Differential Tissue Sensitivity to Elevated Endogenous Insulin Levels During Experimental Peritonitis in Rats

N. Thomas Ryan, George L. Blackburn, and George H. A. Clowes, Jr.

Disturbances of glucose metabolism consequent to experimental peritonitis in rats were studied by measurement of insulin-related metabolism of isolated tissues in correlation with blood insulin and substrate levels. Blood insulin concentrations were threefold higher in infected fasting rats than in normal fasting controls, despite approximately equal blood glucose concentrations, suggesting resistance to the hypoglycemic action of insulin. The increased circulating insulin was associated with a threefold elevation of the insulin-sensitive adipose tissue pyruvate dehydrogenase enzyme complex, and a threefold increase in the rate of conversion of glucose to CO₂ by fragments of epididymal fat pads. Fasting infected animals also had reduced circulating nonesterified fatty acids and relatively less depletion of epididymal adipose tissue, when compared to fasted controls presumably due to the potent action of insulin in opposing lipid mobilization. In contrast, diaphragm pyruvate dehydrogenase was not elevated, nor was diaphragm glucose conversion to CO₂ stimulated in response to the elevated circulating insulin. It is proposed that reduced fat mobilization without concomitantly accelerated glucose oxidation by muscle may result in insufficient metabolic fuel for muscle and this may, in turn, promote amino acid combustion by muscle to meet cellular energy requirements. This suggested mechanism may provide a hypothetical biochemical explanation for the excessive protein catabolism associated with severe infection.

Major injury, particularly when complicated by infection, provokes a sustained breakdown of body protein. The biochemical details of this process are poorly understood. It is known, however, that this period of nitrogen wastage is associated with glucose intolerance and resistance to the action of insulin. Cahill suggests that the sensitivity of insulin-responsive tissues may be decreased after trauma, resulting in less capacity for insulin to suppress muscle proteolysis. Because of the potential clinical significance of these metabolic abnormalities, we have studied the insulin related tissue metabolism of infected rats to gain insight into the cellular basis for the altered metabolic state during infection.

Materials and Methods

Male albino rats (120-150 g) from the Charles River breeding laboratories were maintained in our facilities for 5-7 days prior to experimentation to allow acclimatization. Control-fasted and septic-fasted rats, housed individually, were deprived of food for the 72 hr of the experiment. Animals had free access to water at all times. Studies were performed on normal fed controls, normal 72-hr-fasted controls, and 72-hr-fasted rats with experimental peritonitis. Comparisons were therefore made between normal fed or fasted animals and injured (surgery plus infection) animals. No attempts were
made to differentiate among the influences of the various components of the injury, (i.e., surgery, infection, bacterial products, etc.).

Experimental general peritonitis was induced by ligation of the cecum performed through a 2-cm midline incision. Both operated (septic-fasted) and nonoperated fasted animals were anesthetized with pentobarbital and were fasted beginning the morning of surgery. The infected rats became unpreened, feisty, and walked unsteadily. Postmortem examination demonstrated a gangrenous, necrotic cecum usually accompanied by some degrees of ascites. Gram-negative enteric organisms were cultured from the peritoneal fluid. Moribund or overly active well-preened animals were discarded prior to assay to improve consistency of the degree of illness. Animals prepared in this manner have been shown to exhibit a classical hypercatabolic response, including the associated nitrogen wastage. In one of the experiments, the animals received glucose (0.5 g/kg) by stomach tube 1 hr prior to sacrifice (Table 4).

Pyruvate dehydrogenase activity, chosen for its high sensitivity to insulin was determined by the method of Taylor et al. Total PDH was studied by incubation with 10 mM magnesium. The animals were sacrificed by decapitation and the tissues were excised and frozen in liquid nitrogen within 10–20 sec. Rats used for collection of blood samples were not used for tissue studies. Diaphragm samples were pulverized in a mortar precooled with liquid nitrogen. The resulting powder was weighed to the nearest 5 mg and homogenized to yield approximately 30–50 mg tissue per milliliter medium. The results were calculated on the basis of the measured protein concentration of the homogenate. Adipose tissue samples were frozen in some experiments; however, this was found to be unnecessary and was discontinued in subsequent experiments. Homogenates were prepared to contain 100 mg of tissue per milliliter of medium. Both of the epididymal fat pads were excised and used for preparation of the homogenate. Results were calculated in the same units used for diaphragm, using the measured homogenate protein concentration.

To measure glucose oxidation, hemidiaphragms or uniform fragments of epididymal adipose tissue were incubated in Kreb's Ringer bicarbonate buffer (equilibrated with 95:5 O₂:CO₂, pH = 7.4) containing glucose-1-¹⁴C and unlabeled glucose with a final concentration of 8 mM, for 90 min at 37°C in a Dubnoff metabolic shaker. Incubations were done in a rubber-capped incubation flask with a disposable center well (Kontes Glass). At the end of the assay, 0.2 ml of phenethylamine was injected into a glass fiber filter paper strip contained in one-half of a gelatin capsule (size 0) located in center well. The flasks containing hemidiaphragms were then briefly uncapped and the tissue removed for determination of glycogen deposition by the method of Staubfacher and Renold. Preliminary studies demonstrated no detectable loss of labeled CO₂ results from this brief uncapping. A 0.2-ml aliquot of 10 N sulfuric acid was then injected into the incubation medium to release the CO₂ and stop the reaction. After 20 min of additional incubation at 37°C with shaking, the capsule and filter paper strip were placed in a scintillation vial. Samples were counted in toluene–ethanol (9:1) scintillation fluid (0.4% Omnifluor, New England Nuclear Corp., Boston, Mass.).

Blood was collected in a heparinized tube following decapitation. A portion was immediately deproteinized in perchloric acid (30%) for substrate analysis. Glucose, lactate, beta-hydroxybutyrate and acetoacetate were measured by standard enzymatic techniques. Free fatty acids were measured by the method of Dole and Meinertz. Insulin was determined by radioimmunoassay according to the method of Soeldner, using pork insulin standards. Tissue protein was measured by the biuret method. Adipose tissue samples for biuret protein were extracted with diethyl ether to remove interfering lipid.

### RESULTS

#### Blood Energy Substrates

The whole blood concentrations of a number of blood substrates are shown in Table 1. Circulating free fatty acids in the 72-hr septic-fasted rats were one-half of the 72-hr fasted controls. Similar changes in blood free fatty acids were obtained in a 48-hr experiment using somewhat smaller rats (80–90 g): fasted = 495 ± 138 (12)*, fasted = 1203 ± 489 (11), and septic-fasted = 675 ± 278 (11)

*Mean ± SD, with the number of observations in parentheses.
Table 1. Blood Substrate and Insulin Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Normal Fed*</th>
<th>72-Hr Fasted</th>
<th>72-Hr Septic-Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (µmoles/ml)</td>
<td>7.00 ± 0.24 (21)†</td>
<td>4.69 ± 0.58 (21)</td>
<td>4.45 ± 0.21 (18)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FFA (µeq/liter)</td>
<td>551 ± 32 (30)</td>
<td>771 ± 81 (30)</td>
<td>332 ± 50 (24)</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Lactate (µmoles/ml)</td>
<td>2.31 ± 0.28 (22)</td>
<td>2.35 ± 0.42 (22)</td>
<td>2.25 ± 0.31 (28)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ketones§ (µmoles/ml)</td>
<td>0.16 ± 0.001 (22)</td>
<td>2.59 ± 0.46 (22)</td>
<td>1.55 ± 0.17 (28)</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>91.0 ± 10.7 (10)</td>
<td>10.3 ± 3.0 (18)</td>
<td>30.3 ± 4.5 (18)</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 1. Blood Substrate and Insulin Concentrations

*Postabsorptive.
†Mean ± SE of the mean with the number of observations in parentheses.
§Significance by Student's t test when comparing indicated columns.

μeq/liter. Ketone body (beta-hydroxybutyrate plus acetoacetate) concentrations were also lower in the septic-fasted animals than in the fasted in both the 48- and 72-hr experiments (Table 1), paralleling the free fatty acid levels. The ratio of beta-hydroxybutyrate to acetoacetate was 2.31 for fasting and 1.65 for the infected group. Blood glucose and blood lactate concentrations were the same in the septic-fasted and normal fasted groups. Normal blood lactate levels in the septic group suggests that these animals probably were not seriously hypotensive.18

Blood Insulin

The changes in blood energy substrates were associated with alterations in the blood insulin levels (Table 1). Basal (unstimulated) insulin concentrations were threefold higher in the septic-fasted group than in the control-fasted animals. Both groups were significantly lower than the fed group, 2-4 hr after termination of overnight feeding activity by removal of the food supply.

Adipose Tissue Metabolism

The elevated insulin concentration of the septic-fasted group apparently influenced the metabolic state of adipose tissue from these animals. Pyruvate dehydrogenase activity was significantly higher ($p < 0.001$; significance by Student's test) in the adipose tissue of the 72-hr septic-fasted rats than of the 72-hr fasted controls (0.97 ± 0.23 (32) vs. 0.28 ± 0.15 (28) U/g protein) as shown in Fig. 1. This enzyme has been shown11,12 to be rapidly activated by insulin. Similarly, the oxidation of radioactive glucose to labeled carbon dioxide by fragments of epididymal fat pads from septic-fasted rats was threefold higher than that of normal fasted rats (Table 2).

The adipose tissue mass of the septic-fasted animals was apparently preserved relative to the fasted controls. Gross inspection showed the fat deposits to be
greater in the septic group. The epididymal fat pads of the fasted control rats were reddish and stringy, whereas the infected animals had white, lumpy, and far heavier fat pads. A rough numerical approximation of this observation was obtained by measuring the fresh tissue weights of uniformly dissected epididymal fat pads and expressing the result as mg/100 g of the final body weight (Table 3). In the septic-fasted group, the fat pads represented a greater proportion of body weight than in either the fasted or fed control groups. The absolute weight of the fat pads from infected rats was twice that of fasted controls (Table 3).

**Table 2. Adipose Tissue Glucose Oxidation**

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>72-Hr Fasted</th>
<th>72-Hr Septic-Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmoles glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>converted to CO₂ per hr per mg protein</td>
<td>5.1 ± 1.7 (12)*</td>
<td>1.45 ± 0.75 (16)</td>
<td>4.5 ± 1.6 (15)</td>
</tr>
</tbody>
</table>

*Mean ± SD with the number of observations in parentheses.†Significance by Student’s t test when comparing indicated columns.

**Table 3. Changes in Body Weight and Epididymal Fat Pad Weight During Infection**

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>72-Hr Fasted</th>
<th>72-Hr Septic-Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>125.2 ± 8.1 (36)†</td>
<td>124.5 ± 7.2 (36)</td>
<td>118.4 ± 3.6 (32)</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>136.0 ± 12.9 (36)</td>
<td>90.0 ± 14.2 (36)</td>
<td>83.0 ± 16.4 (32)</td>
</tr>
<tr>
<td>Change in weight as percent initial body weight</td>
<td>8.8 ± 1.3 (36)</td>
<td>-27.4 ± 4.9 (36)</td>
<td>-29.9 ± 9.4 (32)</td>
</tr>
<tr>
<td>Epididymal fat pad weight (mg)*</td>
<td>410 ± 188 (36)</td>
<td>158 ± 141 (36)</td>
<td>349 ± 144 (32)</td>
</tr>
<tr>
<td>Epididymal fat pad weight as percent final body weight*</td>
<td>0.30 ± 0.14 (36)</td>
<td>0.17 ± 0.09 (36)</td>
<td>0.42 ± 0.22 (32)</td>
</tr>
</tbody>
</table>

*Results for two uniformly dissected fat pads.†Mean ± SD with the number of observations in parentheses.‡Significance by Student’s t test when comparing indicated columns.
Muscle Metabolism

Rat diaphragm pyruvate dehydrogenase activity (Fig. 2) was reduced from a value of 12.62 ± 3.01 (15) to 6.47 ± 2.08 (15) U/g protein \((p < 0.001)\) by 72 hr fasting. It was further depressed to 3.97 ± 1.11 (13) \((p < 0.001)\) when infection was superimposed on the 72-hr fast. Total pyruvate dehydrogenase (active plus inactive enzyme), which is known to vary little in different physiologic states, did not show any consistent changes among the groups in either muscle or adipose tissue.

The rate of conversion of glucose U-14C to 14CO2 and labeled glycogen by incubated hemidiaphragms was not significantly different in the septic-fasted when compared to the normal fasted controls (Table 3).

The PDH activity of psoas and gastrocnemius muscle (pooled for assay) showed the same pattern of changes observed for diaphragm, but with greater variability, perhaps due to difficulty in rapidly excising and freezing these samples. The values for PDH activity were, fed: 4.37 ± 1.82 (18); fasted: 1.64 ± 0.9 (18); and septic-fasted: 1.24 ± 1.02 (19). Thus, the PDH activity of the psoas and gastrocnemius muscles, like that of diaphragm, did not increase in response to the elevated endogenous insulin concentration, even though they did not show the significant lowering observed in diaphragm during infection.

Response to Glucose

The PDH activities of diaphragm and epididymal adipose tissues obtained from 72-hr fasted and 72-hr septic-fasted rats given glucose (0.5g/kg) by stomach tube 1 hr prior to sacrifice are shown in Table 5.
Table 5. The Response of Muscle and Adipose Tissue
Pyruvate Dehydrogenase to Glucose Administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>72-Hr Fasted</th>
<th>72-Hr Septic-Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Glucose</td>
</tr>
<tr>
<td>Diaphragm (μmoles)</td>
<td>5.9 ± 1.6 (15)†</td>
<td>9.2 ± 2.1 (15)‡</td>
</tr>
<tr>
<td>Adipose (μmoles)</td>
<td>0.68 ± 0.19 (11)</td>
<td>1.43 ± 0.28 (15)‡</td>
</tr>
</tbody>
</table>

*Administered in equal volumes (about 2 ml) by stomach tube 1 hr prior to sacrifice. Glucose dose was 0.5 g/kg body weight, administered as a 2.5% solution.
†Mean ± SD with the number of observations in parentheses.
‡Significantly different from saline control by Student’s t test, p < 0.01.

The PDH activities of both adipose and muscle tissues were increased in the fasting control group, while only that of adipose tissue responded in the septic-fasted group. The actual magnitude of change between control and infected groups cannot be compared because differences in alimentary glucose absorption rate, or the subsequent insulin response, may exist. However, the different pattern of response of the two tissues is clearly demonstrated.

DISCUSSION

Severe infection is associated with profound abnormalities in body glucose and amino acid utilization. This study demonstrates alterations in muscle and adipose tissue metabolism in association with evidence of reduced lipid mobilization during experimental peritonitis in fasted rats. The observed changes suggest a potential partial explanation for the protein catabolic state associated with serious infection.

Peritonitis in 3-day fasted rats was associated with a threefold elevation of circulating immunoreactive insulin, which may indicate resistance to the effects of insulin on blood glucose. Similar elevations of basal circulating insulin have been observed in man following various forms of trauma.19–23

The observed threefold increase in adipose tissue pyruvate dehydrogenase (PDH) activity (Fig. 1) and in the rate of conversion of glucose to CO₂ (Table 2) by this tissue may be an effect of the elevated insulin concentration on adipose tissue metabolism in the infected rats. The action of insulin on adipose tissue may also result in the lower circulating nonesterified fatty acids and the relative preservation of the epididymal adipose tissue of the infected animals, by virtue of the potent action of insulin in opposing lipid mobilization.24 Reduction of circulating free fatty acids have been also observed in septic pigs,18 septic man,25 and during recovery from hemorrhagic shock in man.24 In addition, increases in insulin in the range observed in this study have been shown to reduce fatty acid mobilization and ketone production in normal fasted man.26

Diaphragm PDH activity (Fig. 2) and glucose metabolism (CO₂ and glycogen formation) (Table 4) were not increased in association with the elevated insulin level observed during infection, in contrast to the changes observed in adipose tissue. Similarly, the increase in PDH that occurred in both muscle and adipose tissue of normal fasted rats following glucose administration (Table 5) did not oc-
cur in the muscle of infected animals, although their adipose tissue responded normally. Muscle, therefore, may have become insensitive to the effects of insulin on glucose oxidation and PDH activity. The glucose effect on PDH may be indirect, since insulin has not been clearly shown to directly modify this enzyme complex in muscle. However, muscle PDH normally rises when blood free fatty acids are low and a failure of this mechanism can be seen in the infected group.

The oxidation of fatty acids by muscle depends upon their circulating concentration, and, therefore, a reduction in the oxidation of fatty acids by muscle probably occurs during peritonitis. However, the data above suggest that the reduced fatty acid availability was not associated with accelerated muscle glucose metabolism. Since energy demand is unlikely to be diminished during infection, these changes would be likely to create a need for metabolic fuel in muscle. Thus, the reduced availability of fatty acids without concomitantly accelerated glucose oxidation may oblige the muscle tissue to utilize alternate fuels for energy production.

In view of the large negative nitrogen balance provoked by serious injury or infection, and the observation that protein may provide an increased proportion of the expended calories after injury, it is tempting to speculate that the protein catabolism associated with serious infection may be provoked by a necessity for direct combustion of amino acids by muscle for energy production.

Normal rats treated with antilipolytic agents during fasting have increased protein breakdown and amino acid deamination supporting the suggestion that reduced lipid mobilization could contribute to nitrogen catabolism.

Reduced fatty acid utilization by muscle during general infection is supported by the observation of Border et al. of reduced carnitine concentration in muscle of septic dogs compared to fasted controls. Carnitine is necessary for fatty acid oxidation, and failure of the normal carnitine adaptation to fasting to occur in the muscle tissue of septic dogs suggests diminished oxidation of fatty acids. A reduced contribution of fat to the overall fuel supply after injury is also indicated by the observation that postsurgical patients have an RQ of about 0.8.

Evidence that mobilization of fat stores may allow the body to curtail the excess protein catabolism associated with trauma has been provided by Blackburn and co-workers studying postsurgical patients. Their studies showed that substitution of amino acid for glucose solutions as fluid replacement was associated with increased circulating nonesterfied fatty acids, low blood insulin concentrations, and a reduced net urinary nitrogen loss. Conversely, reinstitution of the glucose solution was associated with increased blood insulin levels, reduced circulating free fatty acids and the return of a large negative nitrogen balance.

Enhanced amino acid oxidation by muscle has been observed during fasting, diabetes, and following hemorrhagic shock, establishing the potential for increased energy production from these precursors during stress.

In summary, the effects of infection in fasted rats appear to be a decrease in the normal fat mobilization of fasting, without a corresponding increase in muscle glucose utilization. We suggest that these changes could necessitate increased combustion of amino acids by muscle to compensate for the reduced availability of the usual metabolic fuels. Combustion of essential amino acids would drive net protein catabolism by removing a necessary component from the cellular protein
synthetic amino acid pool, particularly in fasted animals. This process could thereby contribute to the excessive nitrogen loss associated with serious infection. This hypothetical biochemical mechanism requires additional study to establish its relative contribution to the metabolic abnormalities observed during the septic hypercatabolic state.

REFERENCES