Effect of Acute Starvation on Rat Diaphragm Function

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Summary The effects of 130 h of acute starvation on diaphragm contractility fatigue were studied in isolated rat diaphragm strip preparations with phrenic nerve stimulation. Compared with controls, starvation produced a reduction in body and diaphragm weights. Twitch and tetanic tensions were reduced by starvation; however, when the force was calculated as the strength (normalized for the weight or muscle cross-section area of the diaphragm), no difference was observed between the control and experimental groups. Starvation induced a significant downward shift in the force-frequency relationship, and also increased diaphragm fatigability, but it had no effect on twitch contraction and relaxation time. We conclude that 130 h of acute starvation decreases diaphragmatic force and endurance, but the strength does not change, because of the reducing diaphragmatic mass.

Key words: starvation, diaphragm function, fatigue.

Studies of undernourished adult patients with chronic respiratory insufficiency have demonstrated that undernutrition reduces diaphragmatic force output and may contribute to ventilatory failure [1]. There have been studies in animal models of acute starvation in different periods [2–5], the longest of which was 108 h [5]. The effects of acute starvation on respiratory muscle function were similar in these reports. The findings that starvation had no significant effect may be due to insufficient starvation period. Therefore, the effect of 130 h of starvation on diaphragm contractility and fatigue resistance of the rat diaphragm were investigated in this study.

Sixteen adult male Swiss albino rats weighing 185–210 g were used in the present study. Experimental animals were maintained according to “Guide to the care and use of experimental animals” by the Canadian Council on Animal Care [6]. Animals were divided into two groups: control and experimental. The rats in the control group were allowed to feed normally. The rats in the experimental
group were allowed to feed on water only ad libitum for 130 h. All animals were placed in separate cages.

After 130 h, rats were killed by decapitation under light ether anesthesia. A muscle strip, with the phrenic nerve intact (0.01 m wide), was prepared from the left midcostal region containing muscle attachments to the ribs and central tendon intact. The rib segment was tied to a fixed hook in a phrenic nerve-diaphragm electrode (Harvard Phrenic Nerve Electrode with Oxygen Bubbled), and the central tendon ligature was attached to a force-displacement transducer (Nihon Kohden Force Displacement Transducer TB 611 T). Phrenic nerve was placed at a sliding jaw in the same electrode. The preparation was placed in an organ bath containing freshly prepared Krebs' solution constantly aerated with 95% O2–5% CO2 with the bath temperature maintained at 32°C.

The muscle was made to contract by applying supramaximal electrical stimuli to the central end of the cut phrenic nerve, administered by a stimulator (Nihon Kohden SEN 3201 Stimulator and SS-201 J Isolation Unit). Muscle length was measured with a scale positioned next to the muscle strip inside the bath and systematically adjusted to an optimal length (L0), which resulted in maximal twitch tension with supramaximal pulses of 0.2-ms duration.

For the measurement of isometric twitch characteristics, supramaximal voltages were delivered and the signal from the force-displacement transducer was amplified and recorded simultaneously on a Digi-Scope converter 500 (Volf Craff) which connected a oscilloscope (Trio Digital Memory Scope MS 1650 B) and a paper recorder (Nihon Kohden WI 681 G) with the paper speed set at 0.2 m/s. From the records, peak twitch tension (Pmax), time to peak tension development (contraction time; CT) and time for peak tension to drop by 50% (half relaxation time; RT1/2) were determined using the average of three contractions. CT and RT1/2 were also determined by Digi-Scope converter and were found to be similar. Force-frequency data were collected using 1.000-ms trains of supramaximal stimuli delivered once every 30 s at a frequency of 10, 20, 50, and 100 Hz. The fatigue resistance of each muscle was determined using a standard brief submaximal contraction (25 Hz, for 160 ms at the rate of 1/s for 45 contraction). The fatigue index was calculated as the ratio of residual force to the initial force after 1.5 min.

After we completed this protocol, muscle strips were weighed. We converted force (N) into stress (N/m²) by dividing force by muscle cross-sectional area (CSA) [7]. Area (m²) was calculated as muscle weight (g) divided by the product of the fiber length (m) times the density of muscle (taken as approximately 1). Data are expressed as means±SD. Statistical analysis was performed with Student's t-test for paired and unpaired data [8]. The collective results of the experiments are shown in Table 1. Fasting also affected the diaphragm force-frequency relationship, as shown in Table 2.

The 23% reduction in body weight observed in starving rats is much greater than that previously reported for rats and hamster after 3–4.5 d of acute starvation [2–5]. The effect of starvation on rat limb muscles depended on the age of the
Table 1. Morphometric data and contractile properties of the diaphragm.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Entry body (g)</td>
<td>231.9±15.6</td>
<td>230.6±10.5</td>
</tr>
<tr>
<td>Final body (g)</td>
<td>233.1±16.9</td>
<td>176.9±8.8*</td>
</tr>
<tr>
<td>Costal diaphragm (mg)</td>
<td>146.3±11.1</td>
<td>112.5±15.0*</td>
</tr>
<tr>
<td>(L_0) (m)</td>
<td>2.06±0.09 ((\times10^{-2}))</td>
<td>2.05±0.07 ((\times10^{-2}))</td>
</tr>
<tr>
<td>CSA (m²)</td>
<td>6.71±0.3 ((\times10^{-4}))</td>
<td>5.20±0.3 ((\times10^{-4}))*</td>
</tr>
<tr>
<td>CT (ms)</td>
<td>27.8±1.5</td>
<td>26.8±1.8</td>
</tr>
<tr>
<td>(RT_{1/2}) (ms)</td>
<td>31.2±2.1</td>
<td>32.0±2.2</td>
</tr>
<tr>
<td>(P_t) (N)</td>
<td>5.6±0.4 ((\times10^{-2}))</td>
<td>4.3±0.5 ((\times10^{-2})*</td>
</tr>
<tr>
<td>(P_o) (N)</td>
<td>18.1±1.3 ((\times10^{-2}))</td>
<td>14.9±0.9 ((\times10^{-2})*</td>
</tr>
<tr>
<td>FI</td>
<td>0.73±0.05</td>
<td>0.55±0.04*</td>
</tr>
</tbody>
</table>

Values are mean±SD. \(L_0\), optimal muscle length; CSA, muscle cross-section area; CT, contraction time; \(RT_{1/2}\), half relaxation time; \(P_t\), peak twitch tension; FI, fatigue index. *\(p<0.01\) for difference between control and experimental groups, **\(p<0.01\) for difference between entry body and final body.

Table 2. Effect of starvation on the diaphragm force-frequency relationship.

<table>
<thead>
<tr>
<th></th>
<th>10 Hz</th>
<th>20 Hz</th>
<th>50 Hz</th>
<th>100 Hz</th>
</tr>
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<tbody>
<tr>
<td>Control (n=8)</td>
<td>8.25±1.4 ((\times10^{-2}))</td>
<td>13.63±0.9 ((\times10^{-2}))</td>
<td>16.63±1.3 ((\times10^{-2}))</td>
<td>18.13±1.3 ((\times10^{-2}))</td>
</tr>
<tr>
<td>Experiment (n=8)</td>
<td>6.38±0.9 ((\times10^{-2})*</td>
<td>8.75±1.1 ((\times10^{-2})*</td>
<td>12.88±1.2 ((\times10^{-2})*</td>
<td>14.88±0.8 ((\times10^{-2})*</td>
</tr>
</tbody>
</table>

Values are mean±SD. *\(p<0.01\) for difference between control and experimental groups.

animal, the degree of adiposity, and the duration of starvation. In general, older rats tolerated longer periods of starvation before the onset of marked protein degradation [3].

This study demonstrated that 130 h of starvation induces reductions in diaphragmatic force in rats, as manifested by a downward shift in the in vitro diaphragm force-frequency relationship, and also increased diaphragm fatigability. However, when the force was calculated as the strength (normalized for the weight or CSA of the diaphragm), no difference was observed between the control and experimental groups. In other words, at the end of the 130h of starvation, the twitch and tetanic tensions per CSA (N/m²) did not change \((p>0.01)\). As is seen in Table 1, there were significant reductions in the total body weight and costal diaphragm weight of the rats in the experimental group at the end of 130 h of starvation. However, the absence of a significant change in \(L_0\) at the end of this starvation period brought about a statistically significant reduction in the calculated CSA.
CT and RT$_{1/2}$, the other isometric contraction characteristics, were found to be similar both when calculated from the recordings with the paper speed set at 0.2 m/s and seen simultaneously on a digital memory oscilloscope. At the end of 130 h of starvation, it was observed that CT and RT$_{1/2}$ did not change significantly ($p > 0.01$).

Shindoh et al. [9] found that 2 d of starvation in the hamster had no effect on diaphragm strength. Lewis and Sieck [10] demonstrated that 4 d of fasting had no effect on the strength of the rat diaphragm and produced only a minor effect on diaphragm fatigability. In contrast, they found that 3 d of fasting induce reductions in diaphragmatic strength in hamster, as manifested by a shift to the right in the force-frequency curve and an increase in diaphragm fatigability [4].

In chronically malnourished animals, there is a significant reduction in both peak twitch ($P_t$) and tetanic tensions ($P_s$) generated by the diaphragm [11–13]. However, when $P_t$ and $P_s$ were normalized for the reduction in diaphragm muscle weight or cross-sectional area of diaphragm muscle fibers, the values were similar in the nutritionally deprived and control animals [11–13]. With acute starvation for 4 d, Dureuil et al. [14] reported that the transdiaphragmatic pressure ($P_{di}$) generated by bilateral phrenic nerve stimulation in rats was significantly reduced at all frequencies of stimulation (10–100 pps) compared with controls. These $P_{di}$ responses were similar to those in the control group when normalized for the weight of the diaphragm [14]. In humans, nutritional deprivation for a period of 7 d was reported to have no effect on tests of respiratory muscle strength [15]. The slightly different findings of these studies probably reflect the different species studied and variations in methodology employed in these different experiments.

The present study demonstrated increased fatigability of the diaphragm with phrenic nerve stimulation in acutely starved animals. Similarly, Dureuil and co-workers [14] reported that, after 4 d of starvation, there was a decrease in endurance of the rat diaphragm, as assessed by the $P_{di}$ response to bilateral phrenic nerve stimulation. Shindoh et al. [9] also reported a minor increase in diaphragm fatigability in hamsters after 2 d of acute food deprivation. In contrast, fatigue resistance of the diaphragm was found to be significantly improved after prolonged nutritional deprivation in rats [12, 13]. This was attributed to selective atrophy of type II fibers with lower oxidative capacity [13], as these fibers most likely belong to motor units that are the most susceptible to fatigue [16]. In humans, acute nutritional deprivation, sufficient to induce mild ketoacidosis and hypoglycemia, had no effect on tests of ventilatory muscle endurance [15].

In short, although we observed the effects of starvation on body and diaphragm weight and strength generation by phrenic nerve stimulation, we were unable to detect significant differences in the isometric twitch characters. Even though the period of starvation used was longer than the previous study [2–5], we found that the effects of starvation on the diaphragm function were similar.

It should be emphasized, however, that our study represents a model of acute unstressed starvation. In is likely that a model of stressed starvation (e.g.,
associated with severe sepsis or trauma) might have a much greater impact on diaphragm structure and function as markedly increased muscle protein catabolism has been described in that condition compared with the unstressed state [17].

REFERENCES