Insulin Reversal of Cancer Cachexia in Rats

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ABSTRACT

The anabolic effects of exogenous neutral protamine hagedorn insulin on tumor-bearing (TB) and non-tumor-bearing (NTB) rats were examined. Exogenous insulin (2 units/100 g/day) produced similar hypoglycemia in TB and NTB rats. Food intake and body weight gain were significantly increased by insulin in NTB rats. In TB rats in an early stage of cachexia, insulin increased food intake and host weight (total body weight minus tumor weight). In TB rats with severe cachexia, insulin increased food intake and stabilized host weight when untreated TB controls were not eating and were losing weight. When daily insulin administration was started at an early stage of tumor growth and continued until death, there was again significant enhancement of host weight and food intake. Heart and adrenal weights were significantly reduced in insulin-treated TB animals. Tumor growth was not stimulated by insulin treatment. Survival time was slightly reduced in TB rats treated with long-term insulin. Survival time in TB rats randomized to insulin during late cachectic decline was not different from untreated TB controls. Insulin did not have any measurable effect on energy expenditure or the motor activity component of energy expenditure in either TB or NTB rats.

Insulin treatment can reverse experimental cancer cachexia. It is a nutritional therapy which preferentially feeds the host over the tumor. As yet, its beneficial effects have not prolonged survival of tumor-bearing animals.

INTRODUCTION

Cachexia is a frequent accompaniment of cancer. Cachexia has two components: anorexia and distant catabolic effects of tumor. Anorexia is the failure of the tumor-bearing host to maintain adequate protein-calorie intake to sustain weight and energy reserves. Distant catabolic effects of tumors include alterations in carbohydrate, protein, and lipid metabolism. Disordered carbohydrate metabolism is manifest by lactic acidemia (1, 2), abnormal glucose tolerance (3, 4), increased gluconeogenesis (4, 5), and Cori cycle activity (6). Tumors grow and incorporate nitrogen at the expense of skeletal muscle protein (6, 7) which is also broken down to provide gluconeogenic precursors (4, 8). Hyperlipidemia and depletion of fat stores are seen in the presence of tumors (9, 10). These metabolic effects contribute to the catabolic decline of the host.

Insulin has anabolic effects that are opposite to many catabolic effects of tumor. In normal animals, administration of exogenous insulin results in hyperphagia, weight gain (11–13), and deposition of body fat (14). Other effects of insulin include inhibition of breakdown of fat and protein, and stimulation of fat and protein synthesis. These qualities make insulin a possible therapy for cachexia (15).

Experimentally, insulin treatment has been found to stimulate food intake and promote host weight gain in three rat strains with different tumor types during early cachectic decline (16, 17). In the aforementioned studies, insulin administration was started when food intake was still adequate to promote total body weight gain, and experimental periods were short (5 days). While treatment reversed cachexia during this period, it is not known whether insulin would have the same effect if given in a more advanced stage of cachexia, when tumor-bearing animals are eating very little or nothing. In this study, the effects of exogenous insulin administration on early and late cachectic decline were examined separately. It would seem logical that if insulin can enhance host weight without stimulating tumor growth, then it may improve survival. We examined this hypothesis in a separate experiment.

In addition to the metabolic decline of the host with tumor growth, motor activity of the host also declines during the growth of several experimental tumors (18–20). This can be regarded as the experimental analogue of asthenia which accompanies cachexia (21). This decline in motor activity appears to be coupled to the tumor-induced decline in food intake (19). Since feeding is a motor activity, it is possible that the decline in food intake might be explained by inability to engage in motor activity (20). Since insulin increases food intake in normal animals and in animals bearing tumors which depress motor activity, it is important to know whether insulin stimulation (or enabling) of feeding occurs by increasing or restoring motor activity. We have used a long-term respiration calorimeter in these experiments to examine this question.

MATERIALS AND METHODS

Animals, Tumors, and Diet. Two hundred fifteen male F344 rats weighing 200–300 g were used in these experiments. The animals were divided into two groups: NTB2 controls and TB. The tumor was a transplantable MCA-induced sarcoma which causes a decline in food intake and usually kills its host in 4–5 wk (see Chart 1). The MCA tumor is a fibrosarcoma which was originally induced in this laboratory by injection of methylcholanthrene into the thigh muscle of a rat. Its cachexia-producing effects are well documented (4, 8, 10, 16, 20, 22, 23). In addition, in the calorimeter experiment (Experiment 4), 18 adult male S-D rats, tumor free and after transplant of a W256 carcinosarcoma, were used. All tumors were transplanted by s.c. inoculation of 1-mm3 viable fragments. When the tumor became palpable (after about 10 days), the animals were fed C-21, a nonscatterable casein-based paste diet which provides 4.85 kcal/g (17). This diet facilitates measurement of food intake. Non-tumor-bearing controls were fed C-21 for at least 7 days prior to the experimental periods. Animals were individually housed and had unlimited access to water. A 12-3:light 12-3-dark cycle was

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maintained throughout experiments. In all experiments, body weight and food intake were measured daily. Tumor size in three orthogonal dimensions was measured every 2–3 days.

Growing tumor weights at points in time prior to excision were estimated by multiplying the product of the three measured tumor dimensions by the ratio of the weight to dimension product at necropsy (24).

\[
\text{Tumor wt}_t = X_t \times Y_t \times Z_t \times \frac{(\text{tumor wt}_t)}{X_F \times Y_F \times Z_F}
\]

(where X, Y, and Z are the measured tumor diameters, t is the time of measurement, and F is final measurement at necropsy. Tumor doubling times over appropriate intervals were assessed as \((T \times \log 2)/(\log F - \log I)\), where T is interval in days, F is final weight, and I is initial weight (25).

Host weight is total body weight minus tumor weight.

Insulin. The insulin used in these experiments was NPH insulin (lletin; Eli Lilly and Company, Indianapolis, IN). Injections were made s.c. after a 1:10 dilution of insulin and saline was made. Unless otherwise stated, injections were given between 10 and 11 a.m.

Experimental Design and Treatment Periods. The MCA sarcoma causes a decline in food intake at 2 wk posttransplant (see Chart 1). Host weight (total body weight minus tumor weight) levels off and starts to decline several days later (early cachectic decline). Total body weight continues to increase until 3–4 wk posttransplant due to tumor weight gain, and then it also starts to decline (late cachectic decline). This decline becomes progressively more severe until the animals become aphagic and die 4–5 wk after transplant. Timing of the experiments is demonstrated in Chart 1.

Effects of Insulin in Early Cachexia (Experiment 1). The effects of insulin administration on TB animals during early cachectic decline were examined. Twenty days post-tumor transplantation during early cachectic decline, 21 TB and 24 NTB F344 rats were randomized to receive daily s.c. injections of either saline, insulin at 1 unit/100 g total body weight/day (13 rats). Group II received insulin s.c. at 1 unit/100 g total body weight twice daily, at 7 a.m. and 7 p.m. (12 rats). Group IV animals received injections of insulin at 2 units/100 g total body weight once daily (14 rats). When food intake during insulin treatment fell below 5 g/day, insulin injections were decreased to 1 unit/100 g once a day in Groups III and IV, in an effort to prevent hypoglycemic death. These injections were continued daily until all animals died. Heart, adrenal, and spleen weights were obtained at death. Survival was measured from date of tumor implantation.

Insulin and Cancer Cachexia

Effect of Insulin on Blood Glucose and Insulin Levels (Experiment 2). Thirty-four F344 rats bearing 2-wk MCA sarcomas and 34 NTB controls were used to determine the effects of exogenous insulin administration on blood glucose and insulin levels. Animals were given either saline (control) or NPH insulin s.c. at 2 units/100 g body weight at 9:00 a.m. At 3:00 p.m., orbital blood samples were obtained (no anaesthesia) (26). Serum glucose levels were determined by the glucose oxidase method (27), and serum insulin levels were measured by radioimmunoassay with antibody raised against rat insulin (28).

Calorimeter Experiments (Experiment 5). This experiment was done with two different rat strains: 18 NTB and 14 TB (W256 carcinosarcoma) male S-D rats; and 16 NTB and 10 TB (MCA sarcoma) F344 rats. Tumor-bearing animals were studied in the third and fourth weeks of tumor growth.

Each rat was maintained, individually for four 24-h periods, in a long-term respiration calorimeter. For the first 2 days in the calorimeter, each rat was untreated; on the third and fourth days, each rat received NPH insulin s.c. at the beginning of the 24-h period (9.00 a.m.) at a dose level of 1.5 units/100 g total body weight for S-D rats and 2 units/100 g total body weight for F344 rats. The rats were allowed food and water ad libitum, and daily intake of food and water was recorded. Environmental temperature in the calorimeter was 27–29°C (thermal neutrality), and a 12-h light, 12-h dark cycle was maintained at all times.

The respirometer continuously measures oxygen consumption and carbon dioxide production of the total organism by comparing composition of inflowing (room) air. The animal chamber, air-flow system, and analyzing cells have been described in detail previously (29). The present version, which records data every minute on magnetic tape cassette, has been described in detail elsewhere (30). Data were processed on a Wang 2200VP programmable calculator using calibration data derived from standard gases, and a version of Weir’s equation (31) modified for the composition of the diet used. The derived minute energy values are integrated over the recorded 24-h period to yield total daily energy expenditure (and, utilizing bomb calorimeter values for food, feces, and urine, to yield total energy balance), or they are plotted on a digital plotter to give continuous, instantaneous energy expenditure over the 24-h period.

It was shown earlier, by comparison with directly recorded motor activity, that all metabolic elevations above a resting base expenditure could be attributed to motor activity (29). The total daily energy expenditure could thus be partitioned into compartments attributable to rest and to motor activity (29). The procedure originally devised for this partition was adapted to the stripchart records of 02 and CO2 then being used. The procedure used here is a computerized variant (30). The average of the recorded 24-h period for each rat was then computed. The average of the all values (V) for each 4-h period within the range min ≤ V ≤ (min + 0.05

\[ V = \frac{1}{4} \sum_{i=1}^{4} V_i \]

3Wang Incorporated, Lowell, MA.
INSULIN AND CANCER CACHEXIA

Table 1
Effects of insulin administration over 5-day period in non-tumor-bearing and tumor-bearing rats during early cancer cachexia (Period 1, Chart 1)

Host weight is total body weight minus tumor weight.

<table>
<thead>
<tr>
<th></th>
<th>NTB rats</th>
<th></th>
<th></th>
<th>TB rats</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline treated (n = 9)</td>
<td>Insulin (1 unit/100 g/day) (n = 5)</td>
<td>Insulin (2 units/100 g/day) (n = 10)</td>
<td>Saline treated (n = 8)</td>
<td>Insulin (1 unit/100 g/day) (n = 4)</td>
<td>Insulin (2 units/100 g/day) (n = 9)</td>
</tr>
<tr>
<td>5-Day food intake (g)</td>
<td>63.8 ± 2.2</td>
<td>70.7 ± 2.5</td>
<td>87.8 ± 3.2</td>
<td>50.3 ± 3.1</td>
<td>82.4 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>Final tumor wt (g)</td>
<td>33.5 ± 4.8</td>
<td>41.3 ± 8.9</td>
<td>35.7 ± 5.2</td>
<td>5.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated host wt change (g)</td>
<td>14.1 ± 1.4</td>
<td>18 ± 1.3</td>
<td>30.4 ± 1.6</td>
<td>10.5 ± 1.6</td>
<td>14.5 ± 1.7</td>
<td>33.4 ± 2.6</td>
</tr>
<tr>
<td>Calculated tumor doubling time (days)</td>
<td>5.2 ± 0.2</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Mean ± SE.
* P less than 0.001 compared to saline-treated NTB rats.
* P less than 0.001 compared to saline-treated TB and NTB rats.
* P less than 0.001 compared to saline-treated TB rats.
* P less than 0.01 compared to saline-treated NTB rats.

Chart 2. Food intake and total body weight change in insulin and saline-treated TB male Fischer rats (Experiment 2). When food intake in cachectic TB rats fell below 5 g/day, rats were randomized to receive either daily NPH insulin (2 units/100 g/day) or saline injections until death. Day of randomization and initiation of treatment is marked Day 0. Points, mean; bars, SE. Differences in food intake and total body weight change between insulin and saline-treated animals are significant (P < 0.001) from Day 1 on.

RESULTS

Effects of Insulin in Early Cachexia (Experiment 1). Data from this experiment are summarized in Table 1. NTB rats demonstrated a significant increase in food intake and weight gain over saline-treated controls when treated with insulin at 2 units/100 g/day (P < 0.001), but not at 1 unit/100 g. Saline-treated TB rats were cachectic as evidenced by a significant decline in food intake and loss of host weight compared to NTB controls (P < 0.001). When insulin was given at 1 unit/100 g/day, no effect was seen in any of these parameters; however, at a dose rate of 2 units/100 g/day, TB animals demonstrated a significant increase (P < 0.001) in food intake to levels comparable to NTB controls. This resulted in a significant increase in host weight compared to saline-treated TB animals (P < 0.001).

Calculated tumor doubling times were similar in all groups (Table 1).

Effects of Insulin in Late Cachexia (Experiment 2). All animals demonstrated a marked decline in food intake and were losing total body weight prior to randomization and treatment (Chart 2). Following randomization, the saline-treated group continued this downward trend and were eating nothing after 2 days; insulin treatment, however, reversed the trend, and these animals showed an increase in food intake and continued to eat until death. There was a profound decline in calculated host weight in saline-treated TB rats (−53 ± 4.4 g) compared to the insulin-treated group (−2.6 ± 3.1 g) (P < 0.001). Survival times from the time of randomization were not significantly different between groups (5 ± 0.6 days for insulin-treated rats versus 6.6 ± 1.8 days for saline-treated rats). Tumor sizes at death were not significantly different (70.8 ± 2.5 g in insulin-treated rats versus 67.3 ± 2.8 g in saline-treated rats).
Effects of Long-Term Continuous Insulin (Experiment 3). Long-term administration of insulin to TB rats at 2 units/100 g/day (Groups III and IV) resulted in significantly greater food intake compared to control (Group I) and rats treated with 1 unit/100 g/day (Group II) (Chart 3). Food intake remained elevated in these rats until Day 34 when large numbers of animals began to die. Total body weight was also significantly elevated in both the 2-unit/100-g/day groups (Chart 4), and, more importantly, calculated host weight rose and remained elevated in Groups III and IV compared to control (Chart 3). Group IV animals (2 units/100 g/day in a single dose) showed less loss of host weight after Day 25 than Group I and IV animals (Chart 3). Group IV animals had a net host weight gain of 50 g compared to 0 g in control TB animals (P < 0.001). Food intake was also significantly elevated in both the 2-unit/100-g groups and remained elevated in Group IV rats until Day 34 (Chart 3).

Table 2

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Survival (days) measured from days of tumor implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (no treatment)</td>
<td>40.2 ± 0.5 ± *</td>
</tr>
<tr>
<td>Group II (1 unit/100 g/day)</td>
<td>36.4 ± 1.0</td>
</tr>
<tr>
<td>Group III (1 unit/100 g/twice/day)</td>
<td>34.5 ± 1.4</td>
</tr>
<tr>
<td>Group IV (2 units/100 g/day)</td>
<td>35.1 ± 1.4</td>
</tr>
</tbody>
</table>

* Mean ± SE.

P < 0.001 compared to Groups II, III, and IV.
In general, tumor growth was not affected by insulin administration (Chart 4). However, untreated TB animals (Group I) had significantly longer (5 days) survival than any of the insulin-treated groups ($P < 0.001$) (Table 2). There were no significant differences in survival between the three insulin-treated groups (Table 2). Adrenal and heart weights were significantly greater in insulin-treated animals than in animals receiving insulin at 2 units/100 g/day (Groups III and IV). This relationship holds for organ weights expressed as a function of either total body or host weight (Table 3). Adrenal and heart weights were significantly greater in untreated rats than in rats receiving insulin at 2 units/100 g/day (Groups III and IV) (Table 3). Insulin levels were significantly higher in all groups of Sprague-Dawley rats compared to Fischer rats. Within each strain, insulin treatment did not change energy expenditure (Table 5).

Activity compartment (percentage of energy expenditure spent on motor activity) was significantly lower in TB than NTB S-D rats, both with and without insulin treatment. Tumor size during the treatment period was $39.7 \pm 7$ g. Insulin did not change activity compartment in either the NTB or TB state. In the F344 rats, on the other hand, the TB state did not depress activity compartment, and again insulin did not influence motor activity (Table 5). Tumor size during the experimental period was $57 \pm 5.6$ g.

### DISCUSSION

These experiments demonstrate the potent anticachectic effects of exogenous insulin: reversal of anorexia and preservation of host weight, even in late cachectic decline. The mechanism of appetite stimulation by exogenous insulin is unclear. It is unlikely that this is a direct or primary effect of insulin, because postprandial satiety is associated with elevated levels of endogenous insulin, and when insulin is infused directly into cerebrospinal fluid, feeding is suppressed (33-35). It is also unlikely to be purely the result of hypoglycemia, since insulin can produce hypoglycemia without stimulation of food intake (e.g., in the presence of hypothalamic damage) (36). Also, insulin-induced feeding can be suppressed by naloxone without affecting hypoglycemia (37). The effect of insulin on food intake is probably of a tertiary or more indirect order.

Insulin influences the growth of some hormone-dependent tumors, typically those mammary tumors induced by 7,12-dimethylbenz(a)anthracene (38-40) and some human breast cancers (41, 42), but there is no useful information in these studies concerning food intake or the nutritional status of the host. It can also slow growth of tumors not known to be hormone dependent (W256) but only if the insulin hyperphagia is simultaneously prevented by restricted feeding (43). The MCA tumor used here and the tumors (W256 and Morris hepatoma 5123) in which the beneficial effects of insulin on the host were previously shown (16, 17) are not known to be hormone dependent, but insulin-treated NTB rats of both strains and in TB S-D rats. Intake in the insulin-treated TB F344 rats was slightly but not significantly higher than in untreated rats.

Energy expenditure, adjusted to constant body weight and caloric intake, was significantly higher in all groups of Sprague-Dawley rats compared to Fischer rats. Within each strain, insulin treatment did not change energy expenditure (Table 5).

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hormone independence has not been exhaustively demonstrated. Increased availability of substrate alone (as achieved with parenteral nutrition) also maintains host weight but accelerates tumor growth (10). The insulin effect, then, may reflect the resultant of two distinct effects of insulin on tumor: a direct hormonal depression of tumor growth opposed by increased availability of substrate from the insulin-induced hyperphagia. The main point of the present results is that, whatever the detailed mechanism may be, it is possible, by use of insulin, to improve the condition of the host without accelerating tumor growth. The questions of whether similar or even more beneficial effects could be shown in hormone-dependent tumors if insulin dosage were adjusted to stimulate hyperphagia, and whether some restriction of food intake might be closely monitored to avoid hypoglycemia, death. If this can be successfully achieved, then perhaps survival can be prolonged.

The calorimeter studies reported here show significant differences between S-D and F344 rats in energy expenditure for both NTB and TB states. These interstrain differences are not unusual and agree with previously reported values (17, 18, 20). The S-D rats demonstrated significantly less motor activity in the TB than in the NTB state, an observation which agrees with previous reports (19). This effect was not seen in the F344 rats, of which previous reports have described a lowering of motor activity in the TB state (20). The effect of insulin on food intake in the F344 TB rats in the calorimeter runs was not as marked as that seen on the S-D rats. This is due to the fact that the F344 rats were treated with insulin several days after the initial untreated calorimeter runs, so cachexia was relatively more advanced. No effect of insulin on energy expenditure or activity compartment of energy expenditure was seen. This held true even in animals whose feeding was significantly enhanced by insulin and suggests that feeding and motor activity are independent effects, as has been described previously (20).

The use of exogenous insulin in tumor-bearing animals is a nutritional manipulation which preferentially feeds the host over the tumor. Further work must document what body compartment (i.e., fat or lean body mass) is enhanced or preserved by insulin treatment, and whether these intuitively beneficial effects can be translated into improved survival by altering the scheduling or dosage of insulin.

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**REFERENCES**


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