Exp. Anim. 40(4), 499— 509, 1991

Effect of the Energy Density of Non-purified Diets on Reproduction, Obesity, Alopecia and Aging in Mice

Toshiaki SUZUKI**' *, Sho-ichi TANA-AMI**, Hiroshi FUJIWARA***, and Teru ISHIBASHI*

**Graduate School of Science and Technology, Niigata University, 8050 Ikarashi-ninomachi, Niigata-shi, Niigata 950-21, **Tsukuba Research Branch, Research Center, Nihon Nosan Kogyo, K. K., 5246 Takura, Tsukuba-shi, Ibaraki 300-33, and ***Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi-shi, Tokyo 186, Japan*

(Received 2 April 1991/Accepted 25 June 1991)

Four diets with graded levels of energy at 24% crude protein were fed to C 57 BL/6 J mice for approximately 3 years to develop pelleted non-purified diets. The nitrogen-corrected metabolizable energy (MEn) of the diets ranged from 2.86 to 3.73 kcal per g of dry matter (DM). Fifteen males and 30 females were assigned to each diet. The mice were housed together for 1 week at 7 week intervals, experiencing 5 matings. After the reproduction stage, they were allowed to complete their life span. Moribund mice after 60 weeks of age were subjected to histopathological examination. The highest energy diet showed the following results in comparison with the lowest energy diet : ① weaning weight of pups increased by 31.6% ; ② males showed slight obesity even during the reproduction stage, but females did not ; ③ both sexes developed remarkable obesity after 50 weeks of age with 41.2% (males) and 49.6% (females) increasing in maximum body weight ; ④ although daily feed intake decreased by approximately 18%, the MEn was slightly over-consumed ; ⑤ females showed higher incidence of alopecia with age ; ⑥ the survival rate after 50 weeks of age decreased earlier and life span was shortened ; ⑦ histopathological changes associated with aging developed earlier. On the other hand, the early death rate of dams at parturition increased with a decrease in dietary energy. It was concluded that at least 2 types of diets are needed throughout the life span of C 57 BL/6 J mice : a high energy diet with an MEn value of 3.73 kcal/g DM for maximum reproduction and a low energy diet with an MEn value of 2.86 kcal/g DM for the long term stage after reproduction to retard obesity and aging most effectively. —— KEY WORDS : aging, alopecia, diet, mice, obesity

In the previous experiment [1], the effect of the energy density of diets on the performance of growing young C 57 BL/6 J mice and gestating or lactating primiparous mice was studied under the condition of sufficient protein ingestion. This was accomplished via 4 pelleted non-purified diets with graded levels of energy at 24% crude protein content. The MEn value of the diets determined with rats ranged from 2.86 to 3.73 kacl/g dry matter (DM). These were considered practically the minimum and maximum. The result showed that a nitrogen-corrected metabolizable energy (MEn) of 3.73 kcal/g DM or more was necessary for maximum performance of growth during the 1 week after weaning and during lactation periods, in terms of growth rates for suckling or weanling mice and in the earlier onset of sexual maturity. For growth after 4 weeks of age and during gestation periods, however, the MEn of 2.86 kcal/g DM was sufficient in terms of the growth rate of young mice and the birth weight of newborn pups.

In the present experiment, the same diets used in the previous experiment were fed to mice of the same strain up to as long as approximately 3 years, in order to study the effect of energy density of diet on repeated

reproduction, obesity, survival rate, life span and histopathological changes in aging mice. The objectives of this experiment were to clarify the relationships between dietary energy density and the response of mice, and to find the variations in the diet needed by mice throughout their life span, thereby leading to the development of practical pelleted non-purified mouse diet (s) to cover all mouse stages.

Materials and Methods

1 . Animals and diets : Four pelleted non-purified diets were fed to conventional C 57 BL /6 J mice 6 weeks of age. Mice of this strain were chosen for their low incidence of tumors, Composition of diets was the same as in the previous work [1]. The nutritional values of the diets determined previously with rats are shown in Table 1. Supplies of diet and water were provided *ad libitum*. The pelleted diets were replaced by newly produced pellets every 6 months. Fifteen male mice and 30 female mice were assigned to each experimental diet. Seven or 8 male mice were housed together in each cage, and 10 female mice were caged similarly. The cages were made of aluminum (22 cm × 32 cm × 12 cm), with sterilized cut rice straw used as bedding. At 10 weeks of age, the mice were housed together, 2 females and 1 male per aluminum cage, and allowed to mate for 1 week. During that time, the impregnated females were selected by daily examination of vaginal plugs, and these mice were housed individually in small plastic cages (12 cm × 19.5 cm × 13 cm) during gestation and lactation periods. Pups were weaned at 21 days of age. The second mating was conducted 7 weeks after the first mating. The mating was repeated 5 times at 7-week intervals, with the mice experiencing 5 reproduction cycles up to 45 weeks of age when the last young were weaned. One reproduction cycle included one week mating, approximately 3 weeks of gestation, and 3 weeks of lactation. At each mating, the body weights of all mice was measured. During reproduction stages, non-gestating and non-lactating mice as well as male mice were caged in groups as described before except during the 1-week mating periods. The other experimental conditions have been described previously [1].

Reproductive performance among dietary treatment from the 1 st to the 5 th reproduction cycle was measured in terms of reproduction rate, weaning weight of pups, total number of pups, litter size, and nursing rate. The reproduction rate was defined as the number of female mice which weaned young divided by the number of mated females × 100 ; litter size was defined as the number of live pups at birth. Nursing rate was calculated as the number of weaning pups divided by the number of live new-born pups.

After the last weaning, all female mice as well as all male mice were caged in groups as described before and allowed to complete their life span. The feeding trial was continued as long as approximately 100 weeks after the last weaning. Feed and water were supplied at least once a week. Bedding was also changed and the number of survivor mice was counted at least once a week. At 53, 86 and 100 weeks of age, all mice were weighed and the feed intake was measured for 2 to 3 weeks. Individual mice of both sexes were examined for alopecia at 24,

Table 1. Digestive nutrients and metabolizable energy (MEn) of experimental diets (dry matter basis)[1]

Diet No.	Rats	Crude protein	Carbo-hydrate[2]	Carbo-hydrate[2]	MEn[3]
		(%)	(%)	(%)	(kcal/g)
1	3	22.6±0.6	6.0±0.4	49.3±1.2	3.73±0.03
2	4	22.3±0.4	4.6±0.1	49.3±0.7	3.51±0.05
3	4	21.6±0.4	2.5±0.1	49.1±0.3	3.28±0.02
4	4	20.5±0.6	1.2±0.1	42.3±0.6	2.86±0.01

[1] Values are means±standard. [2] Nitrogen-free extracts+crude fiber
[3] Nitrogen-corrected metabolizable energy

38, 53, 86 and 100 weeks of age.

2 . Histopathological examination : After 60 weeks of age, moribund and dead mice were subjiected to histopathlogical examination to study the effect of dietary energy on aging in mice. Examination of dead mice was conducted only when there was no gross evidence of autolysis. Examined were 38 male mice and 52 female mice. Moribund mice were decapitated after cervical dislocation, and autopsied. The tissue samples were immediately fixed in 10 % neutral buffer-formalin and imbedded in paraffin. The imbedded samples were sliced to sections 5 μm thick and stained with haematoxylin and eosin for microscopic examination. The periodic acid-Schiff reaction was used to stain kidneys. Pyridine Congo-red staining was also used for amyloid. Organs and regions inspected included the liver, kidneys, lungs, heart, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, adrenals, spleen, genitalia and accessory glands, 3 brain sites, pituitary, salivary gland, thymus, pancreas, urinary bladder, mammary glands, lymph nodes and others.

3 . Statistical analysis : The mean and standard deviation were calculated from the data. Student's T-test was used for statistical comparison of the two mean values ; p values of 0.05 or less were considered significant.

Results

1 . Reproduction : The reproductive performance of mice fed the experimental diets is shown in Table 2 . The reproduction rate generally decreased with the progress of the reproduction cycles except with Diet 4, where the higher rate was observed at the later cycles. There was no clear tendency in the reproduction rate in response to dietary energy change among the dietary treatments. But the highest reproduction rate was obtained with the lowest energy diet. The weaning weight of pups tended to increase with the progress of the reproduction cycle up to the 4 th cycle and then decreased at the 5 cycle. There was tendency in each reproduction cycle for the weaning weight to decrease in response to decreases in the energy density of diets. The difference in the weaning weight among the diets was largest and most significant at the

Table 2. Reproductive performance of mice fed experimental diets[1]

Performance / Diet	Reproduction cycle					
	1st	2nd	3rd	4th	5th	1−5th
Reproduction rate (%)						
Diet 1	43.3(13/30)	24.1(7/29)	34.5(11/29)	37.9(11/29)	28.6(8/28)	33.8(49/145)
2	40.0(12/30)	33.3(10/30)	43.3(13/30)	25.0(7/28)	14.3(4/28)	31.5(46/146)
3	50.0(15/30)	23.3(7/30)	23.3(7/30)	19.2(5/26)	24.0(6/25)	28.4(40/141)
4	36.7(11/30)	33.3(10/30)	48.3(14/29)	58.3(14/24)	47.6(10/21)	44.0(59/134)
Weaning weight (g)						
Diet 1	9.9 ± 1.2^{a}	8.8 ± 0.8^{a}	9.7 ± 1.3^{a}	11.5 ± 1.6^{a}	10.6 ± 1.8^{a}	10.0 ± 1.7^{a}
2	8.4 ± 1.5^{b}	8.6 ± 1.4^{a}	9.5 ± 1.5^{a}	10.5 ± 1.5^{b}	10.3 ± 1.9^{a}	9.2 ± 1.5^{b}
3	7.3 ± 1.2^{c}	7.9 ± 0.9^{b}	7.3 ± 1.4^{b}	10.3 ± 1.6^{b}	9.0 ± 1.6^{b}	7.9 ± 1.3^{c}
4	6.6 ± 0.8^{d}	7.1 ± 0.8^{c}	7.6 ± 1.3^{b}	8.5 ± 1.8^{b}	8.2 ± 1.2^{b}	7.6 ± 1.3^{d}
Number of weaning pups						
Diet 1	98	60	74	62	29	323
2	102	77	102	59	22	362
3	101	51	55	33	13	253
4	87	58	91	72	56	364

[1] Thirty female and 15 male mice were used for each diet. At 10 weeks of age, the mice were housed together for 1 week with 2 females versus 1 male per each aluminum cage and allowed to mate. One week mating was repeated 5 times from 10 weeks of age to 38 weeks of age at 7 week intervals. Reproduction rate was defined as number of female mice which weaned young divided by number of mated female mice (shown in parentheses)×100. Weaning weight values are means±standard deviation. Values with different superscripts are significantly different from others within the same column ($p < 0.05$).

1 st cycle. The average weaning weight of pups for all cycles was significantly different among the diets. The highest energy diet resulted in a 31.6% increase in the weaning body weight of pups in comparison with the lowest energy diet. The number of weaning pups for all cycles was lowest for Diet 3. But there was no clear tendency in this regard among diets. The average litter size for all cycles was 7.7, 8.5, 7.6 and 7.6 for Diets 1 to 4, with an insignificant difference. There was no significant difference in average nursing rate, which was 86.1, 86.6, 71.5 and 82.7% for Diets 1 to 4, respectively. The nursing rate for Diet 3 (22.8%) had a large standard deviation. Also the reproduction started with 30 females for each diet and ended with 28, 28, 25 and 21 females at the 5 th cycle for Diets 1 to 4, respectively. A decrease in dietary energy density increased the death rate in females during reproduction.

2 . Obesity and alopecia : The body weight change of males and females from 6 to 100 weeks of age is shown in Fig. 1 . Males showed a higher growth rate up to 10 weeks of age when fed higher energy diets. There was a significant difference in body weight at the first mating (10 weeks of age) between Diets 1 and 4. After 10 weeks of age, the difference in body weight among diets increased. At 38 weeks of age (the last mating), the average male body weight per diet was 40.9^a, 37.6^a, 35.6^b and 32.1^c g for Diets 1 to 4, respectively (Values with different superscripts are significantly different from others. Hereafter, a significant difference will be expressed in the same manner.). After 38 weeks of age, the difference in body weight was further magnified. Males fed Diets 1 and 2 showed apparent obesity ; their maximum body weight was obtained at 86 weeks of age, when body weights for Diets 1 to 4 were 49.7^a, 50.4^a, 41.9^b and 35.2^c g, respectively. The maximum body weight for the highest energy diet was 41.2% heavier than that for the lowest energy diet. After that age, the body weight of males fed Diets 1 and 2 decreased sharply. Males fed Diet 4, however, showed no sign of obesity, and their body weights kept increasing up to 100 weeks of age. Males fed Diet 3 showed moderate obesity, and did not show any sharp decrease in body weight after 86 weeks of age. But ther e was no significant difference in the female body

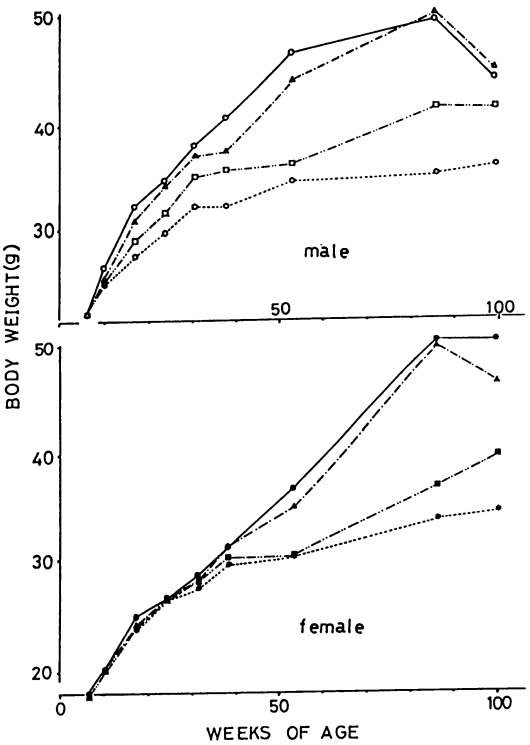

Fig. 1. Body weight change of mice fed experimental diets with graded levels of MEn. ○—○, ●—● : Diet 1, 3.73 kcal/g △—△, ▲—▲ : Diet 2, 3.51 kcal/g □—□, ■—■ : Diet 3, 3.28 kcal/g ⬡—⬡, ⬢—⬢ : Diet 4, 2.86 kcal/g. Average coefficient of variance for all diets was 9.2 and 10.7% for males and 12.2 and 15.2% for females at 53 and 100 weeks of age, respectively.

weights between Diets 1 and 4 up to 38 weeks of age. Females did not show an early difference in body weight among dietsas males did. But after 38 weeks of age, a great difference in body weight emerged, as in the males. Females fed Diets 1 and 2 showed obvious obesity, with their maximum body weight obtained also at 86 weeks of age. Body weights at that age for Diets 1 to 4 were 50.4^a, 49.9^a, 36.9^b and 33.7^c g, respectively. The highest energy diet resulted in a 49.6% increase in the maximum body weight compared to the lowest energy diet. The body weights of females fed Diets 1 or 2 stopped increasing or decreased sharply after that age, whereas females fed Diet 4 did not show obesity and females fed Diet 3 showed only moderate obesity. Females fed Diets 3 or 4

increased in body weight up to 100 weeks of age.

Aging mice increased feed intake with decreases in dietary energy. At 53 weeks of age, the average daily feed intake was 3.5^{a}, 3.7^{ab}, 3.9^{bc} and 4.2^{c} g DM per mouse for Diets 1 to 4, respectively. There was no significant difference in the average MEn intake among diets. Approximately similar results were obtained at 86 and 100 weeks of age.

As shown in Fig. 2, alopecia occurred mainly in females. Alopecic areas were found in the dorsal, lateral and abdominal body, head, face and other regions. The incidence gradually increased with age for the mice fed higher energy diets, whereas Diet 4 repressed the incidence. At 100 weeks of age, the incidence was 66.7, 31.8, 0 and 0% in females for Diets 1 to 4, respectively. Clearly, high energy diets accelerated the incidence of alopecia.

3 . Survival rate : The survival rates for male and female mice is shown in Fig. 3 . Up to 53 weeks of age, the male death rate was very low. But early death occurred in female mice during the reproduction period. Most deaths during this period seemed to be caused by difficulty in parturition. Intravaginal stillborn pups were frequently found in the dead mothers. As the dietary energy was lowered, the death rate rose. The cause of death was not identified. After 53 weeks of age, the survival rate for males decreased sharply. The average life span (days) maximum 20% of males (range in parenthesis) was 875 (858-910), 918 (881-937), 938 (930-946) and 920 (892-939) for Diets 1 to 4, respectively. Higher energy diets tended to decrease survival rate more rapidly. After the reproduction period, the survival rate of females came to resemble that of males. The average life span (days) maximum 20% of females was 839 (800-860), 921 (874-951), 922 (879-1008) and 915 (848-1030) for Diets 1 to 4, respectively.

4 . Histopathological findings : There were no clear signs of infection during the feeding trial except for an unidentified intestinal pinworm. In Table 3, the numbers of mice which had major histopathological changes in the liver, kidney, or both are indicated for each diet. The tumors in the liver consisted of mostly lymphosarcoma, reticulum cell sarcoma and hepatoma. Other than major changes listed in Table 3, bile duct hyperplasia, hemangioma and calcium deposits were found in livers.

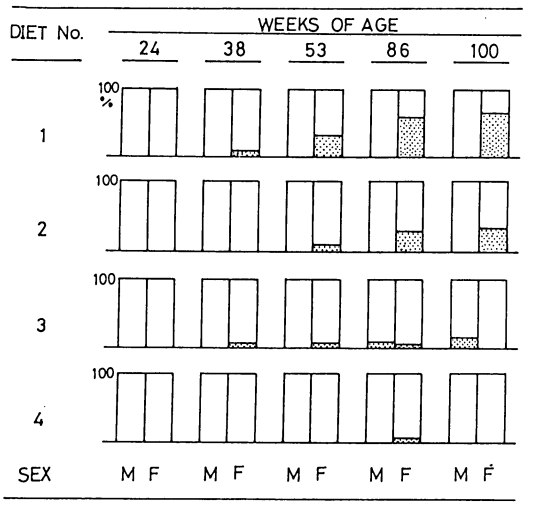

Fig. 2. Incidence of alopecia in mice at various ages fed experimental diets. MEn levels of diets are shown in Fig. 1. M, male ; F, female

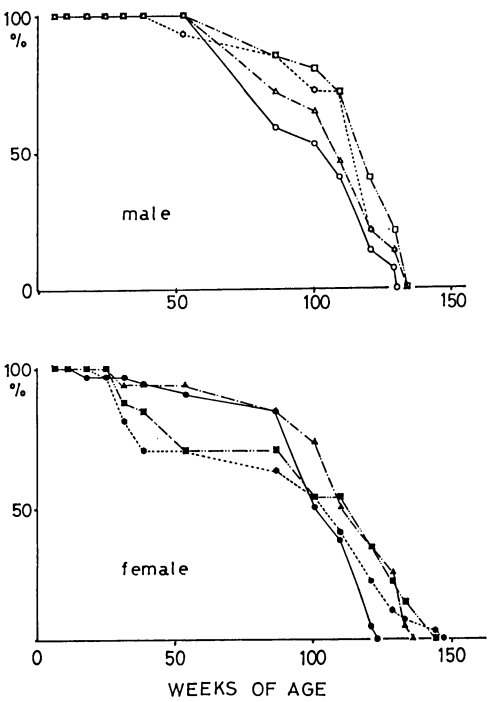

Fig. 3. Survival rate of mice fed experimental diets. Symbols are the same as shown in Fig. 1.

Table 3. Histopathology in liver and kidneys of aging mice fed experimental diets[1]

Lesions	Diet			
	1	2	3	4
Number of examined mice	20	23	26	21
Liver				
Tumor	3	5	8	5
Necrosis	0	2	2	4
Amyloidosis	3	0	1	1
Fatty change	4	0	1	1
Karyomegaly	5	8	6	8
Eosinophilic inclusions	1	0	0	2
Kidneys				
Tumor	1	2	4	0
Amyloidosis	9	6	6	5
Hyaline droplets	0	3	0	1
Proteinous casts	3	7	3	6
Calcium deposits	1	0	2	2
Small round cell infiltration	0	2	0	0
Tubular atrophy	1	2	0	1

[1] Moribund mice examined after 60 weeks of age. Dead mice were included if there was no gross evidence of autolysis. Mice of both sexes were included. The maximum life span of the mice was 910, 951, 1008 and 1030 days for Diet 1, 2, 3 and 4, respectively.

There was no difference in the incidence of the above findings between males and females except in cases of amyloidosis and eosinophilic inclusions : there was no incidence of amyloidosis in females and no eosinophilic inclusions in males. The incidence of fatty change was found only in the liver and not in other organs. Also, the incidence of amyloidosis and fatty change was relatively high in the highest energy diet. In the kidney, tumors comprised mostly lymphosarcoma and reticulum cell sarcoma. Amyloidosis occurred in females at levels of 31, 20, 21 and 30% for Diets 1 to 4, respectively. The incidence for males was 71, 38, 25 and 18% for Diets 1 to 4, respectively. Males showed a higher incidence of amyloidosis, which was more marked with increases in dietary energy. No particular tendency was found in the incidence of other histopathological changes among diets and between sexes.

Histopathological changes in the myocardium consisted of vacuole formation (the presence of fat was suspected in the vacuoles), in filtration of small round cells, and hyaline degeneration. Also, amyloidosis appeared in muscles and blood vessels, necrosis in muscles, periarteritis, infiltration of fibroblasts, and cholesterol-like droplets. In the lungs there was eosinophilic catahral pneumonia associated with eosinophilic crystals in the cytoplasma of the alveolar epithelial cells, lymphosarcoma, reticulum cell sarcoma, adenocarcinoma and adenoma. In the stomach, there was hyperkeratosis of mucosal epithelium, cyst formation in the glandular stomach, and amyloidosis. In the intestines, amyloidosis occurred in the jejunum and ileum. Lymphosarcoma, lymphatic leukamia and reticulum cell sarcoma also appeared. There was no difference among the diets in the incidence of the above changes in these organs.

In 90 of the mice, spontaneous tumors occurred in the following organs (incidence in parentheses) : lymphatic system including spleen and lymph nodes (47), liver (23), lungs (23), intestine (23), kidneys (10), pituitary (7), pancreas (6), adrenals (3), mammary gland (4), omentum (2), skin (3), palpebra (1), skeletal muscle of pelvic limb (2), uterus (3), ovary (1), gall bladder (1), stomach (1) and salivary gland (1). Such tumors were mostly reticulum cell sarcoma and lymphosarcoma. No differencein tumor incidence was found among the diets.

Discussion

Female mice were resistant to obesity during their reproduction stage (10 to 45 weeks of age in this experiment) compared to male mice (Fig. 1). The male mice fed higher energy diets may have already become slightly obese at 10 weeks of age (Fig. 1). Similar results were reported previously [1]. Male mice seem to be more susceptible to obesity at early stages. The presence of the estrus cycle may be associated with a resistance to obesity in female mice during reproduction. Coling and Herberg [2] demonstrated that ovarian hormones can influence food hoarding behavior in female rats. They found that the body weight of rats after ovariectomy rapidly increased for a month in parallel with a sharp increase in food hoarding. Daily intramuscular administration of estradiol benzoate significantly decreased body weight and hoarding activity. Donohoe *et al.* [3] also reported that 17-beta estradiol significantly reduced food in take and body weight in ovariectomised rats.

It is generally accepted that animals eat for energy and can regulate their feed intake in response to the energy density of diets to meet necessary energy demands [4, 5]. If animals can adjust energy intake level, it is difficult to explain why mice developed such a tremendous obesity particularly after 50 weeks of age (Fig. 1), when fed higher energy diets. In Table 4, the intake of feed and MEn is shown together with data obtained in the previous experiment from 3 to 10 weeks of age [1]. From 53 to 100 weeks of age, the daily feed intake of mice almost always decreased with increases in dietary energy density. The highest energy diet on the average resulted in a 18.3% decrease in feed intake compared to the lowest energy diet. But the MEn intake was not significantly different regardless of dietary energy density. This suggests, the ability of mice to adjust energy intake level. However, there was a general tendency in mice fed lower energy diets to reduce the MEn intake slightly and vice versa. In young mice, a similar tendency of a slight reduction in MEn intake with decrease in dietary energy also occurred except at 3 to 4 weeks of age. Young and old mice did not change their response to dietary energy density in terms of intake in feed and energy throughout their life span except for a special stage right after weaning (3 to 4 weeks of age) when there was a large decrease in the energy intake, as in the previous work [1]. It is believed that the ability of animals to adjust energy intake is not precise enough and higeher energy diets result in daily slight over-consumption of energy, the accumulation of which causes obesity in mice at the later stage. Forbes has referred to over-consumption by animals on high energy diets in hisbook [6], citing Jocobs' report that inclusion of 25% fat in diets of rats led to slight but persistent overeating and gradual obesity.

Rothwell and Stock [7] showed that rats over-fed by cafeteria feeding exhibited increased sensitivity to the thermogenic effects of noradrenaline and increased energy expenditure. They suggest that diet-induced thermoge-

Table 4. Daily intake of feed and MEn of mice fed experimental diets[1]

Age of week	Sex	Diet 1		Diet 2		Diet 3		Diet 4	
		Feed	MEn	Feed	MEn	Feed	MEn	Feed	MEn
		g	kcal	g	kcal	g	kcal	g	kcal
3– 4	♂♀	2.0	7.5	2.0	7.5	2.1	6.9	2.1	6.0
4–10	♂	2.8	10.4	2.7	9.5	3.4	11.2	3.2	9.2
	♀	2.5	9.3	2.6	9.1	2.7	8.9	3.1	8.9
53	♂♀	3.5 ± 0.2^{a}	13.0 ± 0.7	3.7 ± 0.2^{ab}	12.9 ± 0.6	3.9 ± 0.2^{bc}	12.7 ± 0.6	4.2 ± 0.3^{c}	12.0 ± 0.9
86	♂♀	2.9 ± 0.1^{a}	11.0 ± 0.4	3.0 ± 0.1^{a}	10.6 ± 0.5	3.2 ± 0.1^{b}	10.5 ± 0.4	3.7 ± 0.3^{c}	10.7 ± 1.0
100	♂♀	4.0 ± 0.5^{a}	14.7 ± 1.8	3.9 ± 0.5^{a}	13.7 ± 1.8	3.7 ± 0.3^{a}	12.1 ± 0.7	4.8 ± 0.5^{b}	13.7 ± 1.5

[1] The data for 3 to 10 weeks of age were obtained in the previous experiment [1]. Except for 4–10 weeks of age, the data of males and females were averaged together, since there were no differences related to gender. From 53 to 100 weeks of age, feed intake was measured for each cage 2) cages for males and 3 cages for females). Values with different superscripts are significantly different from others within the same row ($p < 0.05$) for the various intakes of feed and MEn.

nesis (DIT), involving changes in the activity of the sympathetic nervous system innervating brown adipose tissue, is a mechanism that resists obesity. Sakaguchi *et al.* [8] showed that rats fed high fat diets for more than 3 weeks had depressed activity of the sympathetic nervous system compared to those fed low fat diets. One possible explanation for the suppression of sympathetic nervous activity was a lack of stimulation by carbohydrate in the high fat diets. The effect of carbohydrate in stimulating the sympathetic nervous system has been reported in various studies [9, 10, 11]. In the higher energy diets of this experiment, a smaller part of the total dietary energy came from digestive carbohydrate (Table 1), compared to the lower energy diets. The decrease in DIT might be responsible, in part, for the obesity observed in this experiment. Obesity may be associated with the early onset of tumors, because the stasis or decrease in body weight of mice fed higher energy diets after 86 weeks of age (Fig. 1) was considered to be caused by the presence of tumors. MEn of diets should be less than 2.86 kcal/g DM to avoid obesity in C 57 BL/6 J mice.

Rowlatt *et al.* [12] studied the longevity of C 57 BL/Icrf a′ mice which were maintained in a conventional environment and fed a pelleted diet. Results were almost comparable with the present data except that maximum longevity in their study was slightly longer in males than females. A report by Harrison and Archer [13] referred to the longevity of C 57 BJ/ 6 J male mice maintained in a barrier-sustained condition, where the maximum 10% longevity was 994 to 1172 days, or slightly longer than in the present study. From our data on survival rates (Fig. 3), the highest energy diet with an MEn value of 3.73 kcal/g DM is needed for minimum loss of dams during reproduction, because lower energy diets result in higher incidence of death in dams at parturition (Table 2 and Fig. 3). After the reproduction stage, lower energy diets with an MEn value of 2.86 kcal/g DM or less may allow longer life span.

Rowlatt *et al.* mentioned above also studied the histopathology in their mice at 6, 24 and 30 months of age. Their findings were similar to ours except that they report a high incidence in inflammatory infiltration of livers and lungs and also in duodenal plaques in younger mice, considered a reactive hyperplasia associated with the diet. They reported tumors of a wide variety, amyloidosis in livers and kidneys, hyaline glomeruli in kidneys, crystals in the air spaces in lungs, and others. However, they did not report fatty changes.

Our histopathology findings almost accorded with the changes of aging mice reported by Frith and Ward [14] includig fatty metamorphosis in the livers of old obese mice. Major histopathological changes associated with aging in the examined aging mice were classified into 4 categories. *Tumors* were mostly reticulm cell sarcoma and lymphosarcoma. *Degeneration* was mostly fatty change, amyolidosis, hyaline droplets, and proteinous casts in kidney tubules. *Inflammation* included varieties of inflammation such as eosinophilic catahhral pneumonia, periarteritis and inflammation in myocardium. *Cell damage* included karyomegaly in both liver and myocardial cells. Results are shown in Table 5. Aging mice tended to show early onset of these histopathological changes when fed higher energy diets. On the other hand, lower energy diet repressed the early onset of these 4 categories. From Table 5, it is evident that higher energy diets accelerated the aging process in mice.

Table 5. Onset of histopathological changes associated with aging in mice[1]

Age in weeks	Diet No.	Tumors				Degeneration				Inflammation				Cell damage			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
60~100		0	1	2	0	1	0	1	0	1	0	0	0	0	0	0	0
101~115		8	6	3	2	5	4	1	1	2	0	4	1	2	3	2	2
116~130		6	9	11	9	5	4	4	6	1	2	2	3	1	0	2	2
131~		0	3	2	1	0	3	3	1	0	2	2	1	0	1	0	0

[1] Values are number of mice which showed the histopathological changes at each age interval. Mice examined are described in Table 3.

Furthermore, higher energy diets might increase the incidence of metabolic abnormality such as fatty change and amyloidosis in the liver and kidney (Table 3).

The incidence of alopecia in female mice increased with age and also with increased dietary energy (Fig. 2). Long [15], however, observed alopecia more in C 57 BL males than in females. In each cage, there was usually one mouse whose hair was intact, and this mouse was suspected of nibbling the hair or trimming the whiskers of it's cage mates. Long attributed these traits to indications of a social hierarchy in mice. Militzer and Wecker [16] also reported that alopecia was especially frequent in male mice in pregnant monogamous breeding groups with C 57 BL females. They also studied 10 breeding pairs of C 57 BL /6 J mice through time-lapse photography, and suggested that the cause of alopecia was a slight increase in self-grooming and a dramatic increase in allo-grooming by their female partners. In our experiment, however, the incidence of alopecia (not including trimmed whiskers) was much higher in females. This is probably because of different experimental conditions. Males and females were mostly housed separately in the present experiment except for a 5-week stay together at mating. On the other hand, Finch [17] caged C 57 BL/6 J male mice in groups and allowed them to complete their life span. The pelage of the males remained intact and smooth throughout most of their life-span, and alopecia did not occur even in the aged mice of 40 months or older. It can be attributed to the absence of active allo-grooming females that there were no alopecia among males caged in groups in our and Finch's experiments. Finch also depilated an area of skin of the mice and showed that hair regrowth was retar ded in the male mice 24 months old or older. Therefore, delayed hair regrowth may be associated with the development of alopecia in the last stages of our experiment. It is noted that a low energy diet with a MEn value of 2.86 kcal/g almost completely protected against alopecia. Fur eating or barbering has been reported in rabbits [18] and in guinea pigs [19], and a supply of roughage in diets has been considered somewhat effective to prevent fur eating. Alopecia in mice may have some relevance to fur eating in these hervibores.

The effects of dietary energy density on the performance of repeated reproduction was not clear in this experiment except in regard to the weaning weight of pups and the early death rates of dams (Table 2). The former effect was clearest in the 1 st cycle. The result was similar in the previous experiment with primiparous dams [1]. This is probably because the ingestion capacity of primiparous dams is smaller than that of dams after the 1 st reproduction cycle. The latter effect, an early death rate of dams, showed that diets with very low energy were harmful to female mice at parturition, although the lowest energy diets seemed to increase the reproduction rate in female mice. In the previous report [1], it was concluded that a diet with an MEn of 2.86 kcal/g DM was sufficient for gestating mice in terms of litter size and birth weight of newborn pups. However, this diet can not be applied at the gestation stage in terms of the new criteria.

From the above and the previous results [1], it was concluded that at least two types of diets are needed throughout the life span of C 57 BL/6 J mice : ① a high energy diet with MEn value of 3.73 kcal/g DM for 1 week after weanling to maximize the growth of weanling and during the reproduction stage to reduce difficulty at parturition and maximize milk production ; ② a low energy diet with MEn value of 2.86 kcal/g DM for the growth stage except for 1 week after weaning and during stages after reproduction to delay aging process and maximize life span. The low energy diet is presumably desirable for adult males and non-producing females.

It should be noted, however, that the above conclusion about optimum dietary energy density refers to life time *ad libitum* feeding for C 57 BL/6 J mice. *Ad libitum* feeding of low energy diets may successfully prevent obesity, retard the aging process, and extend longevity in the animals of relatively small sized strains or species. As animal size increases, the ingestion capacity generally increases. Larger animals, rats for instance, may require even a lower dietary energy density than MEn of 2.86 kcal/g DM to obtain the same effects. As discussed in the previous report [1], an MEn of 2.86 kcal may be a practical minimum for pelleted diets of omnivorous animals. To obtain lower energy diets, a larger amount of

roughage such as alfalfa must be included in the diet, somewhat similar to diets for hervivorous animals.

On the other hand, feed restriction has been recognized as remarkably effective with rodents in retarding aging, increasing longevity, and decreasing the incidence of age-related diseases. Numerous works since the first report by MaCay *et al.* [20] in the late 1930 s have been reviewed and categorized [21-23]. Roe [24] and Weindruch *et al.* [25] suggest that feed restriction or meal feeding should be adopted for rodents instead of *ad ilibitum* feeding. In the light of that research, if *ad libitum* feeding of low energy diets is not effective, to prevent obesity, retard aging and increase longevity, it may be a practical solution to adopt meal feeding of low energy diets with an MEn value of 2.86 kcal/g DM or less as a normal feeding regimen.

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飼料のエネルギー含量のマウスの繁殖，肥満，脱毛および加齢に及ぼす影響

鈴木敏明*·**·田名網祥一**·藤原　弘***·石橋　晃*

*新潟大学大学院自然科学研究科
**日本農産工業株式会社中央研究所筑波試験場
***株式会社ヤクルト本社中央研究所

マウス用の固形飼料を考案するため，粗蛋白質が24%と一定でエネルギーを段階的に変えた4種の固形飼料を約3年間C57 BL/6Jマウスに給与した。飼料の窒素補正代謝エネルギー (MEn) は乾物当たり最低2.86最高3.73 kcal/gであった。1飼料区に対し雄15匹雌30匹ずつを割り当て，7週間隔で雄と雌を1週間同居させて5回の交配を経験させた後，寿命まで飼育した。60週齢以後の瀕死期のマウスは病理組織学的検査に供した。最高MEnの飼料においては最低MEnの飼料に較べて，①子マウスの離乳時体重が31.6%増大し，②雄は雌と異なり繁殖期間中から軽度の肥満傾向が現れ，③50週齢以降は雄雌とも著しく肥満して最大体重がそれぞれ41.2および49.6%増大し，④飼料摂取量は約18%減少したがMEnの摂取量はわずかに過剰となり，⑤雌マウスに加齢に伴い高率に脱毛が発生し，⑥50週齢以後の生存率が低下して寿命が短縮し，⑦加齢に伴う病理組織学的変化が早期に出現した。一方，飼料のMEnの低下に伴い，雌マウスの分娩時の死亡率が増大した。以上から，C57 BL/6Jマウスの一生を通じ，MEnが高低2種の飼料，即ち，MEnが3.73 kcal/gDMの飼料を最良の繁殖成績を得るために，2.86 kcal/gDMの飼料を繁殖期以後の長期飼育における肥満と加齢の最も効果的な抑制のために，必要とすることが判明した。