A Nude Mutant Derived from Five Decades-Old Rat Colony with an Immuno-potent Character - A Potential Model for Non-Communicable Diseases.

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Running Title: Immunocompetent nude rat model for metabolic diseases

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Abstract:

Background: A spontaneous mutant rat with a hairless phenotype and an intact thymus was discovered in a long-standing SD/NIN rat colony at a national animal resource facility.

Objective: We conducted extensive phenotypic and biochemical analyses on this mutant strain to determine its suitability as a preclinical model for immunocompetent testing in non-communicable disease research.

Methodology: To accomplish this objective, we subjected the mutant rats to strict and frequent phenotypic and genetic surveillance. We assessed their food intake, body weight, blood cell profile, clinical chemistry, adipose tissue deposition, and bone mineral density using TOBEC and DXA analysis.

Results: Initially, only two hairless mutant rats, a male and a female, were born from a single dam in the SD/NIN rat strain. However, the results indicate that the mutant colony propagated from these unique pups displayed distinct phenotypic features and exhibited differences in feeding behaviour, weight gain, and clinical biochemistry. The food conversion rate (FCR) was significantly higher in nude females (2.8-fold) while 26% lower in nude males. Both sexes of nude rats had significantly higher triglycerides and lower glucose levels in females. However, glucose levels did not change in male nude rats. Furthermore, nude female and male rats had significantly lower fat (TOBEC) and bone mineral content (DXA). Nonetheless, bone mineral density was only slightly lower (7-8%) compared to the heterozygous groups.

Conclusion: These findings indicate that the spontaneous mutant rat has the potential to serve as an immuno-potent and modulatory testing system in PK/PD and toxicology, which can be further explored for therapeutic drug discovery.

Keywords: Nude rat, Sprague Dawley, TOBEC, DXA
Introduction

In animal models, hair can interfere with the observation of various biological processes such as blood vessels, tumours, subcutaneous cell transplantation or device implantation, and lesions. Hairless rat or mouse models are typically used for such experiments, but these models lack a complete immune system, which does not accurately mimic the patient's situation. To study immune responses in such experiments, immunocompetent nude rodents are preferred. Although such models exist in different parts of the world with various background strains, this publication focuses on describing the characteristics of nude rats established in the NIN animal facility.

The nude rat used in this study is a spontaneous mutation in the Sprague Dawley - National Institute of Nutrition (SD/NIN) rat strain, which is a rat colony that has been maintained at the ICMR-NIN animal facility in Hyderabad, India for over 50 years. The mutation was discovered as a spontaneous model in the SD/NIN rat colony, and this identified population was selectively propagated and characterised over the next 50+ generations. The characteristics of the nude NIN/SD rats were analysed in terms of growth curve and serum parameters of liver, kidney and metabolism to obtain baseline data for further research.

Materials and Methods

The NIN animal facility selectively bred and housed mutant nude rats and their parents under controlled conditions. The facility maintained a fresh air supply with an air exchange cycle of 12-15 cycles/hour and controlled humidity and temperature levels between 50-70% and 22-28 degrees Celsius, respectively. The animals were subjected to a 12-hour dark and 12-hour light cycle to maintain their circadian rhythms. After receiving approval from the Institutional Animal Ethics Committee (No. P40F/IAEC/NIN/11/2012), the experiment was conducted at ICMR-NIN, which is a facility authorized by CCSEA for breeding and research purposes.
The study compared male and female animals from four groups of SD/NIN rats, including homozygous (female and male, nude rat), heterozygous (female and males, rat with hair), identified as ‘hr−/−’ and ‘hr+/−’, respectively. A total of 24 rats, including 6 males and 6 females of both homozygous (‘SD/NINhr−/−’) and heterozygous (‘SD/NINhr+/−’) were used in the study. The animals were 35 days old and observed for 90 days for feed intake and body weight gain. The rats were housed in open cages and provided water *ad libitum*. Feed intake was measured by offering 30 grams of standard pelleted diet (18% protein content) and weighing the remaining feed the next day at the same time.

For body composition analysis, injectable anaesthetics (Inj. Ketamine and Inj. Xylazine) were administered to the animals at a dose of 70mg/Kg and 2.5mg/Kg, respectively.[1] Analysis was performed using Total Electrical Body Conductance (TOBEC) and Dual Energy X-ray Absorptiometry (DXA). TOBEC analysis was performed using the instrument "EM - SCAN" (also known as TOBEC) with the tube model "SA - 3000" from TOBEC (Multidetector, Springfield, III, USA) to scan the animals in this study.[2] This approach is non-invasive and measures several parameters such as fat-free mass (FFM), total fat percentage (Fat%), total body sodium (TBNa), total body potassium (TBK) and total body water (TBW). The animals were stabilised with chemical sedation prior to body composition testing.[3] Mathematical calculations were used to determine the body composition parameters.[4] The DXA scan was performed using the "Hologic Discovery Wi" bone densitometer, which provides data on anatomy, bone density, bone content and lean mass.

Afterward, the experimental rats had blood samples extracted from the retro-orbital sinus[5] to conduct a complete blood count and plasma analysis. The plasma was evaluated for liver-specific markers (albumin, total protein, bilirubin), kidney-specific markers (creatinine, urea), obesity-related/metabolic disorder markers (total cholesterol, triglycerides, glucose), and tissue damage-specific enzymes (ALT-alanine transaminase/SGPT-serum glutamate pyruvate transaminase, AST-aspartate transaminase/ SGOT-serum glutamate oxalate transaminase, alkaline phosphatase) using ACE Alera® (Clinical Chemistry System).
The study concluded by euthanising all 24 rats through CO$_2$ asphyxiation,$^6$ followed by gross examinations and organ removal for histopathological evaluations.

**Results**

During necropsy, both homozygous and heterozygous rats had a thymus, which is in contrast to previous findings in nude rats. There were no significant changes in the other organs. The study also compared the body composition and feed intake of nude rats with heterozygous rats. The feed intake was higher in nude rats than in heterozygous rats, and the body weight was also higher in the former. Feed intake increased until day 63 in both nude rats and heterozygous rats of both sexes. The mean food intake was higher in nude rats but lower in heterozygous rats, and the feed conversion ratio was higher in the nude rats. However, the body weight of all groups increased until 63 days of age and remained stable thereafter, but adult males had a higher weight than females (Figure 1A-F). The feed conversion ratio was also higher in nude rats, indicating a higher metabolic rate (Figure 2). Serum biochemistry markers showed high albumin levels in female nude rats, while male nude rats had slightly lower levels of total protein and bilirubin. There were no major changes in creatine, electrolytes, and glucose (Figure 3-4). In addition to above, body composition was assessed using DXA (Figure 5) and TOBEC (Figure 6). Interestingly, the results indicate that fat percentage and bone mineral content was lower in nude rats compared to heterozygous rats.

**Discussion**

During necropsy, the thymus was found to be present in both SD/NIN rats (hr$^{-/-}$ and hr$^{+/-}$), in contrast to the previous literature which described nude rats.$^{7-8}$ The absence of significant alterations in the remaining organs, along with supporting FACS data (which is currently unpublished), renders this model advantageous for research studies that necessitate a robust immune response and require the animals to be housed in a barrier-free environment.
Regarding feed intake and body weight, both homozygous (nude rats) and heterozygous rats of both sexes exhibited a gradual increase in feed intake until day 63\textsuperscript{th} (9 weeks) (Figure: 1A). After 9\textsuperscript{th} week, the mean food intake was higher in nude rats (22.53 grams/day for females and 27.47 grams/day for males), while it was lower in heterozygous rats (16.52 grams/day for females and 20.12 grams/day for males) (Figures: 1B & 1C). In comparison, feed intake was 36\% higher in both male and female nude rats compared to heterozygous rats. Additionally, protein concentration also had an impact on the amount of feed intake. Generally, an adult laboratory rat receives 15 grams of feed per day for maintenance, 15 to 20 grams per day during gestation, and 30-40 grams per day during lactation.\cite{9}

The TOBEC technique was used to assess body composition at various time points after 3 months of age, alongside DXA. In comparison to heterozygous rats, female nude rats showed a low-fat percentage of 37\% in the TOBEC analysis, whereas male rats had a low-fat percentage of 31\% (Figure: 6A & 6B). The fat-free mass (FFM) and lean body mass (LBM) did not change significantly (1.05-fold and 1.03-fold, respectively) between nude and heterozygous female rats, but the percentage of fat was lower (0.63-fold) in nude female rats.

Furthermore, the percentage of total body potassium (TBK), total body sodium (TBNa), and total body water (TBW) were extrapolated from an e-value of TOBEC, and no significant differences were observed between nude female rats (1.05-fold, 1.05-fold, and 1.05-fold, respectively) and heterozygous female rats (Figure: 6D). Similarly, there were no significant differences observed in FFM\% (1.04-fold) and LBM\% (1.03-fold) between male nude and heterozygous rats, but the fat percentage was lower in nude male rats (0.69-fold). Additionally, the electrolytes (TBK and TBNa) and water content (TBW) were similar in nude (1.04, 1.04, and 1.01, respectively) and male NIN/SD rats (Figure: 6C).

Using DXA for body composition analysis, a week after TOBEC, it was found that the nude rats (both female and male) had a slightly reduced fat percentage (Figure 5A & 5B), while their lean body mass remained unchanged (female: 1.06-fold, male: 1.02-fold). Additionally, the DXA analysis showed lower
bone mineral content (BMC) (female: 0.79-fold, male: 0.76-fold) and bone mineral density (BMD) (female: 0.93-fold, male: 0.92-fold) in the nude rats (Figure 5C & 5D). The feeling of cold on bare skin generally requires burning of fat, particularly brown fat, to generate more energy.[10] However, certain animals like the nude mole rat have developed the ability to reduce energy requirements by enduring hypoxia to anoxia for up to 3 hours.[11]

The serum biochemistry markers revealed interesting gender-based differences in nude rats. Females showed a significant increase in albumin levels (1.29-fold), while males exhibited no such change (0.98-fold). Interestingly, total protein (TP) and bilirubin (BIL) remained unaffected in nude females (1.01-fold and 1.02-fold, respectively), while males had slightly lower levels of TP (0.92-fold) and BIL (0.91-fold) compared to heterozygous male rats (Figure: 3A & 3B). Surprisingly, creatine (CRE) levels did not show significant changes in either female (0.93-fold) or male (0.96-fold) nude rats. However, urea levels were increased in both female (1.20-fold) and male (1.12-fold) nude rats compared to their counterparts in heterozygous rats of each gender (Figure: 3C & 3D).

In female nude rats, glucose levels were significantly low (0.77-fold), while male nude rats showed no significant change (1.04-fold). Interestingly, total cholesterol (TC) levels were markedly high in both female (1.31-fold) and male (1.26-fold) nude rats, while serum triglycerides showed no significant changes (female: 1.04-fold, male: 1.08-fold) (Figure: 4A & 4B). Notably, albumin levels were high (29%) in nude females, as was blood urea (20%), while creatine (a renal marker) showed minimal changes. The ratio of urea to albumin was also not significantly different, whether in nude females (11.8) or males (10.2), or heterozygous rats (females: 10.3, males: 8.87).

In comparison to heterozygous rats, both male and female nude animals exhibited higher levels of total cholesterol. However, in female nude animals, glucose levels were 23% lower, which may help to hinder further cholesterol deposition. Some studies suggest that elevated glucose levels can impact cholesterol transport and metabolism,[13] whereas high cholesterol levels can alter glucose metabolism in return.[14]
Despite having a high feed intake, nude female animals maintained low glucose levels and weight, which is surprising and requires further investigation. While numerous studies have linked blood glucose levels to cholesterol levels,[15-16] the nude animals in this study seem to possess a mechanism that challenges this hypothesis.

Additionally, triglyceride levels were normal in both sexes of nude rats, possibly due to the high energy requirements of glucose consumption that prevents triglyceride deposition or keeps them at a normal level, allowing this model to control glucose effectively.[17]

AST (aspartate transaminase) liver enzymes showed no significant changes (Figure: 4C & 4D) between NIN/SD rats and nude animals (female: 1.02-fold, male: 0.98-fold). However, ALT (alanine transaminase) was lower in nude rats (female: 0.87-fold, male: 0.84-fold), which can be caused by factors such as lower muscle mass.[18] It should be noted that a decrease in ALT does not necessarily indicate a negative health condition.

Furthermore, the enzyme ALP (alkaline phosphatase), which is related to bone metabolism, was found to decrease in male nude rats (0.79-fold) but not in female nude rats (0.96-fold). This reduction in ALP levels corresponds with the DXA results showing low BMC and BMD in male nude rats, but not in females. The discrepancy in females could be due to several factors, including multiple myeloma and osteoporosis.

In addition, it is possible that low ALP values in male nude rats could be due to protein deficiency (malnutrition) as they consumed less food than females, although there was no correlation found in TOBEC and DXA (FFM% and LBM% were normal).

The results of the haematological profile showed no changes in the absolute numbers of platelets, erythrocytes, and white blood cells (as seen in Figures 30-31). However, the nude rats exhibited a higher number of monocytes and a lower number of eosinophils, at 1.10-fold and 0.78-fold, respectively. It is important to note that the increase in monocytes may indicate various pathologies, such as chronic
infections, chronic stress on the kidney, an immunosuppressed state,[19] autoimmune diseases,[20] chronic viral diseases,[21] and cancer-related conditions.[22]

The nude rat from the SD/NIN colony displays unique alterations in blood, serum, fat, and bone parameters, making it an excellent candidate for a genetic model in nutritional research. A thorough investigation is required to better comprehend the mechanisms and physiology of nude animals.

Conclusions

Our model does not require a barrier facility, unlike the immunocompromised animals, making it suitable for a range of studies without the need for specialized infrastructure or housing to address morbidity or mortality concerns. Additionally, the nude animal model we offer at our facility is highly resilient, and its distinctive blood profile and excellent feed conversion can prove advantageous in nutritional research.

Further examination is necessary to determine the specific fat type present in our model, conduct behavioral tests, and assess its susceptibility to cancer or metabolic disease. A comprehensive understanding using meta-genomics could offer potential solutions to malnutrition issues.

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Conflicts of interest

There are no conflicts of interest.

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References


Figure 1: Food intake & body weight (Fold increase) in homozygous (nude) animals on comparison to SD/NJNhr+/- (Heterozygous rat).

See image above for figure legend.
Figure 2: Food Conversion Ratio (Fold increase) in homozygous (nude) animals on comparison to SD/NINhr<sup>++</sup> Heterozygous rat.

Figure 2
See image above for figure legend.

Figure 3: Serum Markers (Fold increase) in homozygous (nude) animals on comparison to SD/NINhr<sup>++</sup> Heterozygous rat.
Figure 3

See image above for figure legend.

Metabolic markers in male rats

Metabolic markers in female rats

Enzymatic markers in male rats

Enzymatic markers in female rats

Figure 4: Metabolic and Enzymatic Markers (Fold increase) in homozygous (nude) animals on comparison SD/NINhr+/- Heterozygous rat

Figure 4

See image above for figure legend.
Figure 5: DXA analysis (Fold increase) in homozygous (nude animals) on comparison with respective sex of SD/NINhr+/+ (Heterozygous) rat.

Figure 5

See image above for figure legend.
Figure 6: TOBEC analysis (Fold increase) in homozygous (nude animals) on comparison to SD/NINhrsu (Heterozygous) rat.