Effect of Systemic Hyperinsulinemia in Cancer Patients

Martin J. Heslin, Elliot Newman, Ronald F. Wolf, Peter W. T. Pisters, and Murray F. Brennan

Department of Surgery, Surgical Metabolism Laboratory, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Data defining the isolated effect of insulin on whole body protein and glucose metabolism in cancer patients are limited. Ten normal volunteers (controls), age 55 ± 3 years (mean ± SEM); 8 cancer patients, age 61 ± 3 years, weight loss 2 ± 1% (CANWL); and 8 cancer patients, age 55 ± 2 years, weight loss 18 ± 2% (CAWL), were studied in the postabsorptive state. Whole body leucine kinetics were determined during a baseline and then a study period during which insulin was infused at 1.0 milliunits/kg/min to achieve a high physiological level of 71 ± 6, 83 ± 5, and 64 ± 5 microunits/ml in controls, CANWL, and CAWL, respectively. Whole body net balance equals protein synthesis minus protein breakdown. Glucose disposal (mg/kg/min) is the rate of D30 infusion at steady state.

Glucose disposal of CANWL and CAWL during the study period was significantly (P < 0.05, analysis of variance) less than controls (3.91 ± 0.25 and 2.85 ± 0.29 mg/kg/min in controls; 3.66 ± 0.18 and 2.85 ± 0.16 mg/kg/min in CANWL; and 5.87 ± 0.6 mg/kg/min in CAWL), suggesting resistance to insulin with respect to carbohydrate metabolism. Hyperinsulinemia, under euglycemic and near basal amino acid conditions, significantly reversed the negative postabsorptive leucine net balance (P < 0.05, analysis of variance) by decreasing protein breakdown in controls as well as weight-stable and weight-losing cancer patients, suggesting that cancer patients are not resistant to the anti-catabolic effect of insulin with respect to whole body protein metabolism.

INTRODUCTION

The syndrome of cancer cachexia is characterized by severe weakness, debilitation, and generalized host wasting (1). This malnourished state is associated with decreased survival (2), and in up to one-half of cancer patients cancer cachexia has been implicated as the sole cause of death (3). The etiology of this syndrome is multifactorial in nature, and while decreased nutrient intake is partly responsible, a major contribution comes from the well described abnormalities in host intermediary metabolism of carbohydrate, protein, and fat (4).

Reports documenting abnormal peripheral glucose disposal (5), gluconeogenesis (6), and whole body glucose turnover (7) confirm alterations in carbohydrate metabolism in these patients. Abnormalities of protein metabolism have been reported at the whole body level (8, 9) as well as in skeletal muscle (9) and liver proteins (9). Fat metabolism is also modified as evidenced by abnormal host lipid stores (10), and free fatty acid and glycerol turnover rates (11, 12). The cachectic cancer patient ineffectively utilizes nutrients and furthermore seems to be unable to adapt to the malnourished state as normal humans do by conserving lean body mass (13).

With the advent of TPN,3 the ability to provide adequate nutrient intake in debilitated cancer patients was made possible. However, nutritional support to improve the clinical outcome in this group of patients has not been associated with a survival benefit (4, 14). Part of the explanation for the lack of success with standard nutritional support may lie in the failure of the current nutritional regimens to completely reverse the abnormalities of intermediary metabolism.

A well documented abnormality of metabolism in cancer patients is resistance to insulin with respect to carbohydrate metabolism (15, 16). These studies documented decreased uptake of glucose in peripheral tissues and in the whole body under the influence of insulin. However, data examining the response of cancer patients to insulin with respect to protein metabolism are limited. This is potentially important for 2 reasons. (a) Resistance to the well described antianabolic actions of insulin could be a partial etiology for the increased catabolism of protein stores, which has been previously documented in cancer patients (8, 9). (b) Resistance to insulin with regard to protein metabolism could be responsible for lack of demonstrable survival benefit in patients supported by TPN. The present study was undertaken to examine the isolated effects of hyperinsulinemia under euglycemic conditions with near basal amino acid levels on protein and glucose metabolism in weight-stable and weight-losing cancer patients.

MATERIALS AND METHODS

Subjects. Cancer patients were selected based on a histological diagnosis of cancer, with or without the presence of weight loss at the time of presentation. These patients were selected from the Surgical Service of the Memorial Sloan-Kettering Cancer Center. All were preoperative surgical patients studied before any surgical, radio-, or nutritional therapy except one patient who was studied 6 months after the last dose of a chemotherapy regimen. Cancer patients were divided into 2 groups based on weight loss greater or less than 10% of premorbid weight during the 6 months before diagnosis.

The control group consisted of 10 normal, healthy, weight-stable volunteers within 15% of their ideal body weight (19). Both cancer patients and controls were studied in the postabsorptive state after a 10–12-h overnight fast. Informed written consent was obtained before any testing, and all studies were carried out in the Adult Day Hospital of the Memorial Sloan-Kettering Cancer Center at rest in the supine position under an Institutional Review Board-approved protocol. These subjects had no history of systemic illness, hypertension, ischemic heart disease, or diabetes mellitus. None was taking medication known to alter protein or glucose metabolism. Subjects consumed their usual diet for the 3 days before study. A 24-h urine collection was completed before study that was analysed for total creatinine. Demographic and nutritional indices of the cancer patients and volunteers are listed in Tables 1 and 2.

Isotopes and Infusates. 1-[1-14C]Leucine (50 μCi/mmole) and [14C]-HCO3 (250 μCi/mmole) were obtained from Dupont (Boston, MA). 1-[1-14C]Leucine was diluted in normal saline to a final concentration of 0.56 μCi/ml. [14]HCO3 was also diluted in normal saline to a final concentration of 0.9 μCi/ml. All tracers were filtered through a 0.2-μm sterilizing filter (Acrodisc; GelmanSciences, Ann Arbor, MI) before infusion. The insulin infusate was composed of 100 ml of sterile normal saline, 2 ml of the subject's plasma, and 31 units of regular Humulin insulin (Lilly and Co., Indianapolis, IN) for a final concentration of 300 milliunits/ml. The amino acid infusate was 10% Travasol without electrolytes (Travenol Laboratories, Deerfield, IL), which contains (in mg/100 ml) 730 leucine, 600 isoleucine, 580 lysine, 580 valine, 560

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2To whom requests for reprints should be addressed, at Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

3The abbreviations used are: TPN, total parenteral nutrition; CANWL, cancer patients with weight loss <10% of premorbid weight; CAWL, cancer patients with weight loss ≥10% of premorbid weight; C, control group; Rn, rate of disappearance; Rn, rate of appearance; KIC, α-ketoisocaproic acid.
phenylalanine, 480 histidine, 420 threonine, 400 methionine, 180 tryptophan, 2070 alanine, 1150 arginine, 1030 glycine, 680 proline, 500 serine, and 40 tyrosine. The exogenous glucose infused was 30% dextrose (Abbott Laboratories, North Chicago, IL).

Experimental Protocol. The experiment was divided into two parts: the basal postabsorptive state, which consisted of a 120-min equilibration period (−165 to −45 min) and a subsequent sampling period of 45 min (−45 to 0 min) immediately followed by a euglycemic, hyperinsulinemic, euleucinemic period (0-120 min) followed by a second sampling period of 45 min (120-165 min) (Fig. 1). During the study period, a 10% amino acid solution without electrolytes were started. The rate of amino acid infusion (bolus = 400 milliunits/m², continuous infusion 0.16 μCi/m²/min) was started. After 2-h equilibration period, arterial blood was drawn at 15-min intervals for determination of the same parameters as in the baseline period. The arterial glucose was measured at the bedside at 5- to 10-min intervals, and the dextrose infusion rate was adjusted accordingly. Endogenous glucose production under the influence of insulin at this level has been demonstrated to be completely suppressed in similar control and cancer patients (25) during the equilibration period, therefore the rate of exogenous glucose infusion to maintain euglycemia at steady state was used to determine the rate of whole body glucose uptake.

After a 2-h euglycemic, hyperinsulinemic, euleucinemic equilibration period, arterial and expired gas samples were collected at 15-min intervals for 45 min for determination of the same parameters as in the baseline period. At the termination of the study period, the rate of exogenous glucose and insulin infusions were discontinued, and the glucose infusion was maintained for 30–45 min to avoid rebound hypoglycemia.

Analytical Methods. Arterial blood samples were obtained and allocated to appropriate tubes (Becton Dickinson, Rutherford, NJ) and immediately placed on ice. Arterial blood for immunoreactive insulin determination was collected in plain tubes, centrifuged, and immediately frozen for subsequent analysis by radioimmunoassay (Autopak, Micromedics, Horsham, PA). Arterial blood for immunoreactive glucagon determination was collected into Na-EDTA tubes with 1000 Kallikrein inhibitor units of aprotinin (Trasylol; FBA Pharmaceutical, New York, NY), centrifuged, and frozen for later analysis by standard radioimmunoassay (26) (Diabetic Core Center, Philadelphia, PA). A subaliquot of arterial blood was used for determination of glucose concentrations at the bedside (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). The rest of the blood was collected into tubes containing sodium heparin. After centrifugation, an aliquot of plasma was immediately deproteinized using 50 μl of 50% sulfosalicylic acid/ml of plasma and then frozen at −80°C until extraction of KIC (27) and subsequent determination of the plasma concentration and specific activity. The KIC was separated from 3 ml of plasma and the plasma concentration determined by high performance liquid chromatography using an internal standard (Series 4; Perkin-Elmer, Stamford, CT) with fraction collection. Immediately after collection of the KIC peak, 500 μl were used to determine radioactivity using a liquid scintillation counter (Minaxi Tricarb; Packard Instrument Co., Chicago, IL), and 200 μl of the remaining collected peak were reinjected for determination of the concentration used in the calculation of the specific activities. The remaining plasma was used for determination of amino acid profiles using ion exchange chromatography and post column ninhydrin derivatization (Pickering Laboratories, Mountview, CA). The average percent recovery of KIC was 85% in known standards made in the laboratory.

The percent CO₂ in expired air was determined by gas analysis (Medical Gas Analyzer 1100; Perkin-Elmer, Stamford, CT). The volume of expired air in the Douglas bag was determined by the evacuation time with a calibrated pump. Hydroxide of 10× Hyamine (1 ml) in absolute ethanol (2 ml) with phenolphthalein indicator was used to collect 1 mmol of CO₂ for liquid scintillation counting (22).

Procedures. Skinfolds were measured at 4 classic sites (biceps, triceps, subscapular, and iliac crest) using skinfold calipers (Lange Calipers, Cambridge, MD). By using regression equations that relate skinfold measurements to underwater weight (28), the percent total body fat was estimated. Skinfold measurements were performed by a single investigator to minimize variability.

Calculations. In all calculations, the values from each period were averaged to provide one value per period per subject.

Whole Body Leucine Kinetics. Whole body leucine flux was calculated using a stochastic model for protein metabolism. This analysis

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Table 1 Demographics

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Gender (M:F)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53 ± 3³</td>
<td>5:5</td>
</tr>
<tr>
<td>CANWL</td>
<td>61 ± 3³</td>
<td>6:2</td>
</tr>
<tr>
<td>CAWL</td>
<td>55 ± 2</td>
<td>5:3</td>
</tr>
</tbody>
</table>

² n = 10. ³ Mean ± SE.

Table 2 Nutritional indices

<table>
<thead>
<tr>
<th></th>
<th>% IBW (%)</th>
<th>Wt loss (%)</th>
<th>Albumin (mg/dl)</th>
<th>Cr/Ht *</th>
<th>% fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106 ± 3³</td>
<td>6.4 ± 0.1</td>
<td>6.8 ± 0.4</td>
<td>29 2</td>
<td></td>
</tr>
<tr>
<td>CANWL</td>
<td>103 ± 2</td>
<td>2 ± 1</td>
<td>4.3 ± 0.1</td>
<td>29 1</td>
<td></td>
</tr>
<tr>
<td>CAWL</td>
<td>100 ± 5</td>
<td>18 ± 2²</td>
<td>3.9 ± 0.2</td>
<td>26 3</td>
<td></td>
</tr>
</tbody>
</table>

*a Cr/Ht, urinary creatinine excretion divided by the height. ² Mean ± SE.

Conclusions

The arterial glucose was measured at the bedside at 5- to 10-min intervals, and the dextrose infusion rate was adjusted accordingly. Endogenous glucose production under the influence of insulin at this level has been demonstrated to be completely suppressed in similar control and cancer patients (25) during the equilibration period, therefore the rate of exogenous glucose infusion to maintain euglycemia at steady state was used to determine the rate of whole body glucose uptake.

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Fig. 1. Experimental design. Large arrow, sampling of blood for amino acid and glucose concentrations, KIC specific activities, and expired air. 

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Table 4 Arterial leucine, total arterial amino acid concentration, and KIC specific activity

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th>Leucine (µmol/liter)</th>
<th>TAA a (µmol/liter)</th>
<th>KIC SA (dpm/µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control b</td>
<td>Baseline</td>
<td>116 ± 6d</td>
<td>1521 ± 155</td>
<td>2638 ± 134</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>111 ± 7</td>
<td>2084 ± 218b</td>
<td>2386 ± 148</td>
</tr>
<tr>
<td>CANWL e</td>
<td>Baseline</td>
<td>130 ± 7</td>
<td>1722 ± 105</td>
<td>2446 ± 234</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>131 ± 8</td>
<td>2276 ± 143c</td>
<td>2245 ± 191</td>
</tr>
<tr>
<td>CAWL f</td>
<td>Baseline</td>
<td>123 ± 13</td>
<td>1693 ± 112</td>
<td>2573 ± 277</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>118 ± 11</td>
<td>2221 ± 137d</td>
<td>2327 ± 143</td>
</tr>
</tbody>
</table>

Significance defined as P < 0.05.

a TAA, total arterial amino acids; SA, specific activity.
b n = 10.
c Mean ± SE.
d CANWL is weight loss <10% (n = 8).
e Significance versus baseline.
f CAWL is weight loss >10% (n = 8).

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body net balance of leucine reversed from a net negative value to positive during euglycemic, euheucinemic, hyperinsulinemia primarily by the $R_e$ decreasing below the $R_d$ in both cancer groups, as was also seen in controls (Fig. 4).

**DISCUSSION**

It is widely accepted that cancer patients have abnormalities of intermediary metabolism. This study has documented that preoperative cancer patients who display insulin resistance with respect to glucose metabolism are not resistant to the anticatabolic effect of insulin with respect to whole body protein metabolism.

In normal humans, insulin causes a decrease in hepatic endogenous glucose production and a stimulation of glucose uptake and storage (32). Resistance to insulin, with respect to both hepatic and peripheral glucose metabolism, can be seen in patients with adult onset diabetes mellitus (33), and this results in hepatic overproduction in the fasting state and decreased uptake of glucose in the fed state. Exogenous insulin administration has been shown to reverse these alterations and restore glucose processing in insulin-dependent and non-insulin-dependent diabetes (34). Although there are reports of glucose intolerance (5) and variable insulin sensitivity in cancer patients, there are little data on the nature and mechanism of this tissue insensitivity to insulin. Prior work from our laboratory in weight-losing cancer patients have documented that the endogenous glucose production is reduced to zero at the levels of serum insulin achieved in this study (25), therefore the rate of glucose infusion is probably a good measure of whole body glucose disposal in these controls and cancer patients. A state of insulin resistance with respect to glucose metabolism was demonstrated by the decreased rate of glucose infusion in both cancer groups under similar plasma insulin concentrations.

Clinically, the abnormalities of protein metabolism in cancer patients include decreased plasma proteins, and visceral organ and skeletal muscle atrophy (35). In the tumor-bearing rat, chronic protein malnutrition causes accelerated loss of carcass weight without effect on the growth of the tumor (36), whereas the normal non-tumor-bearing animal put under similar conditions of malnutrition would adapt and conserve nitrogen. This suggests that the tumor will continue to grow at the expense of the host, and is not under the same nutritional constraints as the host. Further investigations using this model with the addition of protein kinetic measurements found that there was an increased rate of whole body protein turnover, increased liver protein synthesis, and decreased muscle protein synthesis (37). Exogenous insulin in the same model has also been shown to decrease carcass weight loss in cachectic animals (38).

In patients with cancer, the existence of abnormal rates of whole body protein synthesis and degradation are not as well established. Most have reported these rates to be elevated compared to postabsorptive normal humans (39), starved normal humans (8), and malnourished benign disease patients (8). However, others have reported no difference compared to postabsorptive humans (40).

The rates of catabolism and synthesis in the present study were approximately 10% elevated in the weight-losing group compared to controls. Prior work has shown that a normal adaptation to a chronically starved state would be to decrease protein turnover and conserve nitrogen (8, 41), therefore no decrease in the rate of catabolism and synthesis in the face of weight loss probably represents a maladaptation in itself.
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Cancer patients are approximately one-half the value that was achieved in the present study. Since the abnormalities in intermediary metabolism are still present with the addition of standard nutritional support, hormonal manipulation using anabolic agents such as insulin may play a role in the management of these patients. Finally, this work sets up the opportunity to study hyperinsulinemia as an anabolic adjunct in clinical trials with the most catabolic cancer patients: those who require TPN because they were unable to take in adequate nutrients in the postoperative period. The use of TPN would be able to maintain adequate levels of amino acids and the glucose present would be able to prevent any insulin-associated hypoglycemia. Perhaps the use of insulin as an anticatabolic agent in TPN would be able to reverse the degradation of body protein stores and allow ongoing synthesis to replete the body.

In summary, we have documented that cancer patients who were resistant to insulin with respect to glucose metabolism were not resistant to the anticatabolic effects of insulin on whole body protein turnover. This work defines a possible avenue of treatment that needs study in controlled trials to determine possible benefit for the pre- and/or postoperative catabolic cancer patient.

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