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A new rat model for the study of obesity

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Introduction

Wistar is the first strain of rat to be introduced in biomedical research, dating back to 1906 (*Lindsey* 1979). Essentially a randombred strain, several lines of this stock have been distributed throughout the world, and currently many institutions are holding Wistar stocks either in randombred or inbred status. A breeding nucleus of the original Wistar stock was brought to our Institute way back in 1920, and they were maintained in the animal facilities, ever since as an inbred stock. In 1978, pedigree analysis of the breeding stock was carried out, which revealed the existence of four parallel inbred lines in the colony. These lines could be traced back to 4 pairs of parents starting from 1965, for which breeding records were available. The strain was then redesignated as WNIN and the parallel lines as A, B, C and D. Recently, we observed a male "fat" rat in one of the litters of "A" subline. This animal had an abnormal body weight for its age and it turned out to be infertile. The parents of this fat rat was then identified and from its subsequent litters, heterozygous "carriers" could be isolated by test crossing. By selective breeding of these carriers a colony of obese rats is now established, containing all the three genotypes : lean (+/+), heterozygous carriers (+/ob), and obese (ob/ob). The colony is currently in F7 generation.

Some of the physiological and biochemical parameters carried out to establish obesity in these mutants are presented here. These include growth, attainment of sexual maturity, food and water intake, and analysis of plasma glucose, insulin, cholesterol and triglycerides. Carcass analysis was also carried out to find out the body composition of these rats.

Materials and Methods

Animals: The rats were housed in standard polypropylene cages at 22 ± 1°C with light – dark cycles of 12 hours duration. A standard rat chow prepared at our facilities (containing 56 % carbohydrates, 18.5 % protein, 8 % fat, 12 % fibre and recommended levels of minerals and vitamins) and water were provided ad libitum to the animals. Sterilised paddy husk was used as the bedding material and this was changed twice a week.

Breeding: The rats were introduced for mating at 90-110 days of age, and harem mating was practised with a ratio of one male to 2 females. Rats were weighed at the time of mating and pregnancy was confirmed by palpation on 10th day and weight increase on 16th day. The females which were confirmed to be pregnant were separated on 16th day and housed singly. Sterilized paper cuttings were provided as nesting materials. Upon delivery, the pups were sexed at birth, and weighed. The young ones are weaned at 21 days and housed as groups of 3-4 males or females.

The animals were examined from birth to adulthood for any abnormality or defects.

Food intake: 20 animals (10 obese and 10 lean) were selected from 14 days and were monitored for food intake from 21-126 days. Weekly records were maintained for both food intake and weight gain. Food efficiency ratios (FER) were also calculated (*Hoover & Nelson* 1985). Growth: 60 animals were used for growth studies, consisting of 30 lean and 30 obese of equal sex. The animals were weighed weekly using an electronic balance (Essae-DIGI, 0.1g sensitivity) from 14 to 105 days. The females were monitored for attainment of puberty, by observing the days taken for opening of vagina. Metabolic studies: 6 adult males and 6 adult females of 200 days of age from obese and lean genotypes were selected and individually housed for a week in metabolic cages (Techniplast, Italy) to study food and water intake, urine and faecal output. Food and water intake were measured daily and records of urine (collected under xylene) and faecal output of individual animals were main-

Table 1. Breeding Data.

Generations	No. of matings	Total pups	Ave. Litter size	Ave. Birth wt.	Lean including carier			Obese			% of Lean	% of Obese
							Total			Total		
F 0	5	35	7.00	5.90	17	10	27	3	5	8	77	23
F 1	14	104	7.28	6.02	50	47	97	3	2	5	95	5
F 2	16	143	8.94	6.19	61	58	119	17	8	25	83	17
F 3	14	88	6.28	6.21	34	38	72	10	6	16	82	18
F 4	14	92	6.57	6.25	34	46	80	7	5	12	87	13
F 5	25	180	7.20	6.11	78	82	160	14	6	20	89	11
Total	88	642	7.29	6.12	274	281	555	54	32	86	86	14

% of male Obese = 63
% of female Obese = 37

tained. Urine was also examined for proteinuria and glycosuria using Ame's multiple reagent strips (*Unistix, Miles inc, USA*). Faecal protein was estimated by macro Kjeldahl's method (*Osler* 1965).
Glucose, insulin and lipid profile: 14 obese and 14 lean rats of 6 months age were used for the estimation of plasma glucose, insulin, cholesterol and triglycerides.
For oral glucose tolerance test, the animals were fasted for 16 hours, following which an oral dose of glucose (250 mg/100 g body weight) was given through gavage. Blood was collected by retroorbital route (*Riley* 1960) before and 1 hour and 2 hour after glucose load. Blood was collected in vials containing sodium fluoride and potassium oxalate and the plasma was separated and stored at -20°C until analysis. Glucose and insulin was estimated in plasma at fasting as well as after one and two hour glucose load. Glucose was estimated by the kit provided by Stangen immunodiagnostics, Hyderabad, India, based on Trinder's method (*Trinder* 1969). Insulin levels were measured by the double antibody method (*Morgan & Lazzaro* 1962) using an insulin radio immuno assay kit provided by Bhabha Atomic Research Centre, Bombay, India, with porcine insulin as the standard.
Cholesterol and triglyceride levels were measured in fasting blood plasma, using the kits provided by Stangen Immunodiagnostics, Hyderbad, India. The kits for cholesterol and triglycerides are based on Allain's modified method (*Allain et al.* 1974) and the method developed by Fossati and Prencipe (*Fossati and Prencipe* 1982) respectively.
Carcass analysis: 6 adult animals of equal sex from obese and lean groups were taken and sacrificed under excess anaesthesia (using anaesthetic ether). The animals were then processed for carcass analysis and estimation of fat as per the procedure of Rathburn and Nellopace (*Rathburn and Nellopace* 1945). Total nitrogen and ash contents estimated using standard methods (*Osler* 1965). Statistical analysis: Values are reported as the mean ± standard error. Significance of differences between variables were calculated using unpaired student's t-test. A p value of 0.05 or less was considered significant.

Results

Breeding: Table 1 shows the breeding data of the colony upto 5th generation. The average litter size of the colony was 7.29 g and average birth weight was 6.12 g. The percentage of obesity in the colony was 14 %, of which males contributed 63 % and females, 37 %.
General observation on "obese" animals: The animals did not show difference in appearance till they attained 35 days of age. From 35th day onwards signs of obesity was visible in weaned rats, in terms of visible and palpable fat and body shape (Retrospectively these animals also showed higher body weights from birth onwards). By 50-60 days, the tails of these animals showed bending at two

Photograph 1. Adult obese rat with its lean litter mate. Note the kinkiness of the tail in the obese rat.

places, proximal as well as at the distal end (Photograph 1). There was no indication of such defect at earlier stage of life and tails at birth appeared normal. As the animals reached adulthood, all the obese animals had a "rotund" appearance, obliterating the neck region altogether (photo 2). Their hair coat especially at the back, appeared yellowish and dirty due to smearing of urine and faeces. The animals moved minimum with in the cages, and they always laid on their backs keeping their head close to food pellets and water spouts. By 100-120 days, the grooming behaviour was totally lacking in these animals.

Food intake: The average food intake of obese and lean rats per week from 3rd to 18th weeks are given in Figure 1 and Table 1. While the average food consumption of lean rats were around 12 g per day this was almost double in obese animals, i.e. 22 g. The FER value of obese rats was significantly higher than that of lean rats (Table 2).

Growth: The average body weights of lean and obese animals from 2 to 15 weeks are given in Figure 2. The animals could be identified as "lean" and "obese" from 35 days onwards with visible signs of "fatness" in the latter. From 5th week onwards, the obese animals had an average weight gain of 5-6 g per day compared to 2 to 2.5 g of lean. Even at 14 days there was significant difference between lean (♂19.9 ± 0.91 g, ♀20.8 ± 0.85 g) and obese (♂26.1 ± 3.5 g, ♀25.5 ± 3.3 g) animals. There was a marked difference in the weights of lean males and lean females from 8th week onwards. However, in obese animals the weight difference became prominent only after 11th week, and even at the end of 15th week the differences in weights between male and female (♂503.1 ± 18.6 g, ♀466.0 ± 3.2 g) was not as high as seen in lean animals (♂302.4 ± 7.6 g, ♀214.2 ± 2.5 g).

Attainment of puberty, as judged by days taken for the opening of vagina in lean and obese female rats is given in Figure 1. While the vagina opened (Pho-

Figure 1. Food intake of lean and obese rats.

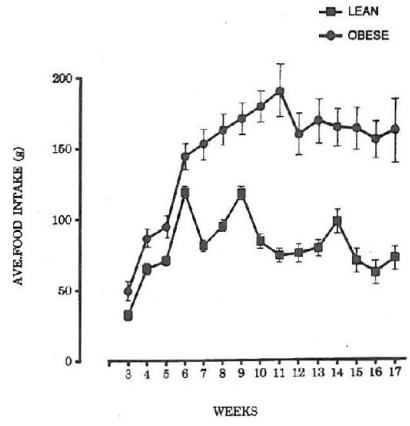
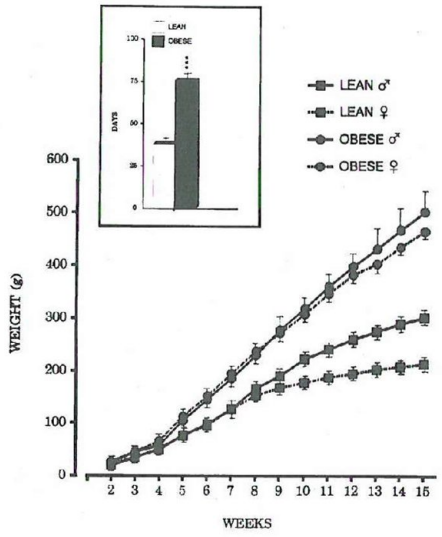


Table 2. Food intake and FER values in Lean and Obese rats.

Groups (n = 10)	Body wt. (g) (d 28)	Body wt. (g) (d 126)	Gain in body wt. (g)	Food intake /week (g)	FER
Lean	35.57 ± 1.42	282.97 ± 8.77	247.40 ± 2.10	80.29 ± 5.37	21.75 ± 3.10
Obese	52.88*** ± 2.13	612.13*** ± 29.78	669.25*** ± 2.30	147.38*** ± 9.75	27.94*** ± 2.99

Values are Mean ± SE. *** $p<0.001$ by students' t-test.

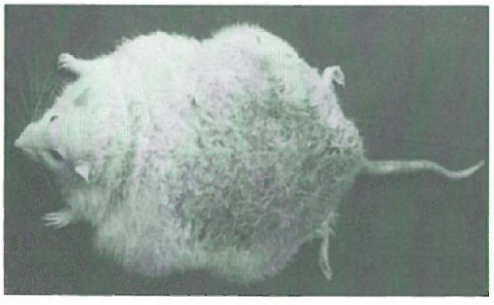
Figure 2. Growth curves of lean and obese rats (♂ &♀). The weights of the obese rats were significantly different ($p<0.001$), from lean rats at 105 days, and even at 14 days ($p<0.001$).



Days taken for opening of vagina in lean and obese rats ($p<0.001$).

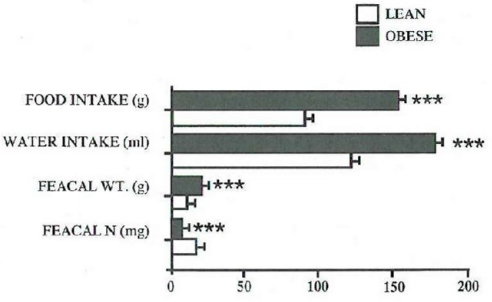
to 3) on 38th day in lean animals (38.3 ± 0.54) this was very much delayed in obese animals, i.e. 76 days (75.6 ± 1.83).

Metabolic studies: The results of the metabolic studies are given in Figure 3 & Table 3. The obese animals consumed more food (151.2 ± 5.83 g) and water (175.7 ± 10.52 ml) compared to lean control



Photograph 2. Adult obese rat, weighing 1300 gms.

Figure 3. Metabolic studies in lean and obese rats. Values were significantly different at $p<0.001$.



(89.1 ± 2.34 g and 120.5 ± 5.8 ml). Faecal output (22.7 ± 0.68 mg) and faecal nitrogen (8.89 ± 0.17 mg) were also significantly differ in obese compared to lean animals (8.58 ± 0.34,15.87 ± 0.62). The urine output between obese and lean rats was not statistically significant (Table 3) though some animals in the latter showed higher urinary volume (50-60 ml). Majority of obese animals (8/12) showed high levels of protein in the urine (Table 3).

Table 3. Urine volume and Proteinurea in lean and obese rats.

Groups (n=12)	Urine volume (ml)	Proteinurea (mg/dl)
Lean	37.58 ± 2.38	28.33 ± 7.25
Obese	41.66 ± 5.01	618.33 ± 232.52[$]

[$]Animals showed large variation. 8/12 animals showed protein values ranging from 100 to 2000 mg, while others showed an average value of 30 mg.

Plasma glucose, insulin, cholesterol, and triglycerides: The analysis of these parameters in lean and obese animals are shown in Figure 4 & Table 4. While the blood glucose was normal at all levels i.e., fasting, as well as 1 and 2 hours after glucose load, for lean and obese animals, the insulin levels were significantly higher in the latter at all time points (Table 4). Serum lactescence, i.e. milky serum was always seen in obese animals. Thus, the cholesterol as well as triglyceride levels were

Table 4. Plasma Glucose and Insulin levels in Lean and Obese rats.

Para-meters	LEAN			OBESE		
	0 hr	1 hr	2 hr	0 hr	1 hr	2 hr
Glucose (mg/dl) (n=12)	78.26 ± 3.23	116.78 ± 5.59	109.85 ± 4.82	72.90 ± 4.67	102.63 ± 5.04	98.49 ± 7.20
Insulin (μU/ml) (n=9)	15.75 ± 1.00	36.74 ± 2.58	24.51 ± 3.11	138.83*** ± 13.04	198.86*** ± 11.16	188.48*** ± 10.35

Values are Mean ± S.E. *** p<0.001. (Students' t-test for Insulin).

found (Figure 4) to be significantly higher in obese (134.1 ± 8.5 and 309.7 ± 32.06 mg/dl) compared to lean controls (81.2 ± 4.9 and 94.1 ± 8.9 mg/dl).

Carcass analysis: The body composition of lean and obese rats as revealed by carcass analysis is given in Table 5. While the fat content increased fourfold in obese (47% in obese vs 9% in lean), the tissue and ash contents were reduced to half (16 and 17% tissue and ash content in obese compared to 27% and 31% in lean). The nitrogen content was similar in both the genotypes.

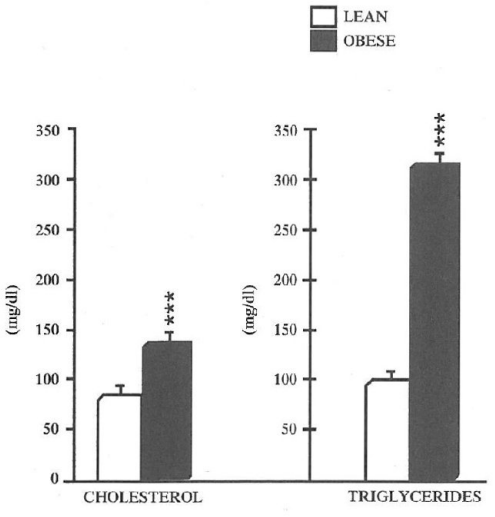


Figure 4. Cholesterol and triglycerides values in lean and obese rats. The values of obese rats were significantly different from lean rats at p<0.001.

Discussion

Historically, the first description of an obese rodent was made by Cuenot (*Cuenot* 1905), wherein he observed that mice with yellow coat colour are obese. Subsequently, Hetherington and Ranson produced obesity in rats by selective electrolytic destruction of regions in hypothalamus (*Hetherington & Ranson* 1940). Since then a number of models of both genetic and experimentally produced obesity have been described in mice and rats and voluminous literature is now available presenting a range of phenotypes similar to that seen in humans. While six single gene mutations contributing to obesity are identified in mouse (*Coleman* 1982), only a single gene mutation i.e. fatty (fa) is identified in rat. The "fa" mutation arose spontaneously in the Zucker (13M) rat strain (*Zucker & Zucker* 1961). A second occurrence of the mutation, originally designated "f" (*Koletsky* 1973) and later renamed Cp (*Greenhouse et al.* 1988) was identified as "fa" allele (*Yen et al.* 1977). All the existing rat obese strains currently known have thus either come from Zucker or Koletsky rats (carrying Cp gene) in combination with other rat strains like Wistar/Kyoto, SHR and Wky (*Greenhouse et al.* 1990). And it should be remembered that Zucker and Koletsky rats have an outbred background to begin with. So far, no obese mutation is observed in any of the existing inbred rat strains. We are reporting for the first time, the occurrence of such a mutation, in our Wistar, inbred colony, viz., WNIN.

Like the Zucker rat, the obese mutation seen here also is carried as an autosomal recessive trait, affecting both the sexes, with preponderance in ma-

Table 5. Carcass analysis of Lean and Obese rats.

Groups (n = 6)	Body wt. (g)	Fat (g)	Tissue (g)	Nitrogen (mg)	Ash (mg)
Lean	381.50 ± 16.73	34.30 ± 5.31	105.23 ± 3.55	11.23 ± 0.40	32.52 ± 1.10
Obese	566.83*** ± 48.03	265.31*** ± 19.28	86.35*** ± 6.27	11.52*** ± 0.38	14.68*** ± 1.07

Values are Mean ± SE. *** $p<0.001$ by students' t-test.

les. Mating between heterozygous carriers always yielded a typical Mendelian ratio of 3:1, lean to obese animals. The true nature of obesity in our obese mutants is revealed by carcass analysis with fat accounting for as much as 47%. The lower tissue and ash contents in animals is a true reflection of the small body frame seen in them. The present mutant shares several traits with that of Zucker rat like hyperphagia, polydipsia, euglycemia, hyperinsulinemia, and hyperlipidaemia. Majority of the animals also showed high proteinuria as seen in Zucker rats (*Shimamura* 1982).

Like Zucker rats, visible and palpable fat is seen only by 5th week in these mutants too. But unlike Zucker and Koletsky rats, significant differences in weights from the lean littermates can be seen in them as early as 14 days, with majority of them even showing higher birth weights. Another striking feature is the lack of significant weight difference between the male and female obese rats even at 15th week of age. Though a marginal difference is seen from 11th week onwards, the average weight gain is more or less same in both the sexes. However, in Zucker rats, the deviation in growth between males and females occur as early as 8th week and by 15th week a significant weight difference is seen between sexes (*Bray* 1970). The peak velocity of growth is reported to differ between male and female rats (*Watson et al.* 1977). While the female show greater growth spurt till puberty, in males this occurs at a later stage. In the present study, while the lean animals showed this pattern clearly, it was not evident in obese animals. This difference between obese and lean rats is also reflected in the delay of sexual maturity seen in the latter, as judged by the days taken for the opening of vagina. The delay seen here is much more than that is reported for Zucker rat (*Saiduddin et al.* 1973). Further, we also observed (data not reported) a longer oestrous cycle of 10 to 12 days (6 days in Zucker rats) with 5-6 days of prolonged oestrous in these mutants. The male obese rats showed reduced testicular size (data not reported) as seen in Zucker males (*Deb & Martin* 1975). The hormone profile of these mutants especially the reproductive hormones is currently under investigation.

The Tail "kinkiness" distinctly visible around 50-60 days is a unique characteristic of these mutants, not shown by any other obese model so far known. This was found to be due to fusion of caudal vertebrae (between 5th-8th mostly, and 13th-14th occassionally) as revealed by x-ray analysis of the tail. The accumulation of fat around the lower abdomen by this age may be responsible for such a defect. Even carrier rats show this trait and it is now used in our colony to identify carriers and

Photograph 3. Difference in the sexual maturity of lean and obese rat. Vagina opened at normal time in lean rat (left), vagina yet to open in the obese (right).

obese animals from lean animals.

The present obese mutant is designated as WNIN/ob, to indicate its origin from an inbred strain of Wistar, maintained at National Institute of Nutrition. The percentage of obesity currently in our colony is around 14%. We hope to improve the production in future, by diligent selection of "carriers" and "willing"obese males (*Ruthkava et al.* 1990) in the breeding programme. We also obtained another, mutant from the same colony showing impaired glucose tolerance (IGT), and efforts are being made to perpetuate this mutation as well.

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Summary

The currently used rat models of obesity and diabetes are derived from either Zucker or from Koletsky rats. Recently, we identified a spontaneous obese rat from our Wistar colony which is maintained as an inbred stock for the past 75 years. Initially, one of the male progeny in a litter was observed to have abnormal body weight for its age. The parents of this rat were identified, the progeny selectively bred, and a colony has been developed. This is designated as WNIN-Ob. The colony is mantained by mating heterozygous animals (+/ob), as the homozygous (ob/ob) were found to be infertile. The trait is carried as an autosomal recessive mutation and the colony is currently in F7 generation.

Obesity is visible in these mutants around 35 days of age. They are hyperphagic and reach a body weight of 500-600 g by 105 days of age. "Kinky" tail is characteristic of this mutant and this is visible around 50-60 days. Sexual maturity is delayed in female obese mutants, as judged by the day of vaginal opening. The animals are euglycemic and show hyperinsulinaemia, hypertriglyceridaemia, and hypercholesterolemia. Another mutant showing hyperglycemia is also obtained from the obese colony. Unlike earlier models which are essentially derived from a random-bred stock, this is the first report of a rat obese model, developed spontaneously from an inbred strain.