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Changes in the Estrous Cycle of Female Rats Fed High Carbohydrate and High Protein Diets

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Abstract- We evaluated the changes in estrous cycle of female rats fed either high carbohydrate or high protein diets for 42 days relatively to commercially available rodent pellets. Animals fed high carbohydrate diet showed a lengthened estrus cycle while those fed with a high protein diet could not continue a healthy estrous cycle. Animals received a protein rich diet showed a lengthened estrus cycle with an atypically extended diestrous stage until protein rich food is withdrawn and a balanced diet was introduced. However, animals fed with a carbohydrate rich diet showed a typical estrous cycle but slightly lengthier. Present findings have shown that nutrition depletion affect on estrous cycle in rats. Interestingly the changes were highly reversible upon replacement of a balanced diet. Results of the present findings suggest that nutritionally balanced diet is important for a healthy estrous cycle in laboratory rats thus diet should be given special attention during animal experimentation.

Index Terms- Diet composition, Nutrient depletion, Estrus behaviour, Growth, Rats

INTRODUCTION

Reproductive processes are energetically costly processes especially in female mammals in which pre and post nataly growth and development is taking place (Flick et al. 1990; Wade et al. 1996), and are characterized not only by cyclic alteration in female reproductive tract and but also in the sexual receptivity. Sexual cycle of mammals other than primates is called estrus cycle (Ganong 1985). Estrus cycle consists of four stages, i.e., (i) proestrus where vaginal smears are dominated by nucleated epithelial cells, (ii) estrus where vaginal smears get cornified, (iii) meta estrus where cornified smears infiltrated by leucocytes, and (iv) diestrus where vaginal smears consisting almost entirely of leucocytes (Hurkaj & Mathur 1976). The recurrent period of receptivity or "heat" is called estrus in which sexual interest of the female is aroused and ovulation is taking place. The duration of estrus cycle varies from animal to animal and may be influenced by extroceptive factors suchas light, temperature, nutritional status and social relationships (Du et al. 1996). Availability of energy have immediate direct effect on reproduction and female mammals will differ or interrupt reproductive attempts in favor of processes necessary for individual survival (Wade et al. 1996), until energy supply become more abundant. This energetic inhibition of reproduction can take a number of forms, including a delay of puberty, cessation of ovulatery cycles, fetal resoroption or infanticide (Bronson 1985; Marstellar et al. 1987; Schncider et al. 1989). Food serves both a proximate and an ultimate factor in regulation of fertility and, therefore food supply is the single most important environmental factor controlling reproduction (Wade 1998; Bronson 1989).

Female rat repeats estrus cycle throughout the year at intervals of four to five days (Ganong 1985; Hurkaj and Mathur 1976), unless subjected to pregnancy, pseudo-pregnancy (after a sterile mating), or disease. The cycle involves whole reproductive tract and it is possible to determine sexual status of the female rat by examining the smears prepared from vaginal fluid. The present study was carried out to investigate the impacts of nutrient depletion on the estrous cycle of female wistar rats. Rats were chosen for this work, since they were by far the most economic model for nutritional studies (Donnelly et al. 2000) and, also they have shown to eat fish and maintain healthy growth rates on fish diets (Arnold et al. 1998).

MATERIALS AND METHODS

Forty-five mature wistar female rats (age 12 ± 2 weeks;weighing 250 ± 20 g) with previously proven standard behaviour of estrous cycle (kept in standard animal house conditions, i.e., Temperature 27-30 ^{0}C; Photoperiod, approximately 12 hours light and 12 hours dark daily) were randomly divided into 3 groups (n=15 per group). Animals were maintained in standard solid bottom rat cages as 5 animals per cage with wood shavings as bedding materials. Each group was given either Boiled rice only (group A) consisting of 91%, 8% and 1% proximate composition of carbohydrates, protein and fat in dry basis, respectively (USDA 2004) or Fish meat only (group B) consisting of 75% and 3% proximate composition of Protein and fat in dry basis, respectively (USDA 2004; Merindol 1969; Lovell 1934) or commercially available readily usable rodents Pellets (group C) consisting of 60% carbohydrates, 28% proteins and 12% fat, *ad libitum* for 42 days. At the end of the initial study period of 42 days, diets of group A and B animals were changed to normal rodent pellets instead of boiled rice or fish meat for further 18 days i.e. as same as in group C animals. Tap water was available at all times and the animals in all groups were provided other environmental conditions similarly.

Amount of leftover food was assessed daily to observe any difference in food preferences in any specified diets and general behavour of rats were recorded for one hour each day during 08.00-12.00 hours. Animals were weighted every other day using a top loading balance (Mettler PE 3600). Vaginal smears were observed daily (between 16.00 - 17.00 hours) to assess status of estrus cycle. Number of estrus cycles and their lengths (number of days per cycle) were computed separately for initial 6 days as well as for next 42 days and then up to day 60. Percentage reduction or increase in body weight of individuals were calculated during the experimental period up to 42 days and from then to 60 days, using initial and final body weights at each stage. Data were statistically analyzed using SPSS statistical package (version 16). Significant level was set at 5% and data was presented as mean ± SD.

RESULTS

Food consumption and general behaviour of rats in group A & B did not show any significant difference compared to those in group C animals fed with standard rodent pellets. However, there was a significant reduction ($p<0.001$) in percentage body weight gain in group B rats during first 42 days. Group A animals did not show any net body weight gain during the initial 42 day study period. In contrarily, percentage body weight in group C animals showed a healthy regular growth throughout the experiment (Figure 1).

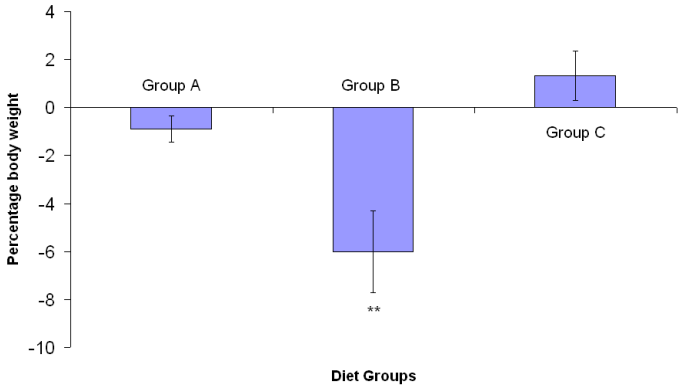

Figure 1:Percentage body weight gain in female rats tested with three different diets (**p<0.01)

Having regular estrus cycle during the initial period, there was no significant difference ($p<0.05$) in length of the estrus cycle in rats tested in three groups during first six days of the experiment. Number of estrus cycles for initial six days in group A, B & C rats were 1.27±0.12, 1.27±0.12 and 1.20±0.11, respectively. However, irregularities in estrus cycle were observed in Group A and B animals after initial 6 days. That is, estrous cycle irregularities occurred as lengthened diestrous stage. It was highly conspicuous in group B animals, i.e., they could not continue a healthy estrus cycle and only diestrus stage could be observed during the experimental period from day 6 to 42 (Figure 2). Although, group A animals could continue the estrus cycle, each cycle took slightly lengthen period (5.71±0.16 days) compared to animals in group C (4.23±0.12 days).

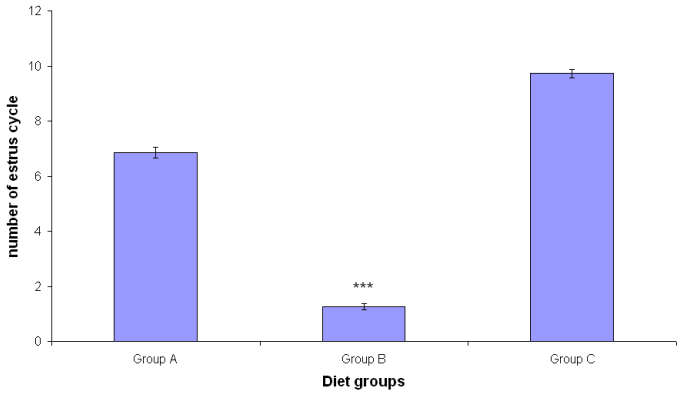

Figure 2:Number of estrus cycles of rats tested in three different diets up to 42 days of the experiment (***p<0.001)

After introducing normal pellet diet since 42 day, group B animals showed significantly higher ($p<0.01$) gain in body weight (29.80±3.46%). Similarly animals in group A showed a regular increase in body weight (1.46±1.45 %) compared to animals in group C (0.89 ± 1.16%). Estrous cycle of group B animals resumed estrous cycle from extended diestrous stage. Number of days per each estrous cycle was significantly higher in group B animals (10.20 ± 0.92 days) compared with that of in group C animals (4.36 ± 0.15 days). Rats in group A also showed a reduction in number of days per estrous cycle (4.64 ± 0.31 days) from its estrous cycle length (4.87 ± 0.19 days) during initial 42 days.

DISCUSSION

Biologically end point data for laboratory rats can be affected by factors such as the light, temperature, social relationships, diet, age and strain. Here, we tested two different diets with increased composition of carbohydrate or protein by depleting protein or carbohydrate and fat composition with reference to commercially available laboratory rodents pellets. Female rats received a protein rich diet showed a lengthened estrus cycle with an atypically extended diestrous stage until protein rich food is withdrawn and a normal balanced diet was introduced.

Animals fed with three diets containing different nutrient composition showed a similar food preference. Rats fed with a high carbohydrate diet containing low amount of protein and fat did not show a significant reduction in body weight throughout the experiment. In contrary, rats fed high protein diet could not maintain their body weight, at least as animals fed high carbohydrate diet. Overall results of individual body weight in present study have clearly shown that nutrition depletion as well as its composition directly affects on animal growth and body weight. However, it is clear that present findings are not in

agreement with Arnold and others (1998) in which authors have found that rats could maintain healthy growth rates on a fish diet.

Similarly to body weight, animals fed either high carbohydrate or high protein diet showed lengthened or ceased ovulatory cycles. Both of these diets in group A and B consisted of low amount of other major nutrients (carbohydrates or proteins and fat) comparatively to a normal rodent diet. It is clear that reduction in balanced diet formula has greatly affected on healthy estrous cycle and changed to an unhealthy and irregular estrous cycle as seen in the experiment. Provision of carbohydrate lack, protein rich diet may have had possible impacts in dramatic reduction of animal growth and changes of their healthy estrous cycle. However, such changes in estrous cycle were reversible, and this was clearly observed during the latter part of the experiment, post day 42. When animals' diets were shifted to standard rodent pellets, estrous cycle shifted towards standard length of the cycle. Animals in the three groups did not show a significant difference in length of estrus cycle during initial six days of the experiment indicating they all had a healthy estrous cycle before commencing the experiment. Differences in estrous cycle we observed in the study were due to direct or indirect effects caused by nutritional depletion. Present study has shown that during the initial period, before the extreme diets were introduced, animals had a healthy estrous behaviour. However, it is not easy to exclude any influence of other nutrients in carbohydrate or protein rich diets. It is presumed that restriction of certain components of diet could target completely unknown endpoints. Food restrictions are known to alter the secretion of pituitary gonadotropins and gonadal steroids. These secretary responses are thought to be preliminary due to suppression of gonadotropin releasing hormone (GnRH) release which is mediated by changing the activity of GnRH neurons in forebrain. Changes in the function of GnRH-secreting neurons have been inferred from circulating levels of LH. Suppression of sexual behaviour appears to be due to decrease in estrogen receptor in the ventromedial hypothalamus (Wade et al. 1996; Berriman et al. 1992). This fact stresses that for a healthy physiological performance in estrous cycle, laboratory rats need a healthy balanced diet. On the other hand, increased availability of carbohydrate or proteins only cannot regulate normal physiological processes in animals. Our results on the length of estrus cycle in animals fed with normal diet is in agreement with previous findings in which rat's ovulatory cycles took place within 4 to 5 days (Ganong 1985; Hurkaj and Mathur 1976).

In previous studies, estrus cycles of hamsters were interrupted by even a brief (48-h) period of food deprivation (Schncider and Wade 1989a; Schncider and Wade 1989b; Schncider and Wade 1990). Therefore, observed irregular behavior of estrus cycle of rats in present study has proven that nutrient composition directly affects on estrus cycle, as in food deprivation. Lengthened and ceased ovulatory cycles in animal groups fed either high carbohydrate or high protein diet are in agreement with Wade (1998) that reproduction was depending on diet and energy availability. Cessation of ovulatery cycle in diestrus stage means that there is no ovulation or sexual receptivity. Therefore, it may directly affect on the population regulation of the individuals. Intensive studies for over a decade have revealed that AlaskanStellerSea lion population decline since the late 1970's in parallel with a shift in the ocean's composition of fish (Calkins et al. 1999). Present findings have shown that lengthened estrus cycles could be reversed nearly to normal length and animals with ceased estrous cycles could re-start the cycle, after introducing the normal diet from 42nd day of the experiment. Therefore, it is clearly evident that nutritional depletion can directly affect on the estrus behaviour and nutritional infertility can be reversed in rats. One possible mechanism for such changes in estrus behaviour of animals may be due to the diet induced changes in LH pulses and changes of the LH pulses can be rapidly restored upon refeeding (Berriman et al. 1992). Furthermore, depletion of available metabolic energy due to increase requirements for thermogenesis or alternatively from mechanisms unrelated to energy expenditure, also inhibits reproduction (Schneider and Wade 1990b). Therefore, it is crucial that diets with sufficient proximate composition of nutrients must need the animals to maintain their physiological processes at optimum level. To achieve better results from the animal experimentation it is necessary to pay attention on nutrient composition in diets along with other environmental factors of animal welfare. Provision of sufficient nutrients to experimental animals reflects reliable results from animal experimentation in different ways. For instance, greater supply of metabolic energy from diets attenuated cannibalism of pups in heavier mothers of hamsters (Schneider and Wade 1989).

CONCLUSION

The present study shows that depletion of balanced nutrient components from daily food supply of laboratory rats could have consequences in disrupted estrous cycle behavior. Although the effects are transient and could be reversed if animals are switched back to normal balanced diet, such consequences could reflect results of other experimentation. Further experimentation is in progress to identify severity of physiological consequences.

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