Nitrogen Excretion in Cancer Cachexia and Its Modification by a High Fat Diet in Mice

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ABSTRACT

Animals transplanted with the MAC16 colon adenocarcinoma showed a loss of body weight as the tumor weight increased, without a reduction in food intake. Both adipose tissue and muscle mass decreased in tumor-bearing animals, although loss of body fat exceeded that of muscle mass for given tumor weight. Urinary nitrogen excretion was significantly elevated when the weight loss did not exceed 3 to 4 g, but above this weight loss there was a conservation of nitrogen and the excretion level fell to or below that found in non-tumor-bearing animals. The presence of a tumor alone was not sufficient to account for the elevated nitrogen excretion, since animals bearing a related colon adenocarcinoma (MAC13) that did not induce weight loss had a nitrogen excretion pattern similar to that of non-tumor-bearing controls. Feeding an isocaloric isonitrogenous diet in which 80% of the calories were supplied as medium chain triglycerides, which significantly elevated plasma levels of ketone bodies, reduced both tumor weight and host weight loss and restored both the nitrogen balance and urea excretion to that of non-tumor-bearing animals. The plasma levels of amino acids, which were reduced in the cachectic state, were also restored to control values in animals fed the medium chain triglyceride diet. These results suggest that excessive nitrogen catabolism in the cachectic state can be prevented by suitable dietary modification.

INTRODUCTION

The principal endogenous energy and nitrogen sources during evolution of weight loss in cancer are adipose tissue triglycerides and skeletal muscle proteins. The total protein content of the muscles is significantly reduced, as is the synthetic ability. Whole-body protein turnover has been shown to be about one-third higher in malnourished cancer patients that in non-cancer patients and fasted normal subjects. The rates of whole-body protein turnover have also been shown to be increased in children with newly diagnosed leukemia or lymphoma. Increased muscle proteolysis in weight-losing cancer patients is also suggested by an increased venous level of alanine from forearm muscles. A negative nitrogen balance has also been demonstrated in both animals and humans with a variety of tumors. Thus even when nitrogen intake was sufficiently high to provide for both the host and the tumor, nitrogen was lost from the carcass to the tumor (8). Hypoalbuminemia is also a feature of cancer cachexia and is the result of both a decreased rate of albumin synthesis and an increased fractional catabolic rate.

Although the biochemical mechanisms underlying these phenomena are still unclear, Lazo (10) has suggested that concentration gradients are established between the free amino acid pools, with a net flux of amino acids taking place towards the tumor cell. Using mice transplanted with Ehrlich ascites tumor cells, Carrascosa et al. (11) have shown a net nitrogen movement from the host to the tumor, which may account for the negative nitrogen balance observed in cancer patients.

As an experimental model of cachexia, we have used the MAC16 adenocarcinoma of the colon transplanted into NMRI mice. This tumor produces extensive weight loss without a reduction in food or water intake. This weight loss, which is directly proportional to the weight of the tumor, is associated with a decrease of both the fat and non-fat carcass mass. Recent results suggest that weight loss may arise from the production by the tumor of lipolytic and proteolytic factors (13). This study investigates the effect of the MAC16 tumor on nitrogen excretion and plasma amino acid levels and the effect of dietary manipulation on these parameters.

MATERIALS AND METHODS

Pure strain NMRI mice were purchased from Banting and Kingman (Hull, United Kingdom) and were fed a rat and mouse breeding diet (Pilsbury, Birmingham, United Kingdom) and water ad libitum. Fragments of the MAC16 tumor (1 x 2 mm) were implanted into the flanks of male NMRI mice by means of a trocar, as described (12). Animals bearing the MAC16 tumor develop their tumors at different times after transplantation and, therefore, the results have been expressed as a function of tumor weight or weight loss rather than days after transplantation. Blood was removed from animals, using a heparanized syringe, by cardiac puncture, under anesthesia with a mixture of halothane, oxygen, and nitrous oxide. Plasma was prepared by centrifuging whole blood in a Beckman microfuge for 30 s and amino acid profiles were obtained by the Macromolecular Analysis Service at Birmingham University, using a Locarte Automatic Amino Acid Analyser.

Dietary Studies. All animals were given free access to rat and mouse breeding diet for 14 days after transplantation, at which time the tumors were palpable but weight loss had not occurred. They were then randomly divided into three groups and weighed. The standard diet contained 50% carbohydrate and supplied 11.5% of the energy as fat. An isonitrogenous isocaloric diet supplying 80% of the calories as MCT was formulated as a paste to minimize food scatter, as previously described (14). One group was given sodium 3-3-hydroxybutyrate in the drinking water, at a concentration of 30 μmol/ml. The average daily water consumption for all groups was 4.1 ml/mouse. Body weights and food and water intake were measured daily during the course of the study and food scatter was subtracted. Body weights were measured at the same time of day. After 8 days the mice were put into Metabowls and urine was collected for 24 h.

Urine Analysis. Urine was collected from the Metabowls and analyzed quantitatively for ammonia and urea using a Sigma diagnostic kit (Sigma Chemical Co., Dorset, U.K.) which depends on the colorimetric determination of ammonia at 570 nm (15). Creatinine nitrogen excretion was determined colorimetrically at 500 nm using a Sigma diagnostic kit (16).

Metabolite Assays. Whole blood (0.2 ml) was used and glucose was determined using the o-toluidine reagent kit (Sigma). Acetoacetate and 3-hydroxybutyrate levels were measured by the method of Mellanby and Williamson (17) and Williamson and Mellanby (18), respectively.

Body Composition Analysis. Each carcass was placed in an oven at 80°C until constant weight was reached. Carcasses were then reweighed and the total fat content was determined by the method of Lundholm et al. (19). The residue was the non-fat mass. The water content was calculated from the wet and dry weights.

The abbreviation used is: MCT, medium chain triglycerides.
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Statistical Analysis. The results were analyzed statistically using the analysis of variance or F ratio.

RESULTS

The effect of the MAC16 tumor on the total body weight and the body composition of male NMRI mice is shown in Fig. 1. Although food intake remained the same (12), total body weight decreased as the tumor weight increased and there was a corresponding reduction in the size of the individual body compartments. The carcass weight loss has been previously shown to be directly proportional to tumor weight ($r = -0.91$) (13). The rate of loss of body fat exceeded that of muscle, particularly at the lower tumor weights. The effect of tumor weight on the thigh plus gastrocnemius muscle dry weights is shown in Table 1. Both muscle weights decreased as the tumor weight increased, suggesting extensive protein mobilization.

Concomitant with the increase in size of the tumor, there was a decrease in the plasma concentration of all amino acids except for taurine, although the percentage decrease varied for the individual amino acids (Table 2). The maximum decrease of 54% was observed for valine and isoleucine. Most of the other amino acids were present in tumor-bearing mice at only 60% of the levels found in non-tumor-bearing animals. One of the hallmarks of chronic malnutrition is an elevation of plasma glycine. However, in the MAC16 tumor-bearing animals glycine levels were reduced to 60% of that of non-tumor-bearing animals, again confirming that the animals were not malnourished.

The total urinary nitrogen excretion in tumor-bearing animals varied according to the extent of weight loss and was significantly elevated at small tumor masses, when the weight loss was 2 to 6 g (Fig. 2). However, above this weight loss there was a conservation of nitrogen and the excretion level fell to or below that found in non-tumor-bearing animals. The total nitrogen output in non-tumor-bearing animals or in animals bearing a related colon adenocarcinoma (MAC13) that did not induce weight loss did not change with time during the course of the study (Table 3) and is not included in Figs. 2 to 6. There was no difference in the nitrogen excretion in the feces between control animals and those bearing the MAC16 adenocarcinoma. Although animals bearing the MAC16 adenocarcinoma remained in positive nitrogen balance throughout the study, there was an initial sharp drop in nitrogen balance when weight loss started to occur, followed by a shift towards a more positive nitrogen balance as weight loss increased (Fig. 3), again suggesting some mechanism for conservation of body nitrogen.

Table 1. Effect of the MAC16 tumor on the thigh plus gastrocnemius muscle weights of male NMRI mice

<table>
<thead>
<tr>
<th>Tumor wt (g)</th>
<th>Wt loss (g)</th>
<th>Muscle wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.179</td>
<td>0.0</td>
<td>0.111</td>
</tr>
<tr>
<td>0.330</td>
<td>3.2</td>
<td>0.080</td>
</tr>
<tr>
<td>0.346</td>
<td>3.5</td>
<td>0.067</td>
</tr>
<tr>
<td>0.350</td>
<td>3.6</td>
<td>0.081</td>
</tr>
<tr>
<td>0.360</td>
<td>3.6</td>
<td>0.092</td>
</tr>
<tr>
<td>0.400</td>
<td>7.1</td>
<td>0.064</td>
</tr>
<tr>
<td>0.509</td>
<td>6.0</td>
<td>0.067</td>
</tr>
<tr>
<td>0.540</td>
<td>7.0</td>
<td>0.077</td>
</tr>
<tr>
<td>0.540</td>
<td>8.8</td>
<td>0.055</td>
</tr>
<tr>
<td>0.604</td>
<td>9.5</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Table 2. Plasma concentrations of amino acids (mean ± SE) in control (C) and tumor-bearing (TB) NMRI mice fed either normal laboratory pellets, 80% MCT, or 80% MCT plus sodium 3-hydroxybutyrate (3HB)

Values have been averaged over the period when weight loss is 2 to 6 g and are the pooled values of 5 animals/group.

Fig. 2. Change in total nitrogen excretion in male NMRI mice bearing the MAC16 tumor. Urine was collected for a 24-h period at times after transplantation corresponding to the stated weight loss. The individual lines represent points from a single animal. Values have been expressed relative to the nitrogen excretion when no weight loss was observed.

Fig. 3. Change in total nitrogen excretion in male NMRI mice bearing the MAC16 tumor. Urine was collected for a 24-h period at times after transplantation corresponding to the stated weight loss. The individual lines represent points from a single animal. Values have been expressed relative to the nitrogen excretion when no weight loss was observed.
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Table 3 Effect of tumor type and dietary modification on weight loss and nitrogen balance

Results are given as mean ± SE for 7 to 9 animals/group. The total nitrogen input was 0.048 ± 0.007 g/24 h and did not vary between the individual dietary groups.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Diet</th>
<th>Initial wt (g)</th>
<th>Final wt (g)</th>
<th>Wt loss (-) or gain (+)</th>
<th>Tumor wt (g)</th>
<th>Total nitrogen output (g/24 h)</th>
<th>Nitrogen balance (g/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Normal</td>
<td>25.38 ± 0.23</td>
<td>26.13 ± 0.2</td>
<td>+0.75 ± 0.23</td>
<td>0.70 ± 0.06</td>
<td>0.032 ± 0.003*</td>
<td>0.016 ± 0.007*</td>
</tr>
<tr>
<td>MAC13</td>
<td>Normal</td>
<td>25.89 ± 0.25</td>
<td>26.08 ± 0.16</td>
<td>+0.19 ± 0.25</td>
<td>0.70 ± 0.06</td>
<td>0.023 ± 0.004*</td>
<td>0.025 ± 0.007*</td>
</tr>
<tr>
<td>MAC16</td>
<td>Normal</td>
<td>25.92 ± 0.19</td>
<td>19.68 ± 0.97</td>
<td>-5.68 ± 0.54</td>
<td>0.66 ± 0.10</td>
<td>0.044 ± 0.003</td>
<td>0.004 ± 0.007*</td>
</tr>
<tr>
<td>MAC16</td>
<td>80% MCT</td>
<td>25.14 ± 0.35</td>
<td>21.69 ± 0.59</td>
<td>-3.46 ± 0.59*</td>
<td>0.23 ± 0.03*</td>
<td>0.028 ± 0.004*</td>
<td>0.020 ± 0.004*</td>
</tr>
<tr>
<td>MAC16</td>
<td>80% MCT + 3HB</td>
<td>24.78 ± 0.38</td>
<td>22.08 ± 0.66</td>
<td>-2.45 ± 0.36*</td>
<td>0.19 ± 0.04*</td>
<td>0.034 ± 0.004*</td>
<td>0.014 ± 0.007*</td>
</tr>
</tbody>
</table>

* *P < 0.05 when compared with MAC16 tumor-bearing animals fed the normal diet.
* *P < 0.003 when compared with MAC16 tumor-bearing animals fed the normal diet.
* *P < 0.01 when compared with MAC16 tumor-bearing animals fed the normal diet.
* *P < 0.0005 when compared with MAC16 tumor-bearing animals fed the normal diet.

animals fed the MCT diets. The effect of the MCT diet was to reduce the total nitrogen output, which did not differ significantly from non-tumor-bearing controls. Also the nitrogen balance was restored to that of non-tumor-bearing controls (Table 3).

The contributions to the total urinary nitrogen output are given in Table 4. Urea is the main end product of nitrogen metabolism and was the main contributor to the total nitrogen output in all groups of animals. Mice bearing the MAC16 tumor have a significantly elevated urea excretion when compared with either non-tumor-bearing controls or animals bearing the MAC13 tumor, but this was reduced to control values when the normal diet was substituted with 80% MCT with or without inclusion of sodium 3-hydroxybutyrate. Ammonia excretion was not significantly elevated in animals bearing the MAC16 tumor but was increased in the urine of animals consuming 80% MCT plus 3-hydroxybutyrate. This coincided with the appearance of both 3-hydroxybutyrate and acetoacetate in the urine and increased levels of these ketone bodies in the plasma (Table 5). Creatinine excretion was significantly elevated in MAC16 tumor-bearing animals fed the MCT diets.

Coincident with the increased plasma concentrations of acetoacetate and 3-hydroxybutyrate, there was an increase in the plasma concentrations of all amino acids, towards control val-
Depletion of muscle protein in animals bearing the MAC16 tumor has been attributed to the production by the tumor of a circulatory proteolytic factor, the activity of which is suppressed by both insulin and 3-hydroxybutyrate (13). In addition to increased protein catabolism, there must be increased amino acid utilization since the plasma concentration of most amino acids decreases at a time when weight loss occurs. However, it is unlikely that the presence of a tumor alone is sufficient to account for the increased nitrogen excretion, since in animals bearing the MAC13 adenocarcinoma, which do not develop weight loss, the tumor mass is not significantly different from that of the MAC16 tumor, although the total nitrogen excretion is similar to that of non-tumor-bearing animals. An increased gluconeogenesis is thought to counteract the tendency towards hypoglycemia in tumor-bearing animals (26), although animals bearing the MAC16 tumor do develop hypoglycemia within 16 days after tumor transplantation (12). The absence of ketosis in cancer patients (27) and in animals bearing the MAC16 tumor would allow continuation of gluconeogenesis, since ketone bodies are thought to protect muscle protein during starvation, possibly by inhibiting the rate of oxidation of branched-chain amino acids in muscle (28).

It has recently been shown (14) that animals bearing the MAC16 adenocarcinoma that were fed a ketogenic diet had a reduction in both weight loss and tumor size when compared with those fed a normal laboratory diet, which is low in fat and high in carbohydrate; this has been confirmed by the present experiments and also by clinical studies on a small group of patients (29), although the latter study did not examine the effect on tumor growth. Such a diet has been shown to increase the plasma concentration of all amino acids towards that found in non-tumor-bearing animals. Induction of ketosis might be expected to inhibit catabolism of muscle proteins and also to inhibit directly the tumor proteolytic activity (13). Thus, in animals fed a diet isonitrogenous with normal laboratory food but in which 80% of the calories are supplied as MCT with or without 3-hydroxybutyrate supplementation, the nitrogen balance and the urea excretion are restored to that of non-tumor-bearing controls, suggesting a decrease in amino acid-catabolizing activity. Ammonium ions are excreted as part of the mechanism of pH regulation and the increased urinary excretion observed in animals fed 80% MCT plus 3-hydroxybutyrate coincides with the excretion of the weak acids acetacetate and 3-hydroxybutyrate. Creatinine excretion bears a direct relationship to the muscle mass and the increased urinary levels found in animals fed the 80% MCT diets are probably related to the increased muscle mass (14). These results contrast with the effect of diet-induced systemic ketosis in a small group of cachectic cancer patients, where no evidence was obtained for an alteration in whole-body nitrogen kinetics or the ability of

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**Table 4** Effect of tumor type and dietary modification on the excretion of urea, ammonia, and creatinine

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Diet</th>
<th>Urea (g/24 h)</th>
<th>Ammonia (10^-3 g/24 h)</th>
<th>Creatinine (10^-3 g/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Normal</td>
<td>0.028 ± 0.003</td>
<td>3.91 ± 0.50</td>
<td>6.44 ± 0.86</td>
</tr>
<tr>
<td>MAC13</td>
<td>Normal</td>
<td>0.015 ± 0.001</td>
<td>13.8 ± 5.1</td>
<td>3.87 ± 0.09</td>
</tr>
<tr>
<td>MAC16</td>
<td>Normal</td>
<td>0.041 ± 0.003</td>
<td>4.15 ± 0.47</td>
<td>8.66 ± 1.15</td>
</tr>
<tr>
<td>MAC16</td>
<td>80% MCT</td>
<td>0.023 ± 0.004</td>
<td>6.14 ± 1.65</td>
<td>14.60 ± 2.52</td>
</tr>
<tr>
<td>MAC16</td>
<td>80% MCT + 3HB</td>
<td>0.028 ± 0.002</td>
<td>14.48 ± 4.21</td>
<td>18.20 ± 3.34</td>
</tr>
</tbody>
</table>

* P < 0.02 when compared with MAC16 tumor-bearing animals fed the normal diet.
* P < 0.001 when compared with MAC16 tumor-bearing animals fed the normal diet.
* P < 0.003 when compared with MAC16 tumor-bearing animals fed the normal diet.
* P < 0.05 when compared with MAC16 tumor-bearing animals fed the normal diet.
* P < 0.006 when compared with MAC16 tumor-bearing animals fed the normal diet.

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**Table 5** Effect of dietary modification on the plasma and urinary concentrations of ketone bodies in animals bearing the MAC16 adenocarcinoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Diet</th>
<th>Glucose (mg/100 ml)</th>
<th>Acetoacetate (mmol/L)</th>
<th>3-Hydroxybutyrate (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Normal</td>
<td>121.8 ± 4.4</td>
<td>0.04 ± 0.002</td>
<td>0.078 ± 0.007</td>
</tr>
<tr>
<td>MAC16</td>
<td>Normal</td>
<td>106.4 ± 9.5</td>
<td>0.039 ± 0.002</td>
<td>0.096 ± 0.015</td>
</tr>
<tr>
<td>MAC16</td>
<td>80% MCT</td>
<td>100.8 ± 6.5</td>
<td>0.074 ± 0.007</td>
<td>0.241 ± 0.022</td>
</tr>
<tr>
<td>MAC16</td>
<td>80% MCT + 3HB</td>
<td>91.3 ± 3.2</td>
<td>0.060 ± 0.004d</td>
<td>0.170 ± 0.012d</td>
</tr>
</tbody>
</table>

* P < 0.025 from non-tumor-bearing animals.
* P < 0.002 from MAC16 tumor-bearing animals fed the normal diet.
* P < 0.0005 from MAC16 tumor-bearing animals fed the normal diet.
* P < 0.01 from MAC16 tumor-bearing animals fed the normal diet.
* P < 0.002 from non-tumor-bearing animals.
* P < 0.00001 from MAC16 tumor-bearing animals fed the normal diet.
the body to retain nitrogen, although all of the patients gained weight (29).

These results suggest that it is possible to reverse the protein-mobilizing effect of a cachexia-inducing tumor by a ketogenic diet. Such a diet should be tolerable clinically and has the added advantage that it causes an increase in host weight while simultaneously causing a reduction in tumor weight. It thus overcomes the potential problem of dietary supplementation causing an increase in tumor growth rate.

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REFERENCES


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