

BODY FAT CONTENT AND NITROGEN EXCRETION  
OF HYPOTHALAMIC OBESE RATS  
DURING STARVATION

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ABSTRACT

Hypothalamic obese rats and their controls were starved to death. During starvation, body weight and nitrogen excretion were studied. Fat content of the carcasses was also analysed. The results show that hypothalamic obese rats may die of starvation while they still contain large amount of body fat. Their body fat was shown to have a better protein sparing action than that of their controls. The cause of death was attributed to the exhaustion of the utilizable body protein.

Obesity caused by hypothalamic lesions was first reported in 1940 (1). Since then this surgically produced obesity has been extensively studied. It is now generally agreed that ventromedial lesions cause a regulatory impairment which in turn leads to obesity (2, 3, 4 and 5). Mayer (6) designated this kind of obesity as "regulatory obesity" and stated that one of the differences between "metabolic obesity" and "regulatory obesity" lies in the fact that the fat content of the body remains high in the former but returns to normal in the latter when their body weight returns to normal. In this laboratory, Han and Young reported that hypothalamic obese rats when fed a restricted amount of food still contained significantly higher percentage of body fat than did their controls, and hence they suggested that the hypothalamic obesity may not be a simple regulatory one (7). However, since the duration of restricted feeding in their experiment was only between 16 to 24 days they

were not sure whether these obese rats might decrease their body fat content to controls' level if the feeding was restricted for a longer period of time.

The present experiment was, therefore, planned to starve both hypothalamic obese rats and normal rats to death and then to analyze their body fat content in order to determine whether hypothalamic obese rats are of the simple "regulatory obesity" nature.

MATERIALS AND METHODS

Eight hypothalamic obese rats of the Sprague-Dawley strain in both sexes were used. They were operated upon 4 to 10 months prior to the start of the experiment. Six rats of the same age, strain and also in both sexes but receiving no hypothalamic lesions served as the controls.

When we picked up these eight hypothalamic obese rats, body weight was used as the only criterion of obesity. Male rats whose body weights were over 450 g and female rats whose body weights were over 300 g were considered obese.

All rats in this experiment were housed in individual cages in a temperature controlled

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rooms (20 to 25 C). Food was not supplied at all but water with vitamins (Weibcon-M, manufactured locally by Soun-fan Pharmaceutical Company containing vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, C, D, E, K and minerals) was supplied all the time. Individual body weights and daily nitrogen excretion were measured for the first fifteen days and then only body weight was followed until the rats died. The study of nitrogen excretion was carried out with the following procedures. Plastic trays were used to collect individual daily excreta. The trays were washed daily in the morning with distilled water and the contents were collected in 250 ml flasks which contained several ml of 10% HCl. Fecal material if present was ground by using a homogenizer and the final homogenized solution was diluted to a known volume (usually 250 ml). Samples from this diluted solution were analysed for their nitrogen content using Kjeldahl's method. From the result of these analyses, the daily excretion

of nitrogen was determined. This value times 6.25 was used to indicate the amount of protein utilized daily during starvation.

The fat content of carcasses was analysed using the method reported previously (7).

## RESULTS

Table I presents the individual data of obese and control rats.

Under starvation, control rats survived from 21 to 30 days with a mean of  $24.7 \pm 4$  days (mean  $\pm$  S. D.). Obese rats survived from 23 to 59 days with a mean of  $40.1 \pm 13$  days. When the two means are compared the obese rats survived a significantly longer period of time than their controls. The duration of survival of obese rats was quite variable and bears no relationship with their initial body weight.

The total weight loss of obese rats was more than that of controls (255 g vs 180 g). This is due to the fact that obese rats survived longer.

TABLE I

*Individual data of obese and control rats*

Group	No.	Sex	Initial body weight, g	Bd. wt. at time of death, g	No. of days survived,	Total weight loss, g	Average weight loss/day, g	Fat % of carcasses	Average daily protein utilization, g	Estimated total protein utilization*, g
Obese	63SN2	F	439	163	42	276	6.6	22.5	0.56	23.6
Obese	63SN3	M	792	480	38	312	8.2	52.4	0.77	29.1
Obese	63SN4	F	330	119	39	211	5.4	1.3	0.62	24.0
Obese	63SN6	F	407	103	52	304	6.5	1.9	0.60	31.2
Obese	63SN7	F	349	131	43	218	5.1	18.0	0.67	28.7
Obese	63SN8	M	504	184	59	320	5.4	0.5	0.76	44.6
Obese	63SN9	M	910	689	23	221	9.1	54.0	0.64	15.3
Obese	63SN12	M	628	428	25	200	8.0	59.15	0.62	15.4
			$544 \pm 212$	$287 \pm 218$	$40.1 \pm 13$	$258 \pm 50$	$6.8 \pm 1.5$	$29.1 \pm 8$	$0.66 \pm 0.08$	$26.5 \pm 9.4$
Control	63SN1	F	290	150	22	140	6.4	0	0.75	16.4
Control	63SN5	F	269	127	29	142	4.9	0	0.59	17.1
Control	63SN13	M	426	194	30	232	7.7	0	1.07	32.0
Control	63SN14	M	407	211	23	196	8.5	0	1.07	24.4
Control	63SN15	M	387	199	23	188	8.2	0	1.23	28.3
Control	63SN16	M	382	198	21	184	8.8	0.5	1.30	27.3
			$360 \pm 65$	$180 \pm 33$	$24.7 \pm 4$	$180 \pm 14$	$7.4 \pm 1.5$	$0.1 \pm 0.8$	$1.00 \pm 0.27$	$24.3 \pm 6.3$
Significance between the two means $p < 0.05$			No	No	Yes	Yes	No	Yes	Yes	No

Values after  $\pm$  are S. D.

\* Estimated total protein utilization = average daily protein utilization  $\times$  number of days survived.

The data on fat content of the carcasses showed that control rats contained almost no fat. In most cases their fat content was not detectable with our method of measurement. Approximately one gram fat was extracted from control rat 63SN16. On the other hand, most of the obese rats still contained large amount of body fat at the time of death. The carcasses of 3 of the obese rats contained even more than 50% body fat (63SN3, 63SN9 and 63SN12). The difference between body fat contents of carcasses of the two groups of rats is also statistically significant.

The data on protein utilization showed that control rats utilized significantly larger amount of protein per day (1.00 g vs 0.66 g). When total protein utilization was calculated, (daily protein utilization  $\times$  number of days survived) the amount of protein utilized by both groups was comparable.

Table II shows the average daily protein utilization of obese and control groups. Control rats utilized significantly larger amount of protein daily in 11 of the 15 days. This shows again that control rat utilized larger amount of protein per day.

TABLE II  
*Average daily protein utilization (N $\times$ 6.25) of obese and control groups*

Starvation day	Obese rats (8)	Control rats (6)	p<0.05
1	1.47 $\pm$ 0.33	1.36 $\pm$ 0.57	No
2	1.24 $\pm$ 0.38	1.78 $\pm$ 0.51	Yes
3	0.88 $\pm$ 0.23	1.44 $\pm$ 0.49	Yes
4	0.61 $\pm$ 0.14	0.77 $\pm$ 0.14	No
5	0.57 $\pm$ 0.08	0.99 $\pm$ 0.24	Yes
6	0.61 $\pm$ 0.12	0.87 $\pm$ 0.26	Yes
7	0.77 $\pm$ 0.25	0.96 $\pm$ 0.19	No
8	0.51 $\pm$ 0.12	1.01 $\pm$ 0.50	Yes
9	0.48 $\pm$ 0.14	0.81 $\pm$ 0.23	Yes
10	0.49 $\pm$ 0.16	0.87 $\pm$ 0.29	Yes
11	0.39 $\pm$ 0.15	0.93 $\pm$ 0.41	Yes
12	0.54 $\pm$ 0.15	0.86 $\pm$ 0.40	No
13	0.44 $\pm$ 0.18	0.84 $\pm$ 0.44	Yes
14	0.34 $\pm$ 0.11	0.77 $\pm$ 0.33	Yes
15	0.35 $\pm$ 0.16	0.77 $\pm$ 0.30	Yes

All values are mean $\pm$ S. D.

#### DISCUSSION

This experiment shows first of all that hypothalamic obese rats still contained significantly larger amount of body fat at the time of death caused by starvation. This finding corroborates Han and Young's findings that hypothalamic obese rats contained a higher percentage of body fat even though their body weight was reduced to control's level. (7),

Control rats might have approximately 11% body fat at the start of starvation (7). The fact that they contained no body fat at death in this experiment showed that they were able to utilize all their body fat as energy sources during starvation. In the obese rats, the situation was

complicated. There was no way to tell how much body fat each obese rat was containing at the beginning of the experiment; it is therefore hard to say from the body fat content of the carcasses alone to what extent the obese rats had utilized their body fat. Nevertheless, the facts that the daily weight loss was comparable in both obese and control groups but the daily nitrogen excretion was less in the obese group indicate that obese rats were able to utilize body fat as energy sources. The fact that the fat contents of carcasses of obese rats 63SN4, 63SN6 and 63SN8 were less than 2% also supports the view that obese rats were utilizing body fat during starvation if obese rats were presumably to have more than 11% body fat at the start of

this experiment. That the obese rats excreted less nitrogen per day than the controls seemed to indicate that they were utilizing their body fat more efficiently. Thus the body fat of obese rats had a better protein sparing action than that of the control rats.

Brobeck and Mayer suggested that the cause of death of these starved obese rats may be the exhaustion of utilizable protein store (8, 9). Hetherington and Weil showed that the total body protein of hypothalamic obese rats is comparable in amount with that of their controls (10). Our data on total protein utilization showed that both obese and control groups had utilized comparable amount of body protein when they died. These data showed that both obese and control rats had utilized a similar fraction of their total body protein at the time of their death. These findings therefore support Brobeck and Mayer's suggestion, and also explain why obese rats contain large amount of body fat when they died of starvation. It is simply because they had exhausted their utilizable protein store before they could have exhausted their fat store.

#### CONCLUSIONS

1. Hypothalamic obese rats may die while still containing large amount of body fat.
2. During starvation, hypothalamic obese rats could utilize their body fat. Their body fat even was shown to have a better protein sparing action.
3. The cause of death of starvation is that the rats have exhausted their utilizable protein store.

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