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Nutrient Intake and Nutritional Indexes in Adults With Metastatic Cancer on a Phase I Clinical Trial of Dietary Methionine Restriction

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Abstract: *Animal studies have shown that dietary methionine restriction selectively inhibits growth of a variety of human tumor xenografts but has relatively few deleterious effects on normal tissues. The objectives of the present study were to determine whether enteral methionine restriction is safe and tolerable in adults with metastatic cancer and whether it reduces plasma methionine levels. Eight patients with a variety of metastatic solid tumors were enrolled in a phase I clinical trial. A commercially available methionine-free medical food served as the primary dietary protein source for all patients. Patients were prescribed diets containing 0.6–0.8 g of protein, 25–35 kcal, and 2 mg of methionine per kilogram per day. Participants remained on the experimental diet for an average of 17.3 wk (range 8–39 wk). Plasma methionine levels fell from 21.6 ± 7.3 to 9 ± 4 μM within 2 wk, representing a 58% decline. Serum albumin and prealbumin levels remained stable or increased. Mean energy intake increased during participation compared with baseline, and protein intake was maintained at target levels. The only side effect was weight loss of ~0.5% of body mass index (0.5 kg) per week. We conclude that enteral dietary methionine restriction is safe and tolerable in adults with metastatic solid tumors and results in significant reduction in plasma methionine levels.*

Introduction

Hundreds of thousands of Americans die each year of the most common malignancies, namely, those originating in the prostate, breast, lung, and gastrointestinal tract. Of these cancers, only lung cancer is readily preventable in the majority of cases. Surgery and radiation treatment save many lives, but far too many people develop metastatic cancer, which is often resistant to chemotherapy and, therefore, lethal. Fortunately, advances in molecular biology in recent years have led to the discovery of several promising targets for cancer treatment, one of which is the methionine dependence of tumors.

Methionine is an essential amino acid that cannot be synthesized from any of the other standard amino acids. Nonetheless, normal mammalian cells proliferate normally in the absence of methionine as long as homocysteine is present in the growth medium (1), and animals fed diets in which methionine has been replaced by homocysteine suffer no ill effects and grow normally (2,3). Homocysteine is a nonstandard amino acid that has the same structure as methionine, except it lacks the methyl group (Fig. 1). Methionine independence of normal tissues is due to remethylation of homocysteine to methionine by the enzymes 5-methyltetrahydrofolate homocysteine methyltransferase and betaine-homocysteine methyltransferase. Although these enzymes are functional in some tumors (4), most tumors are dependent on exogenous, preformed methionine and, therefore, fail to grow, even in the presence of homocysteine (5–8). Dietary methionine restriction causes regression of animal tumors, including human prostate cancer xenografts in nude mice (9,10) and inhibits metastasis in animal models (3,11). Methioninase, an enzyme that degrades methionine and homocysteine, also inhibits growth of solid tumors and leukemia in animals (12–17). One clinical trial of chemotherapy combined with short-term methionine restriction by total parenteral nutrition showed preliminary evidence of activity against gastric cancer (18). The selective antitumor activity of methionine restriction is not due to an absolute difference between benign and malignant tissues, because neither can survive for long in the complete absence of methionine. Rather, tumors are more sensitive than normal tissues to methionine restriction; just as many tumors are more sensitive to chemotherapy and radiation therapy. In contrast, restriction of other essential amino acids is either very toxic or ineffective (19). Methionine restriction, therefore, does not represent indiscriminate "starvation."

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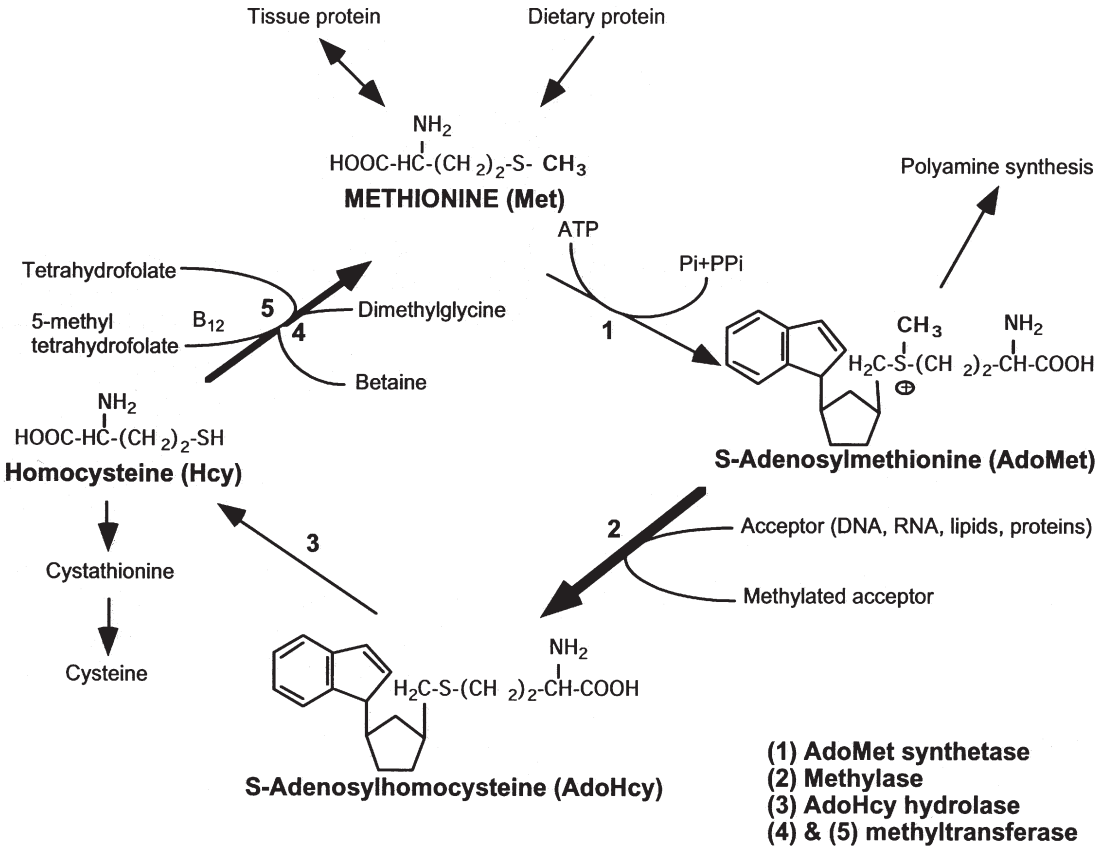

Figure 1. Overview of methionine metabolism.

In light of promising preclinical studies, we undertook a phase I clinical trial of dietary methionine restriction for adults with advanced solid tumors. All patients on the trial were maintained on an enteral diet, because parenteral nutrition is potentially toxic, expensive, and logistically difficult. The primary goals of the trial were to determine whether enteral methionine restriction for several weeks at a time is safe and tolerable and whether it reduces plasma methionine levels.

Subjects and Methods

Patients

Twelve patients at the Houston VA Medical Center with histological or cytological proof of metastatic cancer who were refractory to standard therapy or had a disease for which no standard therapy existed were entered into the study between March 1999 and November 2000. Two patients were taken off the study after only 2 wk because of rapidly progressive cancer and rapid decline in performance status. Two other patients elected to discontinue the study after <1 mo, even though they felt well and remained clinically stable. Data from the remaining eight patients were analyzed. Characteristics of the patients are shown in Table 1. They were on the experimental diet for an average of 17.3 wk (range 8–39 wk). All patients had progressive disease at the time of enrollment. Progressive disease was defined as progressive growth of bidimensionally measurable soft tissue disease or new bone scan lesions. The patient with prostate cancer had a rising prostate-specific antigen on two successive determinations ≥ 1 mo apart and failed an adequate trial of hormonal therapy, which consisted of luteinizing hormone-releasing hormone agonist plus antiandrogen with serum testosterone <50 ng/dl. The patient with prostate cancer remained on the luteinizing hormone-releasing hormone agonist throughout participation and was taken off antiandrogen >2 mo before enrollment. Eligibility require-

Table 1. Patient Characteristics

Diagnosis	Zubrod Performance Status[a]	Time on Trial, wk
Renal cell carcinoma	0	39
Carcinoid	1	14
Sarcoma	2	8
Pancreatic adenocarcinoma	2	8
Renal cell carcinoma	2	16
Prostate adenocarcinoma	2	12
Follicular lymphoma	0	16
Renal cell carcinoma	1	16

a: 0, normal activity, asymptomatic; 1, symptomatic, fully ambulatory; 2, symptomatic; in bed <50% of time.

ments included an estimated life expectancy of ≥12 wk, Zubrod performance status of 0–2, and age >18 yr. Patients received no chemotherapy or radiation therapy for ≥6 wk before study entry. Baseline laboratory parameters included a neutrophil count >1,500/mm^3, platelet count >100,000/mm^3, hemoglobin >9 g/dl, bilirubin ≤1.5 mg/dl, serum aspartate aminotransferase and serum alanine aminotransferase <2.5 times normal, and serum creatinine ≤2.0 mg/dl. Patients with a history of significant cardiac disease, metabolic disorder, infection, or brain metastases were excluded. Only the patient with pancreatic adenocarcinoma experienced weight loss, totaling 15 kg, during the year before enrollment. Written informed consent was obtained from all patients. The study was reviewed and approved by the institutional review board and the General Clinical Research Center at Baylor College of Medicine and the Research and Development Committee at the Houston VA Medical Center.

Patient Monitoring

Before therapy, all patients had a complete history and physical examination. Pretreatment laboratory evaluation parameters included plasma amino acid profile, total plasma homocysteine, complete blood count, platelet count, sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, protein, albumin, phosphorus, uric acid, serum aspartate aminotransferase, serum alanine aminotransferase, bilirubin, lactate dehydrogenase, alkaline phosphatase, lipoprotein panel, prothrombin time, and partial thromboplastin time. The history, physical examination, serum chemistries, and blood counts were repeated every 2 wk. Calcium and lipoprotein panel were repeated every 8 wk. Participants were monitored for side effects according to common toxicity criteria of the National Cancer Institute.

Measurement of Plasma Amino Acids

Plasma amino acid determinations were performed in the Biochemical Genetics Laboratory at Baylor College of Medicine using a Dionix high-performance liquid chromatograph with postcolumn ninhydrin detection. This method involved mixing 110 μl of plasma with 110 μl of Seraprep (Pickering Labs, Mountain View, CA) plus norleucine standard. Samples were then vortexed and allowed to sit for 1 min. Pickering dilution buffer (110 μl), pH 2.2, was then added to the mixture, which was vortexed and centrifuged at 5,000 *g* for 5 min. The supernatant was then placed in a Millipore ultrafree-MC filter (0.45 μm) and centrifuged for 2 min. The resulting protein-free ultrafiltrate was then placed in sample vials in the automatic sample injector. At pH 2.2, the injected sample amino acids were loosely adsorbed to the ion-exchange resin (sulfonated divinylbenzene cross-linked polystrene beads). Depending on their dissociation characteristics, the amino acids were differentially eluted from the column with a series of lithium buffers of increasing pH and ionic strength. Ninhydrin mixed continuously with the column eluent and reacted in a highly specific manner with the separated amines to form colored products, the intensity of which was proportional to the concentration of the amino acid present. An in-line colorimeter measured the absorbance of the amino acid-ninhydrin complex at two wavelengths: 570 nm for primary amino groups and 440 nm for secondary amino groups. A two-channel recorder monitored the colorimeter and provided a record of the primary and secondary amino acid profiles. Amino acids were identified, and concentrations were calculated by a data system that determined the retention times and areas of known concentrations determined previously from calibration standards. Total plasma homocysteine was determined by electrospray tandem mass spectroscopy as previously described (20). Plasma amino acid profile and total plasma homocysteine levels were measured twice per week for the first 2 wk and every other week thereafter.

Development of the Methionine-Restricted Diet

The protocol for implementing the dietary methionine restriction was modified over the course of the study to develop a dietary pattern that could best be used by free-living cancer patients. All patients were placed on Hominex-2 Amino Acid-Modified Medical Food (Ross Products Division, Abbott Laboratories, Columbus, OH), which is approved for treatment of patients with homocystinuria (Table 2). Hominex-2 contains essentially no methionine (Table 3). The quantity of Hominex-2 consumed daily by each patient was calculated to provide 0.6–0.8 g protein/kg body wt. Hominex-2 dose and energy intake were maintained at baseline levels throughout participation, rather than reduced as patients lost weight. Hominex-2 served as the primary dietary protein source for all patients.

The first four patients were placed on a modular diet designed to provide 2 mg of methionine and ~25 kcal per kilogram per day. The diet consisted of low-protein cereals, grains, and breads; fruits; vegetables; margarines and oils; and simple carbohydrates (sodas, hard candies, sugars, and drink mixes). During this period, Hominex-2 was mixed with water and/or low-protein broth in an effort to maximize the amount of regular food that patients could consume. However, over the course of treatment of these initial patients, it became apparent that all were struggling to consume sufficient quantities of food to meet energy needs. The initial patients consistently complained of early satiety and lack of appetite. Commercially available low-protein foods proved difficult for patients to obtain and were not palatable.

The protocol was therefore modified so that Hominex-2 served as the primary source of protein and energy. Hominex-2 mixtures were modified using powdered, citrus-flavored drink mixes, protein-free bouillon, tomato juice, sucrose, and canola oil. These Hominex-2 "shakes" were formulated on the basis of the energy requirements of each patient. By consuming four to five shakes per day, patients met 100% of their protein requirements (0.8 g protein/kg) and ~75% of their energy requirements. Regular food was then used to provide up to 2 mg methionine/kg/day as well

Table 2. Nutrient Composition of Hominex-2 Amino Acid-Modified Medical Food[a]

Nutrients	Amount per 100 g Powder
Protein equivalent, g	30.0
Fat, g	15.5
Carbohydrate, g	30.0
Water, g	1.0
Calories, g	410
Vitamins	
Vitamin A, μg RE	660
Vitamin D, μg	9.88
Vitamin E, mg α-TE	12.1
Vitamin K, μg	60
Thiamine, mg	4.0
Riboflavin, mg	1.8
Vitamin B-6, mg	1.3
Vitamin B-12, μg	9.5
Niacin, mg	26
Folic acid, μg	430
Pantothenic acid, mg	14
Biotin, μg	130
Vitamin C, mg	60
Choline, mg	100
Inositol, mg	70
Minerals	
Calcium, mg	880
Phosphorus, mg	880
Magnesium, mg	225
Iron, mg	13
Zinc, mg	13
Manganese, mg	0.55
Copper, mg	1.0
Iodine, μg	100
Selenium, μg	27
Sodium, mg	880
Potassium, mg	1,370
Chloride, mg	1,160

a: Abbreviations are as follows: RE, retinol equivalents; α-TE, α-tocopherol equivalents.

Table 3. Amino Acid Content of Hominex-2 Amino Acid-Modified Medical Food

Amino Acid	Amount, g/100 g Powder
Alanine	2.98
Arginine	2.08
Aspartic acid	1.53
Carnitine	0.04
Cystine	0.90
Glutamic acid	2.25
Glycine	3.35
Histidine	0.84
Isoleucine	2.16
Leucine	3.36
Lysine	2.00
Methionine	Trace
Phenylalanine	1.76
Proline	2.88
Serine	1.50
Taurine	0.06
Threonine	1.40
Tryptophan	0.34
Tyrosine	1.78
Valine	2.44

as the remaining energy needs. After the reformulation of the beverage, patients were able to consume up to 35 kcal/kg/day.

Methionine is present in most foods as an integral component of dietary protein. After the reformulation of Hominex-2 beverages, dietary methionine exchange lists were developed that allowed patients to select and consume a variety of foods up to their targeted dietary methionine level. Patients could choose small portions of dietary starches (e.g., cereals, potatoes, breads, crackers, canned soups, cookies) and ample portions of fruits and vegetables. Use of protein-free beverages, candies, ices, margarines, and cooking oils served to boost energy intake into target ranges. Patients were counseled not to eat any foods containing animal protein, which is rich in methionine.

Nutritional Intervention and Monitoring

Patients were seen by the study dietitian before initiation of the study. At this session, the dietary regimen was reviewed, and patients were instructed on methods for keeping food records. Patients kept a food record for 1 wk before initiation of the study and weekly thereafter. On initiation of the study, patients were instructed on how to prepare Hominex-2 beverages, given hands-on demonstrations of product preparation, and allowed to sample a variety of beverage flavors. Patients were required to give a return demonstration on use of food scales and product mixing to verify their understanding.

Patients continued to meet with the dietitian every 1–2 wk for the duration of the study. At these sessions, food records were reviewed to enhance accuracy. In addition, patients were weighed, and dietary problems were discussed. Food records from up to 16 wk were analyzed with First Databank Nutritionist Five software.

Results

Macronutrient Intake

Mean total energy intake during the week before initiation of the experimental diet was 23.3 ± 3.3 (SE) kcal/kg/day (n = 8) and increased during the period of methionine restriction (Fig. 2, top left). Mean total protein intake was 0.89 ± 0.24 g/kg/day (n = 8) at baseline and was maintained just below the recommended dietary allowance (RDA) of 0.8 g/kg/day throughout the study (Fig. 2, top right). However, energy and protein intakes were higher for Patients 5–8, who entered after refinements were made in the dietary protocol, than for Patients 1–4 (Fig. 2, middle). Methionine intake fell from a mean of 20.8 ± 5.6 (n = 8) to 2.1 ± 0.74 mg/kg/day (n = 8) on implementation of the experimental diet and remained near 2 mg/kg/day, as intended (Fig. 2, bottom).

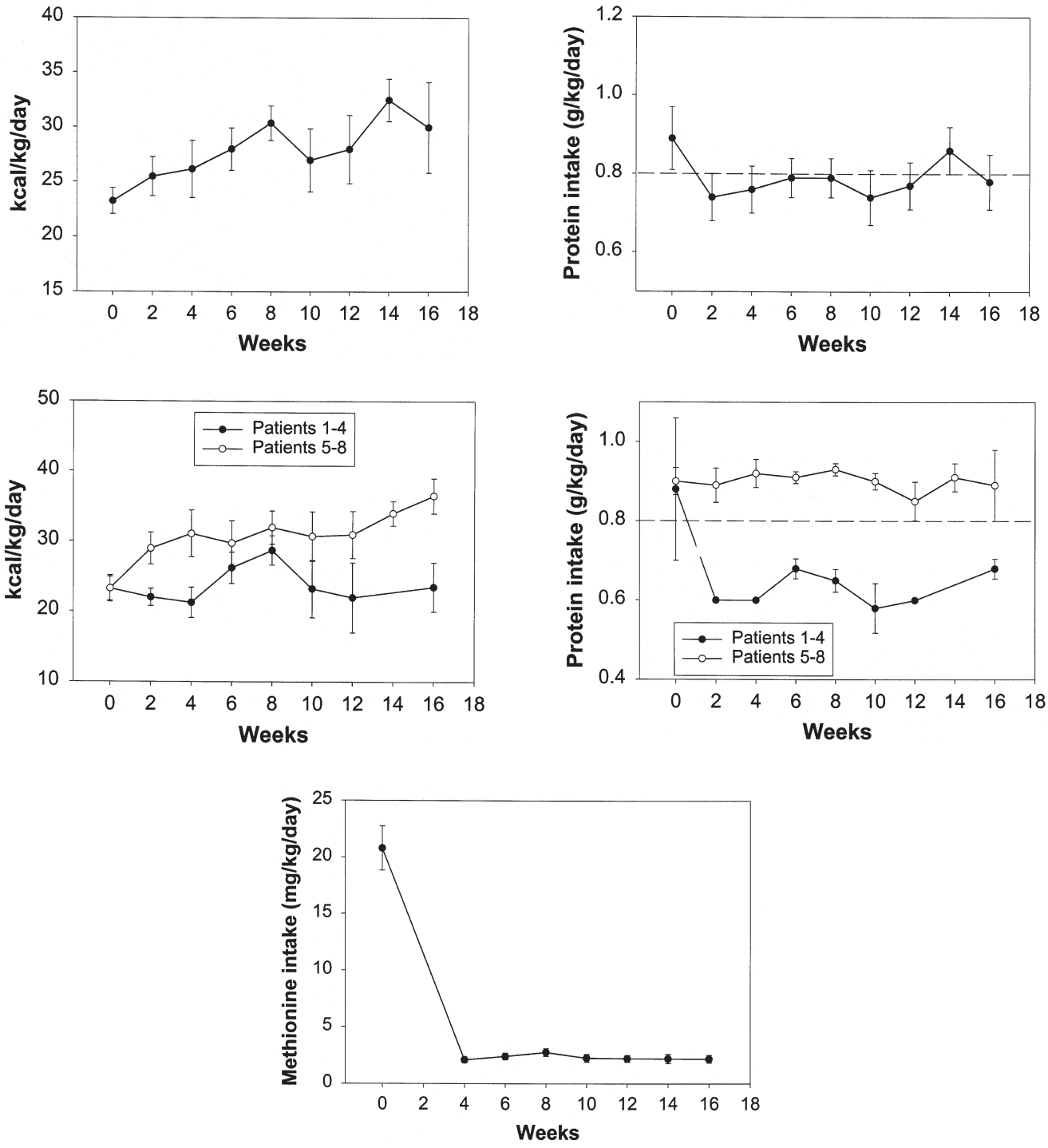

Figure 2. Nutrient intake for patients with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Error bars, SE. Top left: daily energy intake. Top right: daily total protein intake. Middle left: daily energy intake for Patients 1–4 vs. Patients 5–8. Energy intakes for the 2 groups were significantly different at Week 2 ($P = 0.035$) and Week 4 ($P = 0.047$) but were not significantly different at other time points. Only 2 patients remained in each group at Week 16 (see Table 1). Middle right: daily total protein intake for Patients 1–4 vs. Patients 5–8. Error bars are smaller than corresponding symbols for Weeks 2 and 4 (Patients 1–4) and are therefore not visible. Week 12 (Patients 1–4) represents a single patient. Protein intakes for the 2 groups were significantly different at each time point except Weeks 0, 14, and 16. $P < 0.001$ for Weeks 2–8, $P = 0.002$ at Week 10, $P = 0.029$ at Week 12. Bottom: daily methionine intake. Dashed horizontal lines, recommended dietary allowance for total protein intake (0.8 g/kg/day).

Table 4. Dietary Consumption of Lipids, Vitamins, and Minerals at Baseline and During Week 8[a]

	Week 0	Week 8	P^b
Saturated fatty acids, g	25.6 ± 8.4	28.6 ± 9.5	0.315
Monounsaturated fatty acids, g	27.9 ± 8.9	38.9 ± 21.1	0.103
Polyunsaturated fatty acids, g	15.3 ± 6.2	24.5 ± 13.4	0.041
Cholesterol, mg	343.5 ± 272.8	15.1 ± 27.6	0.008
Vitamin A, μg RE	1,257 ± 931	2,007 ± 888	0.058
Vitamin C, mg	61.2 ± 73.6	300.3 ± 165.7	0.009
Vitamin E, mg α-TE	7.4 ± 3.7	35.6 ± 14.4	0.001
Folate, μg	301 ± 170	880 ± 307	0.001
B-12, μg	5.5 ± 2.7	16.7 ± 6.6	0.006
Zinc, mg	12.0 ± 3.3	25.1 ± 9.7	0.009
Copper, mg	1.21 ± 0.7	2.54 ± 0.67	0.006
Selenium, mg	0.1 ± 0.03	0.07 ± 0.02	0.048

a: Values are means ± SD for all 8 patients.
b: 2-tailed, paired Student's *t*-test.

Micronutrient Intake

Consumption of lipids, vitamins, and minerals at baseline and during Week 8 is shown in Table 4. Consumption of polyunsaturated fats, vitamins C and E, folate, vitamin B-12, zinc, and copper increased significantly ($P < 0.05$) during participation. Cholesterol and selenium intakes declined significantly, whereas consumption of monounsaturated fats, saturated fats, and vitamin A did not change significantly. Average selenium intake during Week 8 still exceeded the RDA (50 μg/day), despite the fact that it declined in patients on the experimental diet compared with baseline.

Plasma Amino Acid Levels

Mean plasma methionine levels decreased from 21.6 to 9 μM within 2 wk, representing a 58% decline (Fig. 3, top). Plasma methionine levels fluctuated over time but clearly trended downward throughout the study (Fig. 3, middle). We also measured total plasma homocysteine levels, since methionine demethylation yields *S*-adenosylhomocysteine, which can ultimately be remethylated to methionine or converted to cysteine via transulfuration (Fig. 1). Total plasma homocysteine levels trended downward slightly but fluctuated to a greater extent than methionine levels (Fig. 3, bottom). Levels of other amino acids were not affected significantly by methionine restriction (not shown).

Nutritional Indexes

All patients lost ~0.5% of baseline BMI (0.5 kg) per week at a constant rate throughout the study (Fig. 4, top), regardless of whether they were on the original dietary protocol (Patients 1–4, see **Subjects and Methods**) or the refined dietary protocol (Patients 5–8; Fig. 4, bottom). The diet did not significantly affect serum albumin levels (Fig. 5, top). Serum prealbumin levels were measured for only two of the patients. Prealbumin declined initially in one of the two patients but, thereafter, remained stable and in the normal range, whereas it increased in the other patient over time (Fig. 5, bottom).

Discussion

The present study is the first to show that methionine restriction via an enteral diet for several weeks is safe and feasible in adults with advanced solid tumors. This study lays the groundwork for future cancer treatment trials involving methionine restriction alone or in combination with other modalities, such as methioninase or chemotherapy. If possible, future trials will include a control group consisting of patients fed defined diets identical to the experimental diet but containing normal amounts of methionine.

Weight loss was the only side effect of the diet, and all but one patient regained weight on resumption of a normal diet. The only patient who failed to regain weight after discontinuing the study had cancer cachexia related to pancreatic adenocarcinoma even before his enrollment. Plasma methionine levels and food records indicated that patients adhered to the diet. The high level of compliance displayed by our patients was probably a reflection of their high degree of motivation. We hypothesize that most cancer patients will be similarly motivated if methionine restriction ultimately proves to be effective. Future studies are required to determine whether dietary methionine restriction affects quality of life, although no obvious detrimental effects were observed in the present study.

After observing weight loss in Patients 1–4, we refined the diet to provide increased energy and protein intake. Nonetheless, Patients 5–8, who maintained energy intakes considerably above baseline and protein intakes above the RDA, still lost weight at the same rate as Patients 1–4. One possible explanation for this observation is that 35 kcal/kg/day, which was consumed by Patients 5–8, was still inadequate to maintain positive nitrogen balance. This possibility is supported by early studies showing that energy requirements are considerably higher for patients whose sole nitrogen source consists of purified amino acids than for those

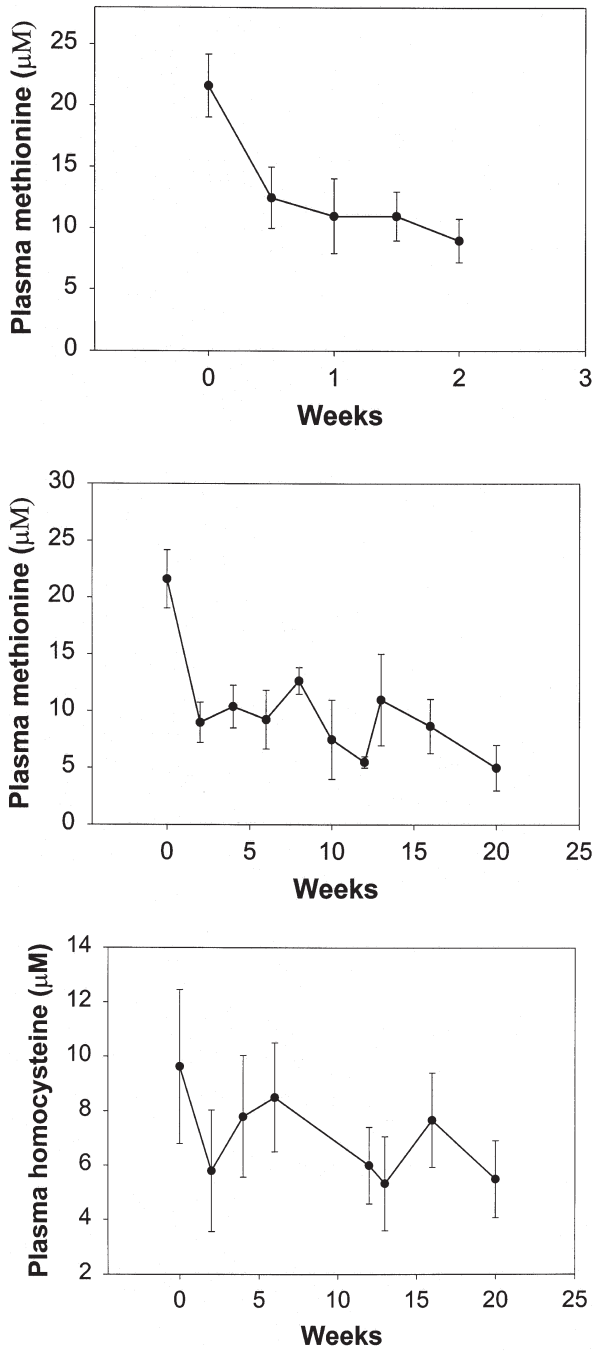

Figure 3. Plasma amino acid levels for patients with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Top: plasma methionine levels during Weeks 1 and 2. Level at Week 2 was significantly different ($P = 0.005$) from baseline; levels at other time points were not.

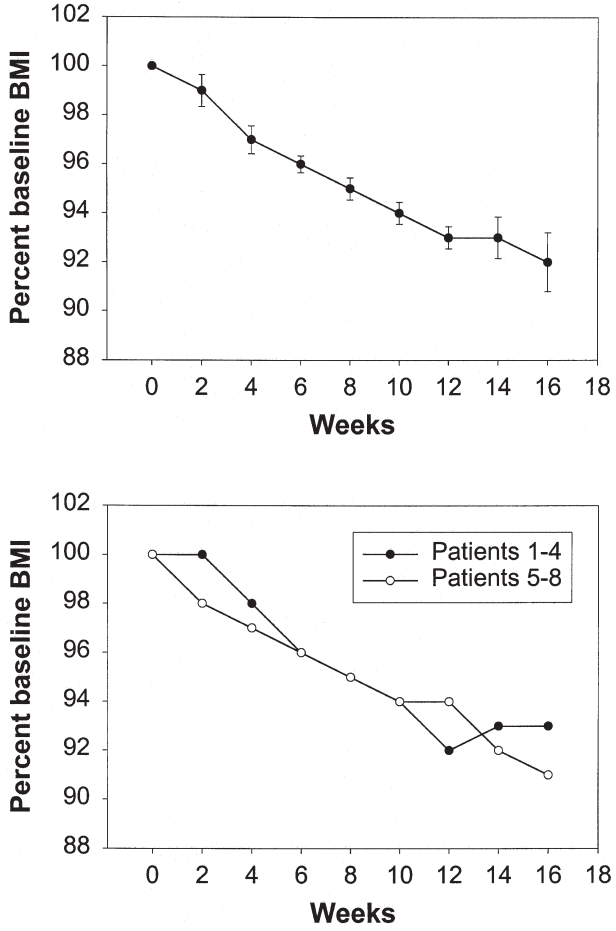

Figure 4. Percent change in body mass index (BMI) for patients with metastatic cancer on a phase I clinical trial of dietary methionine restriction. BMI at each time point except Week 2 was significantly different ($P < 0.001$) from baseline. Top: all patients. Error bars, SE. Bottom: Patients 1–4 vs. Patients 5–8.

who consume intact proteins (21). Alternatively, weight loss experienced by patients in the trial may have been independent of energy intake but, rather, attributable to "obligatory" muscle catabolism related to methionine restriction per se. A recent study designed to quantify dietary methionine requirements in normal subjects sheds light on this issue (22). In that study, stable isotope methods were used to measure obligatory methionine oxidation in normal subjects on a diet completely devoid of sulfur amino acids (methionine and cysteine) for 5 days. Although somewhat controversial (23), obligatory oxidation rates are considered by many to represent the minimum requirement for amino acids, that is, the amount that is oxidized despite maximal body conservation. The obligatory oxidative loss of methionine was 13 mg/kg/day in that study (22). Patients in our trial, who were restricted to 2 mg methionine/kg/day, therefore, consumed 11 mg/kg/day less than the minimum requirement. However, they consumed adequate amounts of cysteine, which is present in Hominex-2. They therefore may have had obligatory methionine oxidation rates <13 mg/kg/day. The fact that all patients reversibly lost weight, despite what would normally be considered adequate energy and protein intake, may actually be encouraging, since it confirms that patients adhered to the diet. The basic premise of this strategy is that dietary methionine restriction will have a greater deleterious effect on tumors than on normal host tissues.

Despite the fact that all patients in this trial lost weight, their serum albumin and prealbumin levels remained stable or increased. Dietary methionine restriction therefore apparently did not cause indiscriminate starvation. Although the

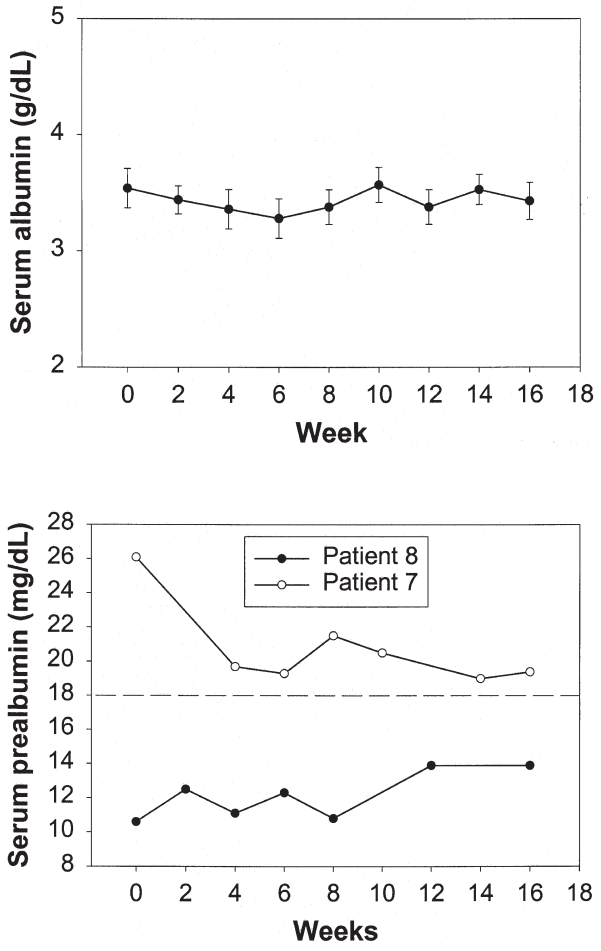

Figure 5. Top: serum albumin levels for patients with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Error bars, SE. Bottom: serum prealbumin levels for Patients 7 and 8. Dashed horizontal line, lower limit of normal for serum prealbumin (18 mg/dl).

mechanisms for the antitumor activity of methionine restriction in animals have not been established, we hypothesize that they may relate to the specialized functions of methionine. This possibility is supported by studies showing that dietary restriction of any of the other essential amino acids has no antitumor effect or results in life-threatening toxicity in tumor-bearing animals (19). Methionine functions as a methyl donor for methylation of DNA and other molecules and as an aminopropyl donor for polyamine synthesis. DNA methylation is known to transcriptionally silence several growth inhibitory genes in tumors (24), and polyamines have far-ranging effects on nuclear structure and cell division (25). Polyamines are more abundant in tumors than in corresponding normal tissues. The antitumor activity of methionine restriction may therefore have little to do with its role in protein synthesis, although future studies are required to clarify this point.

The secondary goal of the present trial was to identify preliminary evidence of antitumor activity. Although preliminary results look promising, it is too early to tell whether methionine restriction has antitumor activity in adults with metastatic cancer and, if so, which tumor types are most sensitive. Many clinical trials will be required to determine whether methionine restriction will be effective alone or in combination with chemotherapy, gene therapy, or other modalities. However, it is clear that enteral methionine restriction for at least several weeks at a time is safe and achievable in patients with metastatic cancer.

Acknowledgments and Notes

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