Annu. Rev. Nutr. 1990. 10:107–32

AMINO ACID METABOLISM IN HUMAN CANCER CACHEXIA

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KEY WORDS: amino acid kinetics, amino acid concentrations

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INTRODUCTION

"Cachexia—a depraved condition of the body in which nutrition is everywhere defective" (96). This description of the clinical condition termed *cachexia* appeared in a biennial review of medicine and allied sciences more than 100 years ago. The word is derived from the Greek words *kakos* (bad)

0199-9885/90/0715-0107$02.00

and *hexis* (condition) and is a general term used for any condition that includes progressive loss and wasting of host tissue.

Cancer cachexia is a complex syndrome present to a variable degree in many cancer patients at some point in their disease (60). Although a variety of physical and biochemical changes accompany cancer cachexia, the syndrome is characterized primarily by progressive host weight loss and diminished nutrient intake (Table 1). Despite these known clinical features, the variable expression of the syndrome across the range of malignancies and within patients harboring pathologically identical neoplasms is important to emphasize. The broad clinical spectrum of patients ranges from otherwise healthy patients who present with weight loss secondary to an unappreciated malignancy to advanced terminal cancer patients in whom the metabolic sequelae of cancer cachexia and starvation coexist to produce all of the clinical features outlined in Table 1.

The prevalence of cancer cachexia depends on the type of malignancy and the sensitivity of the metabolic-nutritional assessment (18, 19, 112). Several studies indicate that weight loss and protein-calorie malnutrition may be present in 50–80% of the general cancer population (13, 23, 85, 108). DeWys et al (32) analyzed the incidence and prognostic effect of weight loss prior to the commencement of chemotherapy in more than 3000 patients followed by the Eastern Cooperative Oncology Group. More than 50% of these patients had lost weight prior to treatment. In 9 of the 12 tumor types studied, stage for stage, median survival in patients with weight loss was significantly shorter than in patients without weight loss. Based on this report and others (41, 85), weight loss seems a clear prognostic indicator of survival time in a variety of malignancies. Moreover, this progressive loss of host body cell mass and associated weakness may be the actual cause of death in up to two thirds of cancer patients (52, 62, 123).

Most studies examining the prevalence of cancer cachexia in patients with malignant disease have, by necessity, used rather insensitive anthropometric

Table 1 Clinical features of cancer cachexia

Symptoms	Physical findings	Laboratory findings
Weakness	Weight loss	Anemia
Fatigue	Skeletal muscle atrophy	Hypoalbuminemia
Malaise	Adipose tissue loss	Glucose intolerance
Anorexia	Myopathy	Deficiency states
		Vitamins
		Minerals
		Electrolytes
		Anergy

and biochemical screening techniques. These techniques, while facile and convenient, are not sensitive indicators of early metabolic disturbance, particularly when applied to patients without weight loss. More sophisticated techniques allow demonstration of early metabolic disturbances in carbohydrate metabolism (28) and amino acid metabolism (56) before the onset of any of the classic clinical features of cancer cachexia.

The alterations in amino acid and protein metabolism in the cancer-bearing host are more easily understood as part of the overall disturbances in host intermediary metabolism. Fundamental disturbances include accelerated rates of gluconeogenesis (73, 104, 127, 128) and hepatic protein synthesis (38, 58, 59, 71, 109, 121, 124, 125). Most likely, a significant number of the abnormalities documented in peripheral amino acid and protein metabolism are best understood in relation to these fundamental changes in the liver. This review concentrates on the techniques used to evaluate amino acid metabolism in human cancer cachexia and the knowledge gained from such work.

TECHNIQUES TO EVALUATE PROTEIN AND AMINO ACID METABOLISM IN HUMANS

A variety of clinical and research techniques are available to study protein and amino acid metabolism in humans (76). As indicated in Table 2, the primarily clinical techniques range from history and physical examination to anthropometry and nitrogen balance; they generally do not provide enough precise information for more than a descriptive, overall assessment of protein and amino acid metabolism. The primarily research techniques by nature are not applicable to nutritional assessment of numerous subjects but provide quantitative information on specific aspects of protein and amino acid metabolism. This review focuses primarily on techniques used to assess amino acid metabolism in normal and cancer-bearing humans.

Nitrogen-Balance Studies

Nitrogen-balance studies are common starting points in evaluating the nutritional effects of disease states that cause weight loss. These studies evaluate net or overall changes and hence provide little insight into the mechanisms responsible for observed changes from normal. The considerable limitations of these rather cumbersome balance studies are reviewed elsewhere (61). They do, however, provide some information on net nitrogen metabolism, but the resultant data are only weakly correlated with more direct isotopic studies of protein turnover (49). Using [^{15}N]glycine infusion in children recovering from protein-calorie malnutrition, Golden et al (49) demonstrated that nitrogen balance bears no relationship to protein catabolic rates and is weakly

Table 2 Techniques to evaluate protein and amino acid metabolism in humans

	Technique	Parameter(s)
Primarily clinical	History	Weakness, fatigue, malaise
	Physical exam	Weight loss, muscle atrophy
	Biochemical screening	Albumin, total protein, prealbumin, transferrin, r binding protein, CRP[a]
	Urinary creatinine	Muscle mass
	Anthropometrics	Triceps skin-fold thickness, midarm muscle circu ence
	Nitrogen balance	Whole-body nitrogen balance
Primarily research	^{40}K	Body composition (lean body mass)
	Isotope dilution (D_2O, $H_2{}^{18}O$)	Body composition (total body water)
	Neutron activation, gamma analysis	Body composition (total body nitrogen)
	3-methylhistidine	24-hr urinary 3-methylhistidine, 3-methylhistine/ creatinine ratio
	Muscle biopsy	Muscle composition, intracellular AA concentratior zyme levels, etc.
	Free AA concentrations	Whole blood, plasma AA concentrations
	Organ/tissue AA balance	Regional AA uptake and release (interorgan AA f
	AA kinetics	Whole-body and regional protein synthesis, degra

[a] Abbreviations: CRP, C-reactive protein; AA, amino acid.

correlated with protein synthetic rates. As such, nitrogen-balance studies may be most useful as a relatively simple clinical tool for evaluating net nitrogen metabolism in groups of patients or in individual patients over time.

3-Methylhistidine Excretion

3-Methylhistidine (3MH) is formed when certain histidine residues in actin and myosin undergo posttranslational methylation. When 3MH is released via protein breakdown, it cannot be reutilized in protein synthesis, since 3MH has no specific amino-acyl tRNA (137). In addition, 3MH is not metabolized to a significant extent; consequently its only metabolic fate is excretion into the urine (138). Thus, investigators (67, 137) have proposed that for subjects on a meat-free diet, the 24-hour urinary excretion of 3MH could be considered to provide an index of skeletal myofibrillar protein breakdown. This technique has in fact been utilized in several clinical studies evaluating skeletal muscle catabolism in disease states (50, 63, 66, 68, 105, 107). Considerable controversy has surrounded the method in the past decade (11, 102). Studies have demonstrated that the urinary excretion of 3MH is not a tissue-specific index

of protein catabolism; data from rat studies suggest that 3MH from splanchnic tissues comprises 20–40% of the total urinary 3MH (78, 126).

Further studies in humans by Rennie et al (99) demonstrated that the increase in urinary 3MH excretion after major operative trauma in humans was not due to increased peripheral release of 3MH as measured by femoral arteriovenous 3MH differences. These authors assumed that the increased urinary 3MH was secondary to increased turnover of the small splanchnic and visceral protein pools. In contrast, Sjolin et al (106) recently measured peripheral and splanchnic 3MH balance in septic patients and estimated that the splanchnic contribution to urinary 3MH excretion in catabolic humans was 8% or less. The relative contribution of the periphery (leg) to urinary 3MH excretion was increased in these septic catabolic patients, directly contradicting the data of Rennie et al (99). Further studies are required to evaluate the metabolism of 3MH in normal and catabolic people. Despite the present controversy, the technique does provide a relatively simple noninvasive measure of primarily muscle protein catabolism for clinical practice. Its utility as a research tool appears limited since its tissue specificity has not been defined in humans, it does not allow evaluation of protein synthesis, and it cannot be used to evaluate factors that regulate protein turnover acutely.

Plasma Amino Acid Concentrations

One of the simplest techniques for investigating amino acid and protein metabolism in altered nutritional states is to measure plasma free amino acid levels. In a 70-kg person, free amino acids represent only approximately 0.5% of the total body amino acid pool (132). This small fraction, however, is the most metabolically active nitrogen in the body, as it is the form in which interorgan amino acid transport occurs. These free amino acids serve as substrates for protein synthesis, gluconeogenesis, ureagenesis, and oxidative catabolism—metabolic processes that may be significantly altered in disease states.

Kinetic studies in normal humans have established that 2–3% of the total body protein pool turns over daily (75, 84, 115, 130). Despite this relatively extensive movement of amino acids between tissues and the plasma compartment, plasma amino acid levels remain stable. As recently reviewed (1), changes in plasma amino acid levels represent the net effect of all factors influencing total body amino acid flux. These factors include influx of dietary proteins from the gut, tissue uptake and efflux, and protein synthesis and degradation. These factors are themselves regulated by a variety of other factors including age (10), sex (9, 93), antecedent diet (79), and the hormonal milieu (8, 44, 64, 103, 135). As such, concentrations of circulating amino acids do not provide specific information on any aspect of nitrogen metab-

olism but can provide comparative information relative to other conditions such as acute and chronic starvation, hepatic failure, and renal failure.

Regional Amino Acid Balance

Utilizing arterial and selective venous catheterization with measurement of regional blood flow, simple measurement of plasma amino acid concentrations can be extended to provide information on tissue or organ amino acid balance (3, 26). The Fick principle can be applied, with knowledge of the regional blood flow and arteriovenous amino acid differences across a tissue or organ bed to evaluate tissue and/or organ amino acid uptake or release. This technique has been used in humans to evaluate amino acid balance across the kidney (42, 91), skeletal muscle (2, 5, 7, 97), and splanchnic bed (6, 17, 46, 120).

In animals, the technique has been extended with selective catheterization of the portal vein, hepatic vein, renal vein, an extremity vein draining a major muscle bed, and an artery to permit simultaneous evaluation of hepatic, extrahepatic splanchnic, renal, and peripheral amino or keto acid balance (4, 36, 65, 134). Catheterization of the portal vein is generally not feasible for human research because of the need for laparotomy but permits the differential evaluation of roles of the gut and the liver in interorgan amino acid exchange. In contrast to circulating amino acid levels, regional amino acid balance studies provide direct information on interorgan amino acid metabolism and have improved understanding of the physiology of interorgan transport of amino acids.

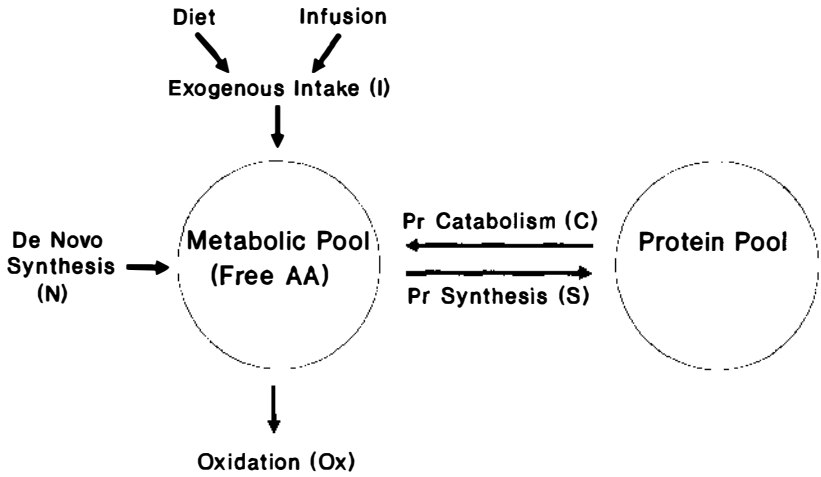
Whole-Body Amino Acid Kinetic Studies

Whole-body amino acid kinetic studies using stable (^{13}C or ^{15}N) and unstable isotopes (^{3}H or ^{14}C) provide significant quantitative information on whole-body rates of protein turnover, synthesis, and degradation. These studies are based conceptually on the two-pool model for whole-body amino acid metabolism (94) (Figure 1). This model evaluates the exchange (via protein synthesis and degradation) between the free amino acid pool and the protein pool, with the inherent assumption that rapid equilibration occurs between extravascular and plasma components of the free amino acid pool. For an essential amino acid, entry into the metabolic or free amino acid pool can be only via exogenous intake (diet or infusion) or by whole-body protein catabolism. Exit from the free amino acid pool occurs via oxidative amino acid disposal or by incorporation into protein via whole-body protein synthesis. Once steady-state tracer-specific activity or enrichment has been attained, whole-body flux or turnover can be evaluated by the ratio of the tracer infusion rate to the steady-state tracer-specific activity or enrichment in plasma or a urinary metabolite (urea or ammonia for ^{15}N studies) containing

the label (131, 133). For an essential amino acid, quantification of any exogenous amino acid intake and of oxidative amino acid disposal then allows calculation of rates of whole-body protein catabolism and rates of nonoxidative amino acid disposal (an index of tracer incorporation into protein via protein synthesis). Selection of stable versus unstable isotopes and position of the label is complex (136); a detailed discussion is beyond the scope of this review. While stable isotopes do not involve radiation exposure to the subject, they must be given in greater than trace amounts to measure their enrichment labels by combined gas chromatography–mass spectrometry. These nontracer levels could theoretically alter membrane-transport kinetics by stimulation of transport (transstimulation) or by competitive inhibition. Analysis of stable isotope enrichment by isotope ratio mass spectrometry allows true tracer doses of stable isotopes to be used but adds considerably to sample size requirements, sample purification difficulties, and instrument analysis time. Unstable isotopes can be given in true trace amounts but involve radiation hazard and cannot be used to evaluate nitrogen kinetics.

Regional Amino Acid Kinetic Studies

Recently, investigators have applied isotopic techniques to the evaluation of regional amino acid metabolism. These methods involve either direct tissue assessment of incorporation of tracer into protein using tissue biopsy (51, 83, 92, 100, 101) or indirect assessment using more advanced regional kinetic



$$\text{Flux} = Q = N + I + C = Ox + S$$

Figure 1 Two-pool model for whole-body amino acid metabolism.

modeling to evaluate amino acid incorporation into and release from tissue (12, 31, 45, 116). The biopsy methods involve labeled (either stable or unstable) amino acid infusion with tissue biopsy to assess the tissue activity or enrichment of the isotope. These methods can directly assess incorporation of the label into tissue proteins and hence allow reasonable comparison of indirect in vivo methods. While offering the possibility of direct tissue assessment, these biopsy methods have the clear disadvantage of requiring tissue biopsy. This requirement is a significant obstacle to widespread use in human studies and makes repeated studies in the same subject difficult.

Recently, Barrett et al (12) and Gelfand & Barrett (45) have described a relatively noninvasive isotopic technique to evaluate regional amino acid metabolism in muscle. This method utilizes a primed continuous infusion of labeled phenylalanine (L-[ring-2,6-^{3}H]phenylalanine) and requires measurement of tissue blood flow with steady-state sampling for amino acid concentration and tracer-specific activity from any artery and a vein draining the muscle bed of interest. The technique assumes no skeletal muscle metabolism of phenylalanine; hence tracer uptake across the muscle bed provides an index of phenylalanine incorporation into protein via protein synthesis, whereas dilution of the label across the muscle bed reflects release of phenylalanine from muscle protein via protein breakdown. This technique provides a readily measurable method of estimating protein turnover in specific muscle beds without requiring muscle biopsy. Perhaps more important, however, is that serial measurements of muscle amino acid kinetics are possible, allowing for the study of the effects of hormones, substrates, and other factors that regulate protein turnover.

AMINO ACID METABOLISM IN HUMAN CANCER CACHEXIA

Introduction

ANIMAL MODELS OF CANCER CACHEXIA A considerable body of literature addresses the metabolic alterations in host metabolism in animal models of cancer cachexia. The common models use rapidly growing, non- or late-metastasizing, transplantable tumors and include the methylcholanthrene (MCA)-induced sarcomas in rats (80, 95) and mice (71, 72, 113), Walker 256 carcinosarcoma in rats (77, 80, 81), and the MAC16 murine colonic adenocarcinoma in mice (16, 74, 117). These studies have all the advantages of animal studies and permit the use of pair-fed control groups. The natural history and development of cancer cachexia can be characterized, and one can completely differentiate between the metabolic effects of cancer and those of starvation or semistarvation to allow definition of cancer-specific effects on intermediary metabolism.

The most frequently used animal models, however, involve nonphysiologic tumor burdens of up to 30–40% of the animals's body weight (82). Norton et al (89) observed that the cancer-specific abnormalities these studies identify are frequently evident only at tumor burdens approaching 10%. This size would be comparable with a tumor larger than the human brain and with a metabolic rate of similar magnitude. Such tumor burdens are rarely seen in human malignancy; most tumors weigh less than 500 g (~0.7%) at diagnosis and seldom exceed 1 kg (29). Thus, the tumor burdens producing cachexia in animal models are usually 10-fold greater than in human malignancy. Morrison et al (82) implanted an inert artificial "tumor" into rats to simulate the increase in mass of the large transplantable tumors and demonstrated that part of the cachectic effects produced by these large tumors arises from mechanical loading of the host with a large mass, regardless of whether the mass is malignant or inert.

Therefore, although the animal tumor models of malignant cachexia allow for precise characterization of alterations in animal-host intermediary metabolism, the conclusions derived from these animal studies may not apply to human cancer cachexia. Nonetheless, given the difficulties inherent in extrapolating data from animal models of cancer cachexia to humans, these studies have provided considerable insight into the host metabolic response to malignancy in humans.

CHOICE OF CONTROLS Data on human cancer cachexia must be carefully evaluated for several potential problems that have affected many of such metabolic studies. First is the choice of a control group. To accurately differentiate cancer-specific metabolic changes from those of semistarvation and/or chronic disease, the ideal control group would be an age- and weight-matched, weight-losing, noncancer group. Such a group is difficult to accrue because relatively few nonmalignant diseases in the elderly are associated with progressive weight loss. More important, modern medical practice does not permit persistent weight loss without intervention with nutritional support. No published study has a homogeneous control group that meets these criteria. Some authors, however, have attempted to address this problem using heterogeneous controls (14, 15, 35, 69) with conditions including anorexia nervosa (usually not age-matched to cancer patients), peptic ulcer disease, postpancreatitis malnutrition, postpancreatectomy malnutrition, postgastrectomy malnutrition, chronic gastric ulceration, various nonmalignant bilious and enteric fistulae, and senile depression.

One way to partially circumvent the problem of accruing weight-losing non-cancer-diseased controls is to study non-weight-losing cancer patients, using age- and weight-matched normal volunteers as controls (54, 56). This technique does not strictly differentiate cancer-specific metabolic events from those of nonmalignant disease, but it does eliminate weight loss and

gross body compositional changes as variables in the interpretation of the findings.

APPROPRIATE PATIENT SELECTION The second problem affecting metabolic studies of human cancer cachexia is the heterogeneity of the cancer patients themselves. Cancer cachexia is a syndrome with variable degrees of expression, even among patients with pathologically identical neoplasms of comparable stage. Hence, studies of patients with heterogeneous disease stage and histopathology may yield a diverse spectrum of responses. In addition, antineoplastic therapy itself (surgery, chemotherapy, radiation therapy, immunotherapy) may induce clinical findings that are also found in cancer cachexia (see Table 1). In fact, Van Eys et al (118) and Donaldson (33) suggested that treatment, rather than the malignancy itself, is the major cause of cachexia in pediatric cancer patients. Thus, recent antineoplastic therapy, the presence of metastatic disease (especially hepatic metastases), associated illness (e.g. diabetes mellitus, hypothyroidism, hypertension), and paraneoplastic syndromes make cancer-specific metabolic alterations difficult to differentiate from postoperative changes, changes secondary to other modalities of cancer treatment, and changes of organ dysfunction secondary to metastatic or associated disease. When carefully analyzed, virtually all of the metabolic studies of human cancer cachexia are affected by one or more of these difficulties in patient or control selection.

Amino Acid Concentrations

Several investigators have reported isolated, specific free amino acid concentrations in cancer patients (14, 53; P. W. T. Pisters, E. Cersosimo, A. Rogatko, M. F. Brennan, submitted). Four studies (summarized in Table 3) have specifically addressed circulating amino acid concentrations in cancer patients (15, 25, 27, 88). These studies raise several issues that deserve comment. First, the previously noted multiple factors influencing plasma free amino acid concentrations should always be considered in evaluations of any data on free amino acid concentrations. Second, three of the four studies present data from patients with heterogeneous tumor types [in the fourth (25), tumor type/stage is not defined for the inpatient and outpatient groups]. Only the study by Norton et al (88) adequately stratifies patients by tumor type. This delineation is critically important in establishing any cancer-specific changes in free amino acid concentrations. For example, patients with head, neck, and gastrointestinal malignancies may have a significant amount of dysphagia and/or malabsorption secondary to mechanical factors, such that the amino acid profiles of these patients may represent the combined effects of malignancy and protein-calorie malnutrition (55). Although no consistent cancer-specific amino acid profile has emerged from these studies, patients with extraintestinal, nonobstructive malignancies appear to have minimal

aberrations in their amino acid profiles, as demonstrated by the nonesophageal subgroups studied by Norton et al (88) and the preoperative (presumed early stage) solid-tumor patients in the study by Ching et al (25).

More profound changes in amino acid concentrations may occur with more advanced malignancy, although this idea has not been demonstrated conclusively. The widespread decreases in free amino acids noted in three studies (15, 25, 27) are difficult to attribute specifically to malignancy, as the subjects were heterogeneous groups of patients with primarily gastrointestinal malignancies. No study to date has measured serial individual amino acid profiles to document the effects of progressive malignant disease, antineoplastic therapy, and/or malnutrition. In summary, the degree of aberration of the amino acid profile in a given cancer patient is likely a function of such variables as the specific malignancy, stage of disease, and the extent of associated protein-calorie malnutrition. Further studies with early-stage neoplasms, extraintestinal later-stage neoplasms, or longitudinal study designs are required to precisely define amino acid deficits in cancer.

Regional Amino Acid Balance

Despite the availability of in vivo techniques for the evaluation of regional amino acid balance across a variety of tissues and organ beds, little is known about interorgan amino acid metabolism in cancer-bearing humans. Once investigators understood that an increased rate of gluconeogenesis was a fundamental metabolic disturbance present in cancer cachexia (48, 53, 60), they focused on altered peripheral tissue metabolism (20) in an effort to demonstrate mobilization of gluconeogenic precursors from peripheral tissues. Bennegard et al (14), however, reported no difference in postabsorptive peripheral lactate, glycerol, and alanine balance across the leg in eight weight-losing cancer patients and five weight-losing controls with benign disease. Since skeletal muscle forms the bulk of peripheral tissue mass, attention was subsequently directed to peripheral amino acid balance. Preliminary measurements of forearm arteriovenous amino acid differences did not demonstrate differences between patients with advanced cancer and weight-losing patients with benign disease (27). Arteriovenous difference measurements, however, do not account for alterations in regional blood flow that could significantly alter peripheral substrate balance despite no apparent change in absolute arteriovenous difference. Cognizant of this fact, in the most complete study reported to date, Bennegard et al (15) measured the postabsorptive flux of amino acids across the leg in 18 weight-losing cancer patients and 8 weight-losing patients with benign disease. No qualitative or quantitative differences occurred between the groups. A separate, acutely ill group with benign disease had increased peripheral release of multiple amino acids, suggesting that the methodology was sufficiently sensitive to detect

Table 3 Free amino acid concentrations in cancer-bearing humans

Reference	Patient groups	n	Decreased	Increased	Comment
27	Weight-stable cancer	7		(Ala, Ile, Lys)[a]	Demographic/anthropometric characteristics of groups not defined
	Weight-losing cancer	11	(-)[b]		
	Weight-losing noncancer	6	(Leu, Val, Pro, Thr, Ser, Met, Lys, Asp/Asn)[a]		Heterogeneous mix of untreated and/or metastatic solid tumors and relapsing hematologic malignancies
25	Solid tumor preop.	13	(-)[a]		High SEMS on AA concs. w gas-liquid chromatography
	Head and neck preop.	7	(Gly, Thr)[a]		Tumor type/stage not defined for in-patient, outpatient groups
	Outpatient w hepatic metastases	7	(Leu, Val, Thr, Gly, Ser, Pro, Met, Ala)[a]		Normal controls age, sex, weight not defined
	Outpatient wo hepatic metastases	8	(Leu, Val, Thr, Gly, Ser, Pro, Met)[a]		Postop/septic pts used for nonmalig. weight-losing comparisons
	Inpatient w hepatic metastases	10	(Leu, Val, Thr, Gly, Ser, Pro, Lys)[a]		
	Inpatient wo hepatic metastases	16	(Leu, Val, Thr, Gly, Ser, Pro, Lys)[a]		

14	Weight-losing cancer	18	(Ala, Glu/Gln, Thr, Ser, Arg, His, Gly)[b]	(Phe)[b]	>7% weight loss Excellent documentation of clinical and anthropometric nutritional status
	Weight-losing noncancer	8			
	Weight-stable acute illness	9	(Glu/Gln, Trp, Gly, Ser, Pro, His, Cit)[a]		Heterogeneous cancer group
88	Lymphoma	11	(Pro, His, Arg)[a]		4% weight loss
	Sarcoma	9	(Pro)[a]		
	Osteosarcoma	8	(Pro)[a]		
	Metastatic sarcoma	21	(His, Arg)[a]		10% weight loss
	Esophageal cancer	6	(Thr, Ser, Pro, Gly, Ala, Tyr, Phe, Lys, His, Arg, Asp/Asn)[a]		22% weight loss

[a] Versus weight-stable, healthy controls.
[b] Versus weight-losing, noncancer controls.

changes in leg amino acid balance. Recent studies from our group in weight-losing cancer patients and normal controls (P. W. T. Pisters, E. Cersosimo, A. Rogatko, M. F. Brennan, submitted) confirm the findings of Bennegard et al (15) for the branched-chain amino acids and the remainder of the amino acids (P. W. T. Pisters & M. F. Brennan, unpublished observations). Two additional investigators have reported postabsorptive peripheral amino acid flux measurements in esophageal cancer patients (43) and extremity sarcoma patients (87). These studies were designed to evaluate other aspects of metabolism in cancer using each patient as his own control and hence did not examine separate noncancer controls. However, the peripheral amino acid flux data reported in these homogeneous groups of cancer patients are comparable with previously reported studies in normal humans (6, 97).

Because peripheral tissue breakdown is so important in the pathogenesis of cancer cachexia, the paucity of information about interorgan substrate metabolism in cancer cachexia is surprising. In contrast to the information on diabetes mellitus (30, 119, 120), for example, no studies have measured splanchnic substrate balance or disposal of exogenous substrates across the splanchnic bed in cancer-bearing humans. Although such techniques are available for human investigation, these complex inpatient metabolic studies are difficult to integrate into the intense antineoplastic treatment these patients face.

3-Methylhistidine Studies

As noted above, enthusiasm for the use of 3MH as an index of muscle protein metabolism has waned as of late, particularly with the advent of more sophisticated techniques to evaluate skeletal muscle catabolism. Although some investigators have used 3MH excretion in studies evaluating total parenteral nutrition in cancer patients (21, 22), only two reports compare 3MH metabolism in normal and cancer-bearing humans. Heber et al (54) found urinary 3MH and creatinine excretion rates in 12 lung-cancer patients and 6 healthy controls to be significantly elevated in the cancer group (106 ± 11 μmol/g creatinine) compared with controls (71 ± 8 μmol/g creatinine). However, in a subsequent study designed to overcome many of the problems associated with measurement of urinary 3MH, Lundholm et al (69) measured the efflux of 3MH from the leg of 20 weight-losing patients with heterogeneous malignancies, 7 weight-losing patients with benign disease, 8 malnourished but acutely ill patients, and 6 normal controls. Well-nourished controls and acutely ill patients had a statistically significant release of 3MH, but cancer patients and malnourished noncancer patients had an insignificant peripheral 3MH efflux. Thus, 3MH-derived data on muscle protein catabolism in human cancer cachexia are conflicting, and more direct and sensitive methods to evaluate muscle protein catabolism are required to

document alterations in skeletal muscle metabolism in cachectic patients with cancer.

Regional Amino Acid Kinetic Studies

Since so little is known about regional amino acid balance and interorgan metabolism of amino acids in cancer-bearing humans, not surprisingly, only a few investigators have evaluated regional amino acid kinetics in such patients. Lundholm et al (70) obtained samples of rectus abdominis muscle at operation from 43 cancer patients and 55 controls. Compared with controls, the cancer group had significant decreases in the in vitro incorporation of [^{14}C]leucine into skeletal muscle protein and increases in the fractional degradation rate of proteins. Subsequently, Emery et al (37) measured rates of skeletal muscle protein synthesis in vivo using [^{13}C]leucine infusion with percutaneous biopsy of quadriceps muscle in five weight-losing, solid-tumor patients and seven normal controls. Compared with normal controls, cancer patients exhibited significantly decreased synthesis of skeletal muscle protein, as demonstrated by [^{13}C]leucine enrichment in quadriceps protein. Statistically significant differences were also observed when the data were expressed per unit of ribonucleic acid; these results suggest the biochemical basis for these findings may be a reduction in the rate of translation of the nucleic acid message.

To date, skeletal-muscle catabolism in a specific muscle bed in cancer patients has not been quantitated in vivo. Indirect in vivo evaluation of primarily skeletal muscle catabolism in cancer patients using estimates of peripheral 3MH efflux has not demonstrated increased skeletal muscle proteolysis (69). Hence, available in vitro and indirect in vivo human data are contradictory. Emery et al (37) speculated that depressed skeletal muscle protein synthesis may be the major cause of the muscle wasting associated with cancer cachexia. Unfortunately, the biopsy techniques do not allow for the in vivo evaluation of rates of muscle protein catabolism. Further studies using the more sophisticated regional kinetic analyses of Gelfand & Barrett (45) or Thompson et al (117) are required for more direct in vivo evaluation of skeletal muscle protein catabolism in cancer cachexia.

Several investigators have suggested that redistribution or translocation of peripheral proteins to support visceral or tumor protein synthesis is an essential feature of amino acid metabolism in cancer cachexia (24, 58). Only two studies have evaluated visceral amino acid metabolism in cancer-bearing humans, however. Lundholm et al (71) demonstrated increased in vitro incorporation of [^{14}C]leucine into homogenized hepatic proteins of cancer patients compared with normal controls. Their study did not report nutritional status, body-weight loss, or tumor size. Subsequent work on hepatocytes isolated from patients with gastrointestinal malignancies confirmed these findings and explored the relationship between nutritional status and hepatic protein synthesis (109). Patients with malignant disease without weight loss

had a threefold higher rate of hepatic protein synthesis (4980 ± 814 pmol/h per 10^5 viable cells) over that of patients with benign disease without weight loss (1278 ± 318 pmol/h per 10^5 viable cells, $p < 0.001$). Interestingly, among 14 patients with gastric cancer, the 8 with weight loss (380 ± 90 pmol/h per 10^5 viable cells) had lower rates of hepatic protein synthesis than those without weight loss (4061 ± 401 pmol/h per 10^5 viable cells, $p < 0.002$), suggesting that cachexia and nutritional status can modulate hepatic protein synthesis in patients with malignancy.

Whole-Body Amino Acid Kinetic Studies

Discrepancy between energy expenditure and nutrient intake has been considered the fundamental thermodynamic imbalance leading to the development of cachexia. Since whole-body protein synthesis has been estimated to account for up to 20% of resting energy expenditure (98), attention has focused on the measurement of rates of protein and amino acid turnover (Q), protein synthesis (S), and protein catabolism (C) in the cancer-bearing host. Over the past decade, many investigators have evaluated whole-body protein metabolism in cancer patients using amino acid kinetic techniques (35, 37, 40, 54, 56, 58, 110, 129). Table 4 summarizes some of the studies that specifically focused on measurement of whole-body protein turnover rates. Other investigators have evaluated the effects of extent of disease (24), major surgery (47, 122), enteral or parenteral nutrition (22, 34, 57, 90), dietary manipulation (39), and antimetabolic therapeutic intervention (114) on rates of whole-body protein turnover. These studies will be discussed subsequently.

With two exceptions, all investigators to date have observed increased rates of whole-body protein turnover, synthesis, and catabolism in weight-losing and weight-stable cancer patients. Emery et al (37) and Glass et al (47) did not observe any difference in Q, S, or C in patients with malignancy. While these differences may be related to patient population, sample size, etc., the patients in both of these studies were evaluated in the fed state. The amino acid–loading and hormonal fluctuations characteristic of the fed state may obscure a difference in whole-body amino acid kinetics that may be more apparent in the postabsorptive state. Interestingly, studies of carbohydrate metabolism in tumor-bearing rats have demonstrated that fundamental alterations that exist at the molecular level in the fasted state are not demonstrable in animals studied in the fed state (86). Alternatively, one might speculate that the change in protein turnover rates produced by feeding in normal individuals may be blunted in cancer patients such that differences between the two groups are not observed in the fed state. Thus, most evidence suggests that cancer patients have alterations in protein kinetics similar to those observed in patients in the catabolic phase of severe trauma or sepsis. Since the rate of

protein synthesis in human tumors is approximately the same as that of the tissue of origin (111) and human tumors rarely exceed 1% of body mass (29), the observed alterations in whole-body protein metabolism are unlikely to be secondary to the tumor itself but rather are likely due to tumor-influenced alterations in host protein metabolism.

Carmichael et al (24) evaluated whole-body protein metabolism as a function of stage of disease in 11 colorectal cancer patients and demonstrated correlation among Q, S, and C and the percentage of labeled leucine incorporated in plasma proteins, which was used as an index of "advancement of disease." The study did not have a noncancer control group, however, and rather than accepting the limitations of conventional primary pool modeling of whole-body leucine kinetics, the authors assumed that the leucine-specific activity at the site of protein synthesis was 80% of the plasma leucine–specific activity in patients with normal rates of protein breakdown. They reduced this factor progressively if increased rates of whole-body protein catabolism were observed. These assumptions introduce a bias that could artifactually establish or augment correlation between kinetic parameters and advancement of disease.

Two groups have evaluated the impact of major oncologic surgery on whole-body protein metabolism. Glass et al (47) examined preoperative and postoperative (12 weeks) rates of Q, S, and C in 11 colorectal cancer patients and found no differences in rates of whole-body protein kinetic parameters before and after tumor resection. There were differences in the ad lib dietary intakes preceding the preoperative and postoperative studies, however, and as noted above, subjects were evaluated in the fed state, in which differences may be obscured. These factors could easily negate the authors' conclusion that the primary tumor itself does not alter the overall rate of whole-body protein metabolism. Ward et al (122) evaluated postoperative whole-body protein metabolism (^{15}N-glycine) in patients with metastatic cancer (n=9), localized cancer (n=10), and benign disease (n=7). Rates of Q, S, and C were significantly higher in postoperative patients with metastatic disease than in subjects with localized or benign disease. Of direct clinical importance, as the authors note, these fundamental disturbances in protein metabolism may contribute to the increased morbidity and mortality associated with surgery in patients with metastatic cancer.

Other investigators have concentrated on defining the effects of various types of nutritional supplementation on whole-body amino acid kinetics. Norton et al (90) and Burt et al (22) evaluated the effects of parenteral nutrition on whole-body protein metabolism in 7 heterogeneous solid-tumor patients and 11 patients with localized esophageal cancer, respectively. They observed that 10–14 days of parenteral nutrition increased protein turnover, probably as a consequence of the exogenous amino acid infusion. Responses

Table 4 Whole-body amino acid kinetic studies in cancer-bearing humans

Reference	Patients (n)	Controls (n)	Isotope	Findings[a] Q	S	C	Comment
128	Heterogeneous solid tumor (6)	MN-N (6)	[U-^{14}C]Leu	Inc	Inc	—	Bolus injection technique
54	Non–small cell lung (12)	N (6)	[U-^{14}C]Lys	Inc	—	Inc	Inc 3MH also observed in patients
35	Heterogeneous solid tumor (7)	MN-N (7)	[U-^{14}C]Tyr	Inc	Inc	—	
57	Heterogeneous solid tumor (7)	MN-N (11)	[^{15}N]Gly	Inc	Inc	—	
37	Bronchogenic (5)	N (7)	[1-^{13}C]Leu	NC	NC	NC	Postprandial measurements Reciprocal pool model for Leu kinetics Muscle biopsies showed decreased S

56	Sarcoma (6)	N (20)	[1-^{13}C]Leu	Inc	Inc	Inc	Noncachectic patients Primary pool model for Leu kinetics
40	Lung	MN-N	[^{15}N]Gly	Inc	Inc	Inc	Weight-losing and weight-stable patients
	(20)	(14)					Inc Q found in weight-stable patients
	Colorectal	N					De novo Gly synthesis assumed to be zero
	(38)	(8)					

[a] Abbreviations: MN-N, malnourished normal controls; N, healthy normal controls; Inc, increased vs control; NC, unchanged vs control; Q, protein and amino acid turnover; S, protein synthesis; C, protein catabolism.

in total-body protein synthetic and catabolic rates were variable, such that changes in S and C were not significant after 10–14 days on total parenteral nutrition. Careful evaluation of the effects of parenteral nutrition on whole-body protein kinetics ([^{15}N]glycine) demonstrated that parenteral nutrition results in a significant decrease in whole-body protein catabolism and no change in whole-body protein synthesis (57). Subsequent studies demonstrated that enteral nutrition suppresses whole-body protein catabolism in cancer patients and weight-losing controls and reduces the elevated rates of whole-body protein synthesis in tumor-bearing patients (34). Thus, recent data evaluating the metabolic efficacy of enteral or parenteral nutrition in cancer patients using whole-body amino acid kinetic techniques suggest beneficial effects on overall nitrogen economy.

Tayek et al (114) evaluated the effects of therapeutic intervention designed to block the increased gluconeogenesis associated with cancer cachexia. In a randomized, prospective double-blind trial, 12 malnourished lung cancer patients were randomized to receive either placebo or hydrazine sulphate (a phosphoenol pyruvate carboxykinase inhibitor) for 30 days. Lysine kinetics, measured with [^{14}C]lysine, were evaluated before and after one month of hydrazine treatment. Postabsorptive lysine flux (an index of whole-body protein catabolism) decreased significantly in the hydrazine-treated group, ostensibly by reducing the demand for gluconeogenic precursors derived via protein catabolism. This study is significant not only because it was well designed and performed but because it represents one of the first attempts to evaluate the metabolic efficacy of therapeutic intervention designed to block a specific metabolic alteration observed in cancer-bearing humans.

SUMMARY AND CONCLUSIONS

Cancer cachexia is a complex syndrome that occurs with variable incidence in patients with solid tumors and those with hematologic malignancies. It is associated with characteristic physical and laboratory findings, and at a more fundamental level, with significant abnormalities in carbohydrate, lipid, and protein metabolism. These alterations in intermediary metabolism are demonstrable early in the syndrome, even before the onset of weight loss, when the more characteristic features of cancer cachexia are evident. Progressive wasting of peripheral protein stores is a major feature of cancer cachexia and often one of the most graphic realities of malignancy for patients and their families.

Unfortunately, significant problems with the animal models of cancer cachexia make conclusions derived from animal studies difficult to extrapolate to humans. Data from human studies indicate that human cancer cachexia is associated with minimal aberrations in circulating free amino acid

concentrations; increased whole-body protein turnover, synthesis, and catabolism; reduced rates of skeletal muscle protein synthesis; and increased rates of hepatic protein synthesis. Whether or not these alterations represent pathologic responses or physiologic adaptation by the host to the presence of malignancy remains to be seen. Future investigations must focus on more careful evaluation of interorgan amino acid metabolism, investigation of skeletal muscle protein catabolic rates in cancer cachexia, and definition of the roles of altered hormonal and cytokine regulation of these processes. Such studies will more precisely define the level at which amino acid metabolism is altered significantly and, we hope, permit more specific therapeutic intervention designed to reverse the debilitating effects of cancer cachexia.

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