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High sucrose diet induced diabetes in WNIN/Gr-Ob obese rats: Biochemical and histological changes

Aruna Potukuchi[1], Uma Addepally[4], Chiranjeevi Potu[2], Malathi Upadrasta[1], Surekha Mullapudi[2], Suresh Pothani[3] & Raghunath Manchala[1]*

[1]Endocrinology and Metabolism Division; [2]Pathology Division; and [3]National Centre for Laboratory Animal Sciences, ICMR-National Institute of Nutrition, Hyderabad-500 007, Telangana, India

[4]Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad-500 085, Telangana, India

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Acceleration of natural ageing occurs due to multiple reasons such as stress, obesity and Type 2 diabetes working in a vicious cycle. In the present study, we tested if superimposing type 2 diabetes in a rat model of obesity accelerates ageing or not. We aggravated insulin resistance/ induced type 2 diabetes by feeding high sucrose diet (HSD) to 9-10 wk old, male, WNIN/Gr-Ob obese rats. We report here the changes in physiological, biochemical and histological parameters after 3 and 6 months of feeding. Rats fed HSD had the highest insulin resistance as evident from increased HOMA IR and AUC insulin during OGTT. Body weight gain and Food efficiency ratio (FER) were also significantly higher in HSD fed than the control rats after 6 months of feeding. Further, liver steatosis and kidney damage were the highest in the HSD fed rats as evident from ORO staining. Interestingly, HSD fed rats also had the highest intensity of ß-cell staining and functioning (as indicated by higher HOMA-ß). The findings indicate that parameters associated with ageing were accelerated in WNIN/Gr-Ob rats fed HSD, implying that aggravating insulin resistance in obese rats may be associated with accelerated ageing.

Keywords: Accelerated ageing, Food efficiency ratio (FER), Homeostatic model assessment (HOMA), HSD, Insulin resistance, Liver steatosis, Obesity, ORO staining, Type 2 Diabetes

Obesity is not just a chronic disease but a worldwide epidemic which is closely associated with metabolic disorders such as diabetes, cardiovascular diseases and cancer[1]. It has received much attention as a major health hazard in developed countries. WHO has reported that obesity is one of four key metabolic changes that increase the risk of noncommunicable diseases (NCDs) which cause 40 million people die each year, 70% of all deaths globally[2]. If the rate of obesity continues to rise at this pace, it is estimated that by 2025, roughly a fifth of men (18%) and women (21%) worldwide will be obese. Further, more than 6% of men and 9% of women will be severely obese[3]. The United Nations General Assembly on the Prevention and Control of Non-communicable Diseases, has committed to 'Halt the rise in diabetes and obesity' as one of the nine voluntary global targets for achievement by 2025[4]. There are several rodent models of obesity[5] which serve as important tools to carry out histological studies in metabolic syndrome that are difficult to perform in humans. The homology between the rodent and human genomes makes these animal models convenient to study the conditions that affect humans and can be simulated in rats[6,7]. Thus, they are convenient for investigating the adaptive changes in different functional, morphological and biochemical factors.

Visceral adiposity and insulin resistance are the basis of all chronic diseases of ageing[8], associated with dysregulation of energy homeostasis. Accelerated ageing, one of the major problems all over the world[9], is a faster-than-normal deterioration in the functioning of the body and mind. Obesity can exacerbate the age related decline in physical function, leading to a number of chronic diseases including diabetes, cardiovascular diseases and cancer[10]. Substantial changes occur in the metabolic function of the fat tissue during ageing, such as decline in insulin, lipolytic and fatty acid responsiveness. Insulin, a master regulator of ageing, determines the rate and expression of ageing in multiple body systems[11].

Although it is known that obesity and diabetes *per se* can lead to ageing, it is not clear whether or not

*Correspondence:
Phone: +91 40 27197235 (O); (Fax): +91 40 27019074/27018380
E-mail: mraghunath55@yahoo.com

superimposing diabetes on obesity accelerates ageing. Considering that in general, obesity and diabetes go hand in hand, it was considered pertinent to study the stimulatory effects if any, on ageing, of superimposing diabetes on obesity. For this purpose, we developed a rat model in which insulin resistance was overtly aggravated in an obese rat.

In this study, WNIN/Gr-Ob rats were used as the obese rat model. Several earlier studies[12,13] have revealed this to be an appropriate model for the study of obesity and metabolic syndrome. WNIN/GR-Ob rats are the mutants developed from the parental WNIN inbred rat strain maintained at the NCLAS, NIN, Hyderabad, India. WNIN/GR-Ob rats are obese at 35 days of age. They are hypercholesterolemic, hypertriglyceridaemic and normoglycemic under fasting conditions but exhibit impaired glucose tolerance and become hyperglycaemic on challenge with glucose orally[14]. Considering that WNIN/GR-Ob is a glucose intolerant obese rat model, we attempted to induce type 2 diabetes in these rats by feeding high sucrose diet (HSD) and evaluated various age associated biochemical, physiological and histochemical changes at different time points of feeding (3 and 6 months) as compared to WNIN/Gr-Ob and WNIN/Ob rats fed starch based control diets.

Materials and Methods

The animal experimentation was reviewed and approved by the "Institute's ethical committee on animal experiments (IAEC)" at the National Institute of Nutrition, Hyderabad, India, and was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Animals

Male, WNIN/Ob-Lean, WNIN/Ob, and WNIN/Gr-Ob rats, approximately 9-10 weeks of age were procured from the National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad.

Housing and Maintenance of rats

The rats 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% with a 12 h light/dark cycle. These rats were given sterile, powdered diet along with distilled water, *ad libitum*.

Grouping of rats

The rats were grouped as: Group-I, WNIN/Ob-Lean (n=6); Group-II, WNIN/Ob (n=6); Groups III & IV, WNIN/Gr-Ob rats (n=6 per group). Rats in Groups I-III received a starch based control (AIN 93) diet *ad libitum*. Rats of Gr. IV received the same AIN 93 diet in which starch was replaced with sucrose (high sucrose diet: HSD) to aggravate insulin resistance and/or induce type 2 diabetes in them. Therefore, Gr.III represented WNIN/Gr-Ob (ST) and Gr.IV denoted WNIN/Gr-Ob (SC). The composition of semi synthetic powdered diet fed to the rats was as follows: casein, 250; starch/sucrose, 545; cellulose, 50; oil, 100; mineral mix*, 40; vitamin mix*, 10; cystine, 3; and choline, 2 g/kg diet; (*salt and vitamin mixtures were prepared according to the AIN-93G formulation).

Food intake, body weight and Food efficiency ratio (FER)

Food intake of animals was recorded daily and their body weights were recorded once in 15 days. Food efficiency ratio was calculated as the ratio of body weight gain (kg) to food intake (in Kcal/day)[15].

Body composition

Body composition of the rats was determined at the end of 3 and 6 months on their respective diets, using TOBEC (Total body electrical conductivity), a non-invasive technique, using a small animal body composition analysis system. (EM-SCAN/ TOBEC, Model SA–3000 Multi detector, Springfield, IL, USA). It uses two different coil ids, one for lean rats (I.D. 3076) and the other for obese rats (I.D. 3011)[16]. The animals were stabilized and monitored for body composition, as per the guidelines described earlier[14,17]. The body composition parameters: lean body mass (LBM), total body fat percentage and fat-free mass (FFM) were obtained mathematically according to the methods of Morbach & Brans[18].

At the end of feeding the respective diets for 3 and 6 months, the animals were fasted overnight, blood was collected from supraorbital sinus via the inner canthus and plasma samples were prepared. An oral glucose tolerance test was then conducted in the rats (as described below in detail), following which they were sacrificed by cervical dislocation and various tissues were quickly excised in their entirety, rinsed in ice cold 1X PBS, weighed, aliquoted, wrapped in aluminum foils, frozen in liquid nitrogen and stored at −80°C till further analysis.

Insulin resistance (IR) and Oral glucose tolerance test (OGTT)

OGTT was performed by administering through an oral gavage, a glucose solution (50 g/100 mL) at a dose of 2.5 g/kg body weight, to overnight fasted rats. Blood samples were collected at 30, 60 and 120 min

after the glucose load and used for determining the glucose and insulin levels as described below. Insulin resistance (IR) was assessed by calculating Homeostasis model assessment for insulin resistance (HOMA-IR) and % β was calculated by HOMA-β which gives the β-cell function[19].

$$\text{HOMA-IR} = \frac{\text{Fasting insulin (}\mu\text{U/mL)} \times \text{Fasting glucose (mmol/L)}}{22.5}$$

$$\text{HOMA-}\beta = \frac{20 \times \text{Fasting insulin (}\mu\text{U/mL)}}{\text{Fasting glucose (mmol/L)-3.5}}$$

The AUCs of glucose and insulin during OGTT were calculated by the trapezoidal method as described by us previously[20]. The animals' postprandial insulin resistance was determined by computing the ratio of Area under the curve (AUC) glucose/AUC insulin during the OGTT.

Biochemical parameters

For glucose estimation, blood was collected in 2% sodium fluoride tubes. Glucose was estimated on the same day using GOD/POD (glucose-oxidase/ peroxidase) kit. Insulin was quantified in the blood plasma, using the RIA kit from BRIT, Mumbai, India. For determination of other parameters, fasting blood was collected in K3-EDTA tubes and plasma was separated by centrifugation at 4000 rpm at 4°C. Total cholesterol and triglyceride levels were estimated by the enzymatic kit method (Biosystems, USA).

Organ weights and Histopathology

Vital organs like brain, liver, spleen, pancreas, mesenteric adipose tissue, epididymal fat, retroperitoneal fat, gastrocnemius muscle and kidneys were collected, blotted and weighed. A small part of the tissue was fixed in 10% neutral buffered formalin while pancreas and testis were fixed in Bouins solution and used for histopathological examination. Four-micron thick paraffin sections were cut from the livers and kidneys, stained with hematoxylin and eosin and examined under a light microscope. Frozen sections of the liver were stained with Oil-Red-O (ORO) to study hepatic steatosis[21].

Immunohistochemistry of Pancreas for insulin

Immunohistochemistry (IHC) of pancreas was performed by Avidin:Biotin complex method for identification of ß-cells containing insulin by specific antigen-antibody interaction[22,23]. Briefly, the antibody was tagged with chromogen, and the islet numbers per slide were counted under a magnification of 100X in a given field area. The complete slide was analyzed for the number of islets. For obtaining ß-cell number, cells showing reddish brown granules (Insulin secreting ß-cells are stained with the colored compound, di-amino benzidine) were counted on each slide. Staining intensity for IHC was graded according to Hawkins *et al.*[24].

Statistical analysis

Statistical significance of differences among the groups was determined by one way ANOVA followed by Least Significant Difference test. IBM SPSS statistics 21 software was used for performing ANOVA and only if the probability value was less than the one listed at 0.05, the difference was considered significant (P <0.05). Statistical significance of histopathological findings was done according to Yates correction.

Results

Food intake and Body weight gain

Feeding high sucrose diet (HSD) significantly increased the body weight of the rats compared to those fed the starch based diet and in line with this, Food efficiency ratio (FER) was significantly higher in HSD fed WNIN-Gr/Ob rats than WNIN-Gr/Ob rats fed the starch based diet, *albeit* at 6 months time-point only (Fig. 1 A and B). In addition, body weight

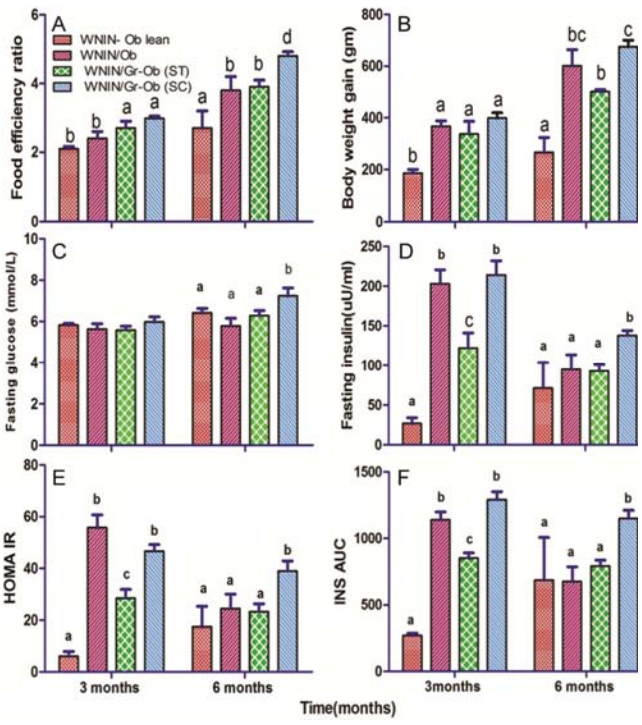

Fig. 1 — (A) Body weights; (B) Food efficiency ratio; (C) Fasting glucose; (D) Fasting insulin (E) HOMA-IR; and (F) INS AUC parameters in different groups of rats after 3 and 6months of feeding.

gain and FER were significantly higher with chronological age in HSD fed WNIN-Gr/Ob rats than those fed the starch based control diet. As one would expect, bodyweight gain and FER of WNIN-Ob lean rats fed starch based control diet were significantly lower than those of the obese rats fed the starch based diet both at 3 and 6 months of feeding.

Body composition

BMI, fat%, LBM%, and FFM% were in general, comparable among the WNIN/Gr-Ob rats fed HSD and starch based control diets. On the other hand, WNIN-Ob lean rats showed significantly lower BMI and fat%, but higher LBM% and FFM% compared to WNIN /Ob and WNIN-Gr/Ob rats fed the control diet. Adiposity index was comparable among the WNIN/Gr-Ob rats fed HSD or starch diet. Interestingly, tissue associated fat% (LBM%-FFM%) was significantly higher in WNIN-Gr/Ob rats fed HSD than those fed starch diet (Table 1) *albeit* at 3 months of feeding but not later. Among the rats fed starch based diet, WNIN/Ob-lean rats had lower BMI, body fat% and AI compared to WNIN/Ob and WNIN/Gr-Ob rats. Considering that body fat% and AI were already significantly high in starch fed WNIN/Gr-Ob rats, HSD feeding resulted in a significant increase in TAF in addition.

Biochemical findings

A significant increase was observed in fasting insulin, HOMA IR and insulin AUC during OGTT in WNIN/Gr-Ob rats fed HSD compared to those fed starch based diet (Fig. 1 D-F), both at 3 and 6 months of feeding. Indeed, fasting glucose values were significantly higher in WNIN/Gr-Ob rats fed HSD than starch based diet fed controls but only at 6 months of feeding (Fig. 1C**)**. Despite feeding for 6 months, AUC glucose values during OGTT were comparable among WNIN/Gr-Ob rats fed starch and high sucrose diets indicating that HSD only aggravated insulin resistance but could not impair OGT. The results suggested aggravation of insulin resistance in WNIN/Gr-Ob rats fed HSD compared to those fed the starch diet.

Notwithstanding the changes observed in the age associated parameters mentioned above, lipid profile was comparable among WNIN/Ob and WNIN/Gr-Ob rats fed starch or high sucrose diets at both the time points studied. Curiously, there was a significant increase in fasting HDL in WNIN/Gr- Ob rats fed high sucrose diet compared to those fed starch diet, *albeit* at only 6 months of feeding but not earlier (Table 2).

Table 1—Changes in body composition parameters in groups of rats after 3 and 6 months of feeding

Time period	Diet & strain	BMI	Fat%	LBM%	FFM%	Tissue fat	AI
3 Months	WNIN/Ob Lean	6.82±0.28^{a}	14.6±0.99^{a}	85.4±0.99^{a}	47.8±0.9^{a}	37.6±0.19^{b}	4±1.3^{b}
	WNIN/Ob	11.0±0.27^{b}	55.4±0.39^{b}	44.6±0.39^{b}	13.8±0.28^{b}	30.8±0.13^{a}	9±0.5^{a}
	WNIN/Gr-Ob(ST)	9.92±0.71^{b}	55.8±0.82^{b}	44.2±0.82^{b}	14.4±0.20^{b}	29.9±0.66^{c}	7.7±1.2^{a}
	WNIN/Gr-Ob(SC)	10.8±0.26^{b}	55.4±0.72^{b}	44.6±0.72^{b}	13.8±0.51^{b}	30.7±0.22^{a}	9.9±0.8^{a}
6 Months	WNIN/Ob Lean	7.33±0.31^{a}	9.16±0.99^{a}	90.8±0.99^{a}	51.7±0.77^{a}	39.2±0.26^{a}	5±0.8^{b}
	WNIN/Ob	13.48±0.48^{b}	52.5±0.75^{b}	47.5±0.75^{b}	15.6±0.58^{b}	31.9±0.18^{b}	12±1.7^{a}
	WNIN/Gr-Ob(ST)	13.68±0.42^{b}	51.8±0.70^{b}	48.1±0.70^{b}	16.1±0.56^{b}	32±0.17^{b}	13.9±1.6^{a}
	WNIN/Gr-Ob(SC)	13.99±0.51^{b}	51.6±0.36^{b}	48.4±0.36^{b}	16.3±0.32^{b}	32.1±0.06^{b}	13.1±2.0^{a}

[*Values given are mean ± SE. Values in a column bearing different superscripts at a given timepoint are significantly different (P <0.05) by One way ANOVA/ LSD test]

Table 2—Changes in lipid profile and glucose AUC during OGTT among groups of rats after 3 and 6 months of feeding

Time period	Diet & strain	TG (mmol/L)	Cholesterol (mmol/L)	HDL (mmol/L)	GLU AUC	GLUAUC: INS AUC
3 Months	WNIN/Ob Lean	0.73±0.05^{a}	1.33±0.22^{a}	1.11±0.07^{a}	914±59.2^{b}	3.4±0.35^{a}
	WNIN/Ob	2.40±0.35^{b}	3.17±0.32^{b}	3.86±0.28^{b}	1274±123^{a}	1.1±0.05^{b}
	WNIN/Gr-Ob(ST)	2.44±0.16^{b}	5.16±0.29^{c}	4.85±0.15^{c}	1327±219^{a}	1.6±0.29^{b}
	WNIN/Gr-Ob(SC)	1.87±0.27^{b}	5.93±0.64^{c}	5.15±0.39^{c}	1485±89.5^{a}	1.2±0.1^{b}
6 Months	WNIN/Ob Lean	0.48±0.08^{a}	2.11±0.42^{a}	1.02±0.89^{a}	892±10.7^{a}	2.5±1.5^{a}
	WNIN/Ob	2.06±0.51^{b}	3.38±0.35^{a}	2.20±0.29^{b}	873±78.3^{a}	1.4±0.2^{a}
	WNIN/Gr-Ob(ST)	4.20±0.71^{c}	5.95±0.67^{b}	3.22±0.53^{b}	914±34.9^{a}	1.7±0.04^{a}
	WNIN/Gr-Ob(SC)	3.12±0.59^{bc}	7.06±0.71^{b}	4.47±0.43^{d}	1275±394^{a}	1.1±0.31^{a}

[*Values given are mean ± SE. Values in a column bearing different superscripts at a given timepoint are significantly different (P <0.05) by One-way ANOVA/ LSD test]

Table 3—Changes in organ weights (g) in groups of rats after 3 and 6 months of feeding

Time period	Diet & strain	Adipose Tissue (AM+AE+AR)	brain	Liver	Kidney	Spleen	Pancreas
3 Months	WNIN/Ob Lean	16.3±6.2[b]	1.6±0.04[a]	9.6±0.7[b]	4.63±0.23	0.89±0.09	1.09±0.12
	WNIN/Ob	62.9±3.9[a]	1.7±0.05	22.5±2.4[a]	6.1±0.21	1.02±0.08	1.2±0.14
	WNIN/Gr-Ob(ST)	44.9±8.5[a]	1.7±0.07	18.4±2.2[a]	5.5±0.17	0.88±0.07	0.96±0.08
	WNIN/Gr-Ob(SC)	55.9±6.4[a]	1.5±0.14[b]	22.6±2.6[a]	5.8±0.13	0.83±0.04	0.98±0.16
6 Months	WNIN/Ob Lean	94.7±6.3[b]	1.8±0.05	12.1±0.7[a]	5.7±0.5[a]	1.1±0.2	1.6±0.1
	WNIN/Ob	24.5±3.9[a]	1.6±0.04	17.9±0.9[a]	6.1±0.29[a]	1.3±0.1	1.1±0.16
	WNIN/Gr-Ob(ST)	116.4±17.8[a]	1.8±0.09	24.7±3.2[b]	6.8±0.9[a]	1±0.2	1.1±0.04
	WNIN/Gr-Ob(SC)	118.4±21.6[a]	1.7±0.06	25.2±2.6[b]	8.4±1.3[b]	1.3±0.1	1.02±0.06

[*Values given are mean ± SE. Values in a column bearing different superscripts at a given timepoint are significantly different (P <0.05) by One-way ANOVA/ LSD test]

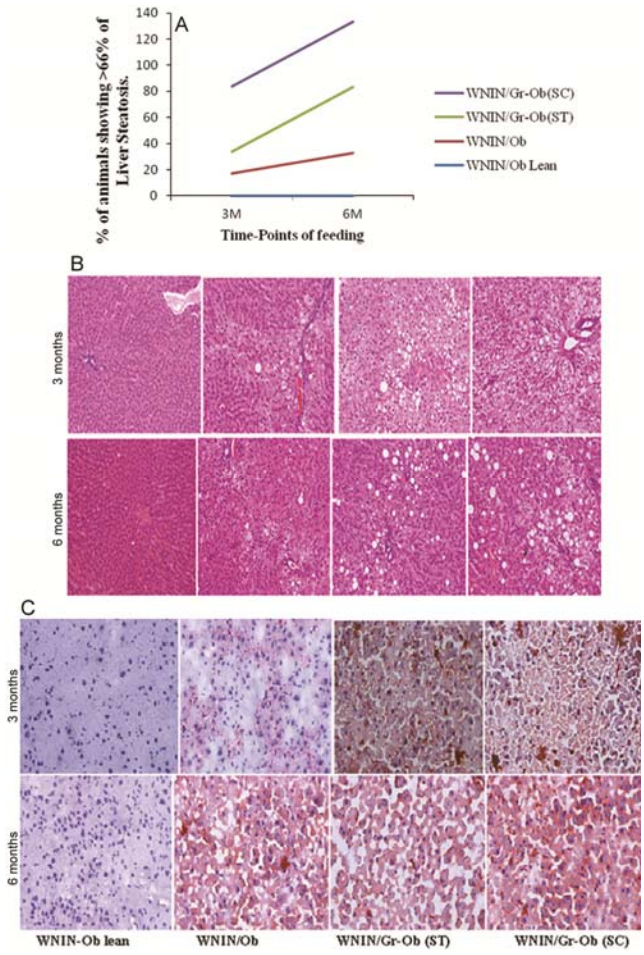

Fig. 2 — Histopathology of Liver tissue. (A) Graph representing liver steatosis; (B) H&E staining of liver tissue; and (C) Hepatic steatosis confirmed by ORO staining.

Changes in organ weights

A decrease in brain wet weight and an increase in liver weight were observed in WNIN/Gr-Ob rats fed HSD compared to their counterparts fed starch diet, at both time points of feeding *albeit* the differences were not significant (Table 3).

Histopathology of organs

Significant liver steatosis (of >66% of liver) was observed in a higher percentage of WNIN/Gr-Ob rats fed HSD compared to those fed starch diet at both time-points of feeding, *albeit* the difference was not significant. This was further confirmed by Oil Red O staining of the frozen sections of liver (Fig. 2 A-C).

As shown by the H&E stained sections of kidneys from different groups of rats, severe degeneration of kidney tubules (of >75%) was observed in 75% of WNIN/Gr-Ob rats fed HSD compared to WNIN/Gr-Ob rats fed starch diet in which only 25% of rats showed such severe degeneration (Fig. 3 A and B).

Immunohistochemistry for insulin

WNIN/Gr-Ob rats fed HSD showed the highest intensity and percentage of pancreatic tissue stained for insulin compared to WNIN/Gr-Ob rats fed starch diet, at both time points of feeding. A significant increase was observed in the % β cell count per islet in WNIN/Gr-Ob rats fed HSD compared to their starch fed counterparts after 6 months of feeding (Fig. 4A). Interestingly, this was in line with the fasting plasma insulin levels which were significantly higher in HSD fed WNIN/Gr-Ob rats. Although it was surprising that WNIN-Ob lean rats showed the highest % of β cells stained per islet, the insulin staining intensity was very low, which coincides with the lower β cell function as calculated from HOMA-β % (Fig. 4B). The β cell function, HOMA-β was significantly higher in WNIN-Gr/Ob rats fed high sucrose diet *albeit* after 3 months of feeding. These findings appear to indicate that despite the low count of β cells in WNIN/Gr-Ob rats fed HSD; there is a maximum efficiency and functioning of β cells (Fig. 5).

Discussion

Obesity is a major cause of insulin resistance, which could progress to T2DM (Type 2 diabetes Mellitus)

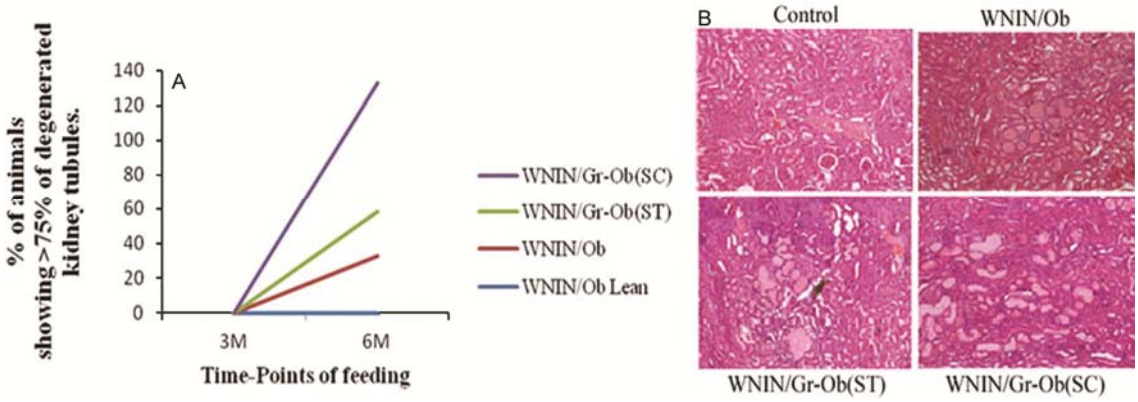

Fig. 3 — Histopathology of kidneys. (A) Graph representing kidney degeneration; and (B) H&E staining of kidney degeneration

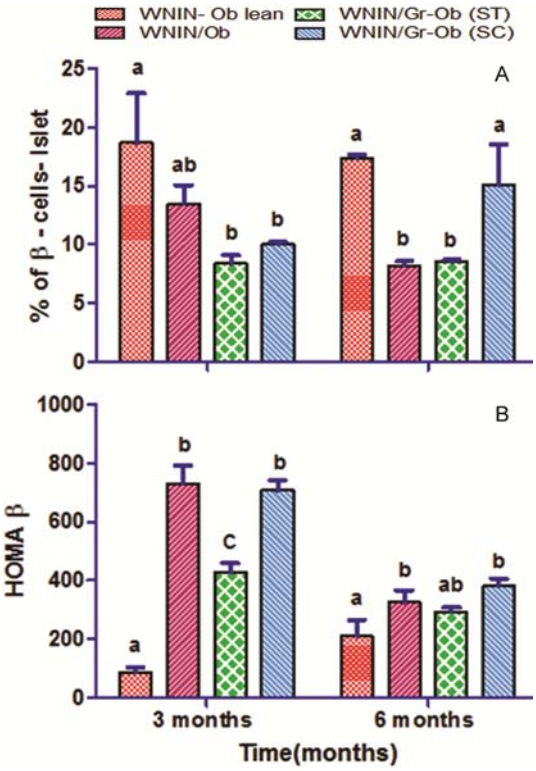

Fig. 4 — (A) % β cell count per islet; and (B) and β-cell function expressed as HOMA-β% in different groups of rats after 3 and 6 months of feeding

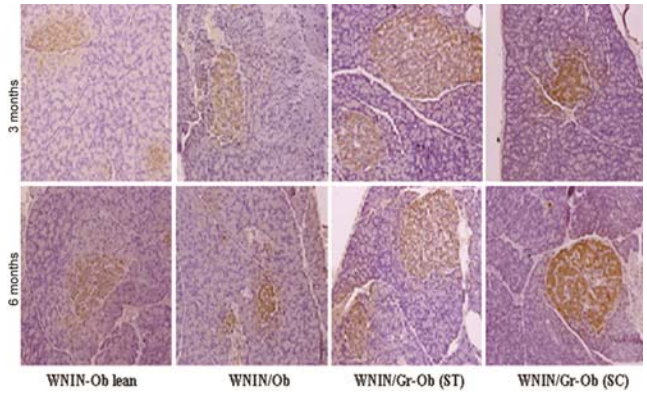

Fig. 5—Immunohistochemistry of pancreas. HSD fed WNIN/Gr-Ob rats shows the highest staining intensity of pancreatic tissue.

and obese are more prone to diabetes[25]. It accounts for 80-85% of the risk of developing T2DM, a disorder of glucose homeostasis that accelerates ageing. Diabetes is one of the most threatening life-style disorders[26]. Obesity and T2DM have a complex relationship, with type 2 diabetes being strongly associated with obesity[27]. Diet, one of the major factors for pre-disposition to T2DM, influences body mass, plasma lipid profile and hormones in circulation. Many studies demonstrate that high sucrose consumption leads to the development of T2DM, which in turn is reported to be associated with obesity and insulin resistance both in humans[28,29] and animal models[30].

Data on the effects of HSD feeding on changes in body weight and associated biochemical and physiological changes are not unequivocal. While some studies reported that HSD feeding induced changes in body weight[31,32], others showed changes in the biochemical and physiological parameters[33,34]. Indeed, some studies in obese rats showed no changes in body weight of rats fed HSD, but only changes in biochemical and physiological parameters[35]. In line with the findings of Toida *et al.*,[36] and our earlier reports[37] of similar nature, we demonstrated here that although feeding HSD to WNIN/Gr-Ob rats had no effect on their bodyweight at 3 months of feeding, but significantly increased the body weight at 6 months of feeding (1.3 folds) and as a consequence the food efficiency ratio was increased (1.2 folds). In addition, it also resulted in changes in biochemical and physiological parameters, compared to their counterparts fed starch diet. These observations are in agreement with those of Malafaia *et al.*[6] and Bocarsly *et al.*[38] who reported an increase in the bodyweight of rats fed HS/HF diets. These findings

probably suggest the requirement of longer duration of feeding HSD to WNIN/Gr-Ob rats, for changes to be seen in their body weights. Alternately, the physical form of sucrose could be one of the reasons for these observed differences[39,40].

Although body weight gain and FER were significantly higher in the WNIN-Gr/Ob rats fed HSD after 6 months of feeding, body fat % as determined by TOBEC (~55%) was comparable to their starch fed counterparts, *albeit* showing a trend towards increase. The failure to observe a significantly higher increase in the body fat % (and visceral adiposity) of HSD than starch fed Gr-Ob rats could be due to the fact that feeding starch diet itself significantly increased the body fat % (specially the visceral adiposity, AI) in WNIN/Ob and WNIN/Gr-Ob rats compared to WNIIN/Ob-lean controls. It is however interesting that WNIN/Ob – lean rats fed starch based diet had significantly higher percent of LBM and FFM than WNIN/Ob and WNIN/Gr-Ob rats, which had significantly higher body fat % and visceral adiposity. These findings confirm the greater conversion of the energy consumed to fat and non-fat tissues (LBM and FFM) in WNIN/Ob & WNIN/Gr-Ob and WNIN/Ob-lean rats, respectively. In this background, it was indeed interesting that HSD but not starch based diet feeding resulted in a significantly higher increase in TAF % in WNIN/Gr-Ob rats. This increase in TAF could be an important factor in the aggravation/acceleration of insulin resistance in the HSD fed WNIN/Gr-Ob rats. Only a trend was observed in the changes in organ weights of WNIN-Gr/Ob rats fed HSD compared to their age matched counterparts fed starch based control diet. Indeed, these findings are in agreement with observations of similar nature, made in earlier studies in WNIN/Gr-Ob rat models at our institute[14,17].

Despite feeding for 6 months, high sucrose diet did not precipitate in T2DM in WNIN/Gr-Ob obese rats. However, it aggravated fasting and postprandial insulin resistance significantly compared to rats fed starch based diet, appears to suggest that it probably requires greater duration of feeding HSD to induce T2D in the WNIN/Gr-Ob rats. Interestingly, our finding that fasting plasma glucose levels were significantly higher in HSD fed rats than their counterparts fed starch diet indicates that HSD feeding aggravated fasting IR as well as impaired the glycemic status of the WNIN/Gr-Ob obese rats which otherwise are euglycemic under fasting conditions. These findings indicate the hyperglycaemic/ diabetes like status of the animals under fasting conditions. Interestingly, fasting plasma insulin and insulin AUC during the oral glucose tolerance test (OGTT) were also significantly higher in WNIN/Gr-Ob rats fed HSD than those fed starch based diet both at 3 and 6 months of feeding, confirming the aggravated insulin resistance but not the hyperglycemic state in these rats when challenged with glucose, akin to the situation in a diabetic condition[41].

Although HSD feeding increased fasting plasma cholesterol and TG levels, the differences (from starch fed controls) were not significant. Surprisingly, we found a significant increase in the plasma HDL-Cholesterol levels of WNIN/Gr-Ob rats fed HSD after 6 months of feeding. Though curious, this finding is in line with that of Ryu *et al*.[42], who reported increased HDL levels in HSD fed rats. However, considering that WNIN/Gr-Ob rats have higher levels of plasma HDL cholesterol than corresponding WNIN controls[43] or the WNIN/Gr-Ob lean rats, the increased plasma HDL levels in HSD fed WNIN/Gr-Ob rats appear to be of little significance.

Whether or not, the higher plasma insulin levels in the HSD fed WNIN/Gr-Ob rats under fasting and postprandial conditions were due to changes in the β cell number and / or function were assessed the β cell number per islet by immuno-histochemical staining of pancreas for insulin production and the determination of HOMA β from the fasting plasma glucose and insulin levels. It was indeed interesting that β cell function, as determined by HOMA β was higher in HSD fed WNIN/Gr-Ob rats compared to those fed starch based diet, both after 3 and 6 months of feeding. It was of further interest that at 3 months of feeding, HOMA β and intensity of IHC staining for insulin were significantly higher but not the % of β cells per islet, whereas at 6 months of feeding, IHC staining intensity for insulin and % of β cells per islet were significantly higher but not HOMA-β. It appears from these findings that at 3 months of feeding, HSD significantly increased insulin production without increasing the β cell number as also corroborated by the significant increase in β cell function (HOMA-β). On the other hand, at 6 months of feeding, the increased insulin production appeared to be due to increased β cell count but not function (HOMA-β). It appears possible that this could be due to the exhaustion of the β cells, to produce insulin. To the best of our knowledge, this is the first report showing the differential effects of HSD feeding to the obese rat on the mechanism of

insulin production, depending upon the duration of feeding.

It is well known that chronic insulin resistance and obesity lead to and are associated with hepatic steatosis and impaired renal function[44,45]. We, therefore, assessed whether or not HSD feeding to WNIN/Gr-Ob rats aggravated these conditions, by histological examination of liver and kidneys. In line with the literature cited above, WNIN/Ob and WNIN/Gr-Ob rats receiving control diet had significantly higher degeneration of kidney tubules and liver steatosis compared to WNIN/Ob lean controls. Interestingly, aggravating insulin resistance/superimposition of diabetes on obesity by HSD feeding appeared to worsen the degeneration of kidney tubules and also further aggravate liver steatosis, which was confirmed further by ORO staining. Indeed a higher percentage of WNIN-Gr/Ob rats fed HSD showed fatty liver confirming that HSD causes fatty liver at a higher rate and this is in line with the report of Ohta *et al*.[46] who observed a severe fatty liver in male ZDF rats by high sucrose feeding (Fig. 2 A-C).

Our findings suggest that feeding HSD to obese rats aggravates IR and impairs glucose tolerance at an earlier age. This appears to be due to qualitative as well as quantitative changes in the insulin producing β cells, in addition to aggravating liver steatosis and kidney tubule degeneration. Considering that these are the changes normally associated with ageing, our findings appear to suggest that HSD feeding to obese rats may accelerate ageing by accelerating/aggravating the age associated mechanisms.

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