

WNIN/GR-Ob - An insulin-resistant obese rat model from inbred WNIN strain

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Background & objectives: WNIN/GR-Ob is a mutant obese rat strain with impaired glucose tolerance (IGT) developed at the National Institute of Nutrition (NIN), Hyderabad, India, from the existing 80 year old Wistar rat (WNIN) stock colony. The data presented here pertain to its obese nature along with IGT trait as evidenced by physical, physiological and biochemical parameters. The study also explains its existence, in three phenotypes: homozygous lean (+/+), heterozygous carrier (+/-) and homozygous obese (-/-).

Methods: Thirty animals (15 males and 15 females) from each phenotype (+/+, +/-, -/-) and 24 lean and obese (6 males and 6 females) rats were taken for growth and food intake studies respectively. Twelve adult rats from each phenotype were taken for body composition measurement by total body electrical conductivity (TOBEC); 12 rats of both genders from each phenotype at different ages were taken for clinical chemistry parameters. Physiological indices of insulin resistance were calculated according to the homeostasis model assessment for insulin resistance (HOMA-IR) and also by studying U¹⁴C 2-deoxy glucose uptake (2DG).

Results: WNIN/GR-Ob mutants had high growth, hyperphagia, polydipsia, polyurea, glycosuria, and significantly lower lean body mass, higher fat mass as compared with carrier and lean rats. These mutants, at 50 days of age displayed abnormal response to glucose load (IGT), hyperinsulinaemia, hypertriglyceridaemia, hypercholesterolaemia and hyperleptinaemia. Basal and insulin-stimulated glucose uptakes by diaphragm were significantly decreased in obese rats as compared with lean rats.

Interpretation & conclusions: Obese rats of the designated WNIN/GR-Ob strain showed obesity with IGT, as adjudged by physical, physiological and biochemical indices. These indices varied among the three phenotypes, being lowest in lean, highest in obese and intermediate in carrier phenotypes thereby suggesting that obesity is inherited as autosomal incomplete dominant trait in this strain. This mutant obese rat model is easy to propagate, and can easily be transformed to frank diabetes model by dietary manipulation and thus can be used for screening anti-diabetic drugs.

Key words Hyperinsulinaemia - hyperleptinaemia - impaired glucose tolerance - lean body mass - TOBEC

The prevalence of obesity is on continuous rise, with nearly half a billion of the global population being overweight or obese¹. It is now widely recognized as a complex and seriously

debilitating nutrition-disorder, associated with increased risk for several major diseases including cardiovascular diseases^{2,3}, diabetes⁴ and osteoarthritis⁵.

Many natural and experimental animal models exist in the world to study obesity and its associated complications. All of these are developed exclusively in the West and in Japan, like Ob/Ob and db/db mouse strains, Zucker and Koletsky rat strains, but none from India. However, in 1994, from our existing inbred Wistar strain of rats (WNIN) - the first stock of rats to be introduced in biomedical research – a mutant obese rat strain (WNIN/Ob) evolved⁶. These mutant rats attain a body weight of more than 1 kg by one year of age. Their average life span is about 1½ year and after one year of life they develop opportunistic infections, tumours, kidney abnormalities, and quite a few of them develop cataract and retinal degeneration. The infertile homozygous obese animals have 47 per cent of fat in the body and are hyperphagic, hyperinsulinaemic, hypertriglyceridaemic and leptin resistant. This strain is maintained through mating of fertile heterozygous carriers (+/-) and is named as WNIN/Ob to indicate its origin from inbred stock of Wistar (WNIN) rats maintained at National Institute of Nutrition, Hyderabad, India⁶. During routine screening of the animals for plasma glucose, it was observed that a few of them develop hyperglycaemia after oral glucose challenge. A pedigree analysis of the latter revealed that, this was due to an accidental mix up of WNIN/Ob rats with WNIN/GR rats [a lean sub strain of WNIN rats showing impaired glucose tolerance (IGT) established at our facility in 1987]⁷. The present set of rats appears to have inherited obesity from the obese parent (WNIN/Ob) and the IGT from the WNIN/GR, and is infertile like its obese parent. Like WNIN/Ob colony, WNIN/GR-Ob colony is also maintained by mating heterozygous carrier (+/-) rats, and are designated as WNIN/GR-Ob.

Both WNIN/Ob and WNIN/GR-Ob rat strains are of major importance for the study of obesity and obesity-related degenerative disorders. Unlike the strains developed in the West, these have arisen from pure inbred background and show all the major metabolic degenerative conditions in one model, thus, making them ideal for studying the metabolic syndrome as a whole. Moreover, once these strains are fully established and characterized, they can be used to study the mechanism(s) of obesity development and also for screening of various anti-obesity and anti-diabetic drugs, before clinical trials. Given the facts that Western models are not easily accessible and highly expensive complete biochemical and molecular characterization of this strain was essential. This study describes our efforts towards physical, physiological and biochemical characterization of WNIN/GR-Ob strain for its obese and IGT nature.

Material & Methods

Housing and breeding: The rats used in the study were housed individually in standard polycarbonate cages with top grill having facilities for holding pelleted feed and drinking water in polycarbonate bottles with stainless steel sipper tubes (Techniplast, Italy) at $22 \pm 2^\circ\text{C}$, with 14-16 air changes per hour with a relative humidity 50-60 per cent with a 12 hour light/dark cycle. The animals were provided with sterile pelleted chow of standard composition established at our institute containing all the recommended macro and micronutrients (56% carbohydrate, 18.5% protein, 8% fat, 12% fiber and adequate levels of minerals and vitamins) needed for rats along with water, *ad libitum*. The rats were introduced for mating at 90-110 days of age at a ratio of 1 (male): 2 (females), and proper records were maintained of mating, deliveries and weaning.

Parameters:

Growth, food intake and metabolic studies - [90 animals by mating carriers (+/-)], consisting of all the three phenotypes [homozygous lean (+/+) 30, heterozygous carriers (+/-) 30, and homozygous obese (-/-) 30] were used for growth studies. The animals were weighed weekly from 14 to 105 days. From this, 12 lean (+/+) and 12 obese (-/-) rats were selected from 21 days and were monitored for food intake up to 126 days. Weekly records were maintained for food intake, weight gain and from these data food efficiency ratios (FER) were derived⁹. Additionally, 6 adult male and 6 females (90-110 days age) from lean (+/+) and obese (-/-) phenotypes were selected and individually housed for a week in metabolic cages (Techniplast, Italy) to study daily water intake, urine and faecal output. Urine was examined for proteinuria and glycosuria, using Ame's multiple reagent strips (Uristix, Miles inc. USA). Faecal protein was estimated by macro Kjeldahl's method⁹.

Body composition - Lean, carrier and obese (12 each of males and females) rats of 120 days of age were selected and their body composition was measured using Total Body Electrical Conductivity (TOBEC) small animal body composition analysis system (EM - SCAN/ TOBEC, Model SA – 3000 Multi detector, Springfield, III, USA). This instrument measures total body electrical conductivity of small animals in a non-invasive manner¹⁰. In lean and carrier rats the estimation was carried out using coil with I.D. 3076 and in obese rats by coil with I.D. 3011. The animals

were stabilized and monitored for body composition, as per the guidelines provided for instrument^{11,12} and the body composition parameters were obtained mathematically¹³.

Clinical chemistry - Lean (+/+), carrier (+/-) and obese (-/-) rats of 21, 28, 35, 50, 100 and 200 days of age (12 males and 12 females from each phenotype) formed the sample of the study. For oral glucose tolerance test (OGTT), the animals were fasted for 17 h and the initial blood sample was collected under mild anaesthesia (anaesthetic ether) with a heparinized capillary tube from the supra-orbital sinus of the eye via the inner canthus by using heparinized microcapillary tubes as illustrated by Riley¹⁴. An oral glucose dose of 250 mg/100 g body weight (bw) was given through a gavage and the blood was drawn under mild anaesthesia after one and two hours, from the time of administration of glucose. The blood collected in tubes containing sodium fluoride (20 mg) was centrifuged to separate plasma and subsequently, kept at -20°C till the analysis.

Plasma glucose and insulin levels were estimated at fasting and after one and two hours after glucose load. Glucose was estimated by kit provided by Stangen immunodiagnosits, Hyderabad, India¹⁵, and insulin levels were measured using a radioimmunoassay kit provided by BARC (Bhaba Atomic Research Centre, Mumbai, India)¹⁶. Physiological indices of insulin resistance were derived using values of fasting glucose, insulin concentrations as per the homeostasis model assessment for insulin resistance (HOMA-IR)¹⁷. Plasma cholesterol and triglyceride levels were measured using the kits (Stangen immunodiagnosits, Hyderabad, India)^{18,19}. Serum leptin levels were measured using radioimmunoassay kit (LINCO Res. Inc. USA).

Glucose uptake studies by diaphragm using U¹⁴C 2-deoxy glucose (2DG) - Six male and 6 female rats of lean and obese phenotypes from WNIN/GR-Ob group were included for glucose uptake studies. Intact diaphragm was removed from the animals by the method described by Kono and Colowick²⁰. The intact diaphragm was trimmed of fat and the central tendon was then bisected to yield two intact hemi-diaphragmatic pieces. The intact hemi-diaphragm pieces were weighed and put into labelled flasks containing Kreb's medium, incubated at 37°C, for 30 min in a water bath with continuous flow of oxygen. A paired experimental design was used for each rat, i.e., one hemi-diaphragm was incubated with insulin and its corresponding pair without insulin. After

incubation, tissues were washed with saline; dried with the help of filter paper and weighed. Subsequently, these tissues were dissolved in solvene (0.8 ml) by placing the vials at 60°C for 4 to 6 h. After ensuring that the tissues were completely dissolved, 10 ml of scintillation fluid was added and kept for overnight incubation at room temperature. The samples were counted in β -scintillation counter (LKB-1219 Rackbeta, USA).

The study was reviewed and approved by the Institutional Animal Ethical Committee (IAEC), and was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Statistical analysis: Student's unpaired "t" test was used to compare the difference between the groups. Multiple ANOVA was carried out with multiple comparisons using Duncan's multiple range test. Time trend analysis, to test the difference in the mean values of data at three different time points (50, 100 and 200 days) by repeated measures of ANOVA was employed. Log transformation of highly variable data was carried out and further tested with repeated measures of ANOVA. $P < 0.05$ was considered significant.

Results

Breeding: A total of 90 females were mated with 45 males in a time span of 5 matings and their reproductive performance was monitored. A total of 671 pups were recorded after the pre-weaning mortality, consisting of 352 males and 319 females. The average litter size of the colony was 7.9 and the average birth weight was 6.3 g. Among the total litters 222 pups were lean (102 males and 120 females), 355 pups were carrier (184 males and 171 females) and 94 pups were obese (66 males and 28 females). These phenotypes were found to be at a ratio of 1 lean (+/+): 2 carrier (+/-): 1 obese (-/-). The average percentage of obese animals (Fig. 1) in the colony was 14.05 per cent, of which males contributed 70.21 per cent and females 29.78 per cent.

Growth: A sudden spurt in the body weight of obese rats was seen between 4th and 5th week of age. From 5th week onwards, the male obese animals showed an average weight gain of 7.25 g per day compared to 3.5 g in lean and 5.0 g in carrier rats. Females had an average weight gain of 5.48 g per day as compared with 3.13 g in lean and 4.25 g in carrier rats. The weights of lean rats of both sexes were significantly different from carrier and obese rats ($P < 0.05$) from 35 days onwards, till the end of 15 wk of observation (Fig. 2A, B).

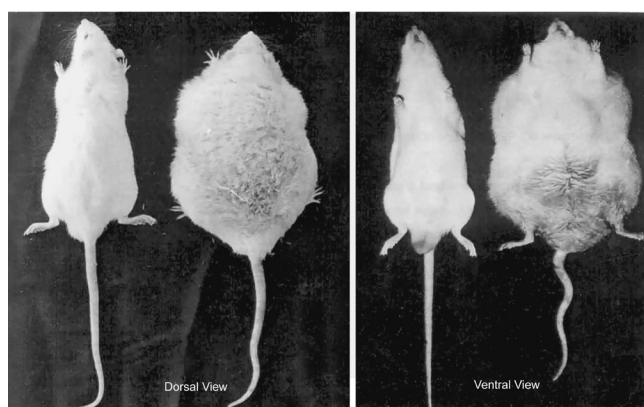


Fig. 1. WNIN/GR-Ob obese mutant rat dorsal and ventral view with lean counterpart.

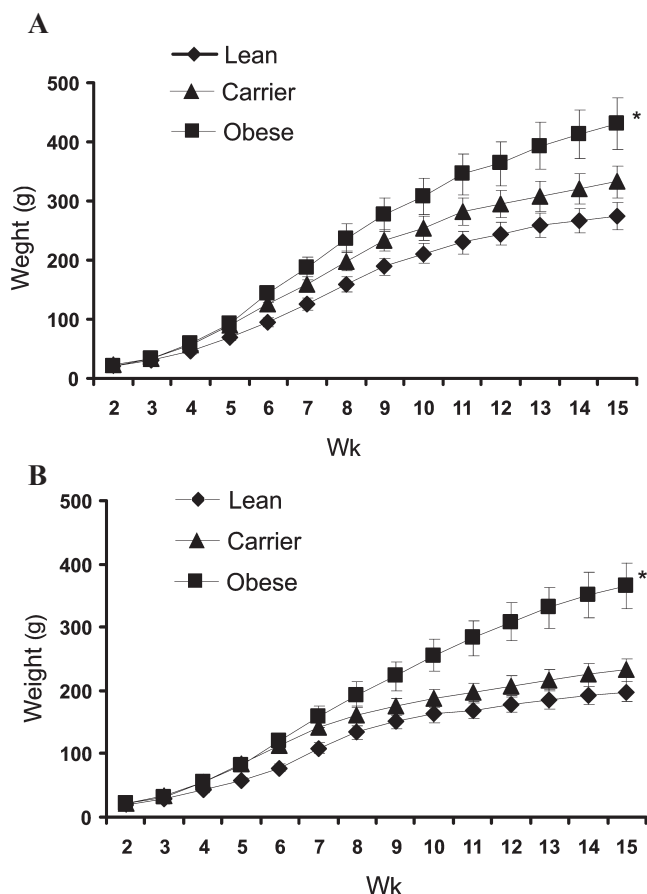


Fig. 2A. Growth pattern of male rats. **B.** Growth pattern of female rats. Each line represents the mean of observations in 30 rats of each phenotype. * $P < 0.05$ compared to lean and carrier rats, by two-way ANOVA followed by post-hoc least significance test.

Food intake: At 21 days, obese rats had significantly high food intake, when compared to lean rats ($P < 0.05$). Further, food intake increased along with increase in age and was found to be significantly different from

that of lean. At 15th week of age obese rats had an average food intake of 143.0 ± 8.93 g/rat/week and lean rats had 97.8 ± 7.09 g/rat/week ($P < 0.05$) (Fig. 3). The average food efficiency ratio (FER) of obese and lean counterparts is shown in Table I, the obese showing significantly higher values as compared to lean rats ($P < 0.05$). Daily water intake (192 ml) and urine output (59 ml) in obese rats were also significantly higher in comparison to those of lean rats (water intake 110 ml and urine output 37 ml) ($P < 0.01$). The per day foecal output in obese rats (25.2 g) was higher than that observed in lean littermates (19.7 g) with no difference in foecal nitrogen content. Besides traces of glycosuria, obese rats (4/6) showed significantly higher proteinuria as compared to their lean littermates ($P < 0.01$).

Body composition: The TOBEC analysis showed that, there was a significant difference in fat between the genders (high in males) in all the three phenotypes of WNIN/GR-Ob rat strain ($P < 0.05$). Obese mutants had significantly higher body fat and low LBM as compared to other two phenotypes (Fig. 4A and 4B). However, LBM of lean and carrier counterparts were not significantly different. In general, there was a significant decrease in total body water, sodium and potassium contents of obese rats of both sexes ($P < 0.05$).

Clinical chemistry: In general, no significant differences were seen between males and females of all the three phenotypes in their clinical chemistry parameters. Therefore, the data pertaining to these parameters are presented by combining the values for both the genders.

Glucose and insulin: With progression of age, insulin levels increased in all the three phenotypes, which was evident after 21 days of age. However, from 28 days

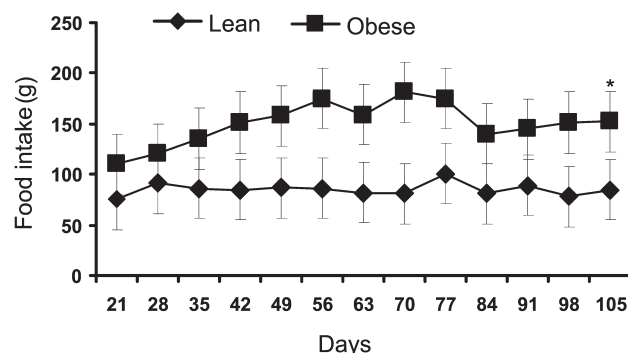


Fig. 3. Food intake of lean and obese animals. Each line represents a mean of observations in 12 rats of each phenotype. Significant differences were seen between the phenotypes at $P < 0.05$.

Table I. Growth table of WNIN/GR-Ob rats (n=12)

Phenotype	Body wt at 28 days (g)	Body wt at 105 days (g)	Weight gain (g)	Average food intake/rat/week (g)	Feed efficiency ratio
Lean	41.50 ± 0.81	290.58 ± 7.93	249.08 ± 14.23	103.28 ± 4.02	19.78 ± 2.65
Obese	67.16 ± 1.16*	513.00 ± 20.32*	445.83 ± 20.35*	175.15 ± 5.16*	25.67 ± 4.82*

Values are mean ± SE. * $P < 0.05$ compared to lean rats

Table II. Selected biochemical parameters in WNIN/GR-Ob rats (n = 12)

Parameters	Age (days)								
	21			28			35		
WNIN/GR-Ob	Lean	Carrier	Obese	Lean	Carrier	Obese	Lean	Carrier	Obese
Glucose (mg/dl)	51.05 ± 6.28	52.01 ± 6.24	92.22 ± 8.05*	57.24 ± 1.02	59.25 ± 2.05	94.00 ± 12.05*	110.10 ± 16.25	108.00 ± 12.02	134.00 ± 14.14*
Insulin (µu/mol)	13.00 ± 4.27	14.02 ± 2.07	14.84 ± 2.87	18.16 ± 2.67	21.05 ± 5.05	72.83 ± 4.67*	31.44 ± 2.09	32.01 ± 1.24	175.63 ± 35.02*

Values are mean ± SE. * $P < 0.05$ compared to respective lean and carrier rats

onwards, obese animals showed significantly higher insulin levels than their lean and carrier counterparts ($P < 0.05$). Further, the obese rats also had higher fasting blood glucose values ($P < 0.05$), compared to lean and

carrier counter parts, which was evident at 21 days, but more pronounced at 35 days of age.

Glucose tolerance curve at 0 (fasting), 1 and 2 h at 200 days for all the three phenotypes are shown in Table III. As per the criteria of impaired glucose tolerance derived by us⁷, only obese rats displayed abnormal glucose response, whereas the levels returned to near fasting levels in both lean and carrier rats. Glucose and insulin responses during the OGTT were calculated by computation of the total AUC of glucose and insulin respectively, using trapezoidal method²¹.

Figure 5A shows plasma insulin levels at 0 (fasting), 1 h and 2 hours after oral glucose load for the three phenotypes at 200 days of age. Obese rats had significantly high levels of insulin at all three time points ($P < 0.001$). While plasma insulin levels in lean and carrier rats returned to near fasting levels, this was not so with the obese rats. Figure 5B gives the average AUC insulin values, during OGTT for the three phenotypes at 50, 100, and 200 days and significant differences were found among the three phenotypes in the HOMA-IR values at time points tested. These were highest in obese (423.8) followed by carrier (77.2) and lean rats (26.6).

Glucose uptake studies: There was a significant reduction in the basal ($P < 0.05$) and insulin stimulated ($P < 0.05$) uptake of $U^{14}C$ -2 deoxy glucose by the diaphragm, in GR-Ob obese (-/-) rats as compared with euglycaemic lean (+/+) littermates (Fig. 6). Glucose uptake in obese rat diaphragm was significantly reduced to half of the basal and 40 per cent of insulin stimulated uptake observed in lean rat's diaphragm ($P < 0.001$).

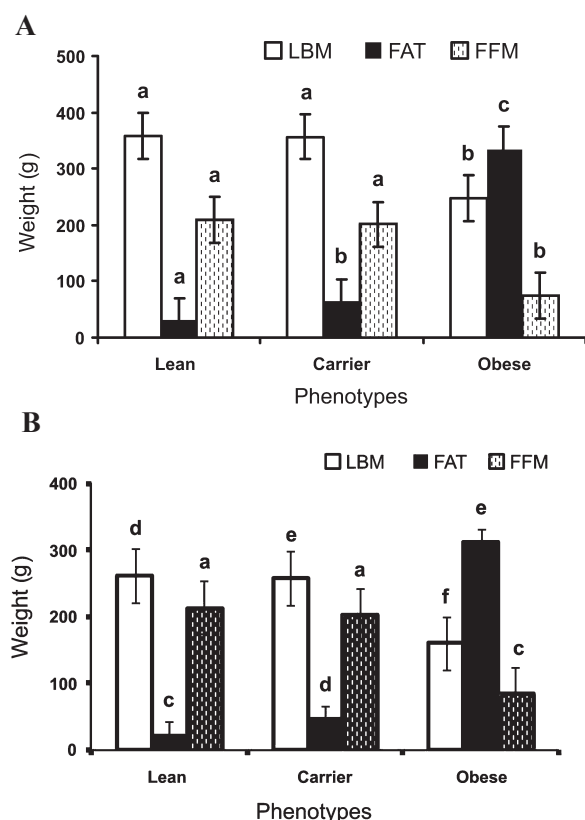


Fig. 4A. Body composition of male rats. **B.** Body composition of female rats. LBM : Lean body mass. Fat : Fat content. FFM : Fat free mass. Each bar represents a mean of observations in 12 rats. The line above each bar is the SEM. Means with different superscript letters are significantly different at $P < 0.05$, by one-way ANOVA followed by post-hoc least significant test.

Table III. Plasma glucose response in WNIN/GR-Ob rats. Significant differences were seen between the phenotypes and means with different superscripts are significantly different at $P < 0.05$, by repeated measures of ANOVA. Values are mean \pm S.E. (n=6) * $P < 0.05$. Time trend analysis at 50D – 50 Days; 100D – 100 Days; 200D – 200 Days.

Phenotypes	Fasting (mg/dl)			1 Hour (mg/dl)			2 Hour (mg/dl)			AUC (mg/dl)		
	50 D	100 D	200 D	50 D	100 D	200 D	50 D	100 D	200 D	50 D	100 D	200 D
Lean (+/+)	70.65 $\pm 2.58^a$	79.90 $\pm 3.44^b$	81.81 $\pm 2.48^b$	91.3 $\pm 3.07^a$	104.49 $\pm 3.83^b$	139.59 $\pm 8.07^b$	83.03 $\pm 3.23^a$	100.91 $\pm 6.02^b$	125.39 $\pm 7.53^c$	168.23 ± 4.79	194.96 ± 7.03	243.06 ± 9.95
Carrier (+/-)	83.05 ± 3.42	86.33 ± 3.67	80.16 ± 3.72	130.47 ± 4.93	119.93 ± 3.26	130.27 ± 7.67	123.59 ± 7.98	122.98 ± 6.05	125.30 ± 8.49	233.83 ± 6.83	223.33 ± 5.73	233.57 ± 12.28
Obese (-/-)	86.11 $\pm 2.83^a$	104.04 $\pm 4.45^b$	116.09 $\pm 4.27^b$	124.58 $\pm 4.49^a$	182.7 $\pm 17.86^b$	179.49 $\pm 9.01^b$	116.85 $\pm 8.02^a$	203.04 $\pm 25.87^b$	194.07 $\pm 12.74^b$	225.10 $\pm 7.02^a$	335.60 $\pm 29.12^b$	338.43 $\pm 13.54^b$

AUC, area under curve

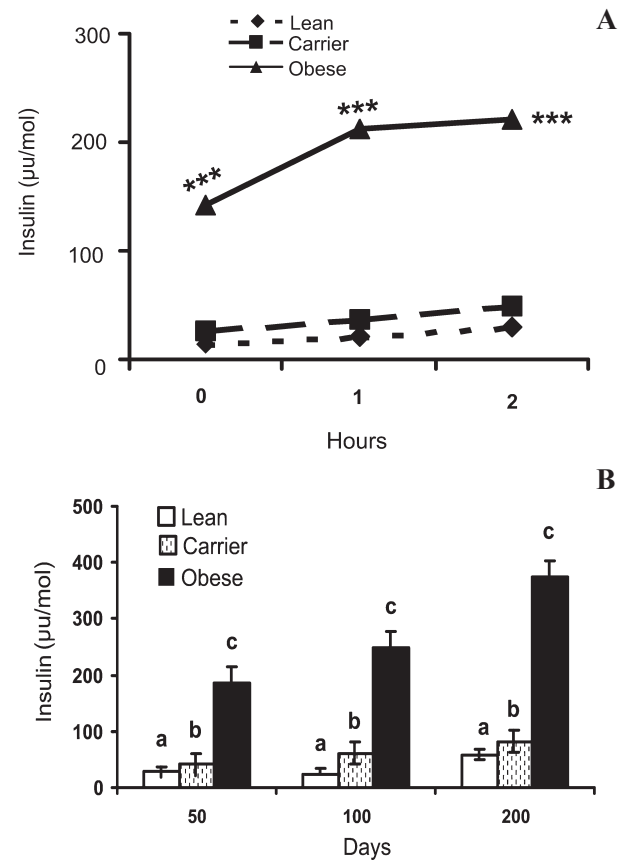


Fig. 5A. Insulin response in WNIN/GR-Ob rats. Each line represents a mean of observations in 12 rats of each phenotype. *** $P < 0.001$ compared to lean and carrier. **B.** Plasma insulin values (AUC) in WNIN/GR-Ob rats. Each bar represents a mean of observations in 12 rats. The line above each bar is the SEM. Means with different superscripts are significantly different at $P < 0.05$, by two-way ANOVA followed by post hoc least significance test.

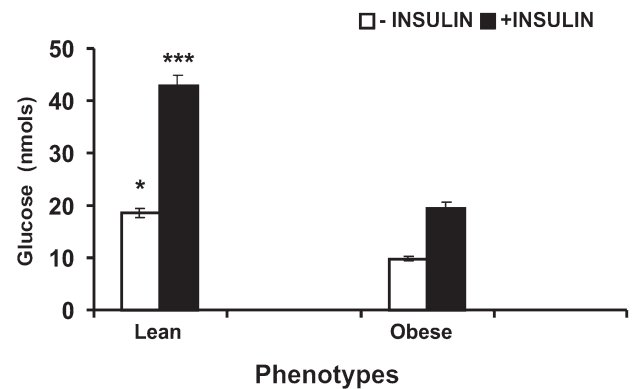


Fig. 6. Glucose uptake by diaphragm in lean and obese rats of WNIN/GR-Ob rats. Each bar represents a mean of observations in 6 rats. The line above each bar is the SEM. $P^* < 0.005$ *** $P < 0.001$ compared to obese.

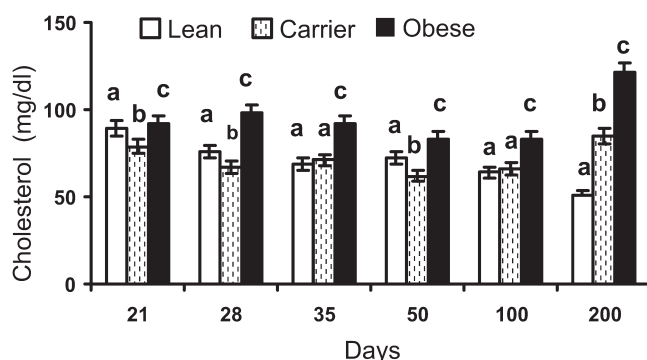


Fig. 7. Serum cholesterol levels of WNIN/GR-Ob rats. Each bar represents the mean of observations in 12 rats. The line above each bar is the SEM. Means with different superscripts are significantly different at $P < 0.05$, by two-way ANOVA followed by post-hoc least significance test.

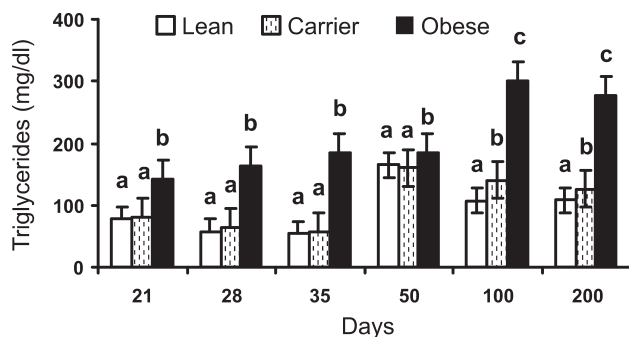


Fig. 8. Serum triglycerides levels of WNIN/GR-Ob rats. Each bar represents the mean of observations in 12 rats. The line above each bar is the SEM. Means with different superscript letters are significantly different at $P < 0.05$, by two-way ANOVA followed by post-hoc least significance test.

Lipids and leptin: Serum lactescence, *i.e.*, milky white serum is always seen in the blood samples of obese rats. Plasma cholesterol as well as triglyceride levels were found (Figs 7 & 8) to be significantly higher in obese rats as compared with their lean and carrier counterparts from 21 days onwards ($P < 0.05$). Routine screening of adult animals for lipid profile revealed that the obese mutant rats reached a maximum at 200 days compared to lean carrier counterparts. This trend was maintained at all ages in obese rats, however, the lean and carrier rats showed decreased plasma lipid levels with advanced age. At 200 days obese rats showed significantly higher serum leptin (19.0 ng/ml) levels ($P < 0.05$) in comparison with lean (0.77 ng/ml) and carrier rats (0.92 ng/ml), and a positive correlation was also established between phenotypes and serum

leptin levels with age ($r = 0.30$) as well as with body weights ($r = 0.74$) of three phenotypes.

Discussion

The Wistar rats, which originated from the Wistar institute of Philadelphia, USA was brought to our institute around 1920, and being maintained ever since as an inbred stock. In 1978, pedigree analysis of the breeding stock has revealed the existence of four parallel inbred lines in the colony, which could be traced back to four pairs of parents starting from 1965, and are shown to be different from each other by a battery of genetic monitoring techniques²². The lines were trimmed to a single line and maintained as an inbred strain, and redesignated as WNIN (to indicate its place of origin and identity). From this WNIN stock, initially, an obese mutant rat strain has emerged in 1993²³, and subsequently, a variant of the same with IGT trait, have been developed.

The sudden spurt in body weight between 4th - 5th week, and a peak by 15 wk (maximum weight of 470 g), was the same as in parental WNIN/Ob shown and similar to other obese rat strains like Zucker, Wistar diabetic fatty (WDF), Zucker diabetic fatty (ZDF) and Spontaneously hypertensive NIH-corpulent (SHR/N Cp)²⁴⁻²⁶. However, like its parent WNIN/Ob, and unlike in Zucker and Koletsky rats (WDF and SHR/N Cp) significant differences from lean littermates were discernable as early as 14 days with majority of them even showing higher birth weights.

Hyperphagia, a characteristic feature of all genetic and non-genetic models was evident in 28 days of age, irrespective of the gender. Although, hyperphagia is a feature of all the major obese mutants, it need not be a precondition to the development of obesity. For example in diabetic Chinese hamster, hyperphagia does not lead to obesity²⁷. However, the present mutant, like its parent obese phenotype showed significantly higher FER than its littermates, and this could be one of the reasons for the observed excessive fat deposits. Metabolic studies revealed polydipsia, polyuria and proteinuria, which are also seen in other obese rodent models. However, the observed proteinuria in obese rats of WNIN/GR-Ob was higher than that observed in obese parent (WNIN/Ob), and they also exhibited glycosuria, unlike WNIN/Ob. Higher dry faecal weight (more than even in its obese parent) observed in obese animals as compared to lean littermate, was a reflection of their hyperphagic nature.

TOBEC analysis of three phenotypes of the present mutant showed differences in LBM, total body fat, and fat percentage. The percentage of fat was very high (47%) in this model as compared to other models²⁸, however, significantly less than its obese parent. The data suggest that the higher fat content contributed to the observed higher weights observed in obese rats.

The present WNIN/GR-Ob mutant was result of a cross between euglycaemic obese WNIN/Ob, and lean WNIN/GR showing IGT trait at 4 months of age²³. These animals display glucose intolerance trait by 50 days of age, with glycoisuria. The onset of this trait is similar to SHR/Ob and ZDF, but different from other obese rat models like Spontaneous hypertension and heart failure/Mcc-corpulent (SHHF/Mcp) and Otsuka Long Evans Tokushima fatty (OLTEF), where there is a delay up to 8-12 months, respectively^{26,29}. Blood glucose levels on fasting were found to be higher in homozygous obese (-/-) animals, in comparison to their carrier (+/-) and lean (+/+) littermates, which became more pronounced from 35 days onwards. However, IGT trait could not be established in them at weaning (21 days), due to oral-glucose induced mortality at that age. By and large, the IGT trait is well established by 50-60 days age and is seen only in obese phenotype. The IGT trait appeared in both the sexes, unlike other models like WDF and SHR/N-Cp rats, where sexual dimorphism was seen with respect to the trait^{5,29}. It is also observed that these mutants are sensitive to dietary manipulations like Vc:WDF/fa-fa³⁰. Purified diets based on glucose, sucrose and starch are shown to increase the fasting blood glucose in these animals to diabetic levels by two to three months. These mutants can thus, replace conventional experimental models based on alloxon/streptozotocin treatment and are ideally suited for screening the potential efficacy of anti-obesity and anti-diabetic drugs (natural as well as synthetic).

Hyperinsulinaemia is an important entity associated with obesity with or without diabetes. The present model showed hyperinsulinaemia as its parental line, and the level was comparable to Zucker⁵, but lower than that of models like WDF, ZDF, SHR/N-Cp and SHHF/Mccp³⁰. While in Zucker rats, the insulin levels increase by 21 days³¹, here the increase was only at 28 days like its parental stock-WNIN/Ob. The spurt in body weight leading to visible signs of obesity occurred at 35 days in these obese mutants, and correspondingly, there was a substantial increase in insulin levels from this age onwards, in both the genders of all the three phenotypes. The HOMA-IR values at 200 days of age,

which are indicative of insulin resistance, were different amongst the three phenotypes, the highest being in obese. This was expected since in obese phenotype, obesity is associated with IGT, and this could be due to defective expression of insulin gene or signaling or both. There seems to be over expression of insulin gene in this model (data not reported), and also the signaling mechanism appears to be defective, as infusion of insulin *in vivo* into the hypothalamus did not elicit any reduction in food intake or weight in WNIN/Ob strain as compared to its lean and carrier counterparts (data not reported).

Basal and insulin-stimulated glucose uptake in the diaphragm showed significant decrease in WNIN/GR-Ob obese rats similar to parental strain²². Basal glucose uptake was severely affected as compared to that of obese rats. Besides this, differences were also seen in insulin-stimulated glucose uptake. This may be due to the fact that while the parental strain WNIN/GR is lean and WNIN/GR-Ob lean littermates (control) were all found to be normal, showing no insulin resistance. In the present WNIN/GR-Ob rats, the gene(s) responsible for IGT seem to be linked to obesity, which was inherited the IGT from the lean WNIN/GR parent and along with it, the insulin resistance from WNIN/Ob parent as well.

In obese rat models, ZDF rats showed the highest levels of cholesterol (474 mg/dl) while the lowest value were recorded in SHHF/Mccp (148 mg/dl)²⁶. WNIN/GR-Ob rats showed a value between 122 to 132 mg/dl at 200 days. Plasma cholesterol levels started to rise between 28-35 days of age in both the genders and obese genotypes. Hypertriglyceridemia, was also present in WNIN/GR-Ob mutants.

The role of leptin in the regulation of food intake is well proven. Except Ob/Ob mouse, all other obese models have high levels of leptin³². In db/db mouse, Zucker and Corpulent rats, leptin resistance is attributed to defective leptin receptor, and in agouti mouse it is due to defective melanin concentrating receptor (MCH) receptors³³⁻³⁵. In WNIN/GR-Ob mutants leptin levels were significantly higher as compared to their age and sex-matched lean and carrier rats. Its correlation with body weight was similar to that of other obese rat models³⁶. Thus, the present obese rats of WNIN/GR-Ob strain appear to be a leptin-resistant with IGT, which mimic human obesity with impaired glucose (pre diabetic or diabetic) metabolism.

Preliminary studies in our laboratory as well as our collaboration with Dr Friedman's laboratory at

USA have shown that the parental obese phenotype of WNIN/Ob strain does not have any defect either in leptin or leptin receptor locus (unpublished data). The available evidence as of now indicates the defect is on chromosome no. 5, located in an area close to leptin receptor, and studies are in progress to identify and sequence this locus.

In conclusion, the data presented here in terms of physical, physiological and biochemical parameters clearly establish the obesity and IGT traits of WNIN/GR-Ob model. The inheritance of obesity appears to be monogenic, incomplete dominant mutation in this model. This indigenous mutant along with its parental WNIN/Ob, are the first natural mutants to be developed from a wistar inbred rat colony and are genetically more pure. It is much easier to propagate our model, as the 'carriers' used for breeding and maintenance of WNIN/GR-Ob strain, are identifiable physically, by body mass index and kinky tail at an early age, even before biochemical differences becomes evident. 'Kinkiness' of the tail is a unique feature of the model, which is faithfully reproduced from its parental line, and is not seen in any other model. Fertility is fully reversible in these mutant obese rats, especially males by simple measures like diet restriction (data not reported). This mutant obese model with its pre-diabetic status can be easily transformed to 'frank' diabetic status by dietary manipulations and therefore, are more suitable for screening anti-diabetic drugs.

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