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Original article

Normal protein anabolic response to hyperaminoacidemia in insulin-resistant patients with lung cancer cachexia☆

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SUMMARY

Background & aims: Insulin resistance of protein anabolism has been speculated to underlie the skeletal muscle wasting characteristic of cancer cachexia. We tested whether insulin resistance is present in cachectic lung cancer patients and if a sustained, physiological elevation of amino acids with hyperinsulinemia would compensate for it.

Methods: Whole-body [^{13}C]leucine and [^{3}H]glucose kinetics were assessed in 10 male non-small cell lung cancer (NSCLC) patients and 10 healthy matched controls during a euglycemic, hyperinsulinemic clamp under conditions of isoaminoacidemia followed by hyperaminoacidemia.

Results: Postabsorptive glucose and protein kinetics were comparable between groups. Glucose uptake was significantly lower in NSCLC patients during hyperinsulinemia. During concurrent isoaminoacidemia, protein breakdown was suppressed in both, but rates were elevated in NSCLC; rates of synthesis did not change, resulting in reduced net protein balance (synthesis − breakdown) in response to insulin in NSCLC. With subsequent hyperaminoacidemia, synthesis increased significantly with no further change in breakdown, resulting in similar increase in net balance between groups.

Conclusions: NSCLC patients with moderate cachexia showed considerable insulin resistance of glucose and of whole-body protein anabolism. Their anabolic protein response was stimulated normally by hyperaminoacidemia. Thus, ample provision of amino acids is a feasible strategy to overcome the protein anabolic failure of cancer cachexia.

1. Introduction

Cancer cachexia is clinically manifested by weight loss, and has been defined as the loss of skeletal muscle, with or without loss of adipose tissue.[1] Muscle wasting predicts poor cancer-associated outcomes: increased fatigue and treatment-induced toxicity, decreased host response to tumor, performance status, and survival.[2–5] Cachexia is highly prevalent at the time of diagnosis in several cancers, including non-small cell lung cancer (NSCLC).[6] In addition to skeletal muscle atrophy, clinical features of cachexia often include anorexia/malnutrition, inflammation, anemia, and hypermetabolism.[1,7]

Insulin resistance is typically defined by a blunted action on tissue glucose uptake and suppression of hepatic glucose production. Insulin resistance of glucose metabolism has been demonstrated in a variety of cancers using the "gold-standard" hyperinsulinemic, euglycemic clamp.[8–10] Normal insulin sensitivity is likely required for optimal muscle protein anabolism to occur during the postprandial state. This response involves the coordinated actions of both insulin and amino acids on stimulating protein synthesis and suppressing protein breakdown. We have shown that when insulin-stimulated glucose disposal is reduced, whole-body protein anabolism is also impaired, in obesity,[11] aging[12] and type 2 diabetes.[13] The contribution of insulin resistance of protein anabolism to muscle wasting in cancer is a commonly proposed mechanism[1] that has not been rigorously tested in humans. To do so, the use of clamp techniques, as well as a control group matched for important variables affecting insulin resistance such as age, sex and adiposity, is imperative to draw relevant conclusions.

Increased muscle proteolysis, mediated by inflammation or tumor-derived catabolic factors, is well established in animal models of cancer cachexia.[14] In human cancer however, one study indicated elevated rates of proteolysis (from urinary 3-methylhistidine excretion),[15] while most reported normal postabsorptive rates.[16–18] On the other hand, muscle protein synthesis was found to be reduced[17] or unchanged.[18] Protein kinetic studies

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examining responses to the main postprandial anabolic stimuli, insulin and amino acids, in cancer cachexia are scarce. Weight-losing cancer patients (mostly bronchial) had reduced rates of muscle protein synthesis during constant, hourly feeding.[19] In patients with lung cancer, whole-body protein synthesis failed to increase during repeated small meals given over 4 h, as was also observed in controls.[20] This approach may not have resulted in typical postprandial hormone and substrate peak concentrations. But, patients with ovarian cancer cachexia undergoing chemotherapy provided with small oral amino acid boluses (total of 40 g) showed the capacity for muscle protein synthesis stimulation, albeit to a lesser extent than healthy older controls. Amino acid feeding had no effect on muscle protein degradation.[18] Overall, these findings suggest a blunted response of protein synthesis to nutrients. Finally, in addition to stimulating and regulating protein synthesis,[21] amino acids, particularly the branched-chain (BCAA) have been shown to decrease glucose disposal, i.e. insulin sensitivity,[22–24] both *in vitro*[25,26] and in healthy humans.[27] But in insulin-resistant conditions such as type 2 diabetes, hyperaminoacidemia did not further attenuate the already impaired glucose disposal.[28]

We chose to study a homogeneous group with advanced stage NSCLC and weight loss, to address the following: (1) does the presence of insulin resistance attenuate protein anabolism, thereby contributing to muscle loss? (2) does hyperaminoacidemia, equivalent to a generous supply of amino acids, combined with hyperinsulinemia have the capacity to stimulate protein synthesis without impairing glucose disposal?

2. Subjects and methods

2.1. Subjects, diet and body composition

Ten patients with NSCLC were recruited from the McGill University Health Centre (MUHC) Oncology Clinics. Inclusion criteria were men ≥18 years with stage III or IV NSCLC, ≥5% weight loss within the previous 12 months,[1] and able to tolerate the metabolic tracer study procedures. Exclusion criteria were: diabetes or diseases affecting glucose and/or protein metabolism, hemoglobin <100 g/L, metastases significantly impairing organ functions, severe pain, ongoing cancer therapy, surgery in the past 3 months, inability to refrain from smoking for a day, and the following medications: anticoagulants, antianginals and antiarrhythmics, high-dose steroids (≥10 mg/d prednisone equivalent), narcotic analgesics and/or NSAIDS.

Nine patients had adenocarcinoma, 2 with brain and 2 with bone metastases (Table 1); 4 were studied before treatment and 6 ≥4 months post treatment. All had an ECOG[29] status score ≤2 and average weight loss was 8%. Healthy control men were specifically matched to patients for age, BMI and smoking habits, all known to influence insulin sensitivity.[11,12,30,31] Controls were weight-stable for more than 6 months, had normal glucose tolerance, and protein intakes within the Dietary Reference Intakes.[32] Medical history, physical examination, laboratory investigation and diet history were performed as previously described.[33] This study was approved by the McGill University Health Centre Research Ethics Board and all subjects provided informed written consent.

Subjects were admitted to our Clinical Investigation Unit the day prior to metabolic study. They received an isoenergetic, isoproteic diet based on diet history and resting energy expenditure measured by indirect calorimetry (Deltatrac, SensorMedics, Yorba Linda, CA) with 1.3–1.6 physical activity level factor. The diet was a liquid formula (Ensure®, Abbott Laboratories, St. Laurent, QC) and standard bran cereal breakfast. Physical activity levels were assessed according to the Physical Activity Scale for the Elderly (PASE).[34]

Table 1
Subject characteristics.

		Control	NSCLC
N		10	10
Diagnosis (SCC/ADC)		–	1/9
Stage (IIIA/IIIB/IV)		–	2/3/5
Weight loss (%/12 mo)		–	7.8 ± 1.3
Age (y)		63 ± 2	66 ± 2
Smoking (pack-y)		22 ± 4	40 ± 11
Quit smoking (y)		17 ± 4	13 ± 5
Weight (kg)		70 ± 2	65 ± 3
Height (cm)		174 ± 2	172 ± 1
BMI (kg/m^2)		23.1 ± 0.5	22.0 ± 0.9
Waist circumference (cm)		89 ± 1	85 ± 3
Thigh circumference (cm)		53 ± 1	48 ± 2[a]
Energy intake	(kcal/d)	2342 ± 140	1891 ± 76[a]
	(kcal/kg LBM d)	47 ± 3	41 ± 2[a]
Protein intake	(g/d)	88 ± 4	71 ± 4[a]
	(g/kg LBM d)	1.7 ± 0.1	1.5 ± 0.1
REE	(kcal/kg LBM d)	29 ± 1	31 ± 1
	(% of predicted)	100 ± 2	105 ± 4
Handgrip strength[b] (kg)		42 ± 1	39 ± 2
Physical activity scale[c] (PASE)		166 ± 18	97 ± 14[a]

SCC: squamous cell carcinoma; ADC: adenocarcinoma; REE: resting energy expenditure; LBM: lean body mass.
[a] $p < 0.05$ versus Control, by independent *t*-tests.
[b] $n = 8$ in NSCLC.
[c] $n = 9$ in Control.

Body circumferences and skinfold thickness were measured with a non-elastic tape and Lange skinfold calipers (Beta Technology Incorporated, Santa Cruz, CA), at standard landmarks.[35] Handgrip strength was measured with the Jamar® hand dynamometer. Body composition was measured by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Healthcare); no patients had edema.[36] Appendicular skeletal muscle index was calculated as lean soft tissue (without bone) of arms plus legs/height.[2] Changes in regional muscle, and visceral, subcutaneous and intramuscular adipose tissue were quantified a posteriori, from digital CT images acquired for diagnostic purposes. Two consecutive scans, prior to and/or overlapping the study day, were analyzed using Slice-O-Matic software (version 4.3, Tomovision, Montréal, Canada), as described in Ref.[37] Because time intervals between scans differed, changes in surface area (cm^2) were calculated as percentage normalized for 100 days.

2.2. Hyperinsulinemic, euglycemic, iso/hyperaminoacidemic clamp protocol

Whole-body glucose and protein kinetics were studied during three consecutive phases of 150 min each: (1) postabsorptive; (2) hyperinsulinemic, euglycemic (5.5 mmol/L), isoaminoacidemic clamp (HyperIns–IsoAA, detailed in Ref.[33]); (3) hyperinsulinemic, euglycemic, hyperaminoacidemic clamp (HyperIns–HyperAA, i.e. plasma BCAA maintained at 700–800 μmol/L (Fig. 1)). These concentrations were previously established as peak postprandial responses of 8 healthy subjects after a 714 kcal liquid mixed-meal with 47% of energy as carbohydrate, 17% as protein (30 g) and 35% as fat. Briefly, after overnight fasting, catheters were inserted in a dorsal hand vein for arterialized blood sampling, using the heated box technique, and in a contralateral antecubital vein for infusions. Primed, continuous infusions of D-[3-^{3}H]glucose (PerkinElmer Inc., Boston, MA) (prime: 22 μCi, infusion: 0.22 μCi/min) and L-[1-^{13}C] leucine (Isotech, Sigma–Aldrich, St. Louis, MO) (prime: 0.5 mg/kg, infusion: 0.008 mg/kg min) were continued throughout the 7.5 h study to calculate glucose and whole-body protein kinetics. A priming solution of NaH $^{13}CO_3$ (MassTrace Inc., Woburn, MA) was administered orally.[33] Following the postabsorptive phase, human