# Changes in Lipogenesis and Lipolysis Associated with Recovery from Reversible Obesity in Mature Female Rats (42893)

RUTH B. S. HARRIS AND ROY J. MARTIN
*Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602*

*Abstract.* **Reversible obesity provides a model for demonstration of weight regulation in mature animals. Changes in body composition and *in vitro* adipose and hepatic lipid synthesis and adipose lipolysis of rats recovering from enforced weight gain were examined to determine whether correction of weight was facilitated by metabolic changes independent of those resulting from hypophagia and negative energy balance. Female Sprague-Dawley rats (200 g) were divided into three groups. Controls ate *ad libitum*, tube-fed control rats were weight matched to controls. Two-hundred percent-fed rats were tube-fed twice control intake. After 26 days tube feeding stopped and a subgroup from each treatment was killed for determination of body composition and *in vitro* tissue metabolism. Further subgroups were examined 5, 10, 15, and 36 days later. At the end of overfeeding 200%-fed rats were hypophagic and had high rates of adipose and hepatic lipid synthesis, which soon returned to normal. Gross changes in body fat mass were corrected by hypophagia and increased adipose lipolysis. The remaining small excess in body fat appeared to be corrected by decreased basal and insulin-stimulated adipose fatty acid synthesis when food intake had returned to normal.**
[P.S.E.B.M. 1989, Vol 191]

The correction of body weight by mature animals to control levels following a period of over- or underfeeding is frequently cited as indirect evidence for the control of body weight. Rats have been made obese by overfeeding (1), by inducing hyperphagia with insulin injections (2, 3), or by electrical stimulation of the hypothalamus (4). When the external stimuli to overeat were removed, the rats were hypophagic until their body weights were similar to those of controls. Regulation of body weight has also been observed in monkeys (5) and humans (6) recovering from overfeeding.

Although it is generally accepted that nongrowing mammals will maintain their weight within a relatively narrow weight range, the mechanisms that normally achieve this regulation have not been clearly defined. As mentioned above, rats that gain weight during a period of enforced overfeeding will return to their control weight once overfeeding stops. Decreased food intake is obviously an important contributor to the weight loss, as the animals go into a state of negative energy balance and catabolize endogenous energy stores. Changes in lipid metabolism that occur following periods of enforced food restriction or starvation in normal weight rats (7) or in humans (8–12) are well documented. However, less information is available concerning changes in adipose and hepatic lipid synthesis and utilization during the period of voluntary hypophagia and weight loss associated with recovery from enforced weight gain.

In this study we examined changes in adipose and hepatic metabolism of rats that were returning to control weight following a period of weight gain that had been induced by overfeeding. We wanted to determine whether weight loss could simply be accounted for by the metabolic consequences of hypophagia or whether there were additional changes in adipose or hepatic lipid metabolism that would facilitate mobilization of fat stores.

## Materials and Methods

Eighty-two female Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing approximately 200 g, were housed in individual hanging wire mesh cages in a temperature-controlled room at 23 ± 1°C. Lights were on for 12 hr each day, from 0530 to 1730 hr. All rats had free access to water and a semi-purified diet, described in Table I, and were weighed

Received September 1, 1988. [P.S.E.B.M. 1989, Vol 191]
Accepted January 23, 1989.

0037-9727/89/1911-0082$2.00/0

**Table I.** Dietary Composition[a]

| | g/kg |
|---|---|
| Casein | 200 |
| Dextrose | 604 |
| Cellulose | 30 |
| Corn oil | 100 |
| Mineral mix[b] | 40 |
| Vitamin mix[c] | 22 |
| L-Methionine | 4 |

[a] The diet was eaten *ad libitum* as a dry powder. It was tube fed in liquid form. Diet was mixed in a ratio of 4:3 with tap water so that 1.4 ml of liquid contained 1 g of dry diet.
[b] Jones Foster Salt Mix (ICN Nutritional Biochemicals).
[c] AIN Vitamin Mixture (ICN Nutritional Biochemicals).

daily. Control food intake, corrected for scatter, was determined on 30 rats for 10 days.

The rats were divided into three groups, matched as closely as possible for weight and for weight gained during the previous week. One group of 30 rats continued to eat *ad libitum* and daily intakes were recorded for these animals. One group of 27 tube-fed control rats received control food intake by stomach tube in three meals a day. The final group of rats was tube-fed 200% control food. Two-hundred percent-fed rats were initially given control intake which was doubled during the next 6 days. Tube-fed rats received the *ad libitum* diet mixed in a ratio of 4:3 with water. Tube-fed meals were given at 0530, 1330, and 2130 hr. Tube-fed control and 200%-fed rats received 7.3 and 14.6 ml/meal, respectively. After 10 days tube-fed control rats had gained more weight than *ad libitum* rats, therefore they were given 85% control intake to keep weight gain the same as that of controls. Tube feeding continued until the overfed rats had received 200% intake for 26 days.

On the last day of tube feeding the rats in each treatment were divided into five subgroups, matched for weight and rate of weight gain during the tube-feeding period. Tube-fed rats received their last meal in the evening. The next morning one subgroup of animals from each treatment was sacrificed for measurement of body fat and *in vitro* adipose and hepatic lipogenesis and adipose lipolysis. The rest of the rats returned to *ad libitum* feeding and daily intakes were recorded. Further subgroups of five or six rats from each treatment were sacrificed 5, 10, 15, and 36 days after the end of tube feeding. The timing of the last sacrifice, at 36 days, was determined by overfed rats having returned to the same body weight and food intake as *ad libitum*-fed and tube-fed control rats.

All rats were fasted for 12 hr before being killed by decapitation between 0830 and 1100 hr. Blood was collected for subsequent analysis of serum glucose and insulin concentrations. The brain was removed for another study. The gastrointestinal tract was dissected out and discarded. Liver and inguinal fat pads were dissected out and weighed. Two slices of hepatic tissue, approximately 100 mg each, were taken using a Stadie Riggs tissue slicer for determination of total lipid synthesis (TLS) and triglyceride fatty acid synthesis (FAS). The rest of the liver was returned to the carcass. Eight 100-mg slices of adipose tissue were taken for determination of TLS, FAS, and measurement of glycerol release as an index of lipolysis. A piece of fat, approximately 50 mg, was fixed in osmium tetroxide solution for subsequent determination of cell number and size distribution by Coulter Counter and Channelizer (13).

Tissue slices for measurement of lipogenesis were weighed and placed in 25-ml Erlenmeyer flasks with 2 ml of incubation medium. Medium was Krebs-Ringer bicarbonate buffer (pH 7.45) containing 5.0 m*M* glucose, 1.0 m*M* sodium acetate, 2% bovine serum albumin, and 1.0 $\mu$Ci/ml [1-$^{14}$C]sodium acetate (60 mCi/mmol). Flasks were gassed with 5% $CO_2$/95% $O_2$, sealed with rubber stoppers, and placed in an oscillating water bath at 37°C for 2 hr. Reactions were stopped by the addition of 0.5 ml of 1.0 *N* $H_2SO_4$.

Tissue slices were transferred to screw-capped tubes containing 5 ml of Dole's solution (5 parts isopropanol, 1 part heptane, and 0.1 part 1.0 *N* $H_2SO_4$). Tissues were extracted for TLS and FAS by a modified Dole's procedure (14). After standing overnight the slices were removed from the tubes, 3 ml of heptane and 2 ml of $H_2O$ were added, the aqueous layer was removed, and the heptane was washed twice with 5 ml of 1.0 *N* NaOH. One milliliter of heptane, containing total lipids, was dried in a scintillation vial. The remaining heptane was transferred to a clean tube with 2 ml of alkaline ethanol (1 ml of saturated KOH/100 ml of 95% ethanol) and saponified at 80°C for 2 hr. The saponified solution was washed twice with 3 ml of heptane and then acidified to methyl red point with 3.0 *N* HCl. Triglyceride fatty acids were extracted with three 3-ml washes of heptane which were combined and dried in a scintillation vial. Ten milliliters of a toluene-based scintillation cocktail (15 g Omnifluor, New England Nuclear, Boston, MA/3.75 liters of toluene) were added to each vial which was counted on a Beckman LS9000 Liquid Scintillation System (Beckman Instruments Inc., Irvine, CA). Rate of lipid synthesis was calculated as nmoles of acetate incorporated per liver or per inguinal fat depot or per $10^6$ adipocytes per hour. Basal rates of lipogenesis were determined on duplicate slices of hepatic and adipose tissue for each rat. In addition, insulin-stimulated lipogenesis was determined on duplicate slices of adipose tissue by adding 1000 microunits/ml insulin (Iletin; Eli Lilly, Indianapolis, IN) to the incubation media. Insulin response of adipose tissue was not measured on the first kill day due to an error in making media.

Basal glycerol release was determined on duplicate slices of adipose tissue. The incubation procedure was as described above except that no radiolabel was present during the 2-hr incubation which was preceded by a

30-min preincubation. Maximum release was measured on duplicate slices incubated in the presence of membrane-permeable 5.0 m*M* dibutyryl cAMP. Incubation medium was deproteinized by addition of 0.2 ml of 30% perchloric acid and centrifugation at 6000*g* for 20 min. Glycerol content of the supernatant was determined by the enzymatic spectrophotometric method of Weiland (15). Glycerol release was expressed as $\mu$moles of glycerol released per fat depot.

Carcass fat was determined by autoclaving the carcasses at 120°C for 45 min and then homogenizing them with three times their own weight of water. Triplicate 10-ml aliquots of homogenate were taken and lipid content was determined by methanol/chloroform extraction, which has been described in detail previously (16). Serum glucose concentration was determined using an enzymatic colorimetric procedure (Sigma Chemical Co., St. Louis, MO). Serum insulin was determined by radioimmunoassay (Cambridge Medical Diagnostics Inc., Billerica, MA) using a porcine insulin standard.

Statistically significant differences between treatment groups sacrificed on the same day were determined by one-way analysis of variance for unequally replicated samples and calculation of least significant difference (17). Initially a two-way analysis of variance had been carried out comparing all of the groups killed at different times, but the large standard errors associated with overfed rats sacrificed on Day 1 of recovery masked differences between groups at later time points.

## Results

Control food intake was found to be 15.6 ± 0.41 g/rat/day. Two-hundred percent-fed rats received 31 g of food per day. Tube-fed controls were initially given 15.6 g/day but this was reduced to 13.3 g/day. During the tube-feeding period *ad libitum* rats ate 15.1 ± 0.14 g/rat/day.

Average food intakes for groups of rats sacrificed at the end of the experiment are shown in Figure 1. Intakes of rats sacrificed earlier were not significantly different from those shown in Figure 1. At the end of tube feeding, *ad libitum* intake of 200%-fed rats fell to approximately 6 g/rat/day and remained significantly lower than that of controls for the next 20 days, during which time a significant amount of excess body weight was lost. The *ad libitum* intakes of tube-fed and *ad libitum* controls were the same on all but the first day of recovery.

Daily body weights of the three groups of rats sacrificed at the end of the experiment are shown in Figure 2. The weights of rats killed earlier were not significantly different from those shown in Figure 2. At the end of tube feeding, 200%-fed rats weighed approximately 150 g more than controls. As soon as tube feeding was stopped, these rats started to lose weight and after 23 days of recovery there was no longer a significant difference between the weights of overfed and control rats. *Ad libitum* and tube-fed control rats weighed the same throughout the recovery period.

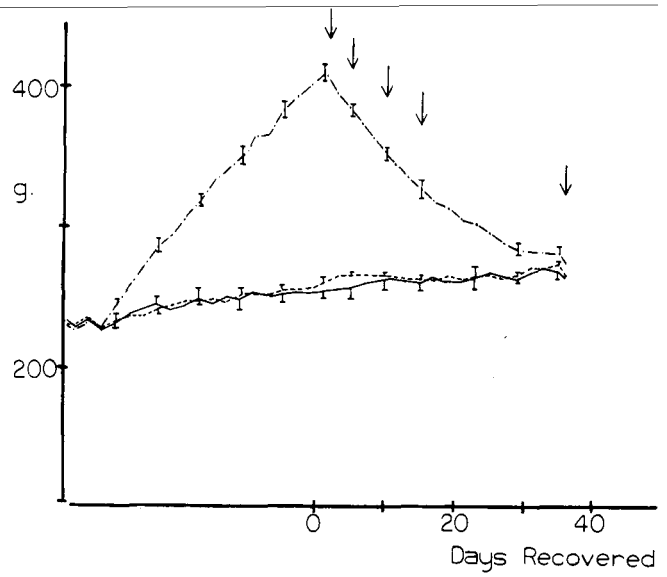


**Figure 1.** Daily food intakes (mean ± SEM) for groups of 200%-fed (-·-·-), tube-fed (——), and *ad libitum*-fed (– – –) rats sacrificed 36 days after the end of tube feeding which ended on Day 0 of the experiment.

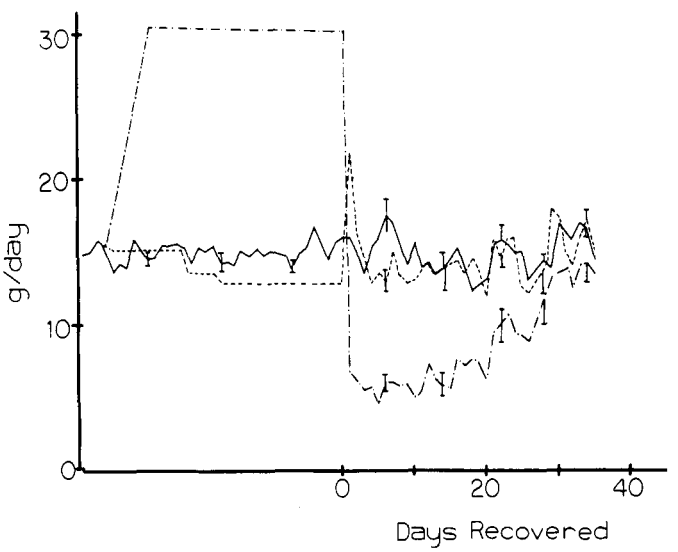


**Figure 2.** Daily body weight (mean ± SEM) for groups of 200%-fed (-·-·-), tube-fed (——), and *ad libitum*-fed (– – –) rats sacrificed 36 days after the end of tube feeding which stopped on Day 0 of the experiment. Arrows indicate the times at which subgroups of animals from each treatment were killed.

Body composition of rats sacrificed at different times during the experiment are given in Table II. Carcass weight reflected differences in live weights of the animals. Livers of tube-fed control rats were slightly enlarged at the end of tube feeding but this was corrected within 5 days of recovery. Livers of 200%-fed rats weighed almost twice as much as those from *ad*

*libitum*-fed rats at the end of tube feeding but had returned to control weight after 15 days of recovery. At the end of tube feeding, 200%-fed rats had about five times as much body fat as controls. Excess fat was lost steadily but overfed rats were still significantly fatter than their controls after 36 days of recovery.

Fat cell size distributions for the different groups of rats are shown in Figure 3. There was no significant difference in the percentage of fat cell size distribution for tube-fed and *ad libitum* control rats at any time during the experiment. A substantial number of cells from the 200%-fed rats killed on all but Day 36 of recovery were larger than the highest diameter measured by the channelizer, which was 160 $\mu$m. With the excessive accumulation of body fat, a second population of small cells appeared in the fat depots of overfed rats, producing a biphasic cell size distribution that was still apparent after 10 days of recovery. The measured cell number per inguinal depot for each group of rats is given in Table III. There was no significant difference in the number of adipocytes per depot for tube-fed and *ad libitum* control rats at any time point of the experiment. There appeared to be an increase in measurable cell number for the overfed rats which was no longer significant at the end of the experiment as the small cells lost lipid and became too small to count by Coulter counting.

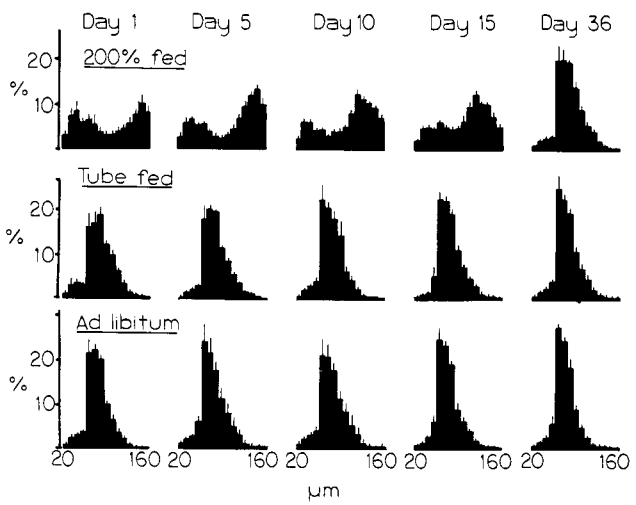


**Figure 3.** Fat cell size distribution (mean ± SEM) for samples of inguinal fat taken from groups of five or six rats after different periods of recovery from tube feeding. Cell size was measured by Coulter Counter and Channelizer and size distribution, from 20 to 160 $\mu$m, was expressed as a percentage of total cells present.

Serum glucose and insulin concentrations are given in Table IV. Both serum glucose and insulin were the same for tube-fed and *ad libitum* controls on all except Day 5 of recovery when the tube-fed rats appeared to be mildly hyperinsulinemic and hypoglycemic. At the end of tube feeding, 200%-fed rats were hyperinsulinemic and hyperglycemic despite a 12-hr fast. Serum glucose was normal by Day 5 of recovery and insulin was normal by Day 10.

Values for hepatic TLS and FAS are given in Table V. Compared with *ad libitum* controls, hepatic TLS was significantly higher for both groups of tube-fed rats at the end of tube feeding. In 200%-fed rats TLS was 7-fold greater than in *ad libitum* controls. By Day 5 of recovery, and all subsequent times tested, there was no significant difference between rates of TLS for tube-fed and *ad libitum* controls. In 200%-fed rats TLS was significantly lower than that of *ad libitum* controls after 10 days of recovery but there was no difference between the treatment groups after 15 and 36 days. Hepatic FAS followed the same pattern as TLS; however, differences between *ad libitum* and 200%-fed rats were not significantly different.

Values for adipose TLS and FAS are given per inguinal depot in Table VI. The parameters were also calculated on a per cell basis and it was found units of expression made no difference to the interpretation of results. These data have not been included as the size

**Table II.** Body Composition[a]

| Parameter | Treatment group | Days following the end of tube feeding | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 15 | 36 |
| Carcass weight (g) | *Ad libitum* | 217 ± 3a | 222 ± 16a | 228 ± 5a | 224 ± 4a | 234 ± 7 |
| | Tube-fed | 221 ± 10a | 230 ± 3a | 231 ± 7a | 226 ± 5a | 232 ± 3 |
| | 200%-fed | 358 ± 6b | 337 ± 2b | 310 ± 5b | 299 ± 8b | 243 ± 3 |
| % Body fat | *Ad libitum* | 8 ± 0.4a | 8 ± 0.6a | 10 ± 0.8a | 8 ± 0.9a | 9 ± 0.6a |
| | Tube-fed | 11 ± 0.5b | 11 ± 0.9a | 9 ± 0.8a | 9 ± 0.6a | 10 ± 0.6a |
| | 200%-fed | 35 ± 0.8c | 31 ± 2.6b | 24 ± 1.0b | 22 ± 1.7b | 13 ± 1.1b |
| Liver weight (g) | *Ad libitum* | 5.6 ± 0.1a | 5.9 ± 0.2a | 6.0 ± 0.2 | 6.1 ± 0.1a, b | 6.5 ± 0.2a |
| | Tube-fed | 6.4 ± 0.1b | 5.8 ± 0.2a | 6.0 ± 0.4 | 5.7 ± 0.2a | 6.4 ± 0.1a |
| | 200%-fed | 10.2 ± 0.4c | 7.1 ± 0.3b | 6.7 ± 0.1 | 6.7 ± 0.2b | 5.8 ± 0.2b |
| Inguinal depot weight (g) | *Ad libitum* | 2 ± 0.1a | 3 ± 0.3a | 3 ± 0.1a | 3 ± 0.3a | 3 ± 0.2a |
| | Tube-fed | 3 ± 0.3a | 3 ± 0.2a | 3 ± 0.3a | 3 ± 0.2a | 3 ± 0.2a |
| | 200%-fed | 15 ± 0.8b | 13 ± 0.9b | 11 ± 0.8b | 10 ± 1.1b | 5 ± 0.6b |

[a] Data are mean ± SEM for groups of five or six rats. Values for a given parameter on a particular day that do not share a common letter are significantly different at $P < 0.05$ as determined by one-way analysis of variance.

**Table III.** Adipocyte Number ($\times 10^6$) per Inguinal Depot[a]

| Treatment group | Days following the end of tube feeding | | | | |
|---|---|---|---|---|---|
| | 1 | 5 | 10 | 15 | 36 |
| *Ad libitum* | 7 ± 0.4a | 7 ± 1.4a | 10 ± 1.5a | 10 ± 1.8a | 9 ± 1.2 |
| Tube-fed | 8 ± 1.2a | 11 ± 0.8a | 9 ± 0.7a | 10 ± 0.9a | 10 ± 1.2 |
| 200%-fed | 18 ± 1.9b | 17 ± 2.6b | 17 ± 1.8b | 17 ± 2.9b | 14 ± 1.3 |

[a] Data are mean ± SEM for five or six rats killed after different periods of recovery from tube feeding. Values for a particular day that do not share a common letter are significantly different at $P < 0.05$ as determined by one-way analysis of variance.

**Table IV.** Serum Insulin and Glucose Concentrations[a]

| Parameter | Treatment group | Days following the end of tube feeding | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 15 | 36 |
| Glucose (mg/100 ml) | *Ad libitum* | 81 ± 5a | 99 ± 6a | 98 ± 5 | 83 ± 6 | 92 ± 8 |
| | Tube-fed | 93 ± 11a, b | 77 ± 7b | 89 ± 4 | 91 ± 6 | 89 ± 6 |
| | 200%-fed | 113 ± 5b | 85 ± 6a, b | 91 ± 5 | 88 ± 1 | 112 ± 11 |
| Insulin (microunits/ml) | *Ad libitum* | 20 ± 2a | 16 ± 2a | 18 ± 2 | 18 ± 2 | 17 ± 3 |
| | Tube-fed | 23 ± 2a | 29 ± 8a, b | 17 ± 2 | 19 ± 5 | 14 ± 1 |
| | 200%-fed | 54 ± 5b | 31 ± 5b | 18 ± 3 | 20 ± 1 | 18 ± 2 |

[a] Data are mean ± SEM for five or six rats killed after different periods of recovery from tube feeding. Values for a given parameter on a particular day that do not share a common letter are significantly different at $P < 0.05$ as determined by one-way analysis of variance.

**Table V.** Hepatic TLS and FAS[a]

| | Treatment group | Days following the end of tube feeding | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 15 | 36 |
| TLS[b] (nmol/liver/hr) | *Ad libitum* | 163 ± 27a | 217 ± 59 | 251 ± 56a | 209 ± 27a, b | 197 ± 29 |
| | Tube-fed | 404 ± 86b | 377 ± 40 | 178 ± 15a, b | 310 ± 68b | 255 ± 26 |
| | 200%-fed | 1172 ± 103c | 181 ± 18 | 111 ± 26b | 115 ± 18a | 170 ± 24 |
| FAS (nmol/liver/hr) | *Ad libitum* | 53 ± 9a | 107 ± 47 | 109 ± 19 | 141 ± 28 | 114 ± 29 |
| | Tube-fed | 113 ± 30a | 165 ± 49 | 98 ± 14 | 211 ± 46 | 154 ± 21 |
| | 200%-fed | 494 ± 77b | 109 ± 10 | 68 ± 16 | 76 ± 17 | 109 ± 26 |

[a] Data are mean ± SEM for groups of five or six rats killed after different periods of recovery from tube feeding. Values for a given parameter on a particular day that do not share a common letter are significantly different at $P < 0.05$ as determined by one-way analysis of variance.
[b] Rates of lipid synthesis were determined by measuring the incorporation of radiolabeled acetate into the appropriate lipid fraction.

**Table VI.** Inguinal TLS and FAS per Depot (nmol/depot/hr)[a]

| Parameter | Treatment group | Days following the end of tube feeding | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 15 | 36 |
| Basal TLS[b] | *Ad libitum* | 110 ± 32a | 81 ± 20 | 78 ± 19 | 55 ± 24 | 90 ± 28 |
| | Tube-fed | 217 ± 39a | 352 ± 127 | 92 ± 46 | 55 ± 17 | 168 ± 78 |
| | 200%-fed | 2082 ± 417b | 364 ± 88 | 51 ± 10 | 44 ± 15 | 18 ± 3 |
| TLS with 1000 microunits/ml insulin | *Ad libitum* | — | 218 ± 76a | 241 ± 85* | 191 ± 68* | 428 ± 93a* |
| | Tube-fed | — | 322 ± 144a | 225 ± 62* | 212 ± 45* | 424 ± 108a* |
| | 200%-fed | — | 836 ± 156b* | 190 ± 51* | 62 ± 15 | 53 ± 14b* |
| Basal FAS | *Ad libitum* | 49 ± 18a | 70 ± 20 | 45 ± 12 | 69 ± 31 | 53 ± 18a, b |
| | Tube-fed | 128 ± 24a | 284 ± 99 | 54 ± 26 | 35 ± 10 | 102 ± 38b |
| | 200%-fed | 1284 ± 364b | 290 ± 69 | 35 ± 5 | 40 ± 13 | 7 ± 1a |
| FAS with 1000 microunits/ml insulin | *Ad libitum* | — | 354 ± 131* | 141 ± 56* | 156 ± 57 | 277 ± 69a* |
| | Tube-fed | — | 524 ± 235 | 133 ± 47* | 166 ± 43* | 315 ± 96a* |
| | 200%-fed | — | 616 ± 164* | 149 ± 44* | 44 ± 13 | 28 ± 10b |

[a] Data are mean ± SEM for groups of five or six rats killed after different periods of recovery from tube feeding. Values for a given parameter on a particular day that do not share a common letter are significantly different at $P < 0.05$ as determined by one-way analysis of variance. An asterisk indicates a significant difference ($P < 0.05$) between basal and insulin-stimulated response, determined by paired $t$ test.
[b] Lipid synthesis was measured by incorporation of radiolabeled acetate into the appropriate lipid fraction.

range of the channelizer may have excluded some large adipocytes in obese rats and resulted in an overestimate of TLS and FAS. Basal rates of adipose TLS and FAS were the same for tube-fed and *ad libitum* controls at all time points. In 200%-fed rats at the end of tube-feeding adipose TLS and FAS were 20-fold higher than in controls. This had been corrected by Day 5 of recovery, although overfed rats were still obese. After 36 days of recovery, when live weight of the 200%-fed rats was normal but body fat was still elevated, adipose TLS and FAS were both significantly depressed in inguinal fat from 200%-fed rats compared with controls. In control rats 1000 microunits/ml insulin increased adipose TLS and FAS 2- to 3-fold at all time points. In 200%-fed rats insulin responsiveness was normal after 5 and 10 days of recovery but was negligible after 15 days of recovery.

Values for glycerol release from inguinal fat are given in Table VII. There was no difference in rate of glycerol release for tube-fed and *ad libitum* controls at any time point but it was 7-fold higher in 200%-fed rats than in controls at the end of tube feeding. This very high rate of release was maintained for at least 15 days and returned to control values by 36 days of recovery. Glycerol release in the presence of 5 m*M* cAMP was approximately three times basal rate for all treatment groups at all time points of the experiment.

## Discussion

In this experiment mature female rats made obese by overfeeding returned to control body weight once overfeeding was stopped. The primary aim of the study was to determine whether recovery of normal body weight could be accounted for by the metabolic consequences of hypophagia or whether additional changes in lipid synthesis or lipolysis enhanced loss of excess body fat.

As tube feeding has been shown to increase the efficiency of energy utilization in rats (18), tube-fed controls were included to distinguish between responses to overfeeding and to meal feeding. The intake of tube-fed controls was reduced to 85% of *ad libitum* intake to maintain a normal rate of weight gain, indicating the degree of change in efficiency of energy utilization. When tube-fed controls returned to *ad libitum* feeding they were hyperphagic for only 1 day, possibly due to the novelty of having dry food available. Increases in body fat content and hepatic TLS apparent at the end of tube feeding were corrected within 5 days and were probably secondary to meal feeding rather than nutrient flux as the rats ate more during the recovery period than they had received during tube feeding.

At the end of overfeeding, 200%-fed rats were obese, hypophagic, hyperglycemic, and hyperinsulinemic and had high rates of hepatic and adipose TLS and FAS. Voluntary food intake of 200%-fed rats was inhibited until body weight returned to control levels, confirming many previous reports of regulation of body weight through the long-term control of food intake (19–21).

Increases in adipose TLS and FAS induced by overfeeding were reversed within 5 days of recovery when the rats were still hypophagic. At the end of the experiment, when body weight and food intake were at control levels but body fat content was still significantly increased, adipose TLS and FAS were depressed in 200%-fed rats, compared with controls. It appeared that food intake could be inhibited by a gross shift in body energy balance, but that the regulatory system was not sensitive to the small increase in body fat that was apparent at the end of the study. The delayed inhibition of FAS and impaired insulin response in the overfed rats was not typical of food-restricted animals. Rats that have been starved for 2 or 3 days have depressed rates of fatty acid synthesis (7, 22), inhibited insulin response (7, 10, 22), and decreased lipoprotein lipase activity (23). The depressed insulin response appears to be a postreceptor effect as insulin binding to adipocytes increases (10, 24). In food-restricted rats the inhibition of lipogenesis has been reported to occur within 7 days (25). In this experiment insulin response was inhibited only after 15 days and TLS and FAS were decreased after 36 days, when food intake had already returned to control levels. As both TLS and FAS were decreased,

**Table VII.** Inguinal Glycerol Release (μmol/depot/hr)[a]

| Parameter | Treatment group | Days following the end of tube feeding | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 15 | 36 |
| Basal glycerol release | *Ad libitum* | 3.4 ± 0.52a | 7.9 ± 1.62a | 4.0 ± 0.56a | 7.5 ± 1.10a | 7.4 ± 1.57 |
| | Tube-fed | 5.4 ± 0.82a | 5.8 ± 1.79a | 6.3 ± 1.77a | 7.1 ± 1.62a | 5.4 ± 1.12 |
| | 200%-fed | 23.1 ± 3.63b | 23.7 ± 7.75b | 29.0 ± 8.56b | 16.7 ± 4.29b | 7.7 ± 0.53 |
| Glycerol release with 5 m*M* dibutyryl cAMP | *Ad libitum* | 10.0 ± 1.56a* | 19.1 ± 3.46a* | 18.5 ± 2.60a* | 22.0 ± 2.48a* | 18.2 ± 2.02* |
| | Tube-fed | 16.8 ± 2.31a* | 18.2 ± 4.14a* | 18.1 ± 2.58a* | 20.8 ± 3.32a* | 17.9 ± 1.69* |
| | 200%-fed | 95.8 ± 24.12b* | 69.7 ± 14.74b* | 75.6 ± 11.21b* | 66.0 ± 12.99b* | 23.1 ± 7.60* |

[a] Data are mean ± SEM for groups of five or six rats killed after different periods of recovery from tube feeding. Values for a given parameter on a particular day that do not share a common letter are significantly different at $P < 0.05$ as determined by one-way analysis of variance. An asterisk indicates a statistically significant difference ($P < 0.05$) between basal and stimulated glycerol release, determined by paired *t* test.

the inhibition was not specific to storage lipid. However, these data may be evidence for a mechanism independent of hypophagia that contributes to loss of fat from the rats. A similar situation has been observed in the parabiotic partners of obese rats which maintain a normal food intake but lose most of their body fat due to inhibition of adipose FAS by a humoral factor originating in the obese animal (13). Feedback inhibition of food intake and FAS may involve humoral messengers that inform the central control system of the state of energy balance of the whole body (13, 26).

At the end of tube feeding, the obese rats were hyperinsulinemic and hyperglycemic and may have been expected to be insulin resistant. It has been established that insulin resistance develops with increased fat cell size (27, 28) and during starvation (8, 10, 29) or food restriction (22). Insulin responsiveness of adipose tissue from 200%-fed rats during the early stages of recovery in this experiment may have resulted from two factors. Obesity was associated with an increased number of small adipocytes which may have masked a depressed insulin response in enlarged cells. By Day 15 of recovery there were few small adipocytes present and insulin did not stimulate either TLS or FAS. By Day 36 of recovery, adipocytes from 200%-fed rats were within the size range found in control rats and responded to insulin although the absolute rate of synthesis was depressed compared with controls. Second, a high (1000 microunits/ml) level of insulin was used to obtain the maximum response from tissue. A dose-response curve would have revealed whether adipose tissue from obese rats was insensitive to insulin.

Although inguinal FAS was normal within 5 days of recovery, adipose glycerol release was still higher in 200%-fed than in control rats after 15 days of recovery. Basal lipolysis in 200%-fed rats was the same as maximum rates in controls but was stimulated by dibutyryl cAMP to the same degree as control tissue, implying that adipocytes from obese rats had an increased capacity for lipolysis which persisted as long as the rats were hypophagic. Mobilization of tissue lipid may be secondary to hypophagia as rats and humans that have been starved or food-restricted have high basal rates of lipolysis (7–9, 11, 12, 22). An enhanced capacity for lipolysis is also apparent in starved humans who give an exaggerated response to adrenergic lipolytic agents (11, 12, 30).

Deposition of excess fat in overfed rats was achieved by both hyperplasia and hypertrophy of measurable adipocytes. The appearance of a second population of small adipocytes with obesity has been observed by other authors (31, 32) but it is not known whether these cells are newly differentiated preadipocytes or whether they are small adipocytes that have accumulated enough lipid to be included in the size range measured by the Coulter Counter. We have previously observed (13) that the cell size distribution remains biphasic, even with increasing body fat content, suggesting that lipid accumulation is limited in the small cells. The small cells disappeared when overfed rats returned to control body weight, presumably because loss of lipid reduced cell diameter to less than 20 $\mu$m rather than because of an actual loss of adipocytes. Metabolic data for adipose tissue was expressed on a per fat depot basis, representing the whole animal response, rather than on a per cell basis as a portion of the cells from obese rats were out of the range of the Coulter Counter. Acetate may not have been the ideal radiolabeled substrate to use for measuring lipid synthesis in rat tissue, as it gives lower values for FAS than are obtained from radiolabeled glucose (33). However, in this experiment all of the comparisons were relative and it has to be assumed that the measurements made with acetate were representative for the different treatment groups.

Hepatic weight TLS and FAS were also increased by overfeeding but were back to normal within 5 days of recovery, indicating a direct relationship between nutrient intake and hepatic lipid synthesis. Greater increases in hepatic FAS may have been observed if measurements had been made postprandially rather than after a fast, as meal feeding induces cycles of lipogenesis and lipolysis (34).

In this experiment rats that had been made obese by overfeeding regained a normal body weight and food intake when overfeeding stopped, but were still significantly fatter than their controls at the end of the study. It is unclear whether the excess fat would have been lost if the recovery period had been extended. However, the results of this study suggest that regulation of body fat content is achieved by at least two mechanisms. Large increases in body energy content are corrected by hypophagia and negative energy balance. Mobilization of fat is facilitated by an increase in the lipolytic capacity of adipose tissue. Correction of smaller changes in fat content may be achieved by inhibition of adipose lipid synthesis that occurs independently of measurable changes in food intake.

This work was supported by Grant DK36890 from the National Institutes of Health.

1. Cohn C, Joseph D. Influence of body weight and body fat on appetite of "normal" lean and obese rats. Yale J Biol Med **34**:598–607, 1962.
2. Panksepp J, Pollack A, Krost K, Meeker R, Ritter M. Feeding in response to repeated protamine zinc insulin injections. Physiol Behav **14**:487–493, 1974.
3. McKay EA, Callaway JW, Barnes RH. Hyperalimentation in normal animals produced by protamine insulin. J Nutr **20**:59–66, 1940.
4. Steffens AB. Influence of reversible obesity on eating behavior, blood glucose and insulin in the rat. Am J Physiol **228**:1738–1744, 1975.
5. Jen K-LC, Hansen BC. Feeding behavior during experimentally

induced obesity in monkeys. Physiol Behav **33**:863–869, 1984.
6. Sims EAH, Danforth E, Horton ES. Endocrine and metabolic effects of experimental obesity in man. Recent Prog Horm Res **29**:457–487, 1973.
7. Owens JL, Thompson D, Shah N, DiGirolamo M. Effects of fasting and refeeding in the rat on adipocyte metabolic functions and response to insulin. J Nutr **109**:1584–1591, 1979.
8. Jensen MD, Haymond MW, Gerich JE, Cryer PE, Miles JM. Lipolysis during fasting. Decreased suppression by insulin and increased stimulation by epinephrine. J Clin Invest **79**:207–213, 1987.
9. Kjellberg J and Ostman J. Lipolysis and glucose tolerance in obese subjects during prolonged starvation. Acta Med Scand **190**:191–198, 1971.
10. Pedersen O, Hjollund E, Schwartz-Sorensen N. Insulin receptor binding and insulin action in human fat cells: Effects of obesity and fasting. Metabolism **31**:884–895, 1982.
11. Ostman J, Arner P, Kimura H, Wahrenberg H, Engfeldt P. Influence of fasting on lipolytic response to adrenergic agonists and on adrenergic receptors in subcutaneous adipocytes. Eur J Clin Invest **14**:383–391, 1984.
12. Wolfe RR, Peters EJ, Klein S, Holland OB, Rosenblatt J, Gary H. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. Am J Physiol **252**:E189–E196, 1987.
13. Harris RBS, Martin RJ. Metabolic responses to a specific lipid-depleting factor in parabiotic rats. Am J Physiol **250**:R276–R286, 1986.
14. Dole VP, Meinertz H. Microdetermination of long chain fatty acids in plasma and tissue. J Biol Chem **235**:2595–2599, 1960.
15. Weiland OHO. Glycerol-UV method. In: Bergermeyer HV, Ed. Methods of Enzymatic Analysis. New York: Academic Press, Vol **3**: p1404, 1974.
16. Harris RBS, Kasser TR, Martin RJ. The dynamics of recovery of body composition following overfeeding, food restriction or starvation of mature female rats. J Nutr **116**:2536–2546, 1986.
17. Steel RGD, Torrie JH. Principles and Procedures in Statistics: A Biometrical Approach. 2nd ed. New York: McGraw-Hill, 1980.
18. Cohn C, Joseph D, Allweiss MD. Nutritional effects of feeding frequency. Am J Clin Nutr **11**:356–361, 1962.
19. Edholm OG. Energy balance in man. J Hum Nutr **31**:413–431, 1977.
20. Hervey GR. Regulation of energy balance. Nature **222**:629–631, 1969.
21. Kennedy GC. The role of depot fat in the hypothalamic control of food intake in rats. Proc R Soc Lond [Biol] **140**:578–592, 1953.
22. Goldrick RB, Hirsch J. Serial studies on the metabolism of human adipose tissue. II. Effects of caloric restriction and refeeding on lipogenesis, and the uptake and release of free fatty acids in obese and nonobese individuals. J Clin Invest **43**:1793–1804, 1964.
23. Fried SK, Hill JO, Nickel M, DiGirolamo M. Prolonged effects of fasting-refeeding on rat adipose tissue lipoprotein lipase activity: Influence of caloric restriction during refeeding. J Nutr **113**:1861–1869, 1983.
24. DeFronzo RA, Soman V, Sherwin RS, Hendler R, Felig P. Insulin binding to monocytes and insulin action in human obesity, starvation and refeeding. J Clin Invest **62**:204–213, 1978.
25. Timmers K, Knittle JL. Effects of undernutrition and refeeding on enzyme activities and rates of glucose catabolism in rat epididymal adipose tissue. J Nutr **110**:1176–1184, 1980.
26. Hervey GR. The effects of lesions in the hypothalamus on parabiotic rats. J Physiol **145**:336–352, 1959.
27. DiGirolamo M, Howe MD, Esposito J, Thurman L, Owens JL. Metabolic patterns and insulin responsiveness of enlarging fat cells. J Lipid Res **15**:332–338, 1974.
28. Olefsky JM. Mechanisms of decreased insulin responsiveness of large adipocytes. Endocrinology **100**:1169–1177, 1977.
29. Newman WP, Bradows RG. Insulin action during acute starvation: Evidence for selective insulin resistance in normal men. Metabolism **32**:590–596, 1983.
30. Kather H, Weiland E, Fischer B, Wirth A, Schlierf G. Adrenergic regulation of lipolysis in abdominal adipocytes of obese subjects during caloric restriction: Reversal of catecholamine action caused by relief of endogenous inhibition. Eur J Clin Invest **15**:30–37, 1985.
31. Allen CE. Cellularity of adipose tissue in meat animals. Fed Proc **35**:2302–2307, 1976.
32. Faust IM, Johnson PR, Stern JS, Hirsch J. Diet induced adipocyte number increase in adult rats: A new model of obesity. Am J Physiol **235**:E279–E286, 1978.
33. Smith S. Lipogenesis in rabbit adipose tissue. J Lipid Res **16**:324–331, 1975.
34. Leveille GA. Control of lipogenesis in fasted and fed meal-fed rats. J Nutr **92**:460–466, 1967.