Body Composition Changes in Rats with Experimental Cancer Cachexia: Improvement with Exogenous Insulin

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ABSTRACT

Exogenous insulin treatment has been shown to improve food intake and host weight of cachectic tumor-bearing (TB) rats, but the composition of the host weight gain has not been quantitated. Sixty-six Fischer 344 rats were randomized to seven groups: early non-tumor-bearing (NTB) (n = 10) who underwent compositional analysis (CA) on the day the methylcholanthrene sarcoma was implanted in TB rats; pretreatment-NTB (n = 10) and pretreatment-TB (n = 10) who underwent CA 25 days later when rats began treatment with saline or insulin; and finally saline-treated NTB (n = 9), saline-treated TB (n = 9), insulin-treated NTB (n = 9), and insulin-treated TB (n = 9), who underwent CA following 5 days of treatment with daily saline or neutral protamine hagedorn insulin 2 units/100 g. Body weight and food intake were measured daily. For compositional analysis, the tumor was separated from the host in TB rats and the entire rat in NTB animals was homogenized, lyophilized and analyzed for fat, water, potassium, sodium, chloride, and protein. The tumor was processed in a similar fashion.

In response to insulin, NTB rats ate significantly more food and had an increase in body weight gain. Compositional analysis of insulin-treated NTB rats indicated a slight, but insignificant, increase in body fat and a similar insignificant decrease in body protein. TB rats ate significantly less than NTB rats during the 5-day experimental period, and insulin treatment significantly increased food intake to levels similar to NTB animals. Compositional analysis indicated that the tumor-bearing state resulted in a significant decrease in total host water, protein, fat, potassium, sodium, and chloride. Insulin administration resulted in preservation of host nitrogen, fat, potassium, sodium, and chloride in cachectic tumor-bearing rats. Insulin treatment did not affect tumor dry weight or composition. The results suggest that exogenous insulin, can preserve normal host composition of TB rats during cachetic decline.

INTRODUCTION

Cancer cachexia is a condition characterized by anorexia and host tissue depletion which is exhibited by malignant tumor-bearing organisms. Experimental cancer cachexia has been extensively studied using the MCA2-induced sarcoma in the Fischer rat (1–3). This tumor reliably and reproducibly produces anorexia, energy reserve depletion, and death within 1 month of implantation (2, 3). Associated abnormalities of carbohydrate metabolism (1), energy expenditure (4), food intake (2, 3), and response to stress (5) have been described. Use of total parenteral nutrition in these animals causes an increase in body weight; however, most of the weight gained is in the form of water and fat, and marked stimulation of tumor growth is produced (6). Administration of the anabolic hormone insulin has been shown to stimulate food intake and body weight gain in TB rats during late cachectic decline (4, 8, 9).

In the present study, we examined the changes in body composition that accompanied cachectic host decline in tumor bearing rats. Whole animal compositional analysis was used to measure body protein, potassium, fat, and water. Measurement of these components at different stages of tumor growth showed that all four significantly decreased during cachetic decline. Exogenous insulin increased food intake in cachetic TB rats and resulted in a significant increase in host weight compared to saline-treated TB controls. Compositional analysis of the insulin-treated TB host indicated that it retained fat, protein, potassium, and water in proportions similar to NTB rats indicating that insulin equally preserved all components of host tissue. Insulin treatment did not change tumor composition. Exogenous insulin can preserve vital host mass during cachetic decline of tumor-bearing rats.

MATERIALS AND METHODS

Animals, Tumors, and Diet. Sixty-six male Fischer 344 rats weighing 220–240 g were used in this experiment. The animals were housed individually and allowed unlimited access to food and water. Diet consisted of AIN76 rat chow pellets which were retrieved daily for measurement of food intake. The dry composition of the AIN76 diet was 20% protein, 65% carbohydrate, 5% fat, 5% fiber, 4.5% minerals, 0.3% methionine, and 0.2% choline as described previously (9). A 12-h light, 12-h dark cycle was maintained throughout the experiment. Body weight, tumor dimensions, and food intake were measured daily.

The tumor used in these experiments was a transplantable MCA-induced sarcoma. Tumors were inoculated as 1-mm³ fragment s.c. into the flank under ether anesthesia.

Insulin. Nonprotamine hagedorn insulin was used (iletin; Eli Lilly and Company, Indianapolis, IN). Injections were made s.c. using a 1:10 dilution with saline. Injections were given between 10 and 11 a.m. Control animals were given injections of similar volumes of sterile saline solution daily during treatment periods. Insulin was given at a dose of 2 units/100 g body weight per day.

Body Composition Analysis. Body composition was analyzed by the following technique. Animals were killed by rapid CO2 asphyxiation. The gut was opened and emptied of stool and undigested food and returned to the carcass. The pelt was removed and frozen in liquid nitrogen at −180°C. The frozen pelt was then shattered into a powder and underwent immediate compositional analysis (early-NTB group).

Treatment significantly increased food intake to levels similar to MB rats during the 5-day experimental period, and insulin treatment significantly increased food intake to levels similar to NTB animals. Associated abnormalities of carbohydrate metabolism (1), energy expenditure (4), food intake (2, 3), and response to stress (5) have been described. Use of total parenteral nutrition in these animals causes an increase in body weight; however, most of the weight gained is in the form of water and fat, and marked stimulation of tumor growth is produced (6). Administration of the anabolic hormone insulin has been shown to stimulate food intake and body weight gain in TB rats during late cachectic decline (4, 8, 9).

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Experimental Design. Sixty-six animals were randomized to treatment groups using a random number method (14). After a 10-day period of acclimation to housing and diet, 10 animals were sacrificed and underwent immediate compositional analysis (early-NTB group). Flank tumors s.c. were implanted in 28 rats and the remaining 28 underwent a sham procedure. When daily food intake in tumor-bearers...
fell below 9 g/day (about 75% of normal intake, occurring 25 days postimplantation), 10 animals underwent immediate compositional analysis (pretreatment TB group). Of the remaining 18 TB rats, half were given a 5-day course of insulin (insulin-treated TB group) and half were given saline (saline-treated TB group). The rats were then killed and compositional analysis was performed. The 28 sham-operated NTB rats were treated in an identical fashion to the TB rats. Ten were killed and underwent compositional analysis at the same time as the pretreatment TB rats (pretreatment NTB group), and the remainder received a 5-day course of either insulin or saline prior to compositional analysis (insulin-treated NTB group, saline-treated NTB group). Timing of NTB rat treatment was designed to parallel TB rat treatment exactly. When a TB rat showed a decline in food intake to 9 g/day or less, and was treated by either immediate compositional analysis or saline or insulin treatment, a NTB rat was randomly selected and similarly treated.

Statistics. All data are presented as mean ± SD. Differences are determined by analysis of variances and t tests corrected for multiple comparisons (Bonferroni Comparison) (15).

RESULTS

Following sham operation, NTB rats maintained a constant daily food intake and gained weight progressively throughout the experimental period. During the 5-day treatment period, insulin-treated NTB rats ate significantly more than did saline-treated NTB rats. This was accompanied by a significant increase in weight gain in insulin-treated NTB animals (Table 1).

TB rats demonstrated a gradual decline in food intake starting approximately 20 days posttumor implantation. Insulin-treated TB rats ate significantly more than saline-treated TB rats, and saline-treated TB rats showed a profound decline in food intake. Insulin-treated TB rats gained weight during the treatment period while saline-treated TB rats demonstrated significant weight loss (Table 1).

Table 2 shows compiled data from compositional analysis of TB and NTB rats from all groups (early, pretreatment, insulin-treated NTB, and saline-treated). Weight in pretreatment NTB rats was significantly greater than in early NTB rats, and after a 5-day course of daily insulin administration, body weight was slightly, but not significantly, greater than in saline-treated NTB rats. Weight of pretreatment TB rats was only slightly higher than that of early NTB rats. Saline-treated TB rats had further significant host weight loss. Insulin-treated TB rats, on the other hand, showed a slight weight gain compared to pretreatment, and weighed significantly more than saline-treated TB rats (P < 0.05). Data from compositional analysis of these animals are also presented in Table 2. Differences in body water, nitrogen, protein, fat, and electrolytes (sodium, potassium, chloride) closely paralleled differences in body weight. Among nontumor bearers, insulin-treated animals had no significant changes in body composition. The tumor-bearing state resulted in a significant decrease in total host water, nitrogen, protein, fat, potassium, sodium, and chloride (pretreatment NTB > pretreatment TB, P < 0.05). Insulin administration to cachectic TB rats resulted in preservation of host nitrogen, protein, fat, potassium, sodium, and chloride compared to saline-treated controls (P < 0.05). No significant differences were noted in the relative contributions of measured body compartments to the whole host organism in any group studied (Table 3). In TB rats, there was a slight increase in body water and protein compartments, and a decrease in body fat, however, these differences were not statistically significant. The relative contributions of water, protein, and fat as a percentage of body weight were not changed by the presence of tumor, or by the treatment with insulin.

Table 4 summarizes compositional analysis of tumors. As expected, tumors in the saline- and insulin-treated groups were significantly heavier than tumors in the pretreatment TB group. Insulin-treated TB rat tumors were slightly heavier and contained more Na+ than saline-treated TB tumors (P < 0.05), but dry tumor weights were not significantly different in the two treatment groups. Tumors were mostly water, which constituted over 80% of their weight. The dry composition of all tumors was over 90% protein, and this finding was similar in all groups. The relative contributions of water and protein to tumor weight were not significantly different in any group. CI- and K+ concentrations were likewise similar in each group. The tumors contained negligible amounts of fat. Insulin treatment did not affect tumor composition.

DISCUSSION

This study examined the effects of cancer cachexia on body composition, and demonstrated the impact of the anabolic
Table 3  Body water, solids, protein and fat expressed as a percentage of body weight in TB and NTB rats before and after treatment with insulin or saline

<table>
<thead>
<tr>
<th></th>
<th>Early NTB (n = 10)</th>
<th>Pretreatment NTB (n = 10)</th>
<th>Saline-treated NTB (n = 9)</th>
<th>Insulin-treated NTB (n = 9)</th>
<th>Pretreatment TB (n = 10)</th>
<th>Saline-treated TB (n = 9)</th>
<th>Insulin-treated TB (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% H2O</td>
<td>54.5 ± 2.7</td>
<td>55.9 ± 1.9</td>
<td>56.7 ± 1.6</td>
<td>55.8 ± 2.1</td>
<td>57.2 ± 1.9</td>
<td>57.5 ± 2.9</td>
<td>56.5 ± 2.6</td>
</tr>
<tr>
<td>% Solid</td>
<td>45.5 ± 2.7</td>
<td>44.1 ± 1.9</td>
<td>43.3 ± 1.6</td>
<td>42.8 ± 1.9</td>
<td>42.5 ± 2.9</td>
<td>43.5 ± 2.6</td>
<td>43.5 ± 2.6</td>
</tr>
<tr>
<td>% Protein</td>
<td>18.8 ± 1.2</td>
<td>18.5 ± 1.3</td>
<td>19.3 ± 0.8</td>
<td>19.2 ± 1.2</td>
<td>20.1 ± 1.6</td>
<td>20.4 ± 1.3</td>
<td>19.8 ± 1.5</td>
</tr>
<tr>
<td>% Fat</td>
<td>21.4 ± 1.1</td>
<td>20.5 ± 1.3</td>
<td>18.9 ± 2.0</td>
<td>20.3 ± 4.0</td>
<td>18.7 ± 4.2</td>
<td>18.5 ± 3.8</td>
<td>19.8 ± 3.5</td>
</tr>
</tbody>
</table>

There are no significant differences noted in any group.

Table 4  Weight, water, nitrogen, protein, potassium, sodium, and chloride content of tumors from rats before and after treatment with insulin or saline

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment tumor (n = 10)</th>
<th>Saline-treated tumor (n = 9)</th>
<th>Insulin-treated tumor (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor weight (g)</td>
<td>55.1 ± 13.0</td>
<td>66.6 ± 7.9*</td>
<td>77.7 ± 12*</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>9.1 ± 2.2</td>
<td>12.6 ± 3.7†</td>
<td>14.0 ± 2.0</td>
</tr>
<tr>
<td>Tumor % H2O</td>
<td>83.3 ± 1.7</td>
<td>80.9 ± 6.8</td>
<td>82.0 ± 1.1†</td>
</tr>
<tr>
<td>Tumor nitrogen (g)</td>
<td>1.2 ± 0.3</td>
<td>1.8 ± 0.5*</td>
<td>1.8 ± 0.3*</td>
</tr>
<tr>
<td>Tumor protein (g)</td>
<td>7.4 ± 1.8</td>
<td>11.0 ± 3.2*</td>
<td>11.5 ± 2.0</td>
</tr>
<tr>
<td>Tumor potassium (mmol)</td>
<td>2.4 ± 0.6</td>
<td>3.0 ± 0.9*</td>
<td>3.1 ± 0.7†</td>
</tr>
<tr>
<td>Tumor sodium (mmol)</td>
<td>3.7 ± 1.2</td>
<td>4.9 ± 1.5*</td>
<td>6.2 ± 1.9*</td>
</tr>
<tr>
<td>Tumor chloride (mmol)</td>
<td>2.6 ± 0.9</td>
<td>4.1 ± 1.6*</td>
<td>4.3 ± 0.7†</td>
</tr>
</tbody>
</table>

* P < 0.05 compared to pretreatment tumors.  
† P < 0.05 compared to saline treatment tumors.  
§ Not significant compared to saline treatment tumors.

The depletion of host protein and potassium in cachectic tumor-bearing rats reflects a breakdown of vital body cell mass secondary to tumor effects. Depletion of body cell mass, which contains skeletal muscle, viscera, and circulating blood elements, seriously impairs the host ability to fight infection and withstand other stresses (16). In normal (NTB) rats, weight loss secondary to limitation of food intake results from preferential utilization of fat as substrate with sparing of protein and body cell mass (17). Weight losing tumor-bearing rats fail to maintain this adaptation. Obligate protein catabolism has been demonstrated in humans with cancer (18), and may be caused by circulating tumor-associated factor or factors (19). Cancer cachexia in tumor-bearing rats has been shown to be transmissible across a parabiotic union (20). Protein catabolism has also been described in sepsis, where it may clearly be due to circulating factors (19, 21).

Insulin treatment of cachectic tumor-bearing rats results in conservation of host weight with sparing of host protein, fat, and potassium. Certainly the increased intake of protein and substrate (food) by the tumor-bearing rat contributes to this host conservation, but in tumor-bearing rats given substrate i.v., protein conservation is not detected, and fat and water deposition predominate (6). In addition, administration of insulin to normal rats results in an increase in food intake similar to that seen in TB rats but does not appear to result in any significant enhancement of body protein or potassium. Recent tracer work also indicates that insulin shifts nutrient flow from tumor to host (22) and insulin reverses the toxic host effects of cachectin/tumor necrosis factor (23) which may be a mediator of cancer cachexia (19). It may be that insulin not only increases food intake in tumor-bearing rats, but overcomes tumor-induced metabolic defects which cause preferential protein catabolism. This metabolic impact of insulin may explain how it can preserve vital host tissue while total parenteral nutrition fails to preserve host composition and increases tumor growth (2, 6, 24). It should, however, be mentioned that total parenteral nutrition is a different diet both in composition and in route of administration and this also may contribute to the different results seen when it is used.

We have previously shown that insulin did not stimulate growth of this MCA sarcoma (3). These data may not be confirmed with a different diet (AIN76). Tumor weights in the insulin-treated group were slightly higher than in the saline-treated group (Table 4); however, dry tumor weights were not significantly different. Tumor composition was likewise similar in all groups, with water making up over 80% of tumor weight.

This work and previous work suggest that insulin is a potent antitumor hormone which can be administered safely to rats over short periods of time (3, 7, 8). Use of insulin in humans with cancer has been suggested (25), but clinical trials have not yet been done.

REFERENCES

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