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METABOLIC ADAPTATIONS TO A "STUFF AND STARVE" FEEDING PROGRAM. I. STUDIES OF ADIPOSE TISSUE AND LIVER GLYCOGEN IN RATS LIMITED TO A SHORT DAILY FEEDING PERIOD *

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Many obese people consume most of their food within a relatively short period each day. These people eat little or no breakfast or lunch but eat voraciously during the evening (1, 2). Stunkard, Grace and Wolff (3) have used the term, "night-eating syndrome" to describe this pattern of eating and suggested that this pattern is the result of psychological factors.

Some strains of rats can be trained to eat sufficient food in 1 hour each day to permit normal rates of growth (4) and studies of the respiratory quotients of these animals indicate increased lipogenesis (5). Tepperman and Tepperman (6) have shown that liver slices from rats trained in this way show increased incorporation of acetate-1-C^{14} and glucose-U-C^{14} *in vitro,* as well as a three- to fourfold increase in hexose monophosphate shunt dehydrogenase activity. However, lipogenesis in liver represents only a small fraction of the total lipid synthesis in the intact animal. Adipose tissue contributes the major portion (7).

These observations coupled with the unusual eating pattern seen in many obese people prompted this study of adipose tissue and liver in rats allowed access to more food than they were able to consume for 2 hours out of each 24, for periods of 1 to 7 days. Studies were made of acetate-1-C^{14} incorporation into lipids by adipose tissue and liver slices *in vitro,* free fatty acid (FFA) content of adipose tissue, glucose-6-phosphate (G-6-P) dehydrogenase and 6-phosphogluconic (6-P-G) dehydrogenase activity of adipose tissue and liver homogenates and liver glycogen.

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MATERIALS AND METHODS

Fifty-six male rats of the Sprague-Dawley strain, weighing 180 to 220 g, were used in the study. These animals were divided into three groups (A, B, and C) and prepared as outlined below. Purina laboratory chow pellets were used as feed throughout the studies. At the time of sacrifice rats were killed by a blow to the head. Adipose tissue and liver slices for the studies indicated below were removed as quickly as possible. Portions of liver for glycogen determination were put into 30 per cent potassium hydroxide immediately after excision.

Group A. Six animals allowed food ad libitum since they were weaned were fasted for 24 hours (from 10:00 a.m. to 10:00 a.m.), sacrificed, and studied as indicated below.

Group B. Forty rats which had been on ad libitum feeding were allowed access to unrestricted amounts of laboratory chow from 8:00 to 10:00 a.m. each day with water ad libitum throughout each day. Groups of 5 or 6 rats were sacrificed at the end of the 2-hour feeding period each day on Days 1–7 of this program and studied as outlined below.

Daily food intake and body weights were determined for the 6 rats in this group which were studied at the end of the feeding period on the seventh day.

Group C. Ten rats were allowed food for only 2 hours per day; five were studied at the end of a 24-hour fast (10:00 a.m. to 10:00 a.m.) after 4 days on this program and the other five after a similar fast after 5 days on the feeding program.

The following studies were done on the animals in Groups A, B, and C. *1)* The incorporation of acetate-1-C^{14} into lipids by epididymal adipose tissue and liver slices was determined by the method of Baruch and Chaikoff (8). Two μmoles of acetate-1-C^{14} containing 2 μc of activity was added to each incubation flask containing approximately 400 mg of adipose tissue or liver, and incubated for 3 hours at 37° C. *2)* Free fatty acid content of adipose tissue was studied by a modification of Dole's method (9). *3)* Liver glycogen was determined by the modified method of Pflueger (10). *4)* Glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activity in cell-free adipose tissue and liver homogenates was studied by the methods described by Kornberg and Horecker (11) and Horecker and Smyrniotis (12). Nitrogen content of adipose and

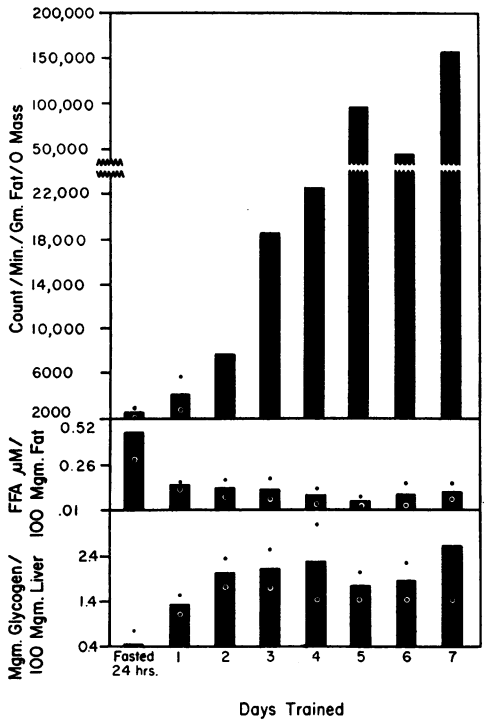

FIG. 1. ACETATE-1-C^{14} INCORPORATION INTO LIPIDS BY ADIPOSE TISSUE, FREE FATTY ACID (FFA) CONTENT OF ADIPOSE TISSUE, AND LIVER GLYCOGEN FROM RATS FED AD LIBITUM AND FASTED FOR 24 HOURS (GROUP A) AND RATS FED 2 HOURS PER DAY FOR FROM 1 THROUGH 7 DAYS (GROUP B). Dots on either side of bars represent 1 standard deviation from the mean.

liver tissue was determined by the micro-Kjeldahl method.

RESULTS

Average food intake for the six animals studied in Group B during the first 2-hour feeding period was approximately 60 per cent of that during the control period in which food was available ad libitum. Food intake during the 2-hour feeding period on the second day was about 95 per cent of the ad libitum control period and remained at this level during the experimental period. The average weight of the six animals fed for 2 hours per day for 7 days decreased for the first 3 or 4 days (average loss about 20 per cent of starting weight) but then began to increase, and by the seventh day all were within 10 per cent of their starting weights. The data on liver glycogen, acetate-1-C^{14} incorporation into lipids by adipose tissue *in vitro*, and FFA content of adipose tissue for Groups A and B are given in Figure 1. From Figure 1 it is apparent that rats fed ad libitum and then fasted for 24 hours (Group A) had little liver glycogen and that adipose tissue from these animals contains relatively large amounts of FFA but incorporates little acetate-1-C^{14} into lipids *in vitro*. In the animals fed for 2 hours per day and studied immediately at the end of the feeding period on Days 1–7 (Group B), acetate-1-C^{14} incorporation into lipids by epididymal fat *in vitro* rose each day and by the fifth day was about 25 times that of animals fed ad libitum, fasted 22 hours, and fed for 2 hours on Day 1. Incorporation of acetate-1-C^{14} into liver slices rose only fourfold during this period. The FFA content of adipose tissue decreased, reaching its lowest level on the fifth day. Liver glycogen rose for the first 3 days and remained relatively stable thereafter.

G-6-P and 6-P-G dehydrogenase activity in adipose tissue rose and on the fifth day was over 200 per cent as high as on the first day of the feeding program (Figure 2). G-6-P and 6-P-G dehydrogenase activity in the livers of these animals was much lower than in adipose tissue and rose only 25 and 40 per cent during the 5-day period.

The data on the animals studied at the end of 24 hours of fasting after being allowed food only 2 hours per day for 4 or 5 days (Group C) are

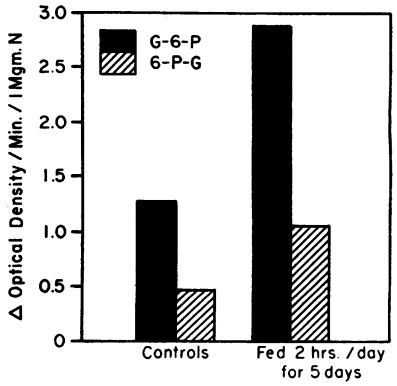

FIG. 2. G-6-P DEHYDROGENASE AND 6-P-G DEHYDROGENASE ACTIVITY IN ADIPOSE TISSUE AFTER THE FIRST 2-HOUR FEEDING PERIOD (CONTROLS) AND AFTER THE FIFTH 2-HOUR FEEDING PERIOD. Values are means for 6 rats in each group. Mean (controls): G-6-P dehydrogenase, 1.23 ± 0.33; 6-P-G, 0.454 ± 0.21. Mean (fed 2 hours per day for 5 days): G-6-P dehydrogenase, 2.88 ± 0.48; 6-P-G dehydrogenase, 1.054 ± 0.29; $p = 0.01$ for both enzymes.

given in Figure 3. Data from these animals are compared with those from animals fed ad libitum and fasted for 24 hours (Group A) and from animals studied immediately after the 2-hour feeding period after 4 or 5 days on the 2-hour feeding program (Group B). Adipose tissue from animals in Group C showed little acetate-1-C^{14} incorporation into lipids *in vitro* and high levels of FFA, as might be expected in a fasting animal. However, these animals had much more liver glycogen than the animals fed ad libitum and fasted for 24 hours in Group A. It is surprising that liver glycogen content in these animals was approximately that of the animals that were studied at the end of the 2-hour feeding periods. G-6-P dehydrogenase and 6-P-G dehydrogenase activity in adipose tissue from the animals fasted for 24 hours after 5 days on the feeding program averaged 0.218 and 0.110 Δ absorbance per minute per mg N, respectively.

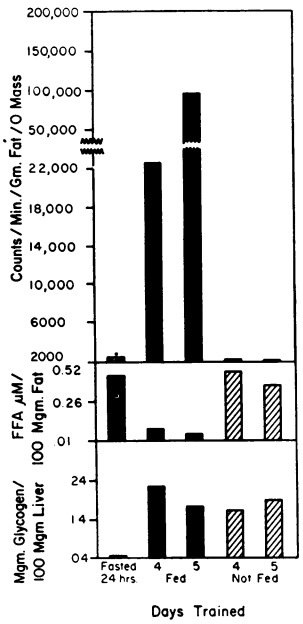

FIG. 3. ACETATE-1-C^{14} INCORPORATION INTO EPIDIDYMAL FAT *in vitro*, FFA CONTENT OF ADIPOSE TISSUE, AND LIVER GLYCOGEN FROM RATS FED AD LIBITUM AND FASTED FOR 24 HOURS (FIRST GROUP OF BARS), COMPARED WITH THOSE FROM ANIMALS AT THE END OF A 2-HOUR FEEDING PERIOD AFTER 4 OR 5 DAYS ON THE 2-HOUR FEEDING PROGRAM (FED) AND WITH ANIMALS FED 2 HOURS A DAY FOR 4 OR 5 DAYS AND THEN FASTED FOR 24 HOURS (NOT FED). Dots on bars represent 1 standard deviation from mean.

DISCUSSION

It is generally accepted that glycogen stores in a well fed animal are limited to the extent that hypoglycemia would occur after a relatively few hours if gluconeogenesis did not occur. In the face of this limited ability to store glycogen it would seem that an animal that eats large amounts in a short period must develop some rapid method of storing this large load of nutrients. In view of the newer concepts of adipose tissue metabolism, one would suspect that adipose tissue might be the site at which the major portion of these nutrients would be metabolized (7).

Data from the present studies support this idea and offer some insight into the mechanisms involved. Adipose tissue from rats limited to a short daily feeding period very quickly develops a markedly enhanced ability to synthesize lipids from acetate *in vitro*. This increased lipid synthesis is associated with greatly increased G-6-P dehydrogenase and 6-P-G dehydrogenase activity in adipose tissue. The activity of these enzymes in adipose tissue from animals fed for 2 hours per day for 5 days increases over 1,000 per cent above the fasting level within 2 hours after the animal starts to eat. The mechanism of this very great and rapid rise in the activities of these dehydrogenases is currently under investigation in this laboratory. Evidence which suggests that this increase in enzyme activity is the result of synthesis of these enzymes will be presented in a subsequent report (13).

The finding of abundant liver glycogen in animals fasted for 24 hours after a short period on this type of feeding program is a puzzling finding. It is possible that rats adapted to this feeding program catabolize fat as their major energy source, limiting glucose utilization to the small amounts required by the central nervous system and possibly a few other tissues. Alternative explanations are that glycogen is formed from protein or fat. However, the latter possibility seems most unlikely.

Increased amounts of liver glycogen have been observed by others (6) in fasted rats fed for 1 hour per day and in chronically undernourished rats (14). These chronically undernourished rats apparently were given a limited amount of food

once daily (limited to the extent that appreciable weight loss occurred) and it is probable that they ate it in a short period, so in effect they were limited to one brief feeding period each day. Eviscerate preparations of chronically undernourished rats (14) showed decreased utilization of glucose, which is consistent with the idea that more fat is being utilized. An increased utilization of fat would protect glycogen stores in the liver without invoking the explanation that glycogenolysis is defective in these rats, as has been postulated (14).

These observations have a number of interesting implications in considering the eating patterns observed in obese people. It seemed reasonable to assume that similar adaptations would occur in humans who consume most of their food within a relatively short period each day. Certainly the adaptive changes in adipose tissue in rats fed in this way occur very quickly. Studies of the long-term consequence of this type of feeding program in rats show that animals fed in this way do get fat and that the feeding pattern tends to be self-perpetuating once established. These studies will be published in a separate report (15).

SUMMARY AND CONCLUSIONS

The observations listed below were made on rats allowed access to more food than they could consume for only 2 hours out of each 24, for from 1 to 7 days.

1. Rats allowed access to unlimited food for only 2 hours each day lost weight for 3 or 4 days, but began to regain and by the seventh day were within 10 per cent of their initial weights.

2. Food intake in the first 2-hour feeding period in these animals fell to about 60 per cent of the 24-hour ad libitum intake but thereafter was approximately 95 per cent of that of ad libitum intake.

3. Incorporation of acetate-1-C^{14} into lipids by adipose tissue *in vitro,* from animals fed only 2 hours per day increased very markedly and by the fifth day of this feeding program was over 25 times that of the first day.

4. Glucose-6-phosphate (G-6-P) dehydrogenase and 6-phosphogluconate (6-P-G) dehydrogenase activity in adipose tissue homogenates from animals fed in this way increased over 200 per cent by the fifth day of this program.

5. Incorporation of acetate-1-C^{14} into lipids by liver slices, and G-6-P dehydrogenase and 6-P-G dehydrogenase activity in liver homogenates increased during this feeding program, but the changes in liver were small when compared with changes in adipose tissue.

6. The free fatty acid content of adipose tissue fell as the rate of lipogenesis in adipose tissue increased.

7. Liver glycogen rose during the first few days of this program and remained relatively constant thereafter. Rats fasted for 24 hours after 4 or 5 days on this feeding program had much higher levels of liver glycogen than did control animals fed ad libitum after a similar fast.

8. The implications of these findings have been discussed.

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