The Effects of Alloxan Diabetes and Insulin on the Oxidative Metabolism of Adipose Tissue*

ALBERT I. WINEGRAD† AND WALTER N. SHAW

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania

(Received for publication, December 1, 1961; revision received, September 4, 1962)

The use of the paired epididymal fat pads of the rat under carefully controlled conditions has revealed the marked sensitivity of this tissue to insulin in vitro or in vivo (1–3) and has permitted the demonstration of numerous abnormalities in the metabolism of adipose tissue from alloxan diabetic rats (4, 5). It has been shown that insulin in vitro or in vivo rapidly increases the depressed oxidation of glucose carbon to carbon dioxide and restores the incorporation of glucose carbon into long chain fatty acid (1, 2, 4) in adipose tissue from alloxan diabetic rats. The studies that form the basis of this report were designed to delineate the effects of alloxan diabetes and insulin on the oxygen uptake of adipose tissue incubated without substrate and with glucose, pyruvate, and various Krebs cycle intermediates.

EXPERIMENTAL PROCEDURE

Male albino rats of the Wistar strain that weighed 125 to 150 g were fed Purina pellets ad libitum. Alloxan diabetes was induced by the rapid intravenous injection of alloxan monohydrate (50 mg per kg) following a 24-hour fast. The animals were not used until at least 2 weeks after treatment by injection, and only if random blood glucose determinations exceeded 300 mg per 100 ml. The rats were decapitated and the epididymal fat pads removed as previously described (1). At no time were the pads exposed to chilled buffer. One pad from each pair of epididymal fat pads served as a control for the other.

To determine oxygen uptake, the epididymal fat pad was incubated in 3.2 ml of Krebs phosphate buffer, final pH 7.4, that contained the appropriate substrate. Incubation was carried out in conventional Warburg vessels of approximately 15 ml total capacity, with one side arm with a vented stopper and a center well in which 0.2 ml of 10% KOH and a filter paper wick were placed. The gas phase was 100% O₂. The medium was kept at room temperature before incubation, and the time between the decapitation of the rats and the beginning of the incubation was kept to 10 minutes or less. In experiments with adipose tissue from alloxan diabetic rats, pooled, paired samples from two rats were used to provide sufficient tissue. The flasks on their manometers were placed in a constant temperature bath at 37°C and equilibrated for 30 minutes. Readings were taken at 10-minute intervals throughout the following 2 hours. Under these conditions, the oxygen uptake of the epididymal fat pads from normal or alloxan diabetic rats remained linear throughout the 2-hour period. When insulin was added, it was placed in the side arm of the flask, so that after tipping, the final concentration in the incubation chamber of the vessel was 0.1 unit per ml. All results were expressed as micromoles of O₂ per milligram of tissue nitrogen per 2 hours.

Incubation with C⁴-labeled substrates was carried out in Stanley-Tracewell vessels as previously described (6). The vessels containing the appropriate substrate in 3.0 ml of Krebs bicarbonate buffer, final pH adjusted to 7.4, were placed in a Dubnoff metabolic shaker set at 37°C, 80 cycles per minute, and exposed to 5% CO₂-95% O₂ before the tissue was added. After the adipose tissue had been placed in the vessels, exposure to the gas phase was continued for an additional 5 minutes. The vessels were then stoppered and kept closed for the remainder of the incubation period. The methods for the determination and calculation of substrate specific activity, carbon dioxide production from labeled substrate, and the incorporation of substrate carbon into long chain fatty acid have been described previously (6). Tissue nitrogen was determined by a micro-Kjeldahl procedure. The results have been expressed as micromoles of labeled substrate carbon isolated in CO₂ per milligram of tissue nitrogen per 2 hours.

Uniformly labeled glucose-C⁴ and α-ketoglutarate-5-C⁴ were purchased from the Nuclear-Chicago Corporation. Pyruvate-2-C⁴, citrate-1,5-C⁴, succinate-2,3-C⁴, and fumarate-2,3-C⁴ were purchased from the Volk Radio-Chemical Company. Glucagon-free insulin was generously provided by the Lilly Research Laboratories through the courtesy of Dr. W. R. Kirtley.

RESULTS AND DISCUSSION

The oxygen uptake of adipose tissue from normal fed rats incubated with pyruvate or oxaloacetate was significantly greater than that of tissue incubated without added substrate (Table I). The oxygen uptake of tissue from normal animals incubated with citrate, α-ketoglutarate, succinate, fumarate, or malate was not greater than that of tissue incubated without added substrate (Table I) and would appear to result primarily from the oxidation of endogenous substrate.

Adipose tissue from normal fed rats converted pyruvate 2 C⁴ to C⁴O₂ at a rate sufficient to account for approximately half of the oxygen consumed (Table II) (cf. Table I). In contrast, the conversion of specifically labeled citrate, α-ketoglutarate, succinate, or fumarate to C⁴O₂ by adipose tissue from normal fed rats (Table II) would account for only a small fraction of the oxygen consumed when these substrates are present in the medium (Table I).

The observations cited above may reflect a very low tissue...
or mitochondrial permeability to exogenous dicarboxylic acids, with oxaloacetate an exception. It is possible, however, that the effect of oxaloacetate on the oxygen uptake of adipose tissue from normal fed rats is dependent upon its prior conversion to pyruvate.

The oxygen uptake of adipose tissue from alloxan diabetic rats incubated without added substrate was reduced to approximately half that observed with tissue from normal fed animals. The oxygen uptake of tissue from diabetic rats incubated with citrate, α-ketoglutarate, fumarate, or malate was not greater than that of similar tissue incubated without added substrate. The effects of added pyruvate, oxaloacetate, or succinate on the oxygen uptake of tissue from diabetic rats (Table I) were possibly greater but statistically inconclusive (p > 0.05 in each case), perhaps owing to variation in severity of the diabetic state. A marked decrease in endogenous respiration is thus found in adipose tissue from alloxan diabetic rats. Impaired utilization of endogenous oxaloacetate and pyruvate may also occur in tissue from diabetic rats, but this point has not been established.

The conversion of specifically labeled pyruvate, citrate, α-ketoglutarate, or fumarate to C402 by adipose tissue from alloxan diabetic rats was decreased (Table II), but the production of C402 from succinate-2,3-C14 was not different from that observed with tissue from normal fed animals (Table II). The interpretation of these data is clouded by the problems of permeability to exogenous substrate and the size of the pools of endogenous substrate, as well as by the possibility of alternate pathways of metabolism. It is of interest, however, that the differences in C402 production from these specifically labeled substrates by tissue from normal and diabetic rats parallel the differences in oxygen uptake observed with the same substrates (Table I).

Insulin in vitro had no effect on oxygen uptake by adipose tissue from normal fed or alloxan diabetic rats incubated with pyruvate, citrate, α-ketoglutarate, succinate, or fumarate (Table I). Insulin in vitro had no effect on oxygen uptake by adipose tissue from normal rats incubated with malate or oxaloacetate (Table I); its effect on oxygen uptake by tissue from diabetic rats incubated with these substrates was not tested. It would appear that insulin in vitro does not affect the endogenous respiration of adipose tissue from normal rats nor the oxidation of exogenous pyruvate or oxaloacetate. Moreover, insulin in vitro does not increase the depressed endogenous respiration of adipose tissue from alloxan diabetic rats or its ability to utilize added pyruvate or oxaloacetate.

The oxygen uptake of adipose tissue from normal fed or diabetic rats incubated with glucose was no different from that of similar tissue incubated without added substrate (Table III) (cf. Table I). The lower oxygen uptake of tissue from diabetic rats incubated with glucose probably reflects the decrease in endogenous respiration. Insulin in vitro had no effect on oxygen uptake by adipose tissue from normal fed or alloxan diabetic rats incubated with glucose.

It has been demonstrated that insulin in vitro increases the conversion of uniformly labeled glucose-C14 to CO2 and long chain fatty acid by adipose tissue from diabetic rats (1). Moreover, in tissue removed from alloxan diabetic rats 3 hours after the administration of insulin, the conversion of uniformly labeled glucose-C14 to CO2 and long chain fatty acid is within normal limits (2). Alloxan diabetic rats were treated with insulin in doses sufficient to produce a significant fall in blood sugar at 1 hour, and to restore it to normal limits when tested at 24 and 48 hours; animals were killed at these time intervals, and their epididymal fat pads were removed and incubated with glucose for the determination of oxygen uptake. One hour after the injection of insulin, there was no change in the depressed oxygen uptake of adipose tissue from diabetic rats in-
Effect of insulin in vitro and in vivo on oxygen uptake of adipose tissue from normal fed and alloxan diabetic rats incubated with 50 mM glucose

All values are expressed as micromoles of oxygen per milligram of tissue nitrogen per 2 hours. Insulin, when present in vitro, 0.1 unit per ml. Rats killed 1 hour after the start of insulin therapy had received 10 units of crystalline insulin intravenously. Rats killed at 24 hours had received 10 units of NPH insulin, and 5.0 units of crystalline insulin subcutaneously at the outset, and an additional 10 units of crystalline insulin 2 hours before killing. Rats killed at 48 hours had received 5 units of crystalline insulin and 10 units of NPH insulin 48 hours previously, 8 units of NPH insulin 24 hours previously, and 2 units of crystalline insulin 2 hours previously.

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Source of tissue</th>
<th>Control (mean ± s.e.m.)</th>
<th>Insulin added</th>
<th>Mean difference ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Normal</td>
<td>7.58 ± 0.35</td>
<td>8.19</td>
<td>+0.62 ± 0.56</td>
</tr>
<tr>
<td>10*</td>
<td>Diabetic</td>
<td>3.81 ± 0.52</td>
<td>3.64</td>
<td>−0.18 ± 0.38</td>
</tr>
<tr>
<td>9†</td>
<td>Diabetic</td>
<td>2.64 ± 0.23</td>
<td>(Pretreated with insulin 1 hr)</td>
<td></td>
</tr>
<tr>
<td>9†</td>
<td>Diabetic</td>
<td>4.58 ± 0.51</td>
<td>(Pretreated with insulin 24 hrs)</td>
<td></td>
</tr>
<tr>
<td>6†</td>
<td>Diabetic</td>
<td>8.23 ± 0.48</td>
<td>(Pretreated with insulin 48 hrs)</td>
<td></td>
</tr>
</tbody>
</table>

* Each paired experiment represents tissue from two rats.
† Each determination was made on two pads from the same rat.

Effect of albumin on oxygen uptake of adipose tissue from alloxan diabetic rats incubated with glucose

Values are expressed as micromoles of oxygen per milligram of tissue nitrogen per 2 hours. Glucose, 20 mM. Bovine serum albumin, when present, 50 mg per ml.

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Control</th>
<th>Albumin added</th>
<th>Mean difference ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6*</td>
<td>2.99</td>
<td>2.21</td>
<td>−0.78 ± 0.40</td>
</tr>
</tbody>
</table>

* Each paired experiment represents tissue from two rats.

### Summary

The oxygen uptake of epididymal fat pads from normal fed and alloxan diabetic rats has been studied. The oxygen uptake of adipose tissue from normal fed rats incubated with pyruvate or oxaloacetate was significantly greater than that of tissue incubated without added substrate, but the oxygen uptake of tissue incubated with citrate, α-ketoglutarate, succinate, fumarate, or malate was not. The endogenous respiration of adipose tissue from alloxan diabetic rats is approximately half of that of tissue from normal fed animals, and the increase on the addition of pyruvate or oxaloacetate to the medium was not statistically significant. Insulin in vitro had no effect on the oxygen uptake of adipose tissue from normal fed or alloxan diabetic rats incubated with pyruvate, citrate, α-ketoglutarate, succinate, or fumarate, nor on the oxygen uptake of tissue from normal animals incubated with malate or oxaloacetate.

The oxygen uptake of adipose tissue from normal fed or alloxan diabetic rats incubated with glucose did not differ from
that of tissue incubated without added substrate. Insulin in vitro had no effect on the oxygen uptake of adipose tissue from normal or diabetic rats incubated with glucose. When insulin was injected in vivo, 24 to 48 hours were required before the oxygen uptake of adipose tissue removed and incubated with glucose was restored to normal. The addition of albumin to the medium did not affect the oxygen uptake of adipose tissue from diabetic rats incubated with glucose.

Acknowledgments—The authors wish to acknowledge the expert assistance of Miss Mary Ann Holzinger and Miss Susan Wolcott.

REFERENCES
The Effects of Alloxan Diabetes and Insulin on the Oxidative Metabolism of Adipose Tissue
Albert I. Winegrad and Walter N. Shaw


Access the most updated version of this article at http://www.jbc.org/content/238/2/524.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/238/2/524.citation.full.html#ref-list-1