Survival and body composition of normal and hypothalamic obese rats in acute starvation

MONTEMURRO, D. G. AND J. A. F. STEVENSON. Survival and body composition of normal and hypothalamic obese rats in acute starvation. Am. J. Physiol. 198(4): 757-761. 1960. — Female hypothalamic obese rats contained approximately 240% more fat, 10% more protein and 187% more water than did their controls, however ratio total water/fat-free mass remained within normal limits in this frank obesity. At death from starvation, the water, fat-free mass and protein compartments were significantly smaller than those of the controls, the fat compartments were not significantly different. Control rats survived starvation for 16.4 ± 1.3 days, obese rats 42.7 ± 1.8 days (P < .001). The resting metabolic rates of normal and obese rats in the fed state were not different and both fell gradually throughout starvation. The prolonged survival of obese rats is due primarily to their excess reserve of energy and not to alterations in metabolic rate. As in the fed state, hypothalamic obese rats drank less water during starvation than did the controls. This is thought to result from direct interference with hypothalamic elements regulating thirst and the spontaneous consumption of water.

The length of time an individual survives acute starvation will depend on the relative reserve of energy, i.e. the amount of tissue which can be sacrificed without stopping vital functions, and the rate at which this reserve is catabolized. Normal rats starved in a temperate environment succumb at 8–15 days (1–4). Death from starvation occurs when the rat has lost between 35 and 40% of its prestarvation body weight (1–4). The liver, spleen and gastrointestinal tract lose weight relatively more rapidly than the whole body, the heart, kidneys and gonads more slowly or in proportion to the whole body, and the adrenals and thyroid lose very little, if any, weight or at the same rate as the whole body (5, 6). The enlargement and discoloration of the adrenals in starved rats appears to be a pathological change rather than simple hypertrophy (6).

In adult male rats the duration of individual survival is not related to the prefasting diet, whether this be high in carbohydrate, fat or protein, nor to the prefasting body weight (4). Rather, Rixon and Stevenson (4) found that survival time was closely related to the daily body weight loss in proportion to the prefasting body weight (gm lost/day/100 gm b.w.), and to the total weight loss sustained before death. They stated: “the individual proportionate rate of weight loss has been correlated with the metabolic rate, indicating that the former reflected the metabolic rate of the animal. The duration of survival in fasting has been correlated with the individual metabolic rate whether measured before or during fasting.”

Rats with bilateral ablation in the region of the ventromedial hypothalamic nuclei develop hyperphagia and obesity (7, 8). In 1946, Brooks and Marine (9) reported that the absolute oxygen consumption of hypothalamic hyperphagic rats falls immediately after placement of the lesions, but then increases gradually to finally surpass that of the controls. In a preliminary communication, Rixon, Montemurro and Stevenson (3) found that hypothalamic obese rats survived acute starvation some two to three times longer than did their much lighter controls. The present study was undertaken to determine the relative importance of the excess fat and reported changes in metabolic rate or oxygen consumption of hypothalamic hyperphagic rats in prolonging their survival in acute starvation.

METHODS

Female Sprague-Dawley rats having an initial weight of approximately 250 gm were used. Obese rats were obtained by placing bilateral electrolytic lesions in the region of the ventromedial nuclei of the hypothalamus with the use of a Horsley-Clarke stereotaxic instrument. Approximately 3 months after operation, these rats demonstrated obesity ranging from moderate (350 gm) to extreme (659 gm). All were housed in individual cages in a room maintained at 21°C ± 1°C with a relative...
humidity of 50%. The day consisted of 12 hours of light and 12 hours of darkness. All rats were fed a synthetic high-fat diet (6.4 Cal/gm) and water ad libitum until the beginning of starvation. Body weights and water consumptions were recorded daily before and throughout starvation.

Oxygen consumptions were determined twice weekly with an apparatus similar to that used by Ferguson and Sellers (10); pure oxygen was used in an environment of 30°C. Most of the animals appeared to sleep in the metabolism chamber throughout the 35-minute period of measurement. Observed oxygen consumptions were corrected to standard temperature and pressure, and expressed in relation to body weight, metabolic size (kg^{3/4}), and surface area. In the prestarvation period, oxygen consumptions were determined in the fed state, not unlike the endogenous diet of starvation.

Total body water was determined in 17 fed rats and in 26 rats after death from starvation. At death, the rats were weighed, frozen at -15°C and later ground in a hand-operated meat grinder. The ground carcass was weighed before and after drying in an oven at 100°C. The dried carcass was then finely minced in a Waring Blender.

Total body fat was determined on 6-11-gm samples of

<p>| TABLE I. Survival of Normal Rats and Rats With Hypothalamic Obesity in Starvation |
|---|---|---|---|
| | No. | Body Weight, gm | Body Weight Loss |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Init.</th>
<th>Final</th>
<th>gm/day</th>
<th>% of Initial</th>
<th>gm/day/100 gm B.wt.</th>
<th>Surviv. Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese</td>
<td>17</td>
<td>439.1 ±23.5*</td>
<td>149.9</td>
<td>6.7</td>
<td>65.1</td>
<td>1.55</td>
<td>42.7</td>
</tr>
<tr>
<td>Controls</td>
<td>9</td>
<td>278.9 +36.6</td>
<td>159.6</td>
<td>7.4</td>
<td>42.7</td>
<td>2.65</td>
<td>16.4</td>
</tr>
</tbody>
</table>

* Means ± S.E.M.

Figure 1. Relation between initial body weight and survival of obese and normal rats in acute starvation.

Figure 2. Relation between body weight and total body fat of hypothalamic obese and normal adult rats in the fed state. Dotted lines represent ±3 S.E. of estimate for body fat.
Rats Fed a High-Fat Diet

TABLE 2. Metabolic Rate of Normal and Hypothalamic Obese Rats Fed a High-Fat Diet

<table>
<thead>
<tr>
<th></th>
<th>Metabolic Rate</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ox Cons.</td>
<td>ml O₂/</td>
<td>ml O₂/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>summation.</td>
<td>min/m²</td>
<td>min/kg²/²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>26</td>
<td>455.6 ± 21.0*</td>
<td>6.78 ± 35</td>
<td>137.4 ± 4.4</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt; .001</td>
<td>&lt; .4</td>
<td>&lt; .2</td>
</tr>
<tr>
<td>Controls</td>
<td>16</td>
<td>282.5 ± 6.0</td>
<td>5.33 ± 1.8</td>
<td>141.6 ± 4.9</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M.

whether expressed per unit of metabolic size (kg⁻¹) or body surface area.

The absolute oxygen consumption and metabolic rate of the obese and normal rats fell sharply in the first week of starvation and then more gradually until death. Both groups fell from a mean of about 1.40 ml/min/m² to about 80 ml/min/m². Approximately 70% of this fall in metabolic rate occurred in the first week of starvation.

Body composition. The composition of the carcasses of obese and normal rats in the fed state is shown in Table 3A. The nine obese carcasses contained 164.3 gm (243%) more fat, 28.7 gm (18%) more water and 39.0 gm (18%) more fat-free mass than did the carcasses of the eight controls. The increase in fat is by far the greatest but not the only change in body composition associated with experimental hypothalamic obesity. When expressed as a percentage of the fat-free body mass, the total water of the obese carcasses was the same as that found in the controls (73.3%). Although the mean weight of the fat-free mass of the obese carcasses was greater than that of the controls, the proportion of the total body mass occupied by the two compartments was smaller (54.4%) in the former than in the latter (77%). The body proteins, calculated from carcass nitrogen, of the obese and control animals did not differ significantly.

Table 3B illustrates the effects of acute starvation on the carcass composition of another two groups of obese and normal rats. Starvation abolished the difference in body weight between obese and normal rats. The greatest change was seen in the fat compartments; the absolute amount and the proportion of fat found at death in the previously obese animals did not differ significantly from that found in the controls. On the other hand, the carcasses of the control rats contained slightly more protein and water than did the previously obese. The proportion of the total body weight occupied by water was greatly increased by starvation, but relative to the fat-free mass it was, if anything, slightly reduced. The proportion of fat-free mass was relatively increased by starvation to 96.0% in the controls and 93.4% in the obese rats.

In the fed state, a correlation coefficient of 0.993 ± .009 was obtained between body weight and total body fat for both obese and control rats (fig. 2). The linear regression equation for this plot, calculated by the method of least squares, was $y = 0.82x - 165.7$, where $y$ = body fat and $x$ = body weight. The standard error of estimate for body fat was 13.6 gm. Using this equation, estimates were made of the initial or prestarvation fat content of the starved rats reported in Table 3B.

The indices measured account for approximately 97% of the total weights of the carcasses; the remaining 3% would include minerals, carbohydrates and other minor components not measured.

Correlative statistics. The following correlation coefficients were obtained between survival (days) and:

- **Prestarvation body weight, gm**: $r = 0.860 ± 0.033$, $P < .001$
- **Prestarvation metabolic rate**
  - $ml O_2$ cons./min/kg²/² = 0.207 ± 0.195, $P < .4$
  - $ml O_2$ cons./min/m² = 0.049 ± 0.024, $P < .9$
- **Prestarvation O₂ cons. ml/min/kg²/²**
  - $(prestarvation bodyweight, kg) = -0.907 ± 0.063$, $P < .001$
- **Prestarvation O₂ cons. ml/min/kg²/²**
  - $(prestarvation bodyweight, kg) = -0.406 ± 0.029$, $P < .001$
- **Prestarvation O₂ cons. ml/min/kg²/²**
  - $(prestarvation bodyweight, kg) = -0.910 ± 0.033$, $P < .001$

Calculation of multiple correlation coefficients added very little to the predictive value of these statistics.

Water consumption in starvation. Hypothalamic obese rats habitually drink less water per gram of food eaten than do normal rats when fed ad libitum (8, 12) or when pair-fed with controls (13). The present observations show that these hypothalamic rats also drink much less water than do normal rats during starvation. The 17 obese rats drank 3.6 ± 0.7 ml/day while the control rats drank 10.5 ± 0.7 ml/day ($P < .001$).

DISCUSSION

The present chemical analysis of the carcasses of hypothalamic obese rats confirms the earlier observation by Hetherington (14), Hetherington and Weil (15) and those based on measurements of carcass specific gravity by Montemurro and Stevenson (16), that the major change in body composition in this condition is the marked increase in fat. Indeed, obese rats in this study contained in excess of 200% more fat than did the controls. In hypothalamic obesity, there is also a slight but significant increase in the fat-free mass and water. Whether or not the whole of this increase can be attributed to protein and other cellular materials accompanying the great increase in fat is not yet clear.

Total body water represents a relatively constant fraction of the fat-free weight. For man, a figure of 70% has been reported by Moleschott (17), and 73% by McCance and Widdowson (18). For the rat, Babineau and Paige (19) gave 72% and Ihut and Ellkinton (20) 71.6% as the proportion of water to fat-free mass. Both of these figures are slightly lower than that found in this investigation—73.3%. The discrepancy is probably due to the fact that the gastrointestinal tract, with its natural...
fluid contents, of the animals reported here was not flushed clean nor discarded (20) nor discarded (19). Table 3 demonstrates that this constant relationship remains unaltered even when fat comprises almost one-half of the total body weight.

In the fed state, the obese rats contained a significantly greater amount of water than did their controls. This was not surprising since they contained more fat and fat-free mass than did the controls. Accepting the values was not surprising since they contained more fat and fat-free mass than did their controls. The water content of the normal and obese rats shown in Table 3 demonstrates that this constant relationship remains unaltered even when fat comprises almost one-half of the total body weight.

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### Table 3. Body Composition of Normal and Obese Rats

<table>
<thead>
<tr>
<th>Body Weight, gm</th>
<th>Water</th>
<th>Fat</th>
<th>Fat-Free Mass*</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml % B. wt.</td>
<td>% FF mass</td>
<td>gm % B. wt.</td>
<td>gm % B. wt.</td>
</tr>
<tr>
<td><strong>Control, 8 rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203.8 ±14.0</td>
<td>75.9 ±3.8</td>
<td>10.7 ±2.4</td>
<td>6.2 ±0.9</td>
<td>1.3 ±0.3</td>
</tr>
<tr>
<td><strong>Obese, 8 rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>483.1 ±41.4</td>
<td>38.3 ±2.1</td>
<td>20.8 ±1.3</td>
<td>34.0 ±1.5</td>
<td>14.3 ±1.7</td>
</tr>
<tr>
<td><strong>P &lt; .001</strong></td>
<td>&lt; .01</td>
<td>&lt; .01</td>
<td>&lt; .01</td>
<td>&lt; .01</td>
</tr>
</tbody>
</table>

Values are means ± S.E. of Mean. *Fat-free mass = Body weight – total fat.

The results of this study confirm the generally-held view that excess fat deposits should prolong survival in acute starvation. The obese rats survived approximately 2.5 times longer than did normal rats. Using the regression equation for the linear relation between carcass weight and carcass fat (fig. 9) it is possible to estimate the initial fat content of the starved rats shown in Table 3B and thus the rate of fat catabolism throughout starvation. Such calculations show that the obese catabolized about 4.3 gm of fat and 0.5 gm protein/day, and the controls 3.5 gm of fat and 0.9 gm protein/day. The controls burned about 33 Cal/day of which 10% came from protein, and the obese 40 Cal/day of which 5% came from protein. The extra fat, as any supply of carbohydrate or fat will do, spared protein.

The extent of body protein loss rather than gross body weight loss, per se, is probably the limiting factor for survival in starvation. The extremely obese rats shown in figure 1 (560–630 gm) survived only a few days longer and died at a higher final body weight than did those obese rats weighing 130 gm less. Their carcasses contained the same amount of fat-free mass and of protein but much more fat than did the carcasses of the lighter members of the previously obese group. There appeared to be an irreducible level of body protein catabolism in starvation and, in the extremely obese rats, this had been attained before all of the fat stores had been fully used.

Rixon and Stevenson (4) found that in a population of normal male rats differences in the innate metabolic rate rather than body weight played a major role in determining the duration of survival in starvation. However, variations in the body weights of the male animals studied by these authors (200–450 gm) were probably not due to obesity. Unfortunately, the body composition of these animals was not determined, but, the proportion of the total body weight occupied by fat, water and fat-free mass in their animals would be expected to vary little outside the physiological changes associated with age, i.e. deposition of protein, slight increase in fat and slight loss of water.

On the other hand, the excess weight of hypothalamic obese rats is, for the most part, fat. The metabolic rate of these obese rats was not significantly different from that of the normal female controls in the fed state or during starvation. Therefore, the prolonged survival of obese rats cannot be attributed to a lower metabolic rate. When comparing the effects of starvation on hypothalamic obese and normal rats, we are comparing rats with essentially the same resting metabolic rate but with vastly different stores of energy. This is reflected in the low correlation coefficient obtained between survival and metabolic rate (ρ = 0.207 ± 0.195 and ρ = 0.49 ± 0.204) and the high correlation coefficient obtained between survival and initial body weight (ρ = 0.060 ± 0.053). It appears that, in an adult population containing both obese and normal animals of the same sex and age and thus a great range in the individual stores of body fat, the magnitude of this store largely determines the duration of survival within the limits of body protein catabolism. If the range of body fat store is relatively small as would occur in the population of normal rats studied by Rixon and Stevenson (4), then the linear relation between the rate of proportionate body weight loss, reflecting metabolic rate, and survival is more apparent. For predictive purposes the best correlation coefficients were obtained in that situation between the ratio presurvival metabolic rate/prestarvation body weight and duration of survival. Equally high and significant correlations were obtained when the metabolic rate in this ratio was expressed per unit of body weight, metabolic size or surface area.

Finally, hypothalamic obese rats drink less water than...
do normal rats during starvation, as they do in the fed state (8). This difference is larger than can be accounted for by the greater fat catabolism and production of metabolic water by the obese animals. Further, the spontaneous water consumption of obese rats is much smaller than their water requirements calculated on the basis of surface area as described by Richter and Brailey (22). It thus seems, as has been suggested on previous occasions (8, 12, 16), that the reduced water intake of hypothalamic-hyperphagic rats is due to direct interference with hypothalamic mechanisms regulating thirst and the spontaneous consumption of water; and this impairment remains patent even in the absence of food intake.

The authors are indebted to Mr. George Boyce and Mrs. F. Thibodeau for their technical assistance in this investigation. The vitamins used in our synthetic diet were generously supplied by Dr. Ruth Wolfe, Hoffman-La Roche, Inc.

REFERENCES