PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 10–14 (1980)

Influence of Meal Frequency on Body Composition and Energy Utilization in Male Weanling Rats (40713)

B. M. SCHOENBORNE AND NANCY L. CANOLTY

Department of Nutrition, University of California, Davis, California 95616

The question of whether or not meal frequency influences body composition and feed efficiency has been of interest to several researchers. A few studies have indicated that body composition has been influenced by alterations in the meal patterns of force-fed rats (1–3); whereas, other studies have indicated that in pair-fed rats (4) and pigs (5) that meal frequency does not alter body composition. The purpose of this study was to determine the influence of restricted meal frequency on body composition, gross feed efficiency, and energy utilization and maintenance requirements in male weanling rats.

Material and methods. Seventy male weanling rats of the Sprague–Dawley strain weighing 50 ± 5 g were randomly placed into one of four groups. Group I consisted of 23 nonfasted male rats which were sacrificed by cervical dislocation at the beginning of the study, Day 0. These animals were used to predict initial carcass weight, lean weight, and body energy for rats in groups III and IV. Group II consisted of 12 male rats which were provided food *ad libitum* for 3 weeks. These rats were used to determine restricted feed intakes for rats in groups III and IV. Rats fed one time per day and four times per day were given 46, 67, and 87% of *ad libitum* food intake. Group III consisted of 18 rats fed one time per day (1 ×/day) at three different restricted levels of feed intake for 21 days. These male rats were given food at 1800 hr. Group IV consisted of 17 rats given food four times per day (4 ×/day) at three different restricted levels of intake for 21 days. These rats were given food at 0600, 1200, 1800, and 2400 hr. Animals in groups III and IV were fed equivalent amounts of the same diet.

Rats in groups II, III, and IV were housed individually in suspended wire cages in animal quarters that were maintained at 27°C and lighted from 0700 to 2100 hr. They were fed a diet containing 24% casein as shown on Table I, and always had free access to water. Rats were weighed weekly and feed intakes were recorded. Paper liners were placed below the suspended wire cages and daily spillage was determined. At the end of 3 weeks, Day 21, these rats were killed by cervical dislocation.

Prior to analyses, gastrointestinal (GI) tracts were removed from all rats. GI contents were discarded and empty GI tracts were returned to the carcasses. The carcasses were then weighed to determine final carcass weights.

For each animal, the metabolic body size ($W^{0.75}$) was computed by raising to the 0.75 power the mean body weights in kilograms during the feeding trial.

Carcasses were lyophilized for 7 days. The dried carcasses were extracted for 7 days with ether and 5 days with acetone. The final fat weights of the rat carcasses were calculated as the differences in the weights of the dried carcasses before and after extraction. The difference between the total weight of a carcass (including moisture) and the weight of the extracted fat was the fat-free carcass weight.

Determining gross feed efficiency. Gross feed efficiency was calculated by subtracting initial body weight (Day 0) from final body weight (Day 21) and dividing by total dry matter intake.

Determining final body, lean, and fat energy. Energy values of samples of extracted fat and portions of dried, fat-free carcass were determined in a bomb calorimeter. Each animal's final fat energy (FE) and final lean energy (LE) were computed by multiplying its fat weight and its dried, extracted carcass weight by 9.360

0037-9727/80/010010-05$01.00/0

TABLE I. COMPOSITION OF 24% CASEIN DIET[a]

Ingredient	Percentage ingredient/100 g diet
Casein[b]	24.00
Corn oil	8.00
Salt mix[c]	6.00
Vitamin mix[d]	2.00
Cerelose[e]	60.00
BHT	0.02

[a] Gross energy = 451.0 kcal/100 g diet; metabolize energy = 89% of gross energy; and dry matter = 90%.

[b] Nutritional Biochemical Co., Cleveland, Ohio.

[c] Composition per kg of diet: (in g) $CaCO_3$, 18.00; K_2HPO_4, 19.50; $CaHPO_4$, 3.60; NaCl, 10.08; $FeSO_4 \cdot 7H_2O$, 1.50; $MgSO_4 \cdot 7H_2O$, 3.88; KI, 0.015; $ZnCO_3$, 0.048; $CuSO_4 \cdot 5H_2O$, 0.018, and $MnSO_4 \cdot H_2O$, 0.138.

[d] Composition per kg of vitamin mix: (in g) choline chloride, 50.0; inositol, 25.0; ascorbic acid, 5.0; calcium pantothenate, 2.5; thiamine hydrochloride, 1.5; pyridoxine, 1.5; nicotinic acid, 1.5; menadione, 1.25; riboflavin, 0.5; *para*-aminobenzoic acid, 0.5; folic acid, 0.03; biotin, 0.0125; Rovimix E-125, 21.4; Rovimix A-325, 2.1; Rovimix AD_3 325/325, 0.23; B_{12} plus mannitol, 1.5, and Cerelose, 885.0.

[e] Cerelose, Corn Products Co., New York, N.Y.

and 4.453 kcal/g, respectively. Total final body energy (BE) was calculated by adding Final FE and Final LE.

Determining carcass, lean, and fat energy deposition coefficients. A regression equation was used to express BE at Day 0 as a linear function of body weight (BW) at that age. Initial BE = 1.6469 + −4.5622 × BW. This equation was used to predict BE at Day 0 in those animals slaughtered at Day 21 by their initial body weights. The body energy change (ΔBE) of an individual animal was calculated to be the difference between BE at Day 0 (predicted) and BE at Day 21 (measured).

A regression equation was used to predict lean weight (LW) at Day 0 from BW at Day 21. Initial LW = 0.7478 + 4.2074 × BW. The change in LW (ΔLW) in an individual animal was the difference between LW at Day 0 (predicted) and LW at Day 21 (measured). The difference between the ΔLW and the total change in BW was taken as the change in the fat weight (ΔFW). The energy value (9.360 kcal/g) of ΔFW indicated the change in fat energy (ΔFE). The difference between ΔBE and ΔFE was the change in lean energy (ΔLE).

For individual animals, energy changes (ΔBE, ΔLE, and ΔFE), and, gross energy intake (GEI) were expressed as per unit of metabolic body size. Regression analysis was then used to express these energy changes as a function of GEI to determine energy deposition coefficients (6–8). From correlation coefficients (r) corresponding t-values were computed to test the statistical significance of correlations. The slopes of the lines, representing the efficiency of gain above maintenance, were determined. Standard errors of energy deposition coefficients were computed and differences among coefficients were determined by Student's t test described by Snedecor and Cochran (9) for comparing slopes of regression lines.

TABLE II. DRY MATTER INTAKE, CHANGE IN BODY WEIGHT AND GROSS FEED EFFICIENCY OF RATS FED ONE TIME AND FOUR TIMES PER DAY

	Level of intake	1 ×/Day (n = 18)	4 ×/Day (n = 17)
DMI (g)	1	126.5 ± 1.0[a]	126.7 ± 0.7
	2	184.4 ± 0.9	186.4 ± 1.1
	3	240.3 ± 1.4[b]	246.0 ± 1.9
ΔBW (g)	1	48.5 ± 1.9	47.8 ± 1.4
	2	83.4 ± 1.4	82.5 ± 1.2
	3	113.4 ± 2.4	117.5 ± 2.8
Gross food efficiency[c]	1	0.38 ± 0.02	0.38 ± 0.01
	2	0.45 ± 0.01	0.44 ± 0.01
	3	0.47 ± 0.01	0.48 ± 0.01

[a] Mean ± SEM.

[b] Significance of difference, $P < 0.05$.

[c] ΔBW (g)/DMI (g).

TABLE III. BODY COMPOSITION OF RATS FED ONE TIME AND FOUR TIMES PER DAY

	Level of intake	1 ×/Day (n = 24)	4 ×/Day (n = 22)
H_2O (g)	1	64.6 ± 1.4[a]	67.0 ± 1.2
	2	86.4 ± 1.7	87.6 ± 1.1
	3	100.7 ± 1.6	110.3 ± 2.3
% H_2O	1	69.3 ± 0.4	69.6 ± 0.5
	2	67.7 ± 0.4	67.3 ± 0.7
	3	67.3 ± 0.5	66.8 ± 0.4
Fat (g)	1	6.3 ± 0.5	5.7 ± 0.5
	2	11.8 ± 0.6	11.8 ± 1.1
	3	15.7 ± 1.2	17.1 ± 1.0
% Fat	1	6.7 ± 0.6	6.0 ± 0.6
	2	9.3 ± 0.5	9.0 ± 0.8
	3	10.0 ± 0.7	10.4 ± 0.7
Fat-free weight (g)	1	86.9 ± 1.8	90.4 ± 1.6
	2	115.8 ± 2.3	118.5 ± 1.4
	3	140.0 ± 2.2	148.0 ± 3.3

[a] Mean ± SEM.

Results and discussion. Dry matter intakes (DMI), changes in body weights (ΔBW), and gross feed efficiencies ($\Delta BW_g/DMI_g$) are shown in Table II. Differences in the DMI between rats fed one time per day and four times per day for level 3 was due to increased spillage for rats fed one time per day. Mean weight gains and feed efficiencies increased as DMI increased. There were no significant differences in body weight changes or gross feed efficiencies when rats fed one time and four times per day were compared. These results are in agreement with other studies (4, 5) which have shown that body weight is not influenced by alterations in meal frequency when animals are pair-fed.

Body water and fat expressed in grams and as a percentage of body weight, as well as fat-free weight are given in Table III. Water, fat, and fat-free weights increased as feed intakes increased for rats fed one time and four times per day. The percentage water remained constant as DMI increased but the percentage fat increased. There were no significant differences in the grams of water, percentage water, grams of fat, percentage fat, and fat-free weight between rats fed one time per day and four times per day. Cohn and Joseph (2) have

TABLE IV. FINAL BE, LE, AND FE OF RATS FED ONE TIME AND FOUR TIMES PER DAY

	Level of intake	1 ×/Day (n = 18)	4 ×/Day (n = 17)
Final BE (kcal)	1	157.7 ± 2.8[a]	158.1 ± 3.1
	2	241.5 ± 5.3	247.4 ± 9.2
	3	303.5 ± 11.4	327.8 ± 5.9
Final LE (kcal)	1	99.2 ± 1.9	104.3 ± 1.9
	2	130.9 ± 2.4[b]	137.3 ± 1.3
	3	156.9 ± 2.8	167.8 ± 4.3
Final FE (kcal)	1	58.5 ± 4.3	53.8 ± 4.4
	2	110.6 ± 5.7	110.2 ± 10.2
	3	146.6 ± 11.3	160.0 ± 8.9

[a] Mean ± SEM.

[b] Significance of difference, $P < 0.05$.

reported that rats force-fed two meals per day accumulate more body fat than *ad libitum* fed rats; whereas, Romsos *et al.* (5) using pair-fed pigs and Ozelci *et al.* (4) using rats have reported that meal frequency did not alter body fat. These conflicting results may be attributed to differences in initial food restriction or differences in feeding techniques.

Final BE, Final LE, and Final FE are given in Table IV. There were no significant differences in final BE and FE in kilocalories between rats fed one time and four times per day, but there was a significant difference ($P < 0.05$) in the final LE for level 2. For both groups, Final BE, LE, and FE increased as GEI increased. These results indicate that alterations in meal frequency did not significantly influence final body energy when rats were fed equivalent amounts of the same diet at three different restricted levels of intake for 21 days.

When $\Delta BE/W^{0.75}$, $\Delta LE/W^{0.75}$, or $\Delta FE/W^{0.75}$ are regressed on $GEI/W^{0.75}$, the slope of the regression line represents the efficiency with which gross energy available (GEA) is utilized for carcass, lean, or fat energy gain, and are referred to as the carcass, lean, or fat energy deposition coefficients, respectively.

There were no significant differences in the carcass, lean, and fat energy deposition coefficients (Table V) for rats fed one time and four times per day. These results indicate that alterations in meal frequency did not influence the efficiency with which GEA was utilized for carcass, lean, or fat energy gain.

The maintenance requirement and the efficiency of energy utilization for rats fed one time per day and four times per day were established by regressing $\Delta BE/W^{0.75}$, $\Delta LE/W^{0.75}$, and $\Delta FE/W^{0.75}$. Maintenance requirement per unit of metabolic body size was calculated from the equation by setting $\Delta BE/W^{0.75}$ equal to zero. The GEI that was required for maintenance during the 21 days was 2092.9 kcal/$W^{0.75}$ for rats fed one time per day and 2480.8 kcal/$W^{0.75}$ for rats fed four times per day. Expressed on a daily basis, the maintenance requirement for total carcass was 99.7 kcal/$W^{0.75}$ for rats fed one time per day and 118.1 kcal/$W^{0.75}$ for

TABLE V. COEFFICIENTS FOR DEPOSITION AND MAINTENANCE OF ENERGY IN THE TOTAL CARCASS, FAT-FREE OR LEAN CARCASS AND CARCASS FAT

Feeding frequency	No. of animals	Total carcass			Carcass lean			Carcass fat		
		Energy deposition coefficient[a]	*r*	Energy maintenance coefficient[b]	Energy deposition coefficient[c]	*r*	Energy maintenance coefficient[d]	Energy deposition coefficient[e]	*r*	Energy maintenance coefficient[f]
1 ×/Day	18	0.34 ± 0.03	0.94	99.7	0.12 ± 0.01	0.96	11.4	0.22 ± 0.02	0.87	141.4
4 ×/Day	17	0.42 ± 0.03	0.96	118.1	0.12 ± 0.01	0.95	17.9	0.30 ± 0.04	0.90	159.6

[a] The slope ± SE of the regression line relating kcal change in carcass energy (ΔCE) per metabolic body size (MBS) and kcal gross energy intake (GEI) per MBS.

[b] Negative *y*-intercept of regression line relating ΔCE/MBS and GEI/MBS divided by slope of that line and the obtained value divided by 21 (the number of days in the feeding trial.)

[c] The slope ± SE of the regression line relating change in lean energy (ΔLE) per MBS and GEI/MBS.

[d] Negative *y*-intercept of the regression line relating ΔLE/MBS and GEI/MBS divided by the slope of that line then divided by 21.

[e] The slope ± SE of the regression line relating change in fat energy (ΔFE) per MBS and GEI/MBS.

[f] Negative *y*-intercept of the regression line relating ΔFE/MBS and GEI/MBS divided by the slope of that line then divided by 21.

rats fed four times per day. There was no significant differences in carcass, lean and fat maintenance coefficients between rats fed one time and four times per day.

Summary. The influence of restricted meal frequency on body composition and energy utilization has been investigated in male weanling rats. Two groups of rats were fed equivalent amounts of a 24% casein diet at three different restricted levels of intake for 21 days. One group was fed one time per day (1800 hr) and the other four times per day (0600, 1200, 1800, and 2400 hr). There were no significant differences in the water, fat, or fat-free weights (g); and final body energy, lean energy, and fat energy between rats fed one time and four times per day. When $\Delta BE/W^{0.75}$, $\Delta LE/W^{0.75}$, and $\Delta FE/W^{0.75}$ were regressed on $GEI/W^{0.75}$, there were no significant differences in the body, lean, and fat energy deposition coefficients between rats fed one and four times per day. In this study, meal frequency did not alter body composition, nor the efficiency with which feed was utilized above maintenance.

---

1. Cohn, C., Joseph, D., and Shrago, E., Metabolism **6**, 381 (1957).
2. Cohn, C., and Joseph, D., Amer. J. Physiol. **196**, 965 (1959).
3. Cohn, C., and Joseph, D., Metabolism **9**, 492 (1960).
4. Ozelci, A., Romsos, D. R., and Leveille, G. A., J. Nutr. **107**, 1768 (1977).
5. Romsos, D. R., Miller, E. R., and Leveille, G. A., Proc. Soc. Exp. Biol. Med. **157**, 528 (1978).
6. Canolty, N. L., and Koong, L. J., J. Nutr. **106**, 1202 (1976).
7. Canolty, N. L., and Koong, L. J., J. Food Sci. **42**, 1130 (1977).
8. Canolty, N. L., and Koong, L. J., J. Nutr. **108**, 1555 (1978).
9. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," Chaps. 6 and 7. Iowa State Univ. Press, Ames (1967).

Received May 21, 1979. P.S.E.B.M. 1980, Vol. 163.