Metabolic Response to Radiation Therapy in Patients With Cancer

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The effect of radiation therapy on substrate metabolism was evaluated in five patients with head and neck or lung cancer. Stable isotope tracer methodology was used to determine urea, amino acid, glucose, and lipid kinetics during postabsorptive conditions before initation, near the midpoint (after receiving 2,672 ± 36 rads), and at completion (after receiving 6,072 ± 307 rad) of a 6- to 8-week course of radiation therapy. Nutritional status was maintained throughout the treatment period by providing supplemental enteral feedings as needed. Postabsorptive plasma insulin, catecholamine, and amino acid concentrations did not change during the course of treatment. Before radiation therapy was initiated, values for the plasma rate of appearance (Ra) of urea (3.35 ± 0.33 μmol · kg^{-1} · min^{-1}), α-ketoisocaproate ([α-KIC] 2.16 ± 0.19 μmol · kg^{-1} · min^{-1}), phenylalanine (0.59 ± 0.052 μmol · kg^{-1} · min^{-1}), and glucose (10.56 ± 1.31 μmol · kg^{-1} · min^{-1}) were in the normal range. However, glycerol and palmitate Ra values (3.11 ± 0.30 and 2.01 ± 0.33 μmol · kg^{-1} · min^{-1}, respectively) were 25% higher than values observed previously in normal subjects. Substrate flux did not change during radiation therapy, and measurements obtained during the midpoint and at completion of treatment were similar to initial values. These results demonstrate that large doses of radiation therapy, administered over 6 to 8 weeks to the upper body, do not cause significant metabolic stress.

THE PRESENCE OF cachexia in patients with cancer predicts a poor clinical outcome.[1,2] It has been hypothesized that abnormalities in substrate metabolism contribute to the development of weight loss and cachexia.[3] Indeed, numerous alterations in protein,[4,5] carbohydrate,[6,7] and lipid[8,9] metabolism have been reported in patients with cancer. Although it has been assumed that these alterations are caused by the presence of cancer itself, the potential effect of cancer therapy on substrate metabolism has not been carefully evaluated.

Radiation therapy is an important treatment modality in the management of patients with cancer. Ionizing radiation damages cellular DNA and causes cell death, thereby controlling tumor growth. Unfortunately, radiation therapy also affects normal cells and could have profound adverse systemic metabolic effects. To our knowledge, the effect of radiation therapy on whole-body protein, carbohydrate, and lipid metabolism in humans has never been studied.

The purpose of the present study was to evaluate the metabolic response to radiation therapy in patients with cancer. Only patients receiving treatment limited to the upper body were studied, to prevent confounding influences on substrate metabolism caused by gastrointestinal toxicity and alterations in nutritional status. Stable isotope tracer infusions were used to assess urea, amino acid, glucose, and lipid kinetics before, during, and after completing a full course of radiation therapy.

SUBJECTS AND METHODS

Subjects

Five patients with newly diagnosed head and neck or lung cancer participated in this study (Table 1). All patients were scheduled to receive radiation therapy to the head and neck or chest area without other concomitant cancer therapy. Patients completed a medical evaluation that included a comprehensive history and physical examination, electrocardiogram, and blood tests. No patient had a history of metabolic diseases such as diabetes, hypertension, or hyperlipidemia, and no patients were taking medications. The study was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Review Committee.

Experimental Design

Patients were admitted to the GCRC at least 72 hours before the first isotope infusion study. Patients were fed a diet containing 1.0 to 1.4 g protein/kg/d and 130% of resting energy requirements, as estimated by the Harris-Benedict equation.[10] In the morning on the fourth day, an isotope infusion study was performed (Fig 1). At 7 AM, after subjects fasted overnight (10 hours), a catheter was placed percutaneously into the antecubital vein of one arm for infusion of stable isotope tracers, and a second catheter was inserted into the contralateral dorsal hand vein, which was heated for sampling arterialized venous blood.[11] At 8 AM, a primed (84.0 μmol/kg)-constant (0.15 μmol · kg^{-1} · min^{-1}) infusion of [$^{15}N_2$]urea (Isotec, Miamisburg, OH) was started and continued for 4 hours. At 9 AM, a primed-constant infusion of [1-^{13}C]leucine (Isotec) (7.2 μmol · kg^{-1} prime; 0.12 μmol · kg^{-1} · min^{-1} constant infusion) and a primed-constant infusion of [ring-$^{13}C_6$]phenylalanine (2.0 μmol · kg^{-1} prime; 0.05 μmol · kg^{-1} · min^{-1} constant infusion) (Isotec) were started and continued for 3 hours. At 10 AM, a primed (18.7 μmol/kg)-constant (0.22 μmol · kg^{-1} · min^{-1}) infusion of [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Andover, MA) was started and continued for 2 hours. At 10:30 AM, a primed (1.5 μmol/kg)-constant (0.1 μmol · kg^{-1} · min^{-1}) infusion of [$^2H_5$]glycerol and a constant (0.04 μmol · kg^{-1} · min^{-1}) infusion of [2-$^2H_2$]palmitate (both Cambridge Isotope Laboratories) were started and maintained for 90 minutes. All isotopes were infused using calibrated syringe pumps (C.R. Bard, North Reading, MA), and the exact isotope infusion rates were subsequently calculated by measuring infusate tracer concentrations. Blood samples were taken before starting the isotope infusion to determine background substrate enrichments, and every 10 minutes during the last 30 minutes of isotope infusion to determine plasma hormone, amino acid, and substrate concentrations, and substrate kinetics. All

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Table 1. Characteristics of the Study Subjects

Patient No.	Age (yr)	Gender (M/F)	Height (cm)	Weight (kg)	Diagnosis
1	42	M	172	71.0	Squamous cell carcinoma of tongue
2	62	F	167	90.5	Adenocarcinoma of lung
3	56	F	164	42.5	Adenocarcinoma of lung
4	61	F	160	50.0	Squamous cell carcinoma of vocal cords
5	50	F	158	70.8	Adenocarcinoma of lung

blood samples were collected in chilled tubes and placed on ice. Blood was collected in heparinized tubes to determine substrate concentration and substrate kinetics, in tubes containing EDTA and Trasylol to determine insulin concentration, in tubes containing EGTA and reduced glutathione to assay catecholamines, and in heparinized tubes containing 15% sulfosalicylic acid to measure plasma amino acids. Blood for amino acid analysis was vortexed and chilled at 4°C for 10 minutes before centrifugation. All other samples were centrifuged within 15 minutes of collection and stored in either a −20°C or −70°C freezer until subsequent processing and analysis.

Radiation treatment was started within 24 hours after completing the infusion study. Treatment was administered daily, 5 consecutive days per week (Monday through Friday), until the total prescribed radiation dose was reached. Patients remained as inpatients in the GCRC during weekdays throughout the entire course of radiation therapy, but were permitted to return home on weekends, if desired. Energy intake and body weight were monitored daily. Consumption of all portions of each meal was encouraged by the nursing staff. Supplemental defined liquid formula feeding was administered orally (Ensure; Ross Laboratories, Columbus, OH) or by nasogastric feeding tube (Osmolite; Ross Laboratories) in patients who were not able to ingest at least 90% of their prescribed diet or who lost more than 2% of their body weight in 1 week. The amount of supplemental feeding was adjusted to meet the patient's total estimated needs, and to maintain constant body weight.

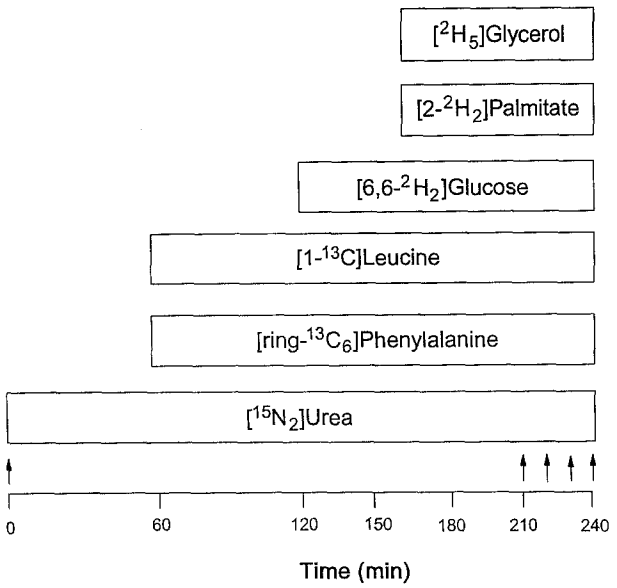

Fig 1. Schematic diagram of the isotope infusion study protocol. Arterialized blood samples were obtained before and during the last 30 minutes of isotope infusion (arrows).

The isotope infusion protocol performed on day 4 was repeated on two occasions. One study was performed near the midpoint of radiation therapy (after patients received 2,580 to 2,700 rad), before receiving treatment on that day. The subsequent study was performed in the morning after completing the entire course of radiation therapy (between 5,760 and 7,300 rad), 6 to 8 weeks after the first treatment.

Analysis of Samples

Plasma samples were analyzed for catecholamines by radioenzymatic assay,[12] and for insulin by radioimmunoassay (Incstar, Stillwater, MN).[13] Plasma glycerol and glucose concentrations were determined enzymatically with an automated analyzer (Technicon, Tarrytown, NY) using glycerol and glucose oxidase methods, respectively. Plasma free fatty acid concentrations were quantified by gas chromatography.[14] Plasma amino acid concentrations were measured by ion-exchange chromatography using an amino acid analyzer (model 6300; Beckman Instruments, Fullerton, CA).

The substrate tracer to tracee ratio (isotopic enrichment) in plasma was determined by gas chromatography–mass spectrometry using an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) with an HP-1 12-m × 0.2 mm fused silica capillary column (Hewlett-Packard). The tracer to tracee ratio of [2-$^2H_2$]palmitate was determined as described previously.[15] Plasma fatty acids were converted to their methyl esters and separated by gas chromatography. Ions at mass-to-charge ratios (*m/e*) 270.2, 271.2, and 272.2 were selectively monitored. The tracer to tracee ratios of [6,6-$^2H_2$]glucose and [$^2H_5$]glycerol were determined as described previously.[15] Plasma proteins were precipitated with barium hydroxide and zinc sulfate, and after centrifugation the supernatant was passed through a mixed cation- and anion-exchange column. Half of the sample was used to form a trimethylsilyl derivative of glycerol, and the other half a penta-acetate derivative of glucose. Glucose enrichment was determined by selectively monitoring ions at *m/e* 200.1, 201.1, and 202.1. Glycerol enrichment was determined by selectively monitoring ions at *m/e* 205.1, 206.1, 207.1, and 208.1. The tracer to tracee ratio of urea was determined as described previously.[16] Plasma proteins were precipitated with 15% sulfosalicylic acid. After centrifugation, the supernatant was passed through a cation-exchange column conditioned with 1N HCl. A bistrimethylsilyl derivative was formed, and ions at *m/e* 189.1, 190.1, and 191.1 were selectively monitored. The tracer to tracee ratio of [1-^{13}C]α-ketoisocaproate (αKIC) was determined by forming a silylquinoxalinol derivative and selectively monitoring ions at *m/e* 232.1 and 233.2 as described previously.[17] The tracer to tracee ratio of [$^{13}C_6$]phenylalanine was determined by forming an *N*-acetyl,*N*-propyl derivative and monitoring ions at *m/e* 250.1 and 256.1.[18]

Calculations

A physiological and isotopic steady state was present during the last 30 minutes of the infusion study. Substrate rate of appearance (Ra) in plasma was calculated using Steele's equation[19] for steady-state conditions, Ra ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) = (F/IE), where F is the isotope infusion rate in $\mu mol \cdot kg^{-1} \cdot min^{-1}$, and IE is the isotopic enrichment (tracer to tracer ratio) of substrate in plasma at isotopic equilibrium.

Statistical Analysis

A one-way ANOVA with repeated measures using time as a factor was used to assess differences in values between studies. A P value of not greater than .05 was considered statistically significant. All data are expressed as the mean ± SD.

RESULTS

Clinical Data

All patients completed their prescribed course of radiation therapy within 6 to 8 weeks. The complications of radiation treatment were typical and included skin changes, mucositis, and xerostomia in patients receiving radiation therapy to the head and neck area, and skin changes in patients receiving radiation therapy to the chest. Isotope infusion studies were performed before initiating radiation therapy, near the midpoint of treatment (after receiving 2,580 to 2,700 rads; mean ± SE, 2,672 ± 37), and after completing the entire course of treatment (after receiving 5,760 to 7,300 rads; mean ± SE, 6,072 ± 307).

Patients with lung cancer maintained a constant body weight throughout the entire study and were able to ingest all of their prescribed meals. However, two patients with head and neck cancer were not able to consume their entire diet because of difficulty swallowing and pain. One patient reached her dietary goals with oral supplements of a defined liquid formula (ENSURE). The second patient required nasogastric tube feedings (OSMOLITE) after the second isotope infusion protocol to maintain adequate nutrient intake. This aggressive approach of monitoring oral dietary intake and providing oral and tube-feeding supplementation was successful in maintaining constant body weights throughout the study. Mean body weights at the time of each isotope infusion study, performed before, at the midpoint, and after completing radiation therapy, were 64.9 ± 8.0, 65.0 ± 8.2, and 63.2 ± 8.4 kg, respectively.

Plasma Hormone Concentrations

Plasma insulin concentrations were similar at each isotope infusion study and were not affected by radiation therapy (Table 2). Plasma insulin levels were normal in four of five patients. However, one patient (who was also obese) had plasma insulin concentrations that were twice the upper limit of normal. Plasma epinephrine and norepinephrine concentrations also remained relatively constant at each isotope infusion study (Table 2).

Table 2. Plasma Substrate and Hormone Concentrations in Patients Before Initiation, at Midpoint, and After Completion of Radiation Therapy

Plasma Variable	Before	Midpoint	After
Glucose (mmol/L)	5.11 ± 0.09	5.01 ± 0.13	4.91 ± 0.20
Free fatty acids (μmol/L)	508 ± 66	478 ± 57	541 ± 28
Glycerol (μmol/L)	62 ± 9	67 ± 2	74 ± 4
Insulin (μU/mL)	9.6 ± 2.7	10.6 ± 4.4	9.4 ± 3.3
Epinephrine (pg/mL)	60 ± 17	37 ± 7	54 ± 15
Norepinephrine (pg/mL)	297 ± 70	208 ± 20	239 ± 64

NOTE. Values are the mean ± SE.

Plasma Amino Acid Concentrations

Plasma essential and nonessential amino acids were in the normal range before beginning radiation therapy in all patients, and did not change significantly during the course of treatment (Fig 2). Mean plasma glutamine concentration was more than twofold greater than the concentrations of other nonessential and essential amino acids.

Plasma Substrate Concentrations

Basal plasma glucose, glycerol, and free fatty acid concentrations were normal in all subjects before beginning radiation therapy (Table 2). Plasma glucose concentration was in the normal range in the patient who had basal hyperinsulinemia. Substrate concentrations were similar at the time of the second and third isotope infusion studies performed during the midpoint and after completion of radiation therapy.

Substrate Kinetics

Urea and amino acid kinetics are shown in Fig 3. Initial urea Ra (3.35 ± 0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was in the lower range of values reported in normal volunteers who were studied using the same isotope tracer methodology.[16] Urea Ra values during the second and third isotope infusion studies, performed at the midpoint and after completion of radiation therapy, respectively, were similar to values obtained during the initial study. The αKIC Ra in plasma (2.16 ± 0.19 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was in the normal range[17] before beginning radiation therapy, and did not change throughout the course of radiation treatments. Phenylalanine Ra (0.59 ± 0.052 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was also in the normal range[20] before radiation therapy, and remained relatively constant throughout the course of radiation treatments.

Glucose, glycerol, and palmitate kinetics are shown in Fig 4. Initial glucose Ra (10.56 ± 1.31 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was normal[15] in all patients and did not change during the course of radiation therapy. One patient who had elevated postabsorptive plasma insulin concentrations did not have an abnormally high glucose Ra. Initial glycerol Ra and palmitate Ra (3.11 ± 0.30 and 2.01 ± 0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively) were approximately 25% higher than values we have observed previously in postabsorptive normal subjects.[15,21-24] Measurements obtained during the second and third isotope infusion study varied by less than 20% from initial values.

DISCUSSION

Alterations in protein,[4,5] carbohydrate,[6,7] and lipid[8,9] metabolism have been reported in patients with cancer. Although it has been hypothesized that the tumor itself is responsible for these metabolic abnormalities, alterations in nutrient intake and nutritional status, concurrent medical complications, and cancer treatment may have been important contributing factors. In the present study, we evaluated the impact of radiation therapy on whole-body protein, carbohydrate, and lipid metabolism in vivo in patients with cancer. The study population was carefully

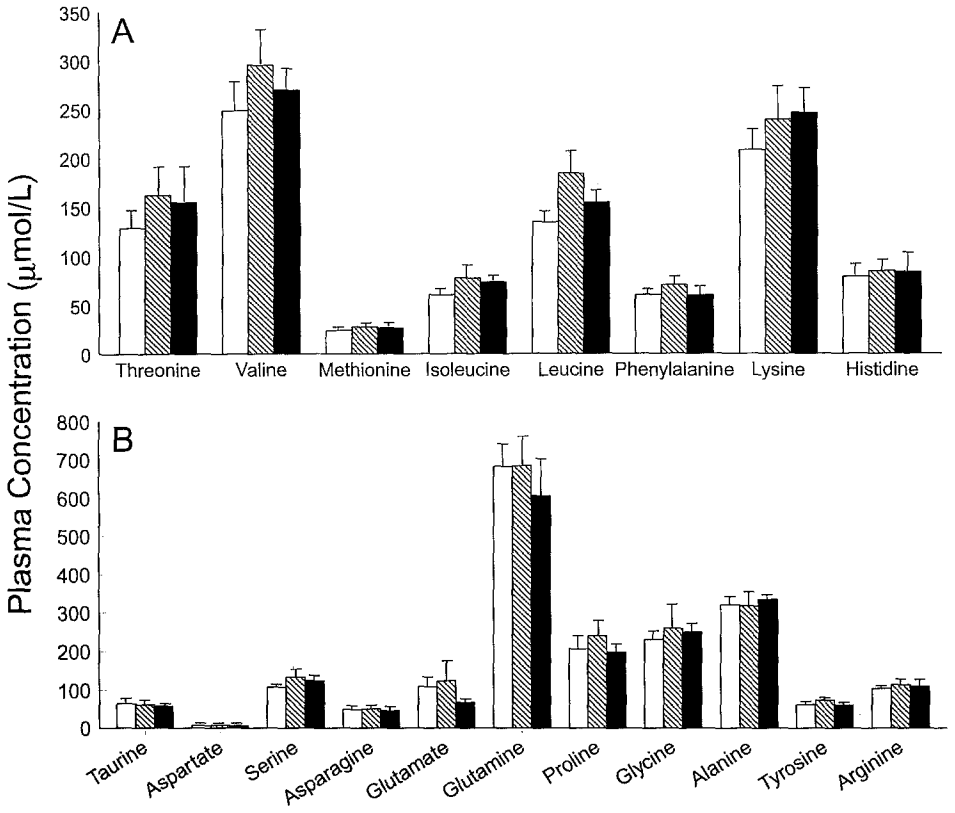

Fig 2. Plasma essential (A) and nonessential (B) amino acid concentrations (□) before beginning radiation therapy (XRT) (▨) near the midpoint of XRT (after 2,672 ± 36 rad), and (■) after completing the entire course of XRT (6,072 ± 307 rad). Values are the mean ± SE.

chosen to eliminate confounding variables that could have influenced the metabolic parameters being measured. Patients with additional medical illnesses or complications were excluded. Furthermore, nutrient intake and nutritional status were maintained throughout the course of radiation treatment by providing supplemental feedings to ensure adequate food consumption. Our data clearly demonstrate that large doses of radiation therapy, administered over 6 to 8 weeks to the upper body, do not cause measurable metabolic stress, as evaluated by plasma hormone concentrations and substrate kinetics.

The breakdown of body proteins and oxidation of released amino acids generates ammonia, which is predominantly incorporated into urea by the liver. Therefore, the rate of urea production is an indicator of net protein catabolism. During periods of severe metabolic stress, protein catabolism and urea production may increase several fold.[25] Initial urea Ra, measured before the start of radiation therapy in our patients, was in the lower range of values reported for normal subjects.[16,26] Urea Ra remained constant after 3 and 6 to 8 weeks of treatment, demonstrating that radiation therapy did not increase net protein catabolism. Protein intake was carefully controlled and kept constant before each isotope infusion study, eliminating the potential influence of dietary changes on the rate of urea production.[27]

The Ra of amino acids in plasma represents another approach for measuring protein breakdown. Two different amino acid tracers were chosen to evaluate amino acid kinetics because of differences in their metabolic fates. Leucine is metabolized principally by skeletal muscle, whereas phenylalanine is almost completely metabolized by the liver. Measuring the enrichment of αKIC in plasma during infusion of labeled leucine provides an index of intracellular leucine Ra, because plasma αKIC is derived only from intracellular leucine.[28] However, plasma αKIC enrichment is slightly higher than intracellular free-leucine enrichment, because it is not in complete equilibrium with the entire intracellular leucine pool.[29] Therefore, measuring αKIC Ra provides a lower limit for endogenous protein breakdown. The data obtained from each of the amino acid tracers in our patients were consistent with each other and with the urea kinetic measurements. The Ra values for αKIC and phenylalanine were in the normal range[20] before beginning radiation therapy, and did not change during the course of radiation treatment. Therefore, radiation therapy did not increase protein catabolism as assessed by amino acid release into plasma. In contrast, during severe metabolic stress such as infection or trauma, αKIC Ra and phenylalanine Ra are consistently increased.[30,31]

Whole-body lipid kinetics, expressed as either glycerol or fatty acid Ra, is a sensitive marker of metabolic stress, because even minimal stimulation of sympathoadrenal activity activates β-adrenergic receptors on adipose tissue and increases lipolytic rates. We have previously found that whole-body lipolytic activity increases progressively with increasing severity of illness and physiological stress.[32] In the present study, patients demonstrated higher baseline glycerol and palmitate Ra values than usually reported in normal volunteers,[15,21-24] suggesting the presence of tumor-associated alterations in metabolic stress or nutritional status.[8] However, glycerol Ra and palmitate Ra did not

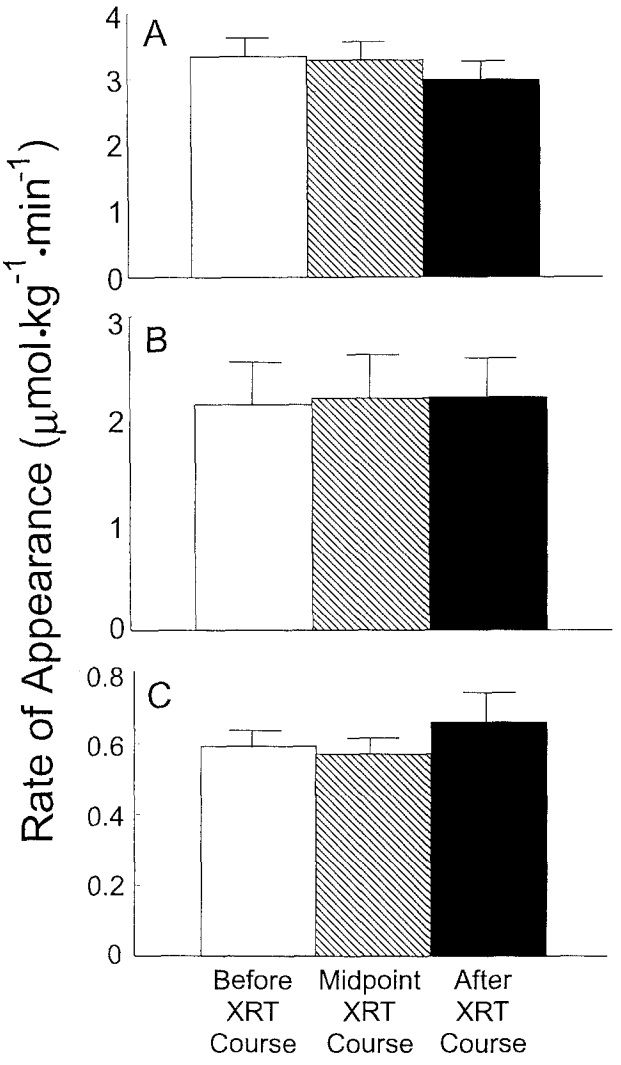

Fig 3. Urea (A), α-KIC (B), and phenylalanine (C) Ra (□) before beginning radiation therapy (XRT), (▨) near the midpoint of XRT (after 2,672 ± 36 rad), and (■) after completing the entire course of XRT (6,072 ± 307 rad). Values are the mean ± SE.

increase during the course of radiation therapy, again supporting the notion that radiation therapy itself does not cause significant metabolic stress.

Most patients who receive radiation therapy lose weight during the course of treatment. In particular, radiation therapy in patients with head and neck cancer causes marked impairment in the ability to eat and in the desire for food, because of alterations in taste, mucositis, sialitis, and xerostomia. In one study,[33] greater than 90% of patients with head and neck cancer lost weight during treatment, with an average weight loss of almost 4 kg. The experience in our patients demonstrates that careful supervision of dietary intake and aggressive enteral nutritional support can prevent weight loss in this patient population.

The rate of energy expenditure often correlates with the degree of metabolic stress.[34] Therefore, if radiation therapy causes metabolic stress, it might increase metabolic rates in patients receiving treatment. Recently, resting metabolic rate (measured by indirect calorimetry) was reported in patients with head and neck cancer at week 0, week 4, and weeks 7 to 8 of radiation therapy.[35] Consistent with our findings, which demonstrate the absence of an effect on protein, lipid, and carbohydrate metabolism, resting energy expenditure did not increase during the course of radiation treatment.

The isotope tracer methodology used in the present study evaluated whole-body protein catabolism, but did not assess protein synthesis. It is possible that radiation therapy affects nitrogen balance, without altering protein breakdown, by inhibiting protein synthesis. To our knowledge, the effect of radiation treatment on whole-body or regional protein synthesis has never been evaluated in humans. However, the effect of radiation administration on hepatic protein synthesis has been studied in adult rats.[36] Net synthesis of albumin was normal, and net synthesis of α1-acid glycoprotein and fibrinogen increased after expo-

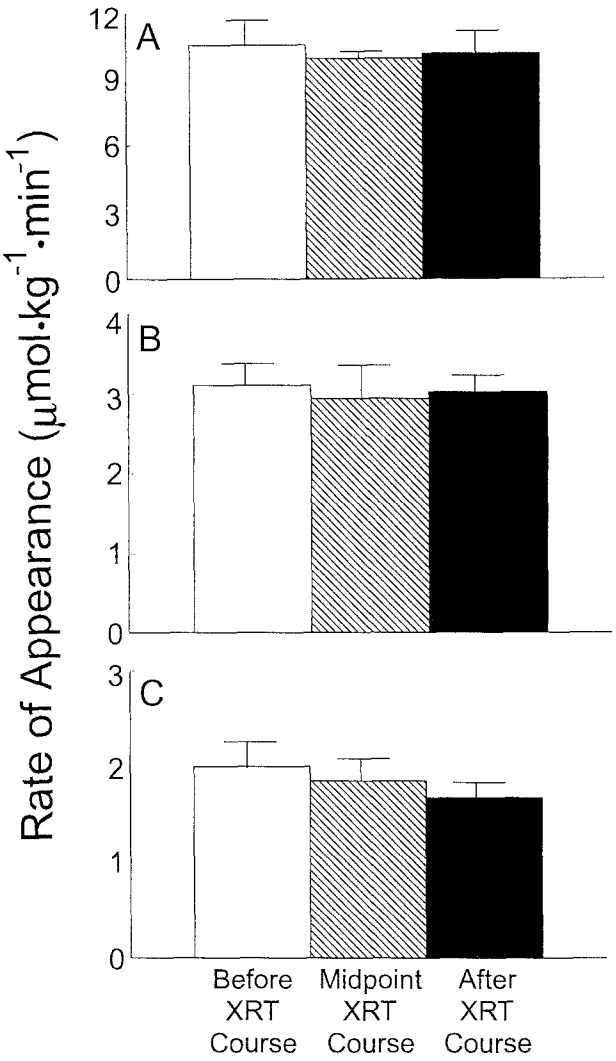

Fig 4. Glucose (A), glycerol (B), and palmitate (C) Ra (□) before beginning radiation therapy (XRT), (▨) near the midpoint of XRT (after 2,672 ± 36 rad), and (■) after completing the entire course of XRT (6,072 ± 307 rad). Values are the mean ± SE.

sure to whole-body irradiation. Therefore, it seems unlikely that radiation therapy itself would cause a negative nitrogen balance in patients with cancer, unless it affected nutrient intake.

Glutamine is the most abundant free amino acid in the body, and is the principal carrier of nitrogen from peripheral tissue to visceral organs.[37] Plasma glutamine concentrations decrease markedly after injury and during catabolic illnesses.[38,39] Furthermore, abdominal irradiation in rats causes a decline in plasma glutamine concentrations.[40] In contrast, radiation therapy to the upper body in patients in our study did not cause significant changes in plasma glutamine concentrations, which remained within the normal range throughout the course of radiation therapy. These results suggest that there may be considerable metabolic differences between abdominal/pelvic irradiation and radiation therapy directed to the upper body. Presumably, these differences are related to radiation-induced mucosal injury, characterized by destruction of crypt cells, decreased villus height, and frank ulcerations.[41]

In summary, the results of the present study demonstrate that upper-body radiation therapy causes minimal metabolic stress and does not affect whole-body protein, lipid, or glucose metabolism. Therefore, radiation therapy should not affect nutrient requirements in this patient population and recommendations for nutritional therapy should be based on standard clinical practices. However, our findings should not be extrapolated to patients receiving abdominal or pelvic irradiation, which may cause considerable damage to intestinal epithelial cells and thereby evoke a much stronger metabolic response.

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