PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 849–854 (1975)

Influence of Alterations in Meal Frequency on Lipogenesis and Body Fat Content in the Rat[1] (38912)

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It has been shown in numerous experiments that rats trained to consume their food in a short daily period (meal-eaters) are more efficient in converting food energy to body energy than animals allowed diet *ad libitum* (nibbler). If the meal-eater is force-fed a quantity of food equal to that consumed by the nibbler, the meal-eater will accumulate more body fat than the nibbler (1–3). If the meal-eater is allowed food for only 2 hr per day, it will consume about 20% less food than the nibbler but will grow at the same rate as the nibbler (4). The reason(s) for the increased energy efficiency observed in meal-fed rats have not been elucidated.

It has been suggested that obese humans may have an increased efficiency for converting dietary energy to body energy. Fabry *et al.* (5, 6) reported that there was an inverse relationship between the number of meals ingested daily and the degree of overweight in 400 adult males. Others (7–10), however, have failed to observe an effect of meal frequency on body weight changes in humans.

Muiruri and Leveille (11) examined the effect of converting rats from meal-eating to nibbling. It was apparent that the previous feeding pattern influenced lipogenic capacity and body weight changes. Rats converted from meal-eating to nibbling gained weight faster and accumulated body fat faster than did control nibbling rats. They also noted that lipogenic enzyme activity in adipose tissue homogenates from converted rats only slowly decreased to the levels observed in the control nibblers. Hollifield and Parson (12) observed similar results.

In the previous study (11) the experiment was terminated 3 wk after the rats were converted from meal-eating to nibbling and the accumulation of body fat was estimated indirectly. In the present study we examine longer term influences of converting rats from meal-eating to *ad libitum* feeding and obtain direct estimates of body fat accumulation. Food intake, body weight gain, body fat content, adipose tissue lipogenic enzyme activities and in vitro rates of fatty acid synthesis were measured at various intervals during the 12-wk study.

Materials and methods. Male Sprague–Dawley rats weighing 250–300 g were used. They were housed in individual cages with raised wire floors in a temperature regulated room. Nibblers had continuous access to food while meal-eaters were allowed to eat from 8 AM to 10 AM. Water was available *ad libitum*. The semi-purified diet supplied approximately 58, 30, and 12% of calories as carbohydrate, protein and fat, respectively (13).

Two hundred sixteen rats were placed on one of four treatments (Table I). One group was meal-fed (meal-eaters) and another allowed to eat *ad libitum* (nibbler). A third group was meal-fed for 3 wk and then allowed to nibble for 9 wk (converted I). The fourth group was meal-fed for 3 wk, allowed to nibble for the next 3 wk, meal-fed from the 6th to 9th wk and then returned to *ad libitum* feeding for the 9th–12th wk of the experiment. Body weights and food intake were recorded weekly. Animals were weighed at 10 AM.

Eight rats from each group were decapitated after 3, 4, 5, 6, 7½, 9, 10½ and

[1] Supported by AM 15847 from NIH. Journal Article Number 7093, Michigan Agricultural Experiment Station.

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TABLE I. EXPERIMENTAL PROTOCOL.

Week	Feeding pattern			
	Nibbler	Meal-eater	Converted I	Converted II
0–3	nibble[a]	meal-eat[b]	meal-eat	meal-eat
4–6	nibble	meal-eat	nibble	nibble
7–9	nibble	meal-eat	nibble	meal-eat
10–12	nibble	meal-eat	nibble	nibble

[a] Allowed continuous access to food.

[b] Allowed access to food from 8 AM to 10 AM each day.

12 wk of the experiment. The epididymal fat pads were rapidly removed and weighed. To determine rates of fatty acid synthesis duplicate 100–150 mg distal pieces of one fat pad were incubated in Krebs-Ringer bicarbonate buffer containing per ml: 1.8 mg glucose, 0.1 μCi glucose-U-^{14}C and 0.1 unit porcine insulin.[4] Procedures for isolation of fatty acids and determination of radioactivity has been described (14).

Another piece of adipose tissue was homogenized in 9 volumes of a buffer containing 0.15 *M* KCl, 4 m*M* $MgCl_2$, 4 m*M* EDTA and 4 m*M* *N*-acetyl-cysteine. The homogenate was centrifuged at 105,000 *g* for 40 min at 4°. Malic enzyme (15), acetyl CoA carboxylase (16), and fatty acid synthetase (17) activities were determined on the supernatant fraction at 25, 37 and 37°; respectively. Protein was determined by the method of Lowry *et al.* (18).

After removing the stomach contents and the epididymal fat pads, the fat content of the remaining carcass was determined gravimetrically according to Mickelsen and Anderson (19). To obtain an estimate of total body fat it was assumed that the previously removed fat pads contained 85% fat. Body fat content was then expressed as a percentage of carcass weight. Body fat gain was calculated by comparing fat content of individual animals with the average fat content of animals within that treatment killed at earlier time intervals.

During the 8th week of the experiment some of the rats developed a respiratory infection. Antibiotics were added to the drinking water during the 9th–12th wk. The incidence of respiratory infection was reduced by the 12th wk and did not appear to affect one treatment group to a greater extent than another. None of the animals died.

Results. Table II shows the body weight gain, food intake and food efficiency of the rats. Meal-eaters gained less body weight ($P < 0.05$) than nibblers over the 12-wk period. These results are in agreement with an earlier report (4). During the initial adaptation period (week 1) meal-fed rats failed to gain weight after which they gained at about the same rate as the nibbler until the 6th wk. During the 6th to 9th week, meal-fed rats gained less ($P < 0.05$) than the nibblers. Total body weight gain for the converted I rats was similar to that of the nibblers while the total gain of the converted II animals was intermediate between that of the nibblers and meal-eaters. During all periods, meal-fed rats consumed less diet ($P < 0.05$) than did nibblers or converted I rats.

Over the 12-wk period the converted I rats gained body weight slightly more efficiently than the other three groups (Table II). The meal-eaters, nibblers and converted II rats were equally efficient in converting dietary energy to body weight gain over the 12-wk period. During the 3- to 6-wk period, meal-eaters gained body weight more efficiently than nibblers; however, these differences were not apparent beyond the 6-wk period. These observations are in agreement with earlier reports (4, 11).

Table III presents the percentage body fat and body fat gain of the rats. As expected, body fat percentage increased with time on experiment. At the end of the 12-wk study converted I rats contained a higher percentage body fat than did the meal-fed rats. Other comparisons among treatment groups were not statistically different.

Table IV presents in vitro rates of fatty acid synthesis, malic enzyme, acetyl CoA carboxylase and fatty acid synthetase enzyme activities in adipose tissue of rats on the four treatments. In agreement with previous reports (20) the lipogenic capacity of adipose tissue from meal-fed rats was

[4] Porcine insulin generously supplied by Dr. R. Chance, Eli Lilly Research Laboratories.

TABLE II. EFFECT OF FEEDING PATTERN ON BODY WEIGHT GAIN, FOOD INTAKE AND FOOD EFFICIENCY IN RATS.[a]

Period-weeks	Feeding pattern			
	Nibbler	Meal-eater	Converted I	Converted II
	—Weight gain, g—			
0–3	119 ± 4	65 ± 6[b]	—	—
4–6	77 ± 4	79 ± 4	102 ± 11[b]	—
7–9	50 ± 4	32 ± 3[b]	58 ± 4[c]	17 ± 3[bc]
10–12	41 ± 4	39 ± 4	51 ± 3[c]	64 ± 3[bc]
4–12	167 ± 6	150 ± 11	203 ± 15[bc]	168 ± 6
0–12	286 ± 6	215 ± 14[b]	284 ± 13[c]	244 ± 10[b]
	—Food intake, g—			
0–3	537 ± 13	359 ± 13[b]	—	—
4–6	532 ± 17	455 ± 15[b]	557 ± 14[c]	—
7–9	555 ± 12	440 ± 13[b]	555 ± 8[c]	438 ± 10[b]
10–12	526 ± 12	432 ± 14[b]	525 ± 12[c]	541 ± 8[c]
4–12	1613 ± 39	1326 ± 39[b]	1638 ± 31[c]	1524 ± 18[c]
0–12	2150 ± 50	1685 ± 50[b]	2009 ± 26[bc]	1892 ± 22[bc]
	—Food efficiency, mg gain/g food—			
0–3	222 ± 7	179 ± 12[b]	—	—
4–6	145 ± 7	173 ± 8[b]	207 ± 14[b]	—
7–9	91 ± 7	81 ± 5	103 ± 6[c]	35 ± 5[bc]
10–12	78 ± 8	93 ± 6	94 ± 4	116 ± 5[bc]
4–12	104 ± 4	113 ± 6	137 ± 6[bc]	120 ± 5[b]
0–12	134 ± 4	127 ± 5	144 ± 4[c]	130 ± 6

[a] Results expressed as mean ± SEM for eight rats. See Table I for description of feeding patterns.
[b] Probability of a significant ($P < 0.05$) effect; nibbler vs. meal-eater, converted I or converted II rats.
[c] Probability of a significant ($P < 0.05$) effect; meal-eater vs. converted I or converted II rats.

TABLE III. EFFECT OF FEEDING PATTERN ON PERCENTAGE BODY FAT AND BODY FAT GAIN OF RATS.[a]

Time-weeks	Feeding pattern			
	Nibbler	Meal-eater	Converted I	Converted II
	—Percentage body fat—			
3	11.31 ± 0.82	11.17 ± 0.07	—	—
6	13.04 ± 0.88	13.31 ± 1.25	13.11 ± 0.92	—
9	14.17 ± 1.07	12.93 ± 0.99	14.34 ± 0.53	12.58 ± 0.97
12	17.06 ± 0.79	15.96 ± 1.08	18.76 ± 0.73[c]	16.05 ± 0.91
	—Body fat gain, g—			
3–12	50 ± 4	34 ± 5[b]	65 ± 5[bc]	46 ± 5

[a] Results expressed as mean ± SEM for eight rats. See Table I for description of feeding patterns.
[b] See Table II.
[c] See Table II.

greater than that from nibblers. Further, the differences tended to increase with time on experiment. This occurred because the lipogenic activity of adipose tissue from nibbling rats declined rapidly as the rats aged; whereas, the lipogenic capacity of adipose tissue from meal-fed rats decreased less with age. It was previously demonstrated that these differences in lipogenic activity of meal-fed and nibbling rats are

TABLE IV. INFLUENCE OF FEEDING PATTERN ON RATE OF *in Vitro* FATTY ACID SYNTHESIS, MALIC ENZYME ACETYL CoA CARBOXYLASE AND FATTY ACID SYNTHETASE ACTIVITIES IN ADIPOSE TISSUE OF RATS.[a]

	Feeding Pattern							
Period-week	Nibbler	Meal-eater	Converted I	Converted II	Nibbler	Meal-eater	Converted I	Converted II
	—Fatty acid synthesis—				—Malic enzyme—			
3	490±42	1000±51[b]	—	—	163±20	292±33[b]	—	—
4	385±36	770±51[b]	612±24[b]	—	147±9	266±9[b]	339±17[b]	—
5	301±38	581±32[b]	368±39[b]	—	114±13	254±12[b]	178±21[b]	—
6	178±21	558±35[b]	306±33[bc]	—	62±8	264±18[b]	119±16[bc]	—
7½	80±3	405±65[b]	174±28[bc]	396±42[b]	28±2	177±14[b]	62±10[bc]	135±11[bc]
9	122±22	433±41[b]	134±18[c]	365±35[b]	39±8	180±32[b]	45±9[c]	111±18[b]
10½	80±27	412±41[b]	82±14[c]	299±30[bc]	21±6	147±15[b]	23±4[c]	116±21[b]
12	67±7	275±30[b]	75±15[c]	160±24[bc]	18±2	136±22[b]	25±4[c]	56±4[bc]
	—Acetyl CoA carboxylase—				—Fatty acid synthetase—			
3	13±1	23±2[b]	—	—	21±2	33±2[b]	—	—
4	12±2	23±2[b]	27±2[b]	—	23±2	36±2[b]	40±1[b]	—
5	11±1	21±1[b]	17±2[b]	—	17±2	30±2[b]	23±2[bc]	—
6	8±1	22±2[b]	14±2[bc]	—	9±1	30±2[b]	15±2[bc]	—
7½	3.7±0.3	15±2[b]	5±1[bc]	13.1±1.6[b]	5±0.5	22±2[b]	11±2[bc]	25±2[b]
9	2.8±0.5	11±2[b]	3.0±0.8[c]	10.4±0.7[b]	5±1	15±1[b]	6±1[c]	14±1[b]
10½	1.5±0.5	6.7±1.0[b]	1.6±0.3[c]	6.4±1.1[b]	3±1	12±1[b]	3.5±0.5[c]	12±1[b]
12	1.7±0.2	8.6±1.4[b]	1.9±0.3[c]	3.9±0.4[bc]	4±0.5	11±1[b]	4±0.5[c]	6±1[c]

[a] Mean ± SEM for eight rats. See Table I for description of feeding patterns. The rate of fatty acid synthesis was expressed as nanomoles of glucose-U-^{14}C converted to fatty acids per 100 mg adipose tissue per 2 hr. Enzyme activities were expressed as nanomoles substrate converted to product per mg protein per min.

[b] See Table II.

[c] See Table II.

apparent whether the results are expressed per mg tissue or per cell (21, 22).

Rats switched from meal-eating to ad libitum feeding (converted I) did not exhibit an immediate return of the *in vitro* rate of fatty acid synthesis to values similar to the nibbling rats (Table IV). In fact, it took 6 wk for the lipogenic rate to return to the nibbling value. Rats returned to meal-eating for the second time (converted II) exhibited a rapid increase in in vitro rate of fatty acid synthesis; within 10 days of returning to meal-eating lipogenic activity of converted II rats equalled that of the rats meal-fed for the entire experiment (Table IV). Again, when converted II rats were returned to *ad libitum* feeding, lipogenic activity decreased only slowly. This suggests that the deadaptation to meal-eating occurs more slowly than does the adaptation to meal-eating. Earlier reports (11, 14) would also support this suggestion.

Changes in the activities of malic enzyme, acetyl CoA carboxylase and fatty acid synthetase were similar to the changes observed in *in vitro* rates of fatty acid synthesis (Table IV). The activities of these enzymes were higher in adipose tissue homogenates from meal-fed rats than from nibbling rats. When rats were switched from meal-eating to *ad libitum* feeding (converted I), lipogenic enzyme activities decreased slowly to reach values similar to the nibbling rats only after 6-wk of *ad libitum* feeding. The return of lipogenic enzyme activities to values similar to those of meal-fed rats occurred within 10 days following the switch from *ad libitum* feeding to meal-feeding (converted II) (Table IV).

Control nibblers normally consume most of their diet during the dark hours. Rats switched from meal-eating (converted I and II) to ad libitum feeding initially con-

sumed some diet when the room lights were on; however, within one week their pattern of intake was similar to that of the control nibblers.

Discussion. A number of metabolic adaptations are induced by meal-eating. In this study we were interested in the deadaptation of meal-fed rats. Changes in body fat content and adipose tissue lipogenic capacity were monitored. Unlike the force-fed meal-eater (1–3) rats allowed to eat for 2 hr per day do not accumulate more body fat than the *ad libitum* fed rats (Table III). These meal-fed rats appear more efficient in converting diet consumed to body weight gain during the first 3–6 wk of meal-eating (Table II); however, longer term studies suggest that this increased energetic efficiency may dissipate after several months of meal-feeding (4). Similarly, rats switched from meal-eating to *ad libitum* feeding exhibit an increased energetic efficiency initially, but not after several weeks of ad libitum feeding (Table II). Common to both of these shifts in feeding pattern is an increase in food intake. Meal-fed rats increased their food intake after the first several weeks of adaptation and rats shifted from meal-eating to *ad libitum* feeding also increased their food intake (Table II and Ref. 11). Heggeness (23) has noted that rats shifted from a restricted maintenance diet to ad libitum feeding also retain a significantly greater fraction of ingested calories than do control *ad libitum* fed rats. In preliminary experiments we have allowed rats to consume diet for 2 hr per day and then, utilizing a feeding apparatus, pair fed the nibbling rats (24). In this experiment food intake and body weight gain were identical. These results suggest that an increase in food intake above some basal level increases food efficiency for a period of time. The mechanism involved in this apparent increased food efficiency remains to be established.

The increase in fatty acid synthesis in rats being adapted to meal-eating occurs within a few days and precedes the increase in malic enzyme and glucose-6-phosphate dehydrogenase enzyme activities (14). This suggested that the activities of these enzymes do not regulate lipogenesis. In the present study, rats (converted II) responded to a second episode of meal-eating with a rapid increase in adipose tissue lipogenic capacity (Table IV). Within 10 days, both rates of fatty acid synthesis and lipogenic enzyme activities reached the level observed in rats meal-fed continuously.

It is apparent that the reversal of the adaptive changes required a longer period of time than that necessary for their induction. In this study approximately 6 wk was required for the deadaptation. The reason(s) for this delay are not readily apparent. Rats reverted from meal-eating to nibbling do not immediately adopt the feeding pattern of control nibbling rats, but require approximately 1 wk for this to occur. This slow reversal of feeding pattern might explain, in part, the delay in metabolic adaptation; however, other factors probably are also involved. The increase in food intake observed immediately after changing rats from meal-eating to *ad libitum* feeding may also be a contributing factor.

The role of meal frequency and shifts in energy intake from a "normal" intake to a restricted intake during weight reduction, followed by a rapid return to "normal" energy intake after weight reduction, in the control of human obesity warrants further study. Metabolic adaptations induced by changes in meal frequency, in some situations, may have long term influences on body composition.

Summary. Rats were allowed to eat only 2 hr per day (meal-fed) or were fed *ad libitum* (nibbler) for 12 wk; another group of animals was meal-fed for 3 wk and then fed *ad libitum* (converted I) while the fourth group of rats (converted II) was meal-fed for 3 wk, allowed to nibble for the next 3 wk, meal-fed from the 6th to 9th wk and then returned to *ad libitum* feeding for the last 3 wk. Body fat gain and food efficiency was increased in converted I rats. The lipogenic capacity of adipose tissue from meal-fed rats was greater than observed in nibbling rats. Changes in adipose tissue lipogenic activity decreased slowly when meal-fed rats were reverted to *ad libitum* feeding whereas lipogenic activity increased rapidly when *ad libitum* fed rats were switched to meal-feeding.

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Received February 5, 1975. P.S.E.B.M. 1975, Vol. 149.