Cellularity and *in Vitro* Fatty Acid Biosynthesis in Adipose Tissue of Meal-Fed and Nibbling Rats[1] (36255)

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Rats trained to consume their food in a short daily period (meal-fed) develop a markedly increased ability to convert carbohydrate to fat (15). The capacity of adipose tissue from meal-fed rats to incorporate a lipogenic substrate into fatty acids *in vitro* is 3- to 5-fold greater than that observed for tissue from control nibbling rats (9). Although it has been assumed that these results represent a true change in the metabolic activity of the adipocyte, it is possible that they are due to an alteration in the size and/or number of adipocytes in meal-fed rats. Recent experiments have demonstrated that the metabolic activity of adipose tissue may be related to the size of the fat cells (13, 16). Results expressed on a lipid or tissue weight basis often are in poor agreement with results expressed on the basis of cellularity (5, 14).

Braun *et al.* (2) found an increase in the DNA content of epididymal adipose tissue of meal-fed rats compared with nibbling animals. This suggested that there was an increased number of fat cells in adipose tissue from meal-fed rats, however, quantititive data on fat cell size and number are lacking.

It is well established that over 50% of the DNA in the epididymal fat pad is associated with cell types other than adipocytes (3, 12) and it has recently been suggested that fat pad DNA content may change without a corresponding change in adipocyte number (3). Consequently, it is necessary to obtain direct estimates of fat cell size and number. The studies reported below were undertaken to evaluate the effect of meal-eating and nibbling on fat cell size and number in rat epididymal adipose tissue and to express the *in vitro* lipogenic capacity of the tissue on a fat cell basis.

Methods. Male Sprague-Dawley rats, weighing approximately 250 g, were used. The animals were housed singly in metal cages having raised wire floors. A semipurified diet (8) supplying approximately 58, 30, and 12% of calories as carbohydrate, protein, and fat, respectively, was fed for at least 4 weeks. The animals were fed either *ad libitum* (nibblers) or were given access to food from 8 to 10 a.m. only (meal-eaters). Water was available at all times. In one experiment (Table II), graded levels of fiber (0, 20, or 60%) were added to the diet of the nibblers to limit body weight gain.

On the day of the experiment, the animals were decapitated and the left epididymal fat pad was removed and weighed. Distal portions of the contralateral fat pad were rapidly excised and treated as indicated below for the various analyses. To determine the *in vitro* capacity for fatty acid biosynthesis, pieces of tissue (weighing about 100 mg) were incubated as previously described (7). The Krebs–Ringer bicarbonate buffer (7) contained half the recommended Ca^{+2}; 10 μmoles of glucose (0.1 μCi of glucose-U-^{14}C) and 0.1 units of porcine insulin/ml. The procedures for isolation and counting of radioactive fatty acids have been described (7). Pieces of adipose tissue, rather than free fat cells, were used in the incubations because of ease of handling. Rodbell (12) has previously shown that fat cells account for virtually all of the lipogenic activity of adipose tissue.

The size and number of fat cells from the

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TABLE I. Body Weight, Epididymal Fat Pad Weight, and Percentage Lipid, Fat Cell Size and Number of Fat Cells per Fat Pad, and *in Vitro* Incorporation of Glucose-U-^{14}C into Fatty Acids by Adipose Tissue of Meal-Eating and Nibbling Rats.

Group[a]	Criteria	Meal-eater	Nibbler	p[c]
1	Body wt (g)	334 ± 6[b]	383 ± 7	<.001
2		354 ± 9	381 ± 8	<.05
3		391 ± 5	411 ± 7	<.05
1	Fat pad wt (g)	1.66 ± 0.09	2.60 ± 0.15	<.001
2		2.19 ± 0.15	2.75 ± 0.11	<.01
3		2.73 ± 0.16	3.32 ± 0.31	ns
1	Lipid (%) in epididymal fat pad	80 ± 1	81 ± 1	ns
2		76 ± 1	80 ± 1	<.05
3		83 ± 4	84 ± 1	ns
1	Fat cell size (mg × 10^{-5} lipid/cell)	8.12 ± 0.43	9.34 ± 0.57	ns
2		10.27 ± 0.57	11.17 ± 0.62	ns
3		9.79 ± 0.68	11.35 ± 0.91	ns
1	Fat cell no. × 10^6/pad	16.55 ± 1.36	22.50 ± 2.10	<.05
2		17.28 ± 1.47	20.07 ± 0.61	ns
3		23.10 ± 1.69	24.60 ± 1.78	ns
1	Fatty acid synthesis/100 mg of tissue[d]	—	—	
2		746 ± 35[b]	270 ± 20	<.001
3		255 ± 21	51 ± 5	<.001
1	Fatty acid synthesis/10^6 fat cells[d]	—	—	
2		970 ± 68	382 ± 40	<.001
3		303 ± 27	67 ± 8	<.001

[a] Animals in groups 1, 2, and 3 were fed the experimental diets for 4, 4, and 14 weeks, respectively.

[b] Mean ± SEM for 10 rats.

[c] Probability of differences between meal-fed and nibbling rats being significant; ns = not significant ($p > .05$).

[d] Results: glucose-U-^{14}C converted to fatty acids (nmoles/2 hr).

epididymal fat pads were estimated by the method of Hubbard and Matthew (3). Fat cells were isolated as described by Rodbell (12). Lipid was extracted from the adipose tissue and free fat cells, and quantitated gravimetrically (11). DNA was extracted from the defatted tissue and cells (11) and quantitated by means of the indole reaction (4).

Results. Data obtained from 3 groups of rats are presented in Table I. Meal-fed rats weighed less and had smaller (in 2 of the 3 groups) fat pads than the nibbling rats. Small differences were observed in percentage of lipid in the adipose tissue (Table I). The heavier fat pads from the nibbling rats tended to have a higher lipid content than the smaller fat pads from the meal-fed animals.

Fat cell size was estimated and data are presented in Table I. There were no significant differences in fat cell size when meal-eating and nibbling rats were compared. The fat pads from nibbling animals had slightly more adipocytes per fat pad than the pads from the meal-eating rats. Only the difference for Group 1 attained significance.

The conversion of glucose-U-^{14}C to fatty acids in epididymal adipose tissue from meal-fed rats was significantly higher than that observed in tissue from nibbling animals, when the results were expressed on a tissue weight basis (Table I). These results were not unexpected and are in close agreement with earlier work (9). The differences in *in vitro* fatty acid biosynthesis observed between groups 2 and 3 for meal-fed and nibbling rats may have resulted from the differences in age of the animals. These results are in agreement with an earlier observation by Leveille (10) on the effect of advancing age on *in vitro* lipogenesis in fat pads from meal-

TABLE II. Body Weight, Epididymal Fat Pad Weight and Percentage Lipid, Fat Cell Size and Number of Fat Cells per Fat Pad, and *in Vitro* Incorporation of Glucose-U-^{14}C into Fatty Acids by Adipose Tissue of Meal-Fed and of Nibbling Rats Fed Graded Levels of Fiber.[a]

Criteria	Meal-fed	Nibbler		
Fiber (%) added to diet:	0	0	20	60
Body wt (g)	450 ± 9[ba]	500 ± 4[b]	451 ± 11[a]	411 ± 4[c]
Fat pad wt (g)	3.08 ± 0.21[a]	4.31 ± 0.22[b]	3.24 ± 0.20[a]	2.09 ± 0.10[c]
Lipid (%) in epididymal fat pad	74 ± 1[a]	78 ± 2[a]	77 ± 2[a]	76 ± 1[a]
Fat cell size (mg × 10^{-5} lipid/cell)	7.63 ± 0.74[a]	8.52 ± 0.51[a]	9.49 ± 0.86[a]	7.12 ± 0.13[b]
Fat cell no. × 10^6/fat pad	30.2 ± 2.0[a]	39.5 ± 3.7[b]	27.1 ± 2.0[a]	22.5 ± 1.1[c]
Fatty acid synthesis/100 mg of tissue[c]	205 ± 22[a]	49 ± 11[b]	53 ± 7[b]	83 ± 14[b]
Fatty acid synthesis/10^6 fat cells[c]	206 ± 23[a]	54 ± 12[b]	65 ± 9[b]	76 ± 11[b]

[a] Rats were fed the experimental diets for 14 weeks.

[b] Mean ± SEM for 8 rats. Mean values on a line followed by the same superscript letter do not differ significantly ($p > .05$).

[c] Results: glucose-U-^{14}C converted to fatty acids (nmoles/2 hr).

fed and nibbling rats.

When the conversion of glucose-U-^{14}C to fatty acids by epididymal adipose tissue was expressed on a fat cell basis, the differences in lipogenic capacity between meal-fed and nibbling rats was nearly the same as when the results were expressed on a tissue weight basis (Table I). This would be expected since the fat cells from the meal-fed and nibbling rats were of similar size.

Varying levels of fiber (0, 20, or 60%) were added to the diets of nibbling rats in an attempt to have nibbling rats that weighed more, the same, and less than meal-fed rats of the same age. Results of this experiment are presented in Table II. The results presented for the meal-fed and the nibbling rats fed no additional fiber are similar to the results presented in Table I. When 20% fiber was added to the diet of the nibbling rats, body weight and fat pad weight, as well as fat cell size and number were similar to that observed for the meal-fed rats (Table II). The addition of 60% fiber to the diet decreased final body and fat pad weight and fat cell size and number to values lower than those observed for the meal-fed rats. The rate of *in vitro* fatty acid biosynthesis was higher in the meal-fed than in the nibbling rats. Results for fatty acid biosynthesis were similar for the nibbling rats fed 0, 20, or 60% fiber.

Discussion. Our results for epididymal fat cell size and number are in accord with the values recorded by Therriault *et al.* (16) and Hubbard and Matthew (3). Salans and Dougherty (13) have recently reported that the mean cell size of adipose cells in the proximal portion of the fat pad is significantly larger than the cells of the distal portion. In the present study, tissue for cell size and lipogenic capacity determinations was obtained from the distal portion of the fat pad. Consequently, the estimates of fat cell number per fat pad (Tables I and II) may be somewhat high. However, this does not affect interpretation of the results pertaining to the lipogenic capacity of the tissue, since all comparisons were made using tissue obtained from the same region of the fat pad. Only 22 to 35% of the adipose tissue DNA was associated with adipocytes (results not presented). These values are similar to those reported by Hubbard and Matthew (3).

It has recently been demonstrated that the *in vitro* rate of incorporation of glucose into triglycerides, in the presence of insulin, is inversely related to fat cell size (3). In the

present study, fat cells from nibbling rats were not significantly larger than those from the meal-fed animals. When 60% fiber was added to the diet of the nibbling rats, fat cells were significantly smaller than those observed in the meal-fed animals (Table II). Still, the lipogenic capacity of the fat cells from meal-fed rats was significantly higher than that of the fat cells from the nibbling animals (60% fiber diet). Under the conditions of these studies, it appears that the small differences in fat cell size did not influence lipogenic capacity to nearly the extent that the meal-eating regime did. Consequently, previously reported effects of meal-eating on *in vitro* lipogenesis are not invalidated by the fact that such results were expressed on the basis of tissue weight rather than cellularity.

Nibbling rats fed 0 or 20% added fiber had significantly larger fat cells than the nibbling rats fed the 60% fiber diet (Table II). Although the results were not significant there was a higher *in vitro* rate of fatty acid biosynthesis in the adipose tissue from nibbling animals with smaller cells (60% fiber diet) than from the nibbling rats with larger fat cells. This observation is in agreement with the recent report of Salans and Dougherty (13).

A marked decrease in *in vitro* fatty acid synthesis capacity per cell occurred between Groups 2 and 3 (Table I), even though cell size was similar. This decrease in fatty acid synthesis capacity may possibly have resulted from the differences in ages of the rats. Although this comparison is subject to criticism because the experiments (Groups 2 and 3) were not conducted concurrently, it is interesting to observe that, in the present study, there was a 76% decrease in *in vitro* fatty acid synthesis with little change in cell size; while in Tables I and V from the study of Salans and Dougherty (13), there was a 26% decrease in triglyceride synthesis associated with a 106% increase in cell size. The reasons for these differences are not readily apparent. The rats used by Salans and Dougherty (13) covered a wider weight range (215–504 g) and they measured the *in vitro* capacity to synthesize triglycerides, while only the incorporation of glucose into fatty acids was determined in the present study. It has recently been shown that, at least in human adipose tissue, radioactive glucose incorporation into triglycerides increased with an increase in cell size (1). Most of the radioactivity was associated with the glyceride–glycerol moiety of the triglyceride. It remains to be established whether there is a differential incorporation of radioactive glucose into the fatty acid and the glycerol moiety of triglycerides as rat fat cells enlarge and/or age.

Summary. Epididymal fat cells were of similar size in meal-fed and nibbling rats. Consequently, the differences in *in vitro* lipogenic capacity of the epididymal fat pads from meal-fed and nibbling rats were of similar magnitude whether the results were expressed on a tissue weight basis or on a fat cell basis. The results of these studies show that the enhanced lipogenic capacity observed in the adipose tissue from meal-fed rats results from a true metabolic adaptation and not a change in cell size.

1. Björntorp, P., and Karlsson, M., Eur. J. Clin. Invest. 1, 112 (1970).

2. Braun, T., Kazdová, L., Fábry, P., Lojda, A., and Hromádková, V., Metabolism 17, 825 (1968).

3. Hubbard, W. W., and Matthew, W. T., J. Lipid Res. 12, 286 (1971).

4. Hubbard, R. W., Matthew, W. T., and Dubowik, D. A., Anal. Biochem. 38, 190 (1970).

5. Knittle, J. L., and Hirsch, J., J. Clin. Invest. 47, 2091 (1968).

6. Leveille, G. A., J. Nutr. 90, 449 (1966).

7. Leveille, G. A., J. Nutr. 92, 460 (1967).

8. Leveille, G. A., and O'Hea, E. K., J. Nutr. 93, 541 (1967).

9. Leveille, G. A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29, 1294 (1970).

10. Leveille, G. A., unpublished data.

11. Novak, M., and Monkus, E. F., Anal. Biochem. 36, 454 (1970).

12. Rodbell, M., J. Biol. Chem. 239, 375 (1964).

13. Salans, L. B., and Dougherty, J. W., J. Clin. Invest. 50, 1399 (1971).

14. Salans, L. B., Knittle, J. L., and Hirsch, J., J. Clin. Invest. 47, 153 (1968).

15. Tepperman, J., Brobeck, J. R., and Long, C. N. H., Yale J. Biol. Med. 15, 875 (1943).

16. Therriault, D. G., Hubbard, R. W., and Mellin, D. B., Lipids 4, 413 (1969).

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