

COLD-INDUCED THERMOGENESIS IN RATS NUTRITIONALLY DEPRIVED EARLY IN LIFE

D. V. MURALIDHARA AND P. S. SHETTY

*Nutrition Research Centre,
Department of Physiology,
St. John's Medical College, Bangalore - 560 034*

Summary : Prewaning nutritional deprivation with or without postweaning partial energy restriction produces animals of small size with defective nonshivering thermogenesis. This is also associated with their inability to tolerate cold exposure to 5°C. Adequate nutritional rehabilitation for a reasonable length of time of 10 days abolishes the defect in cold induced thermogenesis (CIT) although the deficits in body weight or body size are not corrected. This may indicate that the defect may be possibly due to the non availability of enough substrates rather than to a change in the functioning of thermogenic organs such as brown adipose tissue. Sucrose feeding which enhances caloric intake and hence sympathetic activity can reverse the defect in CIT only in older rats suggesting the possibility of delayed maturation of thermoregulatory functions in young rats which are energy deprived. The results of this study possibly indicate that there may be a temporary reduction of the sympathetic nervous system activity during the period of energy restriction which compromises cold tolerance and which is reactivated rapidly following nutritional rehabilitation.

Key words : cold induced thermogenesis
partial energy restriction

preweaning nutritional deprivation
refeeding

INTRODUCTION

Genetically obese (ob/ob) mice die of hypothermia on cold exposure to 4°C despite good thermal insulation (1). This is largely the result of a specific defect in nonshivering thermogenesis (12). Non-shivering thermogenesis (NST) is an important mechanism for thermoregulatory response in animals during cold stress and is mediated through the activity of the sympathetic nervous system on brown adipose tissue (4, 7). The ob/ob mice not only have defective mechanisms related to brown adipose tissue (BAT) mediated thermogenesis, but may also have reduced sympathetic nervous system activity (15), both of which may contribute to the deficiency in cold-induced thermogenesis (CIT) in this species. Since rats which were nutritionally deprived during preweaning period by increasing their litter size, have a diminished capacity for NST (10);

this study was designed to assess the CIT in rats earlier subjected to energy restriction both during the preweaning and in the post weaning period. It was also designed to examine the impact of this early nutritional stress on the reversibility of these processes following adequate nutritional rehabilitation.

MATERIAL AND METHODS

Inbred female Wistar rats were mated to litter in pairs. Litters of rats born within 24 hours of each other were mixed and the litter size was increased to 16 to induce nutritional deprivation during the preweaning period i.e. preweaning nutritional deprivation (PND). The number of pups reduced to 5 with each dam served as controls (14). In the large litter, pups of both sexes were placed in equal numbers, whereas either male or female pups only, were placed with the dam having the small sized litter. The control as well as the PND pups were housed with their dams at $25^{\circ} \pm 2^{\circ}\text{C}$ in polypropylene cages. Food and water available *ad libitum* to all lactating mothers and both the control and PND rats had free access to laboratory rat pellet diet (Energy 15 KJ per gm, protein 20%, crude fiber 4%, Hindustan Lever Foods) following weaning.

In a second series of experiments the degree of nutritional deprivation was intended to be little more severe and prolonged. The following technique was adopted. In addition to inducing PND as mentioned above, energy deprivation was extended in young rats upto the age of 60 days, by partially restricting the food (PND+PR). Since PND animals were smaller with body weights 50% of that controls at three week of age, partial restriction of food in the PND + PR group was of the order to induce a deficit of about 70% in body weight as compared to their mates of small litters at the same age. Following this period of dietary restriction i.e. after 60 days of age, the PND+PR rats had free access to food (rat pellet diet). Once the rats were started on laboratory diet, they were placed in groups of 4 or 5 in polypropylene cages under similar laboratory conditions, mentioned earlier.

Cold exposure: The changes in the body temperature following cold exposure (5°C) was used as an index of cold induced thermogenesis (CIT). CIT was determined at different intervals in experimental animals both before and after nutritional rehabilitation along with their controls. Each control and experimental rat was individually exposed to cold (5°C) by placing them in suitable sized, well ventilated metal chambers which were kept in a sink filled with ice blocks. The metal chambers contained a can of soda lime for removal of CO_2 . A mercury thermometer fitted to the chamber indicated the temperature to which the rats were cold stressed. The capacity for CIT was assessed

by recording the changes in body temperature (oral/rectal) in each animal during cold exposure (5°C) at every 30 min interval for a total of two hours. During each test day, an equal number of control and experimental animals were exposed to cold. The body temperature was recorded using a digital thermometer which was calibrated against a mercury thermometer of 0.1°C sensitivity. The thermometer probe was placed for one minute either in the oral cavity in case of rats of 21 days of age or in the rectum in older rats.

Response to cold stress at 5°C was assessed in the PND rats along with their controls when they were 21, 25, 30, 60 and 90 days old. The PND rats which were partially energy restricted during postweaning period upto 60 days of age (PND+PR) were also cold exposed in a similar manner, when they were 30 and 60 days old (i.e. prior to refeeding) and again after 10 and 30 days of refeeding.

Sucrose feeding and cold induced thermogenesis : Since sucrose feeding is known to enhance caloric intake by 20-30% (8, 9) some of the PND rats were provided with 8% sucrose solution along with the usual rat feed for 72 hours from day 22 and then cold exposed at 5°C on day 25. Similarly, immediately after the partial food restriction was withdrawn at 60 days of age, the PND+PR rats were also provided access to 8% sucrose solution for 72 hours along with *ad libitum* diet following which their responses to cold exposure were studied.

All values are expressed as mean \pm SEM. The body temperatures of both male and female rats of control and experimental are pooled and results given. Statistical significance of differences between groups was determined by students' 't' test and values with P0.05 were considered statistically significant (6).

RESULTS

Body weights : The PND animals weighed 50% less than their controls at weaning and the PND+PR rats at 60 days of age, weighed approximately 65% less than their controls. Both these differences were statistically significant ($P < 0.001$). However, even after adequate nutritional rehabilitation the PND or the PND+PR animals did not have adequate catch-up growth and the body weights remained significantly lower even at 90 days of age.

Response to cold : On exposure to cold (5°C), 21 day old PND rats showed significantly lower body temperatures throughout the exposure time (Fig. 1). The control

rats dropped their body temperature from $35.7^{\circ} \pm 0.2^{\circ}\text{C}$ at zero time to $26.5^{\circ} \pm 0.2^{\circ}\text{C}$ at the end of two hours of cold stress while the PND rats lowered their temperature from $34.2^{\circ} \pm 0.1^{\circ}\text{C}$ at zero time to $21.4^{\circ} \pm 0.7^{\circ}\text{C}$ at the end of 60 minutes of cold exposure. Seven out of 15 PND rats died between 30 and 60 minutes and the remaining 8 rats died between 60 and 90 minutes of cold exposure while none of the control rats succumbed to the two hours of cold stress.

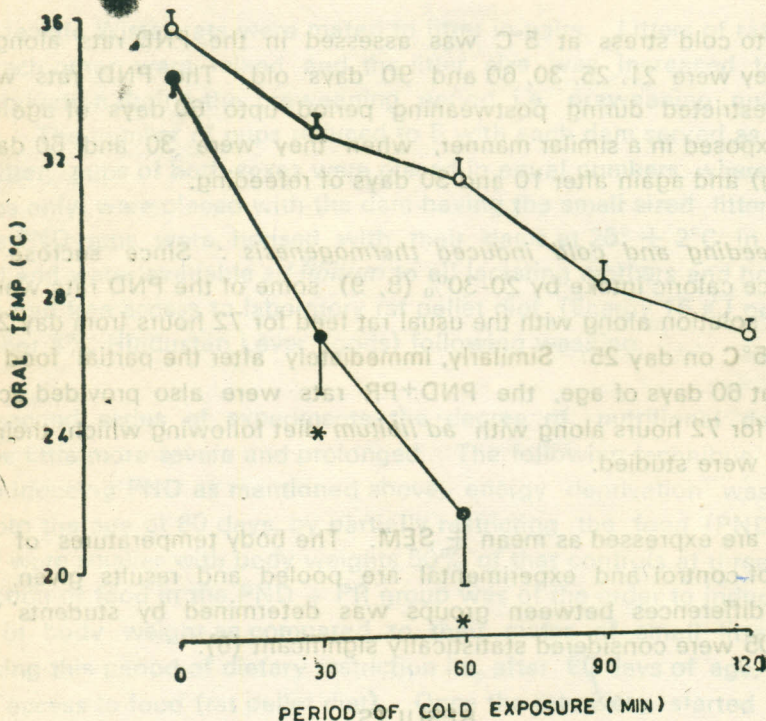


Fig. 1 : Changes in body temperature (oral) following cold exposure (5°C) in control (O) and PND (●) animals. Asterisks indicate statistically significant differences. Means \pm SEM.

The changes in body temperature of PND rats compared with that of their controls during cold exposure after 72 hours as well as 10 days of *ad libitum* feeding are summarised in Table I. The PND rats cold exposed after 72 hours of free access to food had lower body temperatures during the first 60 min and most of the rats died of hypothermia in the next 30 min of cold exposure. The PND rats, who had 10 days of *ad libitum* diet dropped their body temperatures significantly below that of cold exposed controls during the first 60 min of cold exposure, but were able to thermoregulate

TABLE 1 : Body temperature changes following cold exposure (5°C) in² preweaning nutritionally deprived (PND) rats following *ad lib*/sucrose feeding. (Values are mean \pm SEM)

Experimental group	Age (d)	Number of animals (n)	Duration of cold exposure in minutes			
			0	30	60	90
Control	25	6	37.5 \pm 0.1	33.7 \pm 0.2	32.4 \pm 0.3	32.2 \pm 0.6
PND + 72h refeed	25	8	36.4 \pm 0.2*	28.5 \pm 0.5*(1)	22.6 \pm 1.2*(2)	— (5)
PND + 72h Sucrose fed	25	7	36.1 \pm 0.1*	27.2 \pm 0.7*	22.2 \pm 1.2*(3)	— (4)
Control	30	20	35.3 \pm 0.1	31.1 \pm 0.3	29.9 \pm 0.3	28.6 \pm 0.3
PND + 10 days feeding	30	31	34.6 \pm 0.1*	30.2 \pm 0.2*	29.1 \pm 0.2*	28.5 \pm 0.2
						28.2 \pm 0.2

*Statistically significant (minimum P<0.01) compared with their control litter mates.

Figures in parenthesis denote number of rats found dead at the time of interval after cold exposure.

TABLE II : Body temperature changes following cold exposure (5°C) in PND + PR rats following ad lib/sucrose feeding. (Values are mean \pm SEM).

Experimental group	Age (d)	Number of animals (n)	Duration of cold exposure in minutes			
			0	30	60	90
Control	64	8	37.9 \pm 0.3	36.0 \pm 0.3	35.3 \pm 0.4	34.7 \pm 0.5
			37.7 \pm 0.1	33.3 \pm 0.4*	31.1 \pm 0.8*	31.0 \pm 1.0*
PND + PR + 72h Sucrose fed	64	6	38.5 \pm 0.1*	35.5 \pm 0.2	34.8 \pm 0.3	34.3 \pm 0.3
			38.5 \pm 0.1*	35.5 \pm 0.2	34.8 \pm 0.3	34.3 \pm 0.3
PND + PR + 10 days Feeding	70	7	38.5 \pm 0.1*	35.5 \pm 0.2	34.8 \pm 0.3	34.3 \pm 0.4

*Statistically significant (minimum $P < 0.01$) compared to their control litter mates.

without any mortality during next 60 min of exposure to cold. On exposure to cold at 60 days and 90 days of age the PND rats were capable of maintaining their body temperatures at levels similar to that of the controls throughout the period of cold exposure.

The PND+PR rats at 30 and 60 days of age, exposed to cold stress during the period of partial food restriction were unable to maintain body temperatures and died between 60 and 90 minutes of cold exposure (Fig. 2). The 30 day old PND+PR rats recorded lower body temperatures compared to controls at zero time before the start of cold exposure ($35.3^{\circ} \pm 0.3^{\circ}\text{C}$, experimental and $36.2^{\circ} \pm 0.2^{\circ}\text{C}$; control $P < 0.01$). At the

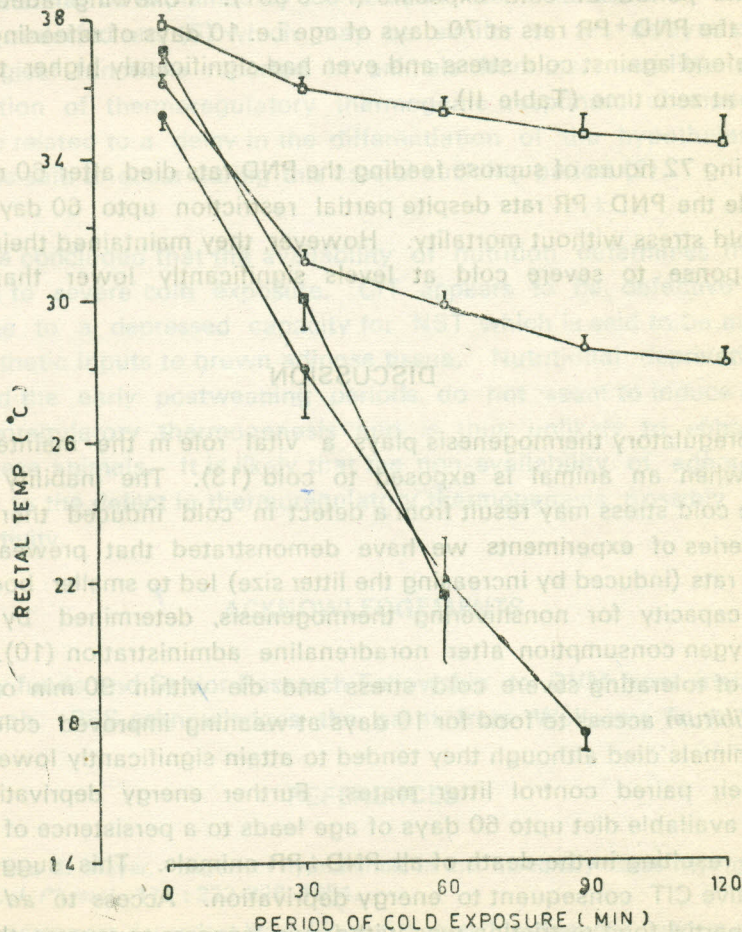


Fig. 2 : Changes in body temperature (rectal) following cold exposure (5°C) in 30 day old control (0) and PND + PR (●) rats and in 60 day old control (□) and PND + PR (■) rats. Means \pm SEM.

end of 90 min of cold exposure the PND+PR showed temperatures of $17.7^{\circ} \pm 0.4^{\circ}\text{C}$ while the controls recorded $28.6^{\circ} \pm 0.3^{\circ}\text{C}$. Four out of 7 experimental rats died between 60 and 90 min while the remaining 3 died by 120 minutes. The zero time body temperatures of 60 day old PND+PR rats was $37.2^{\circ} \pm 0.1^{\circ}\text{C}$ (control: $37.9^{\circ} \pm 0.2^{\circ}\text{C}$) and at the end of 60 min of cold exposure the temperature dropped to $21.6^{\circ} \pm 1.8^{\circ}\text{C}$ (controls: $35.3^{\circ} \pm 0.4^{\circ}\text{C}$) (see Fig. 2). All the 6 rats in this group died between 60 and 90 min of cold exposure. None of the controls, both 30 day old ($n=20$) and 60 day old ($n=8$) died during cold exposure. A statistically significant difference in body temperatures between the controls of 30 and 60 days was also noticed (see Fig. 2) at all points throughout the period of cold exposure ($P < 0.001$). Following adequate nutritional rehabilitation the PND+PR rats at 70 days of age i.e. 10 days of refeeding, were able to completely defend against cold stress and even had significantly higher temperature than their controls at zero time (Table II).

Following 72 hours of sucrose feeding the PND rats died after 60 minutes of cold exposure while the PND+PR rats despite partial restriction upto 60 days of age could survive the cold stress without mortality. However, they maintained their body temperature in response to severe cold at levels significantly lower than their controls (Table I, II).

DISCUSSION

Thermoregulatory thermogenesis plays a vital role in the maintenance of body temperature when an animal is exposed to cold (13). The inability of an animal to survive severe cold stress may result from a defect in cold induced thermogenesis (12). In an earlier series of experiments we have demonstrated that preweaning nutritional deprivation in rats (induced by increasing the litter size) led to smaller body weight with a depressed capacity for nonshivering thermogenesis, determined by measuring the increase in oxygen consumption after noradrenaline administration (10). The PND rats are incapable of tolerating severe cold stress and die within 90 min of cold exposure. However, *ad libitum* access to food for 10 days at weaning improved cold tolerance and none of the animals died although they tended to attain significantly lower body temperatures than their paired control litter mates. Further energy deprivation by partially restricting the available diet upto 60 days of age leads to a persistence of the diminished cold tolerance resulting in the death of all PND+PR animals. This suggests the possibility of defective CIT consequent to energy deprivation. Access to *ad libitum* diet for 10 days after partial food restriction was withdrawn, appears to reverse this thermogenic defect and the animal is able to survive cold exposure, maintaining body temperatures at levels comparable to its paired controls over two hours.

Addition of sucrose in the diet or access to sucrose solution is known to elevate the caloric intake (8, 9) and is also known to stimulate the activity of the sympathetic nervous system within 24 hours of sucrose feeding (11). Sympathetic activity influences brown adipose tissue which is considered to be the main thermogenic organ mediating nonshivering thermogenesis (3). Sucrose over-feeding was used both in weaning PND rats and the partially energy restricted PND+PR rats for 72 hours prior to cold exposure. Sucrose feeding did not improve cold tolerance in the PND rats aged 21 days although it improved the cold tolerance of the partially restricted older animal. This discrepancy in the time required for return to normal thermogenic responses between the PND and PND+PR animals may be explained by the delay in the maturation of thermoregulatory function of the underfed rats (5) which may be similar to the delayed development of other physiological functions reported in animals from increased litter size (14). The slower maturation of thermoregulatory thermogenic responses observed in the PND animals may be related to a delay in the differentiation of the hypothalamic regulatory centres which is said to occur during this crucial suckling period (2).

It may be concluded that the availability of nutrition determines the ability of the rats to respond to severe cold exposure. CIT appears to be defective during energy deprivation due to a depressed capacity for NST which is said to be mediated largely through sympathetic inputs to brown adipose tissue. Nutritional deprivation during the preweaning and the early postweaning periods do not seem to induce any permanent defect in thermoregulatory thermogenesis and is thus unlikely to enhance metabolic efficiency of these animals. It is likely that the non-availability of adequate substrates may contribute to the defect in thermoregulatory thermogenesis possibly by depressing sympathetic activity.

ACKNOWLEDGEMENTS

Aided by funds and Senior Research Fellowship to DVM from Indian Council of Medical Research. PSS acknowledges the grant from Wellcome Trust for the study.

REFERENCES

1. Davis, T.R.A. and J. Mayer, Imperfect hypothermia in the hereditary obese-hyperglycemic syndrome of mice. *Am. J. Physiol.*, **177** : 222-226, 1954.
2. Dorner, G. Environment-dependent brain differentiation and fundamental process of life. *Acta. Biol. Med. Germ.*, **33** : 129-148, 1974.

3. Foster, D.O. and M.L. Frydman. Non-shivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorogenesis induced by noradrenaline. *Can. J. Physiol. Pharmacol.*, **56** : 110-112, 1978.
4. Gale, C.C.A. Neuroendocrine aspects of Thermoregulation. *Ann. Rev. Physiol.*, **35** : 391-430, 1973.
5. Heim, T. and Z. Szelenyi. Temperature regulation in rats semistarved since birth. *Acta. Physiol. Acad. Sci. Hung.*, **27** : 247-255, 1965.
6. Hill, A.B. A short textbook of Medical Statistics. (10th Edition), Hodder and Stoughton (London) 1977.
7. Jansky, L. Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev.*, **48** : 85-132, 1973.
8. Kanarek, R.B. and E. Hirsch. Dietary induced overeating in experimental animals. *Fed. Proc.*, **36** : 154-158, 1977.
9. Landsberg, L. and J.B. Young. Effects of nutritional status on autonomic nervous system function. *Am. J. Clin. Nutr.*, **35** : 1234-1240, 1982.
10. Muralidhara, D.V. and P.S. Shetty. Depressed capacity for non-shivering thermogenesis following preweaning nutritional deprivation in rats. *IRCS Med. Sci.*, **12** : 707-708, 1984.
11. Rappaport, E.B., J.B. Young and L. Landsberg. Initiation, duration and dissipation of diet-induced changes in sympathetic nervous system activity in the rat. *Metabolism.* : **31** : 143-146, 1982.
12. Trayhurn, P. and W.P.T. James. Thermoregulation and non-shivering thermogenesis in the genetically obese (ob/ob) mouse. *Pflungers Arch.*, **373** : 189-193, 1978.
13. Trayhurn, P. and W.P.T. James. Thermogenesis : Dietary and Non-shivering Aspects In : The Body Weight Regulatory System : Normal and Disturbed Mechanisms. (Luigi A. Goffi *et. al.* eds) pp, 97-105, Raven Press, New York, 1981.
14. Widdowson, E.M. and R.A. McCance. Some effects of accelerating growth. I. General somatic development. *Proc. Roy. Soc. Ser. B.*, **152** : 188-206, 1960.
15. Young, J.B. and L. Landsberg. Diminished sympathetic nervous system activity in genetically obese (ob/ob) mouse. *Am. J. Physiol.*, **245** : E148-E154, 1983.