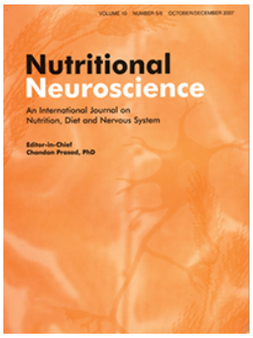

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Increased total DNA damage and oxidative stress in brain are associated with decreased longevity in high sucrose diet fed WNIN/Gr-Ob obese rats

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Background: Obesity and Type 2 Diabetes (T2D) are chronic nutrient-related disorders that occur together and pose a grave burden to society. They are among the most common causes of ageing and death. Obesity and T2D *per se* accelerate ageing *albeit* the underlying mechanisms are unclear yet. Also, it is not clear whether or not superimposing T2D on obesity accelerates ageing. Present study validated the hypothesis, 'super-imposing T2D on obesity accelerates ageing' in WNIN/Gr-Ob, the impaired glucose tolerant, obese rat as the model and evaluated probable underlying mechanisms.
Objectives: To estimate the survival analysis of WNIN/Gr-Ob rats induced with T2D. To determine the extent of DNA damage and oxidative stress in the brain, the master controller of the body, in WNIN/Gr-Ob rats with/without high sucrose induced T2D/aggravation of insulin resistance (IR) after 3 and 6 months of feeding.
Methods: T2D was induced/IR was aggravated by feeding high sucrose diet (HSD) to 9–10 weeks old, male WNIN/Gr-Ob rats. Survival percentage was determined statistically by Kaplan–Meier estimator. Neuronal DNA damage was quantified by the Comet assay while the oxidative stress and antioxidant status were evaluated from the levels of malonaldialdehyde, reduced glutathione, and superoxide dismutase (SOD) activity.
Results and Discussion: HSD feeding decreased longevity of WNIN/Gr-Ob rats and was associated with significantly higher total neuronal DNA damage after three months of feeding but not later. In line with this was the increased neuronal oxidative stress (lipid peroxidation) and decreased antioxidant status (reduced glutathione and SOD activity) in HSD than Starch-based diet (SBD) fed rats. The results suggest that HSD feeding decreased the longevity of WNIN/Gr-Ob obese rats probably by increasing oxidative stress and aggravating IR, a condition that precedes T2D.

Keywords: DNA damage, Neurons, Insulin resistance, Oxidative stress, High sucrose diet, WNIN/Gr-Ob obese rats, Ageing

Introduction

Ageing is a time-dependent accumulation of cellular damage and functional attrition. It is a multifactorial process determined by genetic and various environmental factors, particularly the diet-related ones and the lifestyle being more prominent. Accelerated ageing is a condition where genetic damage, DNA repair capacity, and metabolic changes observed with ageing are pronounced at an early age.[1] The main reasons attributed to this condition are genetic pre-disposition, oxidative stress, diet-related metabolic disorders like obesity, Type II diabetes, cardiovascular diseases, etc. The precise biological and cellular mechanisms responsible for ageing are not known, but according to Fontana and Klein (2007), 'they are likely to involve a constellation of complex and interrelated factors, including oxidative stress – induced protein and DNA damage and inefficient repair mechanisms which in turn contribute to genetic instability.'[2]

DNA, being the central dogma of life, has a key role in the process of ageing and increased macromolecular damage is a critical determinant of the rate of ageing, which alters longevity.[3] DNA damage is reported to be the primary cause of ageing.[4] In mammals, accumulation of DNA damage occurs with age particularly in those cells that do not divide or those that divide slowly, like neurons.[5,6] Indeed, several studies in

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rodents and humans show that ageing is accompanied by accumulation of neuronal DNA damage[7–11] Moreover brain has a direct relationship between accumulation of DNA damage and ageing. The DNA damage in brain cells, particularly the post mitotic cells (neuronal cells), would be a realistic approach and an ideal method to study the extent of DNA damage with respect to the age of the animal. Since, DNA damage is considered as a prime indicator of ageing, in the present study we measured the extent of DNA damage in terms of single (SSBs) and double strand breaks (DSBs).

Oxidative stress has been linked to the pathogenesis of several late onset neurodegenerative diseases[12] and there are several different producers of oxidative stress. However, ageing is a common risk factor for all neurodegenerative diseases and on the other hand, most theories of ageing focus on the cumulative oxidative stress levels in ageing and age-related diseases.[13] Further, oxidative DNA damage by ROS causes multiple lesions, including SSBs and DSBs, apurine/apyrimidine (AP) sites and modified purines and pyrimidines.[14] Since the role of ROS has increasingly been recognized in ageing, in the present study we assessed the oxidative stress and antioxidant status in WNIN/Gr-Ob rats in whom Type 2 Diabetes (T2D)/aggravated insulin resistance (IR) was induced by feeding high sucrose diet (HSD).

Excessive intake of sucrose and high caloric diet contributes to the development of obesity and IR, both in humans and rodents.[15] Moreover, obesity linked IR increases the risk of developing T2D.[16] Further, research on obesity and its impact on brain ageing is gaining importance[17] and emerging evidence indicates a link between T2D and brain ageing.[18,19] Indeed research on the effects of diet and nutrition on metabolic disorders like obesity, T2D, CVD, and ageing is an emerging area of great contemporary relevance. A shift in the dietary patterns, decline in the energy expenditure due to sedentary lifestyle pose an increasing challenge to age-related disorders rather than infectious diseases.[1] Intake of high caloric diet has been reported to elevate 5-hydroxymethyluracil levels in the liver of Fischer rats, indicating oxidative DNA damage.[20] This accumulated DNA damage is considered to be an important factor underlying ageing.[21]

Based on the foregone literature it is clear that obesity and T2D *per se* induces/increase ageing of brain. But it is not clear whether superimposing T2D on obesity accelerates ageing. So in the present study, we hypothesized that ''High Sucrose feeding to WNIN/Gr-Ob obese rats accelerates ageing by aggravating the oxidative DNA damage and stress mechanisms.' To validate/negate the hypothesis we determined the changes in longevity by the Kaplan–Meier survival plot and assessed the underlying/associated parameters like progressive oxidative damage to cellular macromolecules by determining DNA damage (SSBs and DSBs), an important contributor to the ageing process. Whether or not altered oxidative stress and/or antioxidant status (enzymatic and non enzymatic) underlie these changes were assessed in the brain, a key regulator of the body.

Experimentation design/study design

Animals, grouping, feeding, housing and their maintenance

The animal experimental procedure was approved by the 'Institute's ethical committee on animal experiments' at National Institute of Nutrition, Hyderabad, India (P10/NCLAS/IAEC-25/7/2012). Nine to ten weeks old, male rats of the following strains: WNIN/Ob-lean, WNIN/Ob, and WNIN/Gr-Ob were procured from NCLAS, NIN, Hyderabad. They were housed individually in standard polycarbonate cages at 22 ± 2°C, with 14–16 air changes per hour at a relative humidity of 50–60 per cent with a 12 hour light/dark cycle.

WNIN/Ob and WNIN/Gr-Ob are obese mutant rats developed from the parental Wistar (NIN)/WNIN inbred rat colony that has been maintained at NIN, Hyderabad for more than 85 years. WNIN/Ob rats are morbidly obese, euglycaemic, hyperinsulinemic, hyperleptinemic, and insulin resistant. In addition they are susceptible to a variety of opportunistic infections and metabolic diseases[22–24] WNIN/Gr-Ob rats have all the features as above and in addition exhibit impaired glucose tolerance when challenged with glucose orally.

On the other hand WNIN/Ob-lean are the lean littermates of WNIN/Ob rats, which are not obese and do not exhibit IR or impaired glucose tolerance. The WNIN/Ob and WNIN/GR-Ob rats in general mimic the obesity condition.

Keeping in mind that the WNIN/GR-Ob rats are obese, insulin resistant and had impaired glucose tolerance, it was considered prudent to use them to superimpose diabetes/aggravate IR (by high sucrose feeding) on their obesity. Accordingly the following four groups of rats were used in these studies and were given *ad libitum,* a sterile powdered diet along with water, for a period of 6 months:

Group 1: WNIN/Ob Lean controls.

Group 2: WNIN/Ob rats acts as obese controls.

Group 3: WNIN/Gr-Ob (SBD) rats represent diet controls.

Group 4: WNIN/Gr-Ob (HSD) rats represent superimposed condition.

Table 1 Composition of the diet used in the study

	Weight (g/Kg diet)
Ingredients	
Casein	250
Starch/Sucrose[a]	545[a]
Cellulose	50
Oil	100
Mineral mix	40
Vitamin mix	10
Cystine	3
Choline	2

The salt and vitamin mixtures were prepared according to the AIN-93G formulation.
[a]To aggravate IR/induce T2D in the rats, starch was replaced by an equal amount of sucrose in the diet.

While rats in groups 1–3 received starch-based control diet (AIN 93G), those of group 4 received a high sucrose diet (HSD), to aggravate IR and/or induce T2D in them. The composition of the diet used is given in Table 1. Rats were sacrificed by cervical dislocation at the end of three and six months of feeding respectively, after the determination of their body composition and performing an oral glucose tolerance test. Major organs like liver, brain, and white adipose tissue were quickly excised, rinsed in ice cold normal saline and stored frozen as aliquots at −80°C till further analyses. The stored aliquots of the brain were used for determining the oxidative stress markers.

Kaplan–Meier plot of longevity and cumulative hazard plot

Kaplan–Meier estimator was used to determine the longevity by Survival plots and cumulative hazard plot analysis.[25,26] In the present study survival analysis was done statistically to measure the longevity and survival rate of the WNIN-Ob lean, WNIN/Ob, and WNIN/Gr-Ob rats fed starch-based control diet in relation to WNIN/Gr-Ob rats (n=12) fed HSD after feeding for nine months.

Neuronal DNA damage

Comet assay for DNA DSBs, DNA SSBs and total DNA damage

The comet assay (single-cell gel electrophoresis), an established method for measuring DNA damage in eukaryotic cells, was carried out under alkaline and neutral conditions in the isolated neuronal cell suspension. The extent of the total DNA damage was assessed in three independent batches with two rats per each group. All the experiments were carried out under identical conditions. The comet assay under alkaline and neutral conditions detected SSB's and DSB's respectively.

Preparation of neuronal cell suspension

The neuronal cell suspension was isolated from the cortical region of the brain as described by our group earlier.[27] In brief, cortical region of freshly isolated brain was minced in isolation medium and incubated with trypsin to dissociate the cells. The dissociated tissue was strained through nylon meshes of decreasing pore size of 110, 80, and 48 μm successively. Further separation and purification of neurons enriched fractions is achieved by centrifugation on ficoll gradient. The neurons isolated showed 80–90% viability and purity by trypan blue exclusion test.

Comet assay under alkaline conditions

Neuronal cells were suspended in ice cold phosphate buffered saline at a concentration of 1×10^5 cells/ml. The comet assay was performed according to Swain *et al.*, 2012.[27] Briefly, the cells were sandwiched between different concentrations of low melting agarose and transferred to pre-chilled lysis solution. A denaturation step was performed in alkali solution at room temperature under dark conditions. Further, the slides were subjected to electrophoresis in dark conditions and washed with neutralization buffer. These slides were fixed in ice cold 100% ethanol, air dried and stained with SYBR Green dye. The stained slides were analyzed using Zeiss Axio Imager Apotome fluorescence microscope. Observations of 50 randomly chosen comets were made at 400× magnification using 38HE (GFP) filter. The DNA damage was scored as tail moment in terms of the mobility of DNA in the tail of the comet using Comet Score Free Software. Tail moment is expressed as the product of % DNA in tail and tail length measured in arbitrary units.

Comet assay under neutral conditions

The procedure was same as described above, but with minor modifications. Under neutral conditions, the slides were washed with 1× Tris-borate EDTA buffer solution, pH 8.3 (TBE) and electrophoresis was carried out at room temperature. Further the slides were washed with deionized water and immersed in ice cold 100% ethanol. Subsequently, the slides were air dried; DNA was stained with SYBR Green dye for analysis.

Oxidative stress and antioxidant status in the brain

Preparation of brain tissue homogenate

After 3 and 6 months on their respective diets, the rats were killed by cervical dislocation. For determining the oxidative stress and antioxidant status of the brain, different aliquots of the whole brain tissue (n = 6) were used. An aliquot of brain homogenate was centrifuged at 1000 g and used for the estimation of lipid peroxidation. A second aliquot was centrifuged[28] at 12 000 g and the supernatant was used to determine the levels of reduced glutathione and the activity of superoxide dismutase (SOD).

Protein content of tissue homogenates

The amount of protein present in different aliquots of the tissue homogenate/supernatant was estimated by the bicinchoninic acid (BCA) method using Pierce BCA Protein Assay Kit.

Lipid peroxidation

The levels of lipid peroxides (MDA) were measured spectrophotometrically in 1000 g supernatant fraction of the brain homogenate according to Gupta *et al.*, 2009.[29]

SOD activity

SOD activity was measured according to Marklund and Marklund (1974)[30] in 12 000 g supernatant fraction of the brain homogenate. In this method the increase in absorbance was measured spectrophotometrically at 550 nm for 5 min.

Reduced glutathione levels

Reduced glutathione levels were estimated by the method of Ellman (1959)[31] using spectrophotometric method at 412 nm.

Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA) followed by LSD (Least Significant Difference) post hoc test using IBM SPSS software (version 21.0). A probability value of $P < 0.05$ was taken to indicate a significant difference between means. All results are reported as mean ± SE.

Results

Survival analysis to determine the longevity of the rats

A plot of the Kaplan–Meier estimate of the survival function was performed. The Kaplan–Meier estimator, also known as the product limit estimator, is an estimator for estimating the survival function from lifetime data. Kaplan–Meier estimate is one of the best options to be used to measure the fraction of subjects living for a certain amount of time after treatment. We observed a significant decrease in the survival rate of the rats fed HSD when compared to their counterparts fed starch-based diet (SBD) after nine months of feeding (Fig. 1A).

Surprisingly, WNIN/Ob rats showed decreased lifespan when compared to WNIN/Gr-Ob rats fed HSD, which is also evident from the log cumulative hazard plot as shown in the Fig. 1A and B.

DNA damage (SSBs, DSBs, and total DNA damage)

The single strand and double strand damaged DNA comets are presented in Fig. 2A and B. The results indicate that the WNIN/Gr-Ob obese rats fed HSD

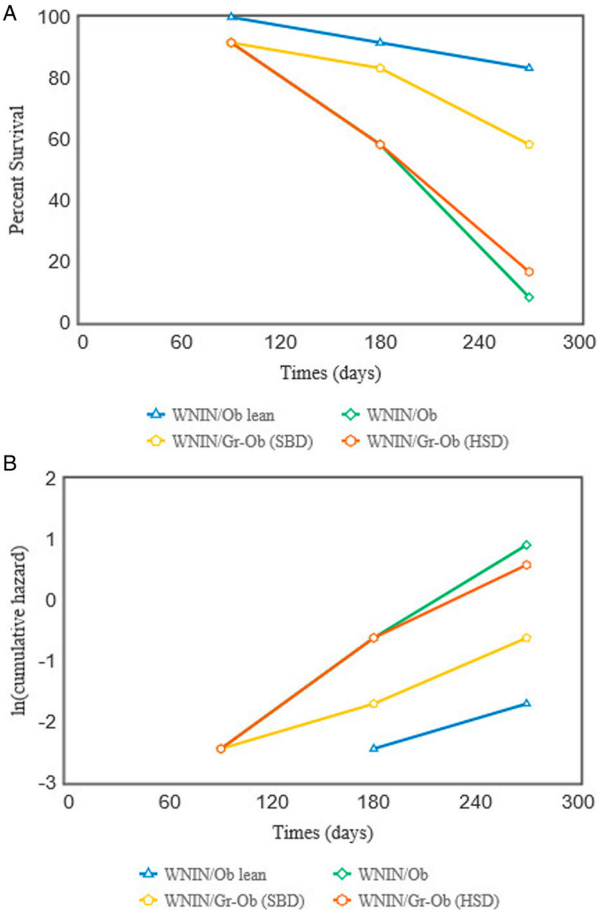

Figure 1 Kaplan–Meier analysis for survival function. (A) Survival percentage of different groups of rats after 3, 6 and 9 months of feeding on their respective diets. (B) Log cumulative hazard plot of different groups of rats after 3, 6 and 9 months of feeding on their respective diets.

showed a trend of increased DNA damage in brain cells, in terms of SSBs and DSBs, when compared to their counterparts fed SBD (Fig. 3A and B).

Nevertheless, total neuronal DNA damage was significantly higher in WNIN/Gr-Ob rats fed HSD compared to their SBD fed controls after 3 months of feeding (Fig. 3C). It was surprising that the neuronal DNA damage was higher in WNIN/Ob than WNIN/Gr-Ob rats fed HSD both after 3 months and 6 months of feeding their respective diets. Also it is evident from the Fig. 3A, B, and C, that in each group of rats, the DNA damage was significantly higher after 6 months of feeding compared to 3 months on their respective diets.

Lipid peroxidation

Oxidative stress in brain, expressed in terms of lipid peroxidation was significantly higher in WNIN/Gr-Ob rats fed HSD than WNIN/Gr-Ob rats fed starch control diet after 3 months of feeding. These levels were 1.8 times higher than that in their lean counterparts (Fig. 4A).

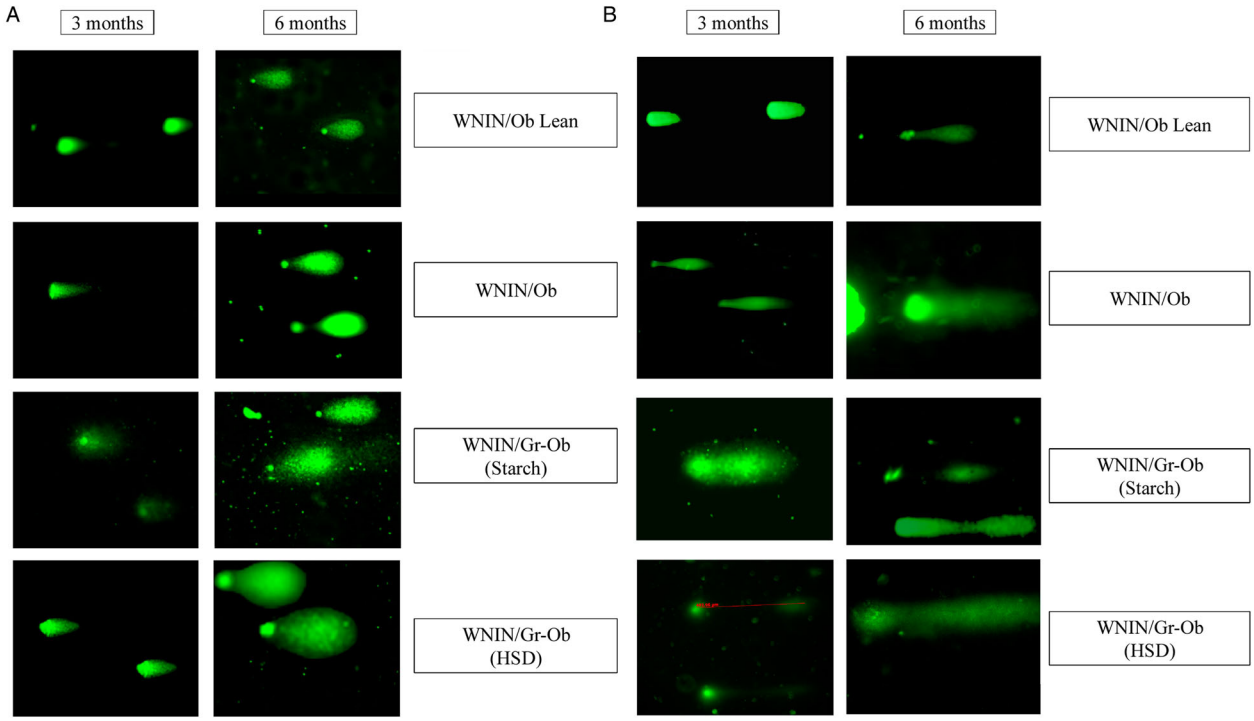

Figure 2 Pictorial presentation of DNA comets representing DNA damage. (A) Fluorescence photographs of the comets of neuronal cells of different groups of rats assayed under alkaline condition (pH > 13). (B): Fluorescence photographs of the comets of neuronal cells of different groups of rats assayed under neutral condition (pH-8.3).

Antioxidant defense

The enzymatic activity of brain SOD was significantly lower in WNIN/Gr-Ob rats fed HSD compared to their counterparts fed SBD, but only after 6 months on their respective diets. The activity of SOD was 1.5 times lower in WNIN/Gr-Ob rats fed HSD compared to those fed starch-based control diet but it was 5.2 times lower than lean rats (Fig. 4B). The levels of the non enzymatic antioxidant, reduced glutathione in the whole brain of WNIN/Gr-Ob rats fed HSD were significantly lower by 1.2 times than WNIN/Gr-Ob rats fed starch control diet after 3 months of feeding (Fig. 4C).

Discussion

Ageing is associated with increased oxidative stress[32,33] and damage of macromolecules including the accumulation of damaged DNA,[28,34,35] decreased DNA repair capacity[36] etc. Ageing could be accelerated by several factors like diabetes, cardiovascular diseases, cancer, genetic pre-disposition etc., and advanced ageing is strongly associated with obesity and T2D Mellitus,[37] which in turn are associated with increased oxidative stress and early onset detrimental consequences.[38] However, the effects of these etiological factors of ageing on cellular senescence and their associated mechanisms are still unclear, let alone whether superimposition of these factors accelerates ageing (including cellular senescence) and the underlying mechanisms. In the present study we assessed whether inducing diabetes in an obese rat accelerates its ageing/cellular senescence, and also deciphered the probable role of oxidative stress (which underlies normal ageing) in their accelerated ageing.

We reported recently that aggravating IR in the obese, WNIN/Gr-Ob rats by feeding HSD increased cellular senescence as indicated by decreased telomere length in their brain.[39] We also showed that ageing associated changes such as liver steatosis, kidney degeneration, increased β cell number and function were advanced and higher in WNIN/Gr-Ob rats fed HSD than SBD.[40] However the accelerated ageing/decreased longevity if any in the HSD fed obese rats and the associated/underlying biochemical and molecular mechanisms still remain to be deciphered.

Indeed accelerated ageing can be better estimated by the measurement of lifespan. Therefore we determined the effect of high sucrose feeding to WNIN/Gr-Ob rats on their survival percentage, using Kaplan–Meier statistical analysis method. Survival plot and log cumulative hazard plots indeed showed a significant decrease in the survival percentage of WNIN/Gr-Ob rats fed HSD compared to starch fed WNIN/Gr-Ob controls after 9 months of feeding but not earlier. This could be due to the finding that rats fed HSD did not developT2D but only showed aggravated IR, a pre-diabetic condition; suggesting that the HSD fed obese rats were in a pre-diabetic state and on the way to become diabetic.[40]

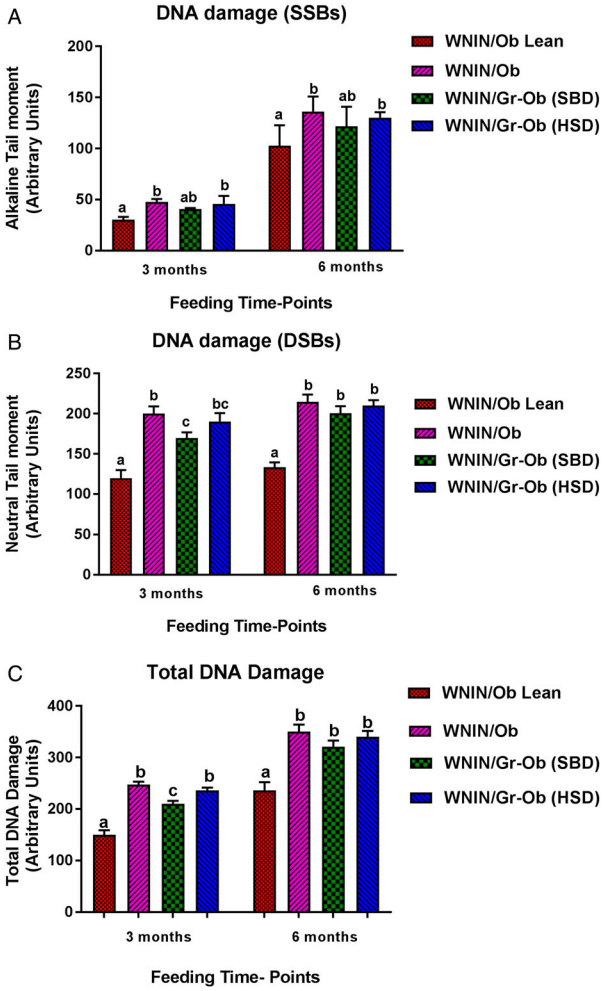

Figure 3 Quantification of DNA damage. (A) Quantification of SSBs measured as Tail moment. Legend: Values given are Mean ± SE. Bars bearing different superscripts are significantly different from one another by one-way ANOVA and post-hoc LSD test. At 3 months DNA damage (SSBs) under alkaline conditions are expressed as alkaline tail moment, one-way ANOVA F-value = 3.12; a vs b = $P <$ 0.05(0.02–0.03) by post hoc LSD test. At 6 months, one-way ANOVA F-value = 3.1; a vs b = $P < 0.05$(0.02–0.04 by post hoc LSD test. (B) Quantification of DSBs in terms of Tail moment. Legend: Values given are Mean ± SE. Bars bearing different superscripts are significantly different from one another by one-way ANOVA and post-hoc LSD test. At 3 months DNA damage (DSBs) under neutral conditions are expressed as neutral tail moment and one-way ANOVA F-value = 15.25; a vs b = $P < 0.01$ by post hoc LSD test. At 6 months, F-value = 24.07; a vs b = $P < 0.01$ by post hoc LSD test. (C) Quantification of Total DNA damage expressed as Tail moment. Legend: Values given are Mean ± SE. Bars bearing different superscripts are significantly different from one another by one-way ANOVA and post-hoc LSD test. At 3 months of total DNA damage, one-way ANOVA F-value = 42.05; a vs b = $P < 0.01$and b vs c = 0.026 by post hoc LSD test. At 6 months, F-value = 22.15; and a vs b = $P < 0.01$ by post hoc LSD test.

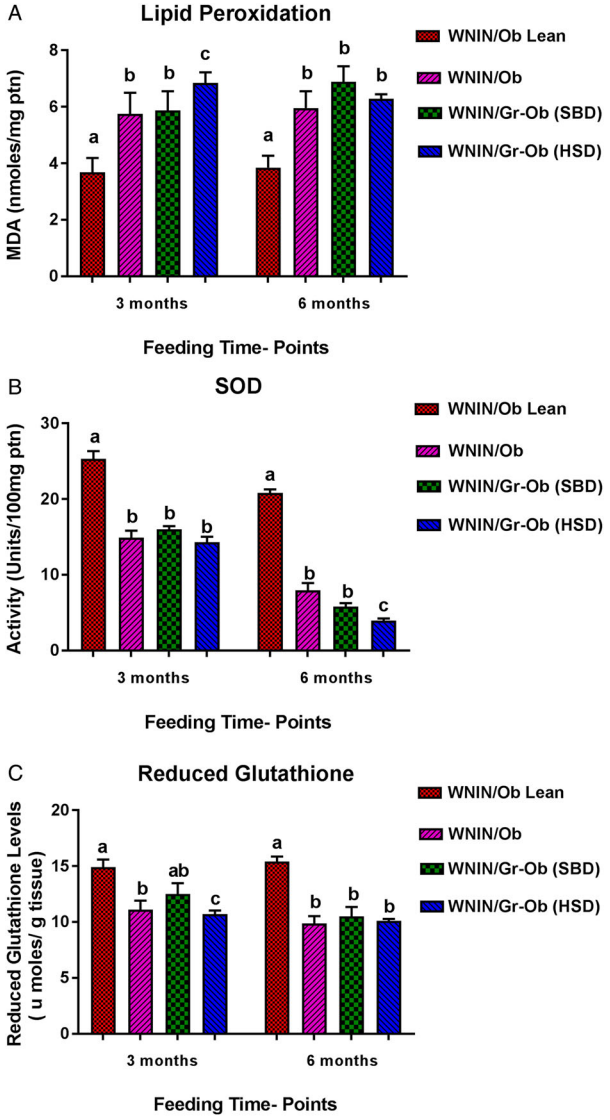

Figure 4 Oxidative stress markers and Antioxidants (Enzymatic and Non enzymatic) Defense Mechanism. (A) Lipid peroxidation of different groups of rats after 3and 6 months of feeding on their respective diets. Legend: Values given are Mean ± SE. Bars bearing different superscripts are significantly different from one another by one-way ANOVA and post-hoc LSD test. At 3 months one-way ANOVA F-value = 5.647; a vs b = 0.01 (0.014–0.04) and b vs c = 0.04 by post hoc LSD test. At 6 months, F-value = 10.6; and a vs b = $P \leq 0.01$ by post hoc LSD test. (B) Antioxidant activity of SOD after 3and 6 months of feeding on their respective diets. Legend: Values given are Mean ± SE. Bars bearing different superscripts are significantly different from one another by one-way ANOVA and post-hoc LSD test. At 3 months one-way ANOVA F-value = 145.45; a vs b = $P < 0.01$ by post hoc LSD test. At 6 months, F-value = 121.56; a vs b = $P < 0.01$ and b vs c = 0.03 by post hoc LSD test. (C) Non enzymatic levels of reduced glutathione of different groups of rats after 3and 6 months of feeding on their respective diets. Legend: Values given are Mean ± SE. Bars bearing different superscripts are significantly different from one another by one-way ANOVA and post-hoc LSD test. At 3 months one-way ANOVA F-value = 15.1; a vs b = $P \leq 0.05$ (0.01–0.05) and b vs c = 0.05 by post hoc LSD test. At 6 months, one-way ANOVA F-value = 50.26; a vs b ≤ 0.01 by post hoc LSD test.

Increased macromolecular damage plays a causative role in ageing and the onset of age-associated diseases.[41] DNA damage accumulates with age and is one of the hallmarks of ageing pathology.[42] Further, the magnitude of cellular DNA damage as indicated by the accumulation of damaged DNA and the type of damage (SSBs and/or DSBs), constitute a key marker of ageing. Therefore, we assessed the SSBs and DSBs of DNA in neuronal cells by comet assay under alkaline and neutral pH. Our observation that total DNA damage was significantly higher in HSD than SBD fed WNIN/Gr-Ob rats after 3 months of feeding and that the DNA damage after 6 months of feeding was greater than that after 3 months of feeding indicates that HSD feeding advanced/accelerated ageing in the obese rats. But the finding that the difference between HSD and SBD fed rats was not statistically significant at the latter time point; we feel could be due to the DNA damage in HSD fed rats reaching the peak as early as 3 months of feeding. That this indeed was true is evident from our finding that DNA damage in the groups of obese rats, at 3 months of feeding, was similar to that in lean controls at 6 months of feeding. Interestingly, this is in line with our earlier observations that right at 3 months of age, WNIN/Ob rats had as much DNA damage as that seen in 15-month-old lean controls.[28] Further this is also corroborated by our observation that total DNA damage in HSD fed rat brains at six months of feeding was only 1.4-fold higher than that at 3 months of feeding, whereas it was 1.6-fold higher in SBD fed controls. These findings once again support greater macromolecular damage, perhaps due to increased oxidative stress, in the obese phenotypes compared to their lean controls of comparable age. Notwithstanding the significant differences observed among groups in total DNA damage, it was intriguing that single strand and double strand DNA breaks, despite being higher in HSD fed rats compared to starch fed controls, were not significantly different between HSD and SBD fed rats, appears to suggest an increase in total DNA damage but not the type of DNA damage by HSD feeding.

Overall it appears that as compared to the neurons from control rats, obese rat neurons are in a greater state of struggle between degeneration and repair. The normal cells fight the stress by up regulating proteins that serve as protective mechanisms[43] but in the present study the obese rats in general and those fed the HSD in particular appear incapable of raising their protective (enzymatic and non enzymatic antioxidant) mechanisms against the oxidative damage of macromolecules (DNA).

To confirm whether the above inference is true or not, we checked lipid peroxidation to determine the oxidative stress levels in brain created mainly by the high levels of fat accumulated in the adipose tissue and also assessed whether this was due to compromised antioxidant status (enzymatic: SOD and non enzymatic: Reduced glutathione antioxidants) of WNIN/Gr-Ob rats fed HSD versus those fed the SBD. In line with reports of similar nature,[44] we found significantly increased lipid peroxidation in HSD fed rats compared to their SBD fed controls after 3 months of feeding. This was further corroborated by a significant decrease observed in the activity of the enzymatic anti oxidant: SOD and the levels of non enzymatic antioxidant: reduced glutathione, indicating a decreased antioxidant defense activity, both of which together could result in greater macromolecular damage. Our present results on increased oxidative stress and decreased antioxidant (enzyme and non enzymatic) status in HSD than SBD fed rats are in agreement with our similar findings in the WNIN/Ob rats reported earlier.[28] Further they suggest a probable causal relationship between the increased DNA damage observed here and the reduction in the telomere length, we reported earlier in the brain of HSD fed obese rats.[40]

Taken together with our earlier reports which showed that feeding HSD to obese WNIN/Gr-Ob rats increased their food intake, body weight gain, aggravated IR, had deleterious effects on major organs like liver, kidney, and pancreas[39] and was associated with decreased telomere length in the neuronal cells[40] our present findings suggest that aggravation of IR in an obese rat by high sucrose feeding decreased their survival as evident from the Kaplan–Meier analysis. This is further corroborated by the increased total DNA damage which appears to be due to increased oxidative stress in them. Interestingly the increased oxidative stress appears to be due to a compromised enzymatic as well as non-enzymatic antioxidant status in the HSD fed obese rat compared to those fed SBD controls. It appears from our findings that accelerated ageing/decreased longevity in obese rats due to high sucrose feeding may involve mechanisms that underlie normal ageing *albeit* at a much advanced and aggravated state by feeding HSD compared to control diet.

However, ageing being a multifactorial disease, further studies at the molecular level clearly are needed to help us understand better the mechanisms of genome maintenance and underlying factors. Unique features of WNIN/Gr-Ob obese mutant rat make it a novel animal model to study not only the metabolic syndrome, but also the reduced longevity/accelerated ageing vis a vis the brain functioning and

mechanisms associated with these phenomena. Considering that our group established earlier the WNIN obese mutant rat as an appropriate model for reduced longevity/accelerated ageing and also deciphered the probable underlying/associated mechanisms,[28] the present study demonstrates that aggravating IR in obesity may accelerate their ageing/reduce their longevity further.

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Disclaimer statements

Contributors None.

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Conflicts of interest None.

Ethics approval The animal experimental procedure was approved by the 'Institute's ethical committee on animal experiments' at National Institute of Nutrition, Hyderabad, India (P10/NCLAS/IAEC-25/7/2012).

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