparently cause the decrease in LMWAs in each organ, resulting from increased oxidative stress, regardless of hyperglycemia. The diabetogenic high-sucrose (72%) diet in CD rat model was copper poor (1.2 ppm), a requirement for CDs to develop the full diabetic phenotype [12]. Fructose-containing sugars (ie, sucrose) possess hypercholesterolemic properties, especially together with a copper-deficient state, and can increase the level of blood lipids [19,20]. The activity of hepatic enzymes regulating lipogenesis and gluconeogenesis is increased by diets having fructose-containing sugars in place of starch [21,22]. We have no explanation to the lack of changes in LMWAs in the plasma.

Unlike the changes in LMWA levels, the antioxidant enzymes' activities did not show any consistent changes in response to HSD-induced dietary stress except for the pancreas. The activity of SOD and CAT increased in the pancreas of the CDs rats.

Changes in the activity of SOD, CAT, and GSH-related enzymes have been previously shown to affect the oxidative stress in the diabetic tissues [23,24]. However, there was no agreement among different laboratories regarding the activity change of these enzyme because some found an increase and others found a decrease [25]. The opposing results are probably related to the degree of oxidative stress because, in slight to moderate oxidative stress, the activity of these enzymes tends to increase, whereas under severe oxidative stress, the activity decreases.

### 4.2. Changes in oxidative injury

Despite the known limitations of MDA assay measured by TBARS, it is the most commonly used marker of oxidative injury, even in diabetes. Normalization of MDA levels after antioxidant treatment in diabetes was seen in the kidney, liver, heart, brain, intestine, lung, and pancreas [26-29]. In the present study, there were no changes in MDA levels and in protein carbonyl content under HSD-induced dietary stress, except for the pancreas of HSD-fed hyperglycemic CDs that showed an increase, emphasizing that, in these animals, there was a difficulty to cope with the HSD-induced oxidative stress. This might stem from genetic differences between this strain and the other animals, rendering the CDs rats more susceptible toward oxidative stress–induced pancreatic injuries and leading to diabetes.

Protein carbonyl content is one of the most general and well-used biomarkers of severe oxidative protein damage [30]. Oxidative modifications of enzymes and structural proteins were found to play a role in the etiology and/or progression of several human diseases including diabetes [31-33].

### 4.3. Redox homeostasis in the pancreas of hyperglycemic CDs rats

The results of pancreatic redox status in HSD-fed hyperglycemic CDs rats demonstrate decreased LMWA level, increased oxidative injuries (MDA/protein carbonyl group), and increased activities of SOD/CAT.

Cells and tissues have several mechanisms for reestablishing the original redox state after a transient exposure to increased oxidative stress. In this quasi-stable state, LMWAs can play a primary role in maintaining the redox homeostasis. However, imbalance by chronic and severe oxidative stress like hyperglycemia might trigger the redox sensitive signal cascades that lead to increased expression of antioxidant enzymes [34]. Therefore, the increase in SOD/CAT activities can represent chronic imbalance of redox homeostasis in the pancreas of HSD-fed CDs rats in vivo. However, this was not sufficient to protect the pancreas from the damage induced by increased oxidative stress as evidenced by the increase in MDA and protein carbonyl groups.

The pancreas of HSD-fed CDr rats showed no decrease in LMWA levels compared with RD-fed CDr rats, unlike the significant decrease in LMWA levels in HSD-fed Sabra and HSD-fed CDs rats. These results raise the possibility that genetic resistance to DM pathogenesis in CDr strain might

Table 2
Results of protein carbonyl assay in the different tissues of Sabra, CDr, and CDs female rats fed RD and HSD

| | Liver | Pancreas |
|---|---|---|
| Sab RD | 7.5 ± 0.3 | 6.9 ± 0.8 |
| Sab HSD | 6.5 ± 0.1 | 6.0 ± 0.9 |
| CDr RD | 7.2 ± 2.5 | 7.0 ± 0.7 |
| CDr HSD | 6.4 ± 1.1 | 7.0 ± 0.3 |
| CDs RD | 5.7 ± 0.9 | 6.3 ± 0.7 |
| CDs HSD | 5.9 ± 0.9 | 9.2 ± 0.8* |

Protein carbonyls were measured spectrophotometrically using the method of Reznick and Packer [16]. Data are presented as means ± SEM. Unit is nanomoles per milligram protein. Statistical significance was set up at $P < .05$ or less ($^*P < .05$). Significance vs RD in the same strain and same tissue.

result from rapid replenishment of LMWAs after HSD-induced oxidative stress. Decreased levels of GSH and elevated MDA levels were consistently observed in diabetes despite inconsistent results reported about the changes of antioxidant enzymes' activities under diabetic environment [25]. Moreover, the apparent importance of LMWAs in inhibition of the development of DM may highlight the potential of the use of LMWAs for the prevention of the disease. In this context, it is important to mention that the teratogenic effects of diabetes were indeed prevented by LMWAs in several studies [35].

There is a lot of evidence that hyperglycemia-induced oxidative stress mediates diabetic complications, such as vascular complications, retinopathy, neuropathy, and nephropathy [36-38]. Many molecular mechanisms underlying oxidative stress–induced diabetic complications were elucidated, including overproduction of superoxide radicals by the mitochondrial electron-transport chain, increased polyol pathway flux, increased advanced glycosylation end product formation, and activation of protein kinase C.

High-sucrose (72%), low-copper (1.2 ppm) diet has been sufficiently diabetogenic in the CDs rats to develop pancreatic damage and the full diabetic phenotype after 4 weeks [13]. The results of the present study about the pancreatic redox status just at the onset of DM may point to a possible mechanism for the induction of pancreatic damage by HSD-induced oxidative stress in these animals. Meira et al [39] also showed that a diet similar to ours—fructose with copper deficiency—is responsible for hyperlipidemia, which leads to increase in oxidative stress.

The involvement of oxidative stress in the development of diabetes has been demonstrated in several animal models. For example, several studies have shown [6] that ROS are involved in pancreatic damage after the use of alloxan in vivo. Another commonly used diabetogenic agent is streptozotocin, and it was demonstrated that nitric oxide contributes to the cytotoxicity of streptozotocin toward $\beta$-cells [40]. In diabetes-prone animals such as the nonobese diabetes mouse and the Biobreeding rat, islet cell infiltration of immune and inflammatory cells occurs before $\beta$-cell death. Destruction of the insulin-producing $\beta$-cell was thought to result from direct exposure to ROS produced by the immune cells [40-42].

The pancreas is the main target organ where the pathogenesis of DM is observed primarily. Previous studies showed that the levels of several key enzymes in defense against ROS are unusually low in pancreatic islets compared with other tissues, suggesting that the islet cells are uniquely susceptible to oxidative stress–induced damage [43,44]. Previous studies showed that the gene expression levels of the CuZn-SOD and the Mn-SOD in the pancreatic islet cells were 60% to 70% lower than the levels found in the liver. For example, Tiedge et al [45] found that glutathione peroxidase expression was only 15% and CAT gene expression was not detectable in pancreatic islets. The oxidative injury (increased MDA and protein carbonyls) in the pancreas of hyperglycemic CDs rats was demonstrated at the onset of diabetes, at a stage when diabetic complications were not developed yet. Hence, in all organs tested, the redox status was normal. However, at the latter stages of diabetes, DM complication-prone organs such as the kidney, brain, and heart may also show the alterations in oxidative injury similar to the pancreas. However, such a study was not performed by us yet.

An interesting question is why oxidative-induced damage to the pancreas leads to type 2 DM. Reduced insulin sensitivity in response to oxidative stress was observed in skeletal muscles by Blair et al [46]. This resulted from an inhibition of insulin-stimulated glucose transport and glycogen synthesis and suggested that activation of the p38 mitogen-activated protein kinase pathway plays a central role in the oxidant-induced inhibition of insulin-regulated glucose transport. In addition, increased insulin resistance was also induced by the increase in the amount of fatty acids [47]. Indeed, in a recent study [48] on the morphologic changes of the pancreas in the CDs rats fed HSD, lipid depositions and interleukin-1$\beta$ positive macrophage infiltration in the exocrine pancreas were observed; but the islets' morphology was preserved. Although the insulin content of the pancreas was similar to controls, there was a decreased glucose-stimulated insulin secretion both in vivo and in isolated perfused pancreas in vitro. Thus, we can presume that increased oxidative stress in the CDs rats produces fatty degeneration of the pancreas; and this causes insulin resistance and the development of type 1 DM.

### 4.4. Conclusions

We have demonstrated changes in oxidative damage biomarkers in HSD-fed hyperglycemic CDs rats' pancreas at the beginning of DM, indicating the involvement of oxidative stress in the pathogenesis of the disease. It indicates that antioxidants may decrease or even prevent pancreatic damage and thus the development of the disease. Moreover, in this rat model for type 2 DM, the pancreas seems to have genetic susceptibility toward increased oxidative stress/reduced antioxidant capacity induced by HSD feeding, rendering this strain prone to dietary/environmental factors–induced DM.

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