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Original article

# High sucrose diet feeding aggravates age related changes in oxidative stress and antioxidant status of WNIN/Gr-Ob obese rats

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### ABSTRACT

**Background:** Ageing is a multifactorial process in which physical and physiological changes accumulate over time, leading to gradual deterioration and death. Obesity and T2DM (Type 2 Diabetes Mellitus) *per se* accelerate ageing but the underlying mechanisms are not clear yet, nor it is known whether superimposing T2DM on obesity accelerates ageing further. The present study validated the hypothesis, "Induction of T2DM in obese rats by feeding high sucrose diet accelerates ageing by aggravating oxidative stress and / or compromising the antioxidant status ". **Materials and Methods:** WNIN/Gr-Ob rats, an established obese rat model was fed with high sucrose diet for the induction of T2DM / aggravated insulin resistance (IR), a pre-diabetic condition. Oxidative stress and antioxidant markers were determined in the liver homogenates by spectrophotometric methods. **Results:** Induction of T2DM / aggravation of insulin resistance (IR) in WNIN/Gr-Ob obese rats by feeding High Sucrose Diet (HSD) significantly increased tissue oxidative stress (protein carbonyls) and decreased antioxidant status: enzymatic (superoxide dismutase and catalase) and non-enzymatic (reduced glutathione) after six months of feeding, as compared to those of control rats fed starch based diet (SBD). **Conclusion:** Taken together with our recent report of aggravated insulin resistance, increased DNA damage and decreased telomere length in the brains of HSD fed obese rats, the present findings suggest that feeding HSD aggravated ageing associated systemic oxidative stress perhaps due to impaired anti oxidant status and may underlie the aggravated insulin resistance, which in turn accelerated their ageing.

KEYWORDS: Obesity, Diabetes, Ageing, Oxidative stress, High Sucrose Diet, WNIN/Gr-Ob rats.

### INTRODUCTION

Altered food habits, current lifestyle and nature of work have led to the deterioration of the quality of life as evident from greater incidence of a variety of age related disorders eg, Obesity, Type 2 Diabetes Mellitus etc, rather than infectious diseases, in developing countries such as India [1]. The increase in technology and the ever changing urbanization have contributed much to the increased prevalence of obesity. Increasing consumption of diets rich in carbohydrates, fats etc, is a very common and popular food habit. The high caloric diets cause inflammation, oxidative stress and insulin resistance which in turn could accelerate ageing, thereby decreasing longevity [2].

Ageing is characterized by a progressive decline in biological functions of tissues with time and a decreased resistance to multiple forms of stress, as well as an increased susceptibility to numerous diseases [3]. The basic mechanisms underlying Ageing are multifactorial and reactive oxygen species (ROS) are one of the important contributing factors. At present, there is increasing evidence for the positive correlation between oxidative stress and Ageing [4].

Oxidative Stress (OS) plays a key role in the pathogenesis of various risk factors for metabolic syndrome, including obesity and type 2 diabetes mellitus (T2DM). OS can be best described as a double edged sword [5] because of its known association with multiple physiological systems during ageing. OS induces cellular senescence which may be an important consequence of obesity and/or contribute to senescence inducing effects of obesity on aged vessels than young arteries [6]. Irreversible and progressive molecular oxidative damage has been well recognized to be the main cause of Ageing process [7].

The National Centre for Laboratory Animal Science (NCLAS), at the National institute of Nutrition, Hyderabad, has one of the oldest, inbred, Wistar rat colony maintained since 1920: Wistar NIN (WNIN). By pedigree and back cross analysis, a mutant strain was established for the obesity trait, designated as WNIN/Ob. By crossing WNIN Glucose Resistant rats and WNIN/Ob carrier, the WNIN/Gr-Ob mutant rats were developed and have been maintained as another well established animal model for obesity [8]. Interestingly, WNIN/Gr-Ob rats are obese, euglycemic and exhibit impaired glucose tolerance when challenged with glucose [9]. Like WNIN/Ob, the WNIN/Gr-Ob rats have a shorter lifespan and also develop opportunistic infections after one year of life. 15-20 % of rats develop cataract and retinal degeneration [10], mammary tumors, lipomas, kidney abnormalities [11] and develop hypertension as well [12].

Considering that obesity and diabetes *per se* promote ageing via ROS mechanisms and the known importance of oxidative stress in accelerating ageing [13], in the present study we determined whether or not, diet induced diabetes on obesity, aggravates ageing associated hepatic oxidative stress, perhaps contributing their accelearetd ageing. The present study assessed at different time points of age, the hepatic oxidative stress in WNIN/Gr-Ob obese rats superimposed with diabetes / aggravated insulin resistance (IR), a condition pre requisite for T2DM, by feeding High Sucrose Diet (HSD).

#### MATERIALS AND METHODS

#### Animal Experimentation

Animal experimentation reported in this manuscript was approved by the "Institute's ethical committee on animal experiments" at National Institute of Nutrition, Hyderabad, India (P10/NCLAS/IAEC-25/7/2012). Male rats, 9-10 weeks of age, belonging to WNIN/Ob-lean (n = 12), WNIN/Ob (n = 12) and WNIN/Gr-Ob (n =24) strains were procured from NCLAS, NIN, Hyderabad. They were housed individually in standard polycarbonate cages at  $22 \pm 2^{\circ}$ C, with 14-16 air changes per hour, at a relative humidity of 50-60 per cent with a 12 hour light/dark cycle. The rats were given *ad libitum*, a sterile, powdered diet along with water, for a period of 6 months.

Group1 – WNIN/Ob-Lean: lean controls for obese rats

Group2 - WNIN /Ob: Obese rats

Group3 – WNIN/Gr-Ob: Obese, IGT rats fed Starch based Diet (control)

Group4 – WNIN/Gr-Ob: Obese, IGT rats fed High Sucrose Diet (obesity with diet induced T2DM)

Rats in groups 1-3 received control (AIN 93G) diet *ad libitum*, while rats in group 4 were also fed the AIN 93G diet but in which starch was replaced by Sucrose (HSD) to aggravate insulin resistance and / or induce T2DM in them. Groups of rats (n= 6) from each group were sacrificed at the end of three and six months of feeding on their respective diets.

# Preparation of liver homogenates for Oxidative stress and antioxidant status

At the end of 3 and 6 months on their respective diets, the rats were killed by cervical dislocation. The livers were dissected out quickly, washed thoroughly with ice-cold phosphate-buffered saline (PBS) (pH 7.4), blotted and weighed immediately. Appropriate number of liver slices were quickly excised, rinsed in saline and stored frozen in liquid nitrogen at  $-80^{\circ}$ C till further analyses.

About 0.1 g liver was weighed, minced and homogenized (10% w/v) in ice cold 50 mM phosphate buffer (pH=7.0) and the homogenate was processed by differential centrifugation to obtain post-mitochondrial and cytosolic sub-fractions as reported earlier [14, 15]. The appropriate fractions were used for the quantification of lipid peroxidation, protein oxidation, reduced glutathione and the activities of antioxidant enzymes: catalase and superoxide dismutase.

### Protein estimation of different sub cellular fractions

The amount of protein present in different sub cellular fractions was estimated by the bicinchoninic acid (BCA) method using Pierce BCA Protein Assay Kit from Thermo Scientific.

#### **Oxidative Stress Markers**

#### Lipid peroxidation

Lipid peroxidation was measured in 1000g supernatant according to Ohkawa et al, [16] and Gupta et al, [17] using thiobarbituric acid. In this assay Malonaldialdehyde (MDA) reacts with thiobarbituric acid to produce a pink colored complex which is read at 532 nm colorimetrically. Tetramethoxypropane was used as an external standard. The levels of lipid peroxides are expressed as nmoles of MDA formed/mg protein.

#### Protein Oxidation

Protein carbonyl content was measured spectrophotometrically in the post-mitochondrial supernatant using 2, 4-dinitro-phenyl-hydrazine according to Reznick and Packer [18]. In this method the absorbance of DNP- protein adducts was measured at 360 nm and the protein oxidation levels are expressed as nmoles/mg protein.

#### Enzymatic Antioxidant Defense Mechanism

#### Super Oxide Dismutase (SOD) activity

The activity of SOD was measured in the liver cytosolic fraction according to Marklund and Marklund [19]. In this method the total SOD activity was assayed by monitoring the rate of inhibition of pyrogallol reduction. One unit of SOD represents the amount of enzyme required for 50% inhibition of pyrogallol reduction per minute. The activity of SOD is expressed as Units /100mg protein/min.

#### Catalase activity

Catalase activity was assayed in 20,000g supernatant spectrophotometrically by monitoring the disappearance of  $H_2O_2$  at 240 nm according to Aebi [20]. One unit of catalase represents the decrease of 1 µmol of  $H_2O_2$  per minute. The activity is expressed as Units /mg protein/min.

Non Enzymatic antioxidants

#### Reduced Glutathione

Reduced glutathione was determined by the method of Ellman [21]. In this method, the tissue homogenate was mixed with Ellmans reagent and the OD of the reaction mixture was measured at 412 nm against blank and values were read from a standard curve of GSH.

#### RESULTS

Oxidative stress markers in liver

Lipid peroxidation, expressed as the concentration of MDA, was the highest in HSD fed WNIN/Gr-Ob rats among all groups and higher than SBD fed WNIN/Gr-Ob controls, at

both the time points of feeding studied, *albeit* the differences were not significant. [Figure. 1a] The MDA levels of all the obese rats in groups 2, 3 & 4 were significantly higher than that of the lean control rats (group 1) after 3 months on their diets, while at 6 months of feeding MDA levels were significantly higher in SBD and HSD fed WNIN/Gr-Ob rats than in groups 1 and 2.

Figure 1a: Lipid peroxidation of different groups of rats after 3 and 6months of feeding on their respective diets.



Lipid peroxidation levels expressed in terms of MDA concentrations in the liver homogenate of rats fed different diets at 3 and 6 months of feeding. All the values given are mean  $\pm$  SE. Bars sharing different superscripts are significantly different (p<0.05) by One-way ANOVA/ LSD test.

At three months of feeding protein carbonyl levels were comparable among all the four groups. However at 6 months of feeding HSD fed WNIN/Gr-Ob rats had significantly higher protein carbonyl levels than SBD fed WNIN/Gr-Ob control rats. [Figure.1b] Not withstanding these findings, the fold increase in protein carbonyl levels at 6 months compared to that at 3 months of feeding, were in general significantly higher in obese rats (groups 2-4) than lean controls (group 1). Indeed the highest fold increase was seen in HSD fed WNIN/Gr-Ob rats (group 4).

#### Figure 1b: Levels of Protein oxidation of different groups of rats after 3 and 6 months on their respective diets



Protein oxidation in the liver homogenate of rats fed different diets at 3 and 6 months of feeding. All the values given are mean  $\pm$  SE. Bars sharing different superscripts are significantly different (p<0.05) by One-way ANOVA/LSD test.

# Antioxidant Enzymatic activity and levels of non enzymatic antioxidant

Though lower than lean controls, catalase activity was comparable among groups at 3 months of feeding. However at 6 months of feeding, the activity was significantly lower in HSD fed WNIN/Gr-Ob rats than those fed the SBD.[Figure. 2a] At 3 months of feeding SOD activity was significantly lower (than lean controls: group1) in SBD and HSD fed WNIN/Gr-Ob rats (groups 3 and 4) but not in WNIN/Ob rats (group 3). Interestingly at six months of feeding SOD activity of all obese rats (groups 2-4) was significantly lower than that of lean control rats (group 1). Indeed HSD fed WNIN/Gr-Ob rats had the lowest of SOD activity among all groups at 6 months of feeding.[Figure. 2b]

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The levels of reduced glutathione (non enzymatic antioxidant) were significantly lower in HSD fed WNIN/Gr-Ob rats compared to SBD fed WNIN/Gr-Ob rats, at both time-points of feeding. While reduced glutathione levels of SBD fed WNIN/Ob (group 2) and WNIN/Gr-Ob (group 3) were comparable to those of lean controls (group 1) after 3

months of feeding, they were significantly lower at 6 months of feeding. [Figure 2c] As one would expect, the enzymatic and non enzymatic antioxidant defense mechanisms reduced significantly with age (value at 6 versus 3 months of feeding) in all groups of rats regardless of the type of diet fed.[Figure 2a, b, c].

Figure 2a: Catalase activity in different groups of rats after 3 and 6 months of feeding.



Activity of catalase expressed in units/mg/min in the liver homogenate of rats fed different diets at 3 and 6 months of feeding. All the values given are mean  $\pm$  SE. Bars sharing different superscripts are significantly different (p<0.05) by One-way ANOVA/LSD test.

#### Figure 2b: Activity of SOD in different groups of rats after 3 and 6 months of feeding their respective diets



SOD activity was expressed in units/100mg ptn in the liver homogenate of rats fed different diets at 3 and 6 months of feeding All the values given are mean  $\pm$  SE. Bars sharing different superscripts are significantly different (p<0.05) by One-way ANOVA/LSD test.



Reduced glutathione levels in the liver homogenate of rats fed different diets at 3 and 6 months of feeding. All the values given are mean  $\pm$  SE. Bars sharing different superscripts are significantly different (p<0.05) by One-way ANOVA/LSD test.

#### DISCUSSION

Obesity and T2DM per se accelerate ageing and both these conditions occur together very frequently [22, 23]. Ageing is associated with increased oxidative stress [24, 25] and advanced ageing is strongly associated with obesity and Type 2 Diabetes Mellitus [26], which in turn are associated with increased oxidative stress and early onset detrimental consequences [27]. In fact, earlier studies have demonstrated the effects of ageing, obesity and diabetes, per se, on ROS mechanisms. However it is not clear whether superimposing diabetes on obesity accelerates ageing; and the biochemical and molecular mechanisms underlying / associated with their decreased longevity are not clear. We reported recently that aggravating insulin resistance in WNIN/Gr-Ob obese rats by feeding HSD, increased cell senescence as indicated by increased DNA damage and decreased telomere length in their brain [28].

We also showed that Ageing associated changes such as liver steatosis, kidney degeneration, increased  $\beta$  cell number and function were aggravated in WNIN/Gr-Ob obese rats fed HSD than SBD [29]. Considering the major role of oxidative stress and antioxidant mechanisms in the metabolic dysregulation of obesity, diabetes and ageing, in the present study we have examined the hepatic oxidative stress and anti-oxidant status in WNIN/Gr-Ob obese rats at two different time points of feeding high sucrose diet (to induce diabetes / aggravate insulin resistance) in comparison with age and sex matched obese rats fed SBD control diet.

It was interesting that only protein carbonyls were significantly higher in HSD than SBD fed rats that too at 6 months of feeding but not earlier. On the other hand the increase in MDA levels was not significant in HSD fed rats than SBD controls at both the time points. This is in line with the finding of Busserolles et al [30] who reported that liver susceptibility to peroxidation was not significantly higher on feeding high sucrose diet and suggested a greater protection of liver against oxidative stress. The differences in oxidative stress could also be due to alterations in lipid composition. Considering that lipids are the primary targets of the oxidative damage [31], these findings appear perplexing. Nevertheless they appear to suggest the differential effects of HSD feeding on different parameters of oxidative stress in WNIN/Gr ob rats.

Alternately, lack of significant increase in lipid peroxidation, could be due to the fact that at the level fed, the HSD had only aggravated insulin resistance but not induce T2DM in these rats [29]. Further, the lack of significance could also be due to smaller sample size in each group. Interestingly, the significantly higher levels of protein carbonyls in HSD than SBD fed rats could be due to the impaired glucose tolerance (IGT) in them, which is known to increase protein glycosylation and this in turn increases their oxidation [32].

To assess whether the increased oxidative stress in HSD fed rats was due to / associated with impaired anti oxidant status, we determined the enzymatic and non enzymatic anti oxidants in the liver tissue. In line with the differential effects observed on different parameters of oxidative stress, Activity of SOD was comparable in HSD than SBD fed WNIN/Gr-Ob rats both at 3 and 6 months of feeding, whereas catalase activity was significantly lower after 6 months of feeding. But, the levels of reduced glutathione, the non enzymatic anti oxidant were significantly lower in HSD than SBD fed rats after both time-points of feeding. Interestingly, the differential effects of HSD feeding observed on different parameters of oxidative stress and anti oxidant status (enzymatic and non enzymatic) are similar to those we reported earlier in WNIN rats fed micronutrient deficient diets [33,34].

These observations indicate that HSD fed obese rats exhibited moderately higher hepatic oxidative stress which was probably due to moderate changes seen in enzymatic and non enzymatic anti oxidants. It was interesting that while there was a 2.2 fold increase in oxidative stress (protein carbonyls) in WNIN/Ob-lean rats at 6 months of feeding compared to that at 3 months, in HSD fed Gr-Ob rats the increase was 5.5 fold. That this could be due to appropriate, time dependant modulations in anti oxidant activities is suggested by the finding that SOD activity of HSD fed Gr-Ob rats decreased only by 1.1 fold with time, whereas in lean rats this decrease was 1.5 fold. Nevertheless, the absolute values of these antioxidant parameters were significantly lower in HSD fed Gr-Ob rats than lean controls at both the time points. Thus, despite a significantly higher percent of change with time in lean than WNIN/Gr-Ob rats, the actual antioxidant status was lower in HSD fed Gr-Ob rats at both time points and hence higher oxidative stress in them.

In the light of the findings reported here, future research on obesity, diabetes and ageing will be an emerging area. The focus shall mainly be to decipher molecular mechanisms, regulation of gene expression and the role of environment specially the dietary habits in determining inflammatory process triggered oxidation, which could accelerate ageing. It will also be challenging to understand the therapeutic strategies from the prospect of decreased longevity.

#### CONCLUSION

Feeding high sucrose diet to the obese rat aggravated the systemic oxidative stress associated with ageing, probably due to impaired anti oxidant status (enzymatic and non enzymatic). This may underlie the aggravated insulin resistance and the consequent reduction in longivity / acceleration of the ageing in the WNIN/Gr-Ob obese rats.

#### DISCLOSURE

The authors declare that there are no competing interests regarding the publication of this paper.

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