The Effect of Insulin on Intact Muscle from Normal and Alloxan-diabetic Rats*

JAMES C. HALL†

From the Newark College of Arts and Sciences, Rutgers, The State University, Newark 2, New Jersey

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The premise that insulin affects the oxidative phase of carbohydrate metabolism originated with the work of Krebs and Eggleston (1). However, much contradictory evidence has been reported and the whole problem has been reviewed by Starke (2). Reports of Villee and Hastings (3), Frohman et al. (4, 5), Sacks (6), Lee and Williams (7), Demis (8) and others have indicated that citric acid cycle activity is decreased in diabetic tissue and have led to the postulate that the condensing reaction is possibly involved (5). Experiments by Hall et al. (9) and Gourley and Fisher (10) have shown that insulin affects the oxygen consumption of intact frog muscle; and Gourley (11) and Hall (12) have noted that insulin can increase the oxygen uptake of mammalian muscle under certain circumstances. The data to be presented here show the effect of insulin in vitro on the oxygen consumption and substrate utilization of relatively intact skeletal, diaphragm, and cardiac muscle from normal and diabetic rats.

EXPERIMENTAL

A total of 180 adult white rats, male, of the Sherman strain, weighing 200 to 300 g were used. One-half of that number were made diabetic by the injection of 180 mg/kg of alloxan subcutaneously after starving for 24 hours. Mortality was markedly decreased by administration of the dose in two parts, 24 hours apart. The animals were considered diabetic if they showed glycosuria and blood sugar levels of 0.25 g per 100 ml or higher after ad libitum feeding of Purina chow. They were used after 2 weeks or more of established diabetes.

The rats were killed by cervical fracture. Small pieces of muscle were removed rapidly, weighed on a torsion balance and immersed in 30% KOH for glycogen determinations by the method of Seifter (13). The muscles for the respiration experiments were then removed and placed in ice-cold Ringer's solution. The skeletal muscles used were the sternomastoideus, sternohyoideus, and scalenes. Thin slips, about 1 by 3 cm and 1 mm thick were obtained by careful teasing. The diaphragm was trimmed and cut into four equal portions, the thicker caudal portion being used for the initial glycogen analysis. The ventricles were cut transversely into slices 0.5 to 0.8 mm thick. Oxygen consumption was measured by standard manometric procedure in 15-ml Warburg vessels gassed with O2 in a medium of Krebs-Ringer-phosphate buffer (14). No more than 10 minutes elapsed between excision and equilibration with O2 especially in the case of heart muscle. Readings were taken for 1 hour previous to tipping and for 3 hours afterwards. At the end of the experiment the muscles were removed for glycogen analysis and 5% trichloroacetic acid was added to the medium. Aliquots of the protein-free filtrate were analyzed for citrate by the method of Natelson et al. (15) and for lactate by the method of Barker and Summerson (16).

RESULTS

The data obtained are summarized in Tables I to III.

Table I—The initial oxygen consumption is lower in diabetic than in normal tissue of all three types. This difference, which is highly significant (p < 0.01), is in direct contradiction to the earlier work of Pearson et al. (17) who found that diabetic heart muscle slices respired 28% higher than normal slices. There is considerable variation, but when large numbers of observations are averaged (between 50 and 70 in each case) the respiration of diabetic tissue is 12 to 20% lower, with the skeletal muscle showing the greatest difference. The respiratory rates are not as high as some that have been reported (Pearson et al., 17) especially in the case of heart muscle, because no substrate was present.

The oxygen uptake of the controls (no additions) falls off in each instance, skeletal muscle showing the greatest decline, and cardiac muscle the least. There is no significant difference between normal and diabetic muscle in this regard. Insulin has no effect on the respiration of normal muscle. In contrast, insulin increases the oxygen consumption of all three types of diabetic muscle and appears to prevent any marked decrease in rate with time. The result is that diabetic tissue with added insulin is respiring at the end of the 3 hours at the same rate as normal muscle at the end of 3 hours is returned to that of normal muscle.

Citrate, in all cases, maintains the respiration near its initial rate, and there is little difference between normal and diabetic tissue. Possibly there is a difference between normal and diabetic heart muscle in this regard but the significance is not striking (p = 2%). When insulin and citrate are both present the response is always greater in diabetic tissue since their combination produces an effect equal to or slightly greater than the sum of the responses when they are present separately. Thus when citrate and insulin are present the oxygen consumption of diabetic muscle at the end of 3 hours is returned to that of normal muscle during the first hour. Only in normal heart muscle is the rate appreciably elevated above the initial rate.

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† A paper, based on this work, was presented at the 1958 meeting of the American Society of Zoologists in Bloomington, Indiana.
**Table I**

**Oxygen consumption**

O₂ consumption calculated as μmole/hour/g wet weight. The first column shows the respiration for the hour before tipping. The remaining six columns show the averaged respiration for the 2nd and 3rd hours after tipping. The medium was Krebs-Ringer-phosphate buffer, pH 7.2 (14). Total volume was 2 ml. Vessels were gassed with oxygen and incubated at 37°. Final concentrations of the materials when added: Na citrate, 0.01 M; Na lactate, 0.01 M; glucagon-free insulin, 0.5 I.U./ml.

Each figure is the mean of 40 to 50 observations ± standard error, where the standard error = \[ \sqrt{\frac{\Sigma p}{n(n - 1)}} \]

The figures in parentheses show the percentage of stimulation when compared with the control values.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Initial O₂ uptake</th>
<th>Control</th>
<th>+ Insulin</th>
<th>+ Citrate</th>
<th>+ Lactate</th>
<th>+ Lactate + Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal, normal</td>
<td>15.4 ± 0.2</td>
<td>11.2 ± 0.5</td>
<td>12.3 ± 0.3</td>
<td>13.5 ± 0.4</td>
<td>11.4 ± 0.3</td>
<td>13.6 ± 0.4</td>
</tr>
<tr>
<td>Skeletal, diabetic</td>
<td>12.0 ± 0.3</td>
<td>8.6 ± 0.2</td>
<td>11.3 ± 0.2</td>
<td>10.8 ± 0.3</td>
<td>8.4 ± 0.4</td>
<td>11.2 ± 0.4</td>
</tr>
<tr>
<td>Diaphragm, normal</td>
<td>24.4 ± 0.6</td>
<td>20.5 ± 0.5</td>
<td>20.2 ± 0.5</td>
<td>23.6 ± 0.5</td>
<td>23.2 ± 0.5</td>
<td>23.4 ± 0.4</td>
</tr>
<tr>
<td>Diaphragm, diabetic</td>
<td>21.6 ± 0.6</td>
<td>17.5 ± 0.6</td>
<td>20.1 ± 0.6</td>
<td>20.3 ± 0.4</td>
<td>20.5 ± 0.5</td>
<td>21.6 ± 0.5</td>
</tr>
<tr>
<td>Heart, normal</td>
<td>40.0 ± 0.7</td>
<td>37.0 ± 0.8</td>
<td>38.8 ± 0.7</td>
<td>44.4 ± 0.8</td>
<td>45.0 ± 0.9</td>
<td>41.0 ± 0.8</td>
</tr>
<tr>
<td>Heart, diabetic</td>
<td>34.8 ± 0.6</td>
<td>28.8 ± 0.6</td>
<td>30.6 ± 0.7</td>
<td>33.4 ± 0.7</td>
<td>33.4 ± 0.7</td>
<td>36.5 ± 0.7</td>
</tr>
</tbody>
</table>

**Table II**

**Glycogen content**

Glycogen content of muscles at beginning and at end of experiment expressed as mg/100 g wet weight. Each figure is the mean of 12 or more determinations ± standard error.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>0 time</th>
<th>Control</th>
<th>+ Insulin</th>
<th>+ Citrate</th>
<th>+ Lactate</th>
<th>+ Lactate + Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal, normal</td>
<td>622 ± 5</td>
<td>191 ± 11</td>
<td>221 ± 8</td>
<td>243 ± 9</td>
<td>386 ± 7</td>
<td>403 ± 12</td>
</tr>
<tr>
<td>Skeletal, diabetic</td>
<td>427 ± 7</td>
<td>120 ± 8</td>
<td>138 ± 16</td>
<td>182 ± 11</td>
<td>235 ± 16</td>
<td>170 ± 11</td>
</tr>
<tr>
<td>Diaphragm, normal</td>
<td>394 ± 5</td>
<td>144 ± 9</td>
<td>206 ± 12</td>
<td>170 ± 7</td>
<td>250 ± 10</td>
<td>416 ± 14</td>
</tr>
<tr>
<td>Diaphragm, diabetic</td>
<td>402 ± 9</td>
<td>160 ± 9</td>
<td>132 ± 7</td>
<td>174 ± 8</td>
<td>220 ± 10</td>
<td>157 ± 14</td>
</tr>
<tr>
<td>Heart, normal</td>
<td>375 ± 6</td>
<td>123 ± 6</td>
<td>123 ± 6</td>
<td>170 ± 10</td>
<td>183 ± 6</td>
<td>160 ± 12</td>
</tr>
<tr>
<td>Heart, diabetic</td>
<td>530 ± 9</td>
<td>120 ± 8</td>
<td>130 ± 8</td>
<td>123 ± 8</td>
<td>123 ± 9</td>
<td>140 ± 11</td>
</tr>
</tbody>
</table>

Lactate maintains the respiratory rate of heart and diaphragm, both normal and diabetic but not of skeletal muscle. When lactate and insulin are both present the results are similar to those for citrate and insulin but tend to be smaller, except in diabetic heart muscle where the oxygen consumption is restored to the normal initial level.

**Table III**

**Utilization of citrate and lactate**

Each figure is the mean of 12 or more determinations ± standard error, expressed as μmoles/hour/g wet weight. In the case of lactate the uptake was calculated by subtracting the amount left in the medium at the end of the experiment from the sum of the lactate added and an amount equal to the weight of the glycogen disappearing.

In the control and insulin vessels, where no lactate was added, the lactate in the medium at the end of the experiment was less than the amount of glycogen disappearing in all cases.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Citrate 0 Insulin</th>
<th>+ Insulin</th>
<th>Lactate 0 Insulin</th>
<th>+ Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal, normal</td>
<td>4.7 ± 0.5</td>
<td>6.6 ± 0.7</td>
<td>14 ± 0.5</td>
<td>14 ± 0.7</td>
</tr>
<tr>
<td>Skeletal, diabetic</td>
<td>4.5 ± 0.6</td>
<td>4.3 ± 0.6</td>
<td>10 ± 0.9</td>
<td>10 ± 1.2</td>
</tr>
<tr>
<td>Diaphragm, normal</td>
<td>6.1 ± 0.3</td>
<td>7.8 ± 0.4</td>
<td>18 ± 0.9</td>
<td>18 ± 1.3</td>
</tr>
<tr>
<td>Diaphragm, diabetic</td>
<td>6.3 ± 0.3</td>
<td>7.0 ± 0.3</td>
<td>14 ± 0.7</td>
<td>14 ± 1.4</td>
</tr>
<tr>
<td>Heart, normal</td>
<td>9.9 ± 0.3</td>
<td>10.2 ± 0.4</td>
<td>32 ± 1.5</td>
<td>32 ± 1.5</td>
</tr>
<tr>
<td>Heart, diabetic</td>
<td>5.8 ± 0.2</td>
<td>6.1 ± 0.3</td>
<td>31 ± 1.6</td>
<td>31 ± 1.6</td>
</tr>
</tbody>
</table>

* Glucagon-free zinc insulin courtesy of Eli Lilly & Co., Indianapolis, Indiana; assayed 25 I.U./mg.
Insulin combined with lactate is partially effective in normal skeletal and heart muscle and in diabetic skeletal and diaphragm muscle. It is rather strange that diabetic heart muscle, which at the start has an elevated glycogen content should lose it so rapidly and fail to replace or retain it. There does not seem to be any correlation between glycogen retention and increased \( \text{O}_2 \) consumption of diabetic tissue for 3 hours at the initial rate of normal muscle.

**Table III**—Insulin increases the utilization of lactate in the three normal tissues, and of citrate in skeletal and diaphragm, but not in cardiac muscle. The uptake of citrate is the same in normal and diabetic muscle with the exception of heart muscle, where it is lower, and insulin does not increase this significantly. In contrast the uptake of lactate is lower in all three diabetic tissues and insulin increases it to or above normal levels, an increase of approximately 100%.

**DISCUSSION**

These experiments have shown that some basic difference in diabetic muscle tissue exists which results in decreased oxygen consumption and that insulin in vitro is able to correct it. This action of insulin cannot be explained by an increase in the permeability of the cell membrane to substrate, for, in some cases no substrate was added; and when substrate was present, the effects of insulin and substrate are additive. Likewise, it cannot be a protective protein effect, because, although the data are not shown, several tests were made with other proteins, bovine serum albumin, egg albumin, and rat plasma, with uniformly negative results. This, therefore, is strong evidence that some step in oxidation is defective in diabetic muscle and can be restored to normal by insulin in vitro. Since oxidation occurs within mitochondria this indicates that insulin, in a physiologically active form, can penetrate the cell membrane and influence mitochondrial activity. Lee and Williams (7) have drawn the same conclusion. Moreover, when substrate is present the effect of insulin is not necessarily accompanied by a corresponding increase in the amount of substrate disappearing from the medium. The uptake of lactate is increased, that of citrate is not.

Another interesting feature, which these results emphasize, is the variability between the three types of striated muscle. For instance, lactate, although taken up, does not increase the \( \text{O}_2 \) consumption of skeletal muscle as compared with its action in diaphragm or cardiac muscle; and diabetic heart muscle is apparently incapable of retaining its glycogen content under any circumstances tested. Even normal heart muscle does not retain glycogen when lactate is present to the same extent as the others do. These experiments lend weight to the theory that insulin directly or indirectly increases the activity of the Krebs’ tricarboxylic acid cycle. This may occur because of a block in diabetic tissue at the level of the condensing reaction which is sensitive to insulin. The fact that lactate uptake is depressed in the diabetic and that insulin increases the uptake of lactate, but not of citrate, tends to support this. Clearly the oxidative machinery of the cell is somewhat deranged in diabetic tissue and can be affected by insulin. Work with mitochondrial enzyme systems would eliminate such problems as cell permeability and show more precisely where insulin acts biochemically. Such experiments are now in process and will be reported later.

**SUMMARY**

1. The respiratory rate of diabetic mammalian muscle is depressed below normal.
2. Insulin added in vitro can return the respiration of diabetic tissue to its normal level.
3. Insulin combined with substrate can maintain the \( \text{O}_2 \) consumption of diabetic tissue for 3 hours at the initial rate of normal muscle.
4. Citrate or lactate can prevent the decline of respiration with time in both normal and diabetic muscle except in skeletal muscle where lactate is ineffective.
5. Aerobic incubation for 4 hours reduces glycogen content to one-third of the initial values. Citrate and insulin reduces this decrease to one-half in skeletal and diaphragm muscle, but not in heart muscle. Lactate and insulin are much more effective in maintaining the glycogen content of skeletal and diaphragm muscle, but not of heart muscle. The response of diabetic muscle is much less in this regard.
6. The utilization of citrate is not increased in the diabetic and is not increased by insulin, whereas that of lactate is depressed in the diabetic tissue and is doubled by insulin.
7. Neither the uptake of lactate or citrate from the medium nor the glycogen sparing effect is correlated with the effects of insulin on \( \text{O}_2 \) uptake.
8. The significance of these findings in relation to modern theories of insulin action is discussed.

**REFERENCES**

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