centrations on day 0 between the group given naftidrofuryl before operation and the controls, and it may have been an artefact of selection. Catecholamines cause an increased production of lactate from the muscle and an acceleration of the alanine shuttle. If the critical factor after trauma is a cellular, rather than a hormonal, modification affecting nitrogen conservation then the utilisation of pyruvate decarboxylation and fatty acid β oxidation as sources of acetyl coenzyme A may be reduced. Under these conditions of immediate postoperative injury—namely, increased muscle lactate production from glycogen, lipolysis, and glucose intolerance—then a rise in elevation of blood ketone, lactate, and pyruvate concentrations would be expected. Smith *et al* showed that early ketoadaptation is associated with improved conservation of nitrogen.[15] In contrast, an agent that is able to increase the utilisation of carbohydrate and fat in the citric acid cycle and reduce the need for protein degradation, as has been suggested for naftidrofuryl,[5] would therefore be expected to diminish the increases in both lactate and total ketone concentrations that occur at this stage. This would indicate a resetting of the regulation that links lactate and pyruvate with ketone production, and the usual inverse relations would still operate. The result reported here for the diminished rise in lactate concentration, although without a change in the ratio of lactate to pyruvate concentrations, and the possibility of a diminished rise in the total ketone concentration on day 1 are not inconsistent with this mode of action on day 1, although it has not been possible to show any nitrogen sparing effect. We believe that further investigation with other nutritional regimens is warranted, and this is currently being undertaken. Nevertheless, the claim that naftidrofuryl alone will reduce the loss of nitrogen postoperatively cannot be generally applied.

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Protein synthesis in muscle measured in vivo in cachectic patients with cancer

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Abstract

Rates of synthesis of protein were measured in vivo in skeletal muscle and in the whole body of cachectic patients with cancer and in normal healthy men, using a tracer infusion of leucine labelled with a stable isotope. Synthesis of protein in muscle was significantly reduced in the patients with cancer (0·030 v 0·198%/hour; p <0·01), whereas whole body rates of protein synthesis and degradation did not differ significantly between the two groups. Thus depression of synthesis of protein in muscle appeared to be the immediate cause of muscle wasting in cancerous cachexia.

Any therapeutic intervention that aims at preventing the onset of cachexia should be designed to stimulate the synthesis of protein in muscle, and measurement of turnover of protein may be used to evaluate such treatment provided that rates of protein synthesis are measured directly in specific tissues.

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Introduction

The most common cause of death in patients with cancer is the generalised wasting of body tissues known as cachexia.[1] This is also one of the most distressing features of cancer both for the patients and for their relatives. Skeletal muscle makes up more than 40% of the body, and wasting of the muscles is a prominent feature of cachexia in cancer. Indeed, the outcome of the disease is most often determined by the wasting of the respiratory muscles and the consequent inability to overcome respiratory infections.

The mechanism by which this muscle wasting occurs is at present poorly understood. Because of the phenomenon of protein turnover the same degree of muscle wasting could

theoretically result from either an increase in the rate of protein degradation or a decrease in the rate of protein synthesis in that tissue. Until recently this question could not be resolved because of the technical difficulty of measuring the rates of synthesis and degradation of protein in muscle in vivo in man. With recent methodological advances, however, the rate of protein synthesis in vivo in human muscle can now be measured directly, by infusing an amino acid labelled with a stable isotope and sampling muscle by percutaneous needle biopsy.[2 3]

A review of the evidence from in vivo studies in experimental animals and man has shown that many conditions associated with muscle wasting, including Duchenne type muscular dystrophy, myotonic dystrophy, and postoperative catabolism, are characterised by a reduced rate of protein synthesis in muscle.[4] We report here that the rate of protein synthesis in skeletal muscle of patients with cancerous cachexia is also appreciably reduced. This implies that nutritional and pharmacological interventions aimed at ameliorating cachexia should be directed at ways of stimulating synthesis of protein in the muscles and that the efficacy of such regimens could be monitored by measuring their effect on this process.

TABLE I—*Clinical state of five patients with cancerous cachexia at the time of measurement of protein synthesis*

Case No	Age (years)	Diagnosis	State of disease	Results of liver function tests	Serum albumin (g/l)	Weight (kg)	Recent loss of weight
1	63	Hypernephroma	Extensive			53	6 kg/6 weeks
2	50	Small cell carcinoma of the bronchus	Extensive	Abnormal	38	52	13 kg/6 months
3	67	Undifferentiated carcinoma of the bronchus	Extensive	Normal	39	64	5 kg/8 weeks
4	59	Small cell carcinoma of the bronchus	Extensive	Normal	40	63	6 kg/6 weeks
5	64	Squamous cell carcinoma of the bronchus	Limited	Abnormal	38	63	3 kg/2 weeks

TABLE II—*Whole body turnover of leucine and synthesis of protein in muscles in five cachectic patients with cancer and seven healthy controls. (Values are mean (SE))*

	Controls (n = 7)	Patients (n = 5)	p
Body weight (kg)	80·8 (7·3)	59·0 (2·7)	<0·05
Age (years)	36 (5)	61 (3)	<0·01
Creatinine excretion (g/d)	1·57 (0·13)	0·96 (0·14)	<0·01
Muscle protein synthesis (%/hour)	0·198 (0·020)	0·030 (0·007)	<0·01
Muscle protein synthesis (g/hour/g RNA)	0·91 (0·11)	0·15 (0·11)	<0·01
Leucine flux (μmol/kg/hour)	194 (8)	183 (11)	NS
Leucine intake (μmol/kg/hour)	55 (2)	38 (3)	<0·01
Leucine oxidation (μmol/kg/hour)	40 (3)	40 (7)	NS
Whole body protein synthesis (μmol leucine/kg/hour)	154 (11)	143 (4)	NS
Whole body protein degradation (μmol leucine/kg/hour)	139 (8)	145 (10)	NS
Plasma insulin (μU/ml)	24 (3)	31 (6)	NS

Patients and methods

We studied five men, aged 50 to 67, with cancer who had all lost weight during the period immediately before the investigation (table I). We compared findings in them with those in seven normal healthy men aged 22 to 65. The study was approved by the ethical committee of University College Hospital. All patients gave informed, written consent. Cancer had been recently diagnosed in all five patients, and none had received any form of antitumour treatment before the study. They had all lost weight before the study (table I) and continued to do so afterwards. All patients reported a loss of appetite: their mean customary energy intake (92 kJ/kg, assessed by dietary history) was 60% of the recommended daily intake for the group.[5] None of the patients was vomiting or haemorrhaging at the time of the study and none was bedridden, although all patients and controls remained in bed during the infusion study.

On the day of the investigation the subjects were fed hourly meals of a milk based liquid diet designed to supply two thirds of each subject's normal daily intake of protein and energy over 10 hours (range 0·4-0·8 g protein and 4-8 kJ/kg/hour). Two hours after beginning the feeding regimen baseline samples of venous blood and expired air were taken. A priming dose (1 mg/kg) of l-leucine labelled in the carboxyl group with carbon 13 (^{13}C) (99%; KOR Inc, Massachusetts) and ^{13}C labelled sodium bicarbonate (0·08 mg/kg) was then injected intravenously and a constant infusion of carboxyl-^{13}C labelled l-leucine (1 mg/kg/hour) into an antecubital vein started. Samples of venous blood, drawn from the other arm, and expired air were collected at regular intervals (every half hour for the first two and a half hours, then every hour), and after seven hours a single sample of quadriceps muscle was taken by percutaneous needle biopsy under local anaesthesia.[6]

Plasma from blood samples was analysed for enrichment of ^{13}C labelled α ketoisocaproate by gas chromatography and mass spectrometry[7]; expired air was analysed for enrichment of carbon-13 dioxide by isotope ratio mass spectrometry; protein from the muscle samples was analysed for enrichment of ^{13}C labelled leucine by isotope ratio mass spectrometry of carbon-13 dioxide liberated by reacting ninhydrin with leucine separated by gas chromatography from the protein hydrolysate.[8] Total production of carbon dioxide was measured by infrared absorbance of timed samples of expired air collected quantitatively in a Douglas bag on at least three occasions during the infusion. The methods were essentially similar to those used in a previous study of the effects of Duchenne type muscular dystrophy.[2] Plasma insulin concentration was measured in at least six samples taken throughout each study, using a radioimmunoassay method (Amersham International, UK). Ribonucleic acid and protein were measured in the muscle biopsy specimens by conventional techniques.[9]

Results and discussion

The rate of protein synthesis in muscle, calculated by dividing the final enrichment of ^{13}C labelled leucine in muscle protein by the average enrichment of α ketoisocaproate in the plasma over the seven hours of infusion,[2] was much lower in the patients with cancer than in the controls (table II). An equally severe depression in rate of protein synthesis was seen when the results were expressed per unit of ribonucleic acid, suggesting that the biochemical basis for this disturbance is a reduction in the rate of translation of the nucleic acid message. This depression of the synthesis of protein in muscle is probably a major cause of the muscle wasting that is suffered by patients with advanced malignant disease. It may indeed be the most important cause of the loss of muscle protein as we have evidence from studies in other patients with cancer that muscle proteolysis is not increased.[10] We have previously shown that a reduced rate of protein synthesis in muscle is characteristic of patients with inherited myopathies causing muscle wasting[2] and also of cachectic mice with tumours.[11]

The patients in the present study were on average older than the controls. Golden and Waterlow have suggested that rates of protein turnover decrease with age,[12] on the basis of the finding that the rate of whole body protein synthesis in six elderly men (aged 66-91, mean 74, years) was 34% lower than the rate previously reported for four middle aged surgical patients (aged 52-75, mean 59, years),[13] measured using similar, though not identical, methods. On the other hand, studies in vitro have suggested that the rate of incorporation of leucine into muscle protein in man is actually greater in patients over 60 than in those under 60.[14] Our data do not show any tendency towards a decreased rate of protein synthesis in muscles with increasing age within either group, and age seems unlikely to be the major cause of the difference between the patients and controls.

The chronically low intake of food by our patients may have been partly responsible for the depression of muscle protein synthesis; we know, however, that complete fasting over 18 hours reduces the rate of protein synthesis in muscle in healthy subjects by only 50%,[3] whereas protein synthesis in muscle in our patients (who were being fed 70% as much as the controls during the study) was reduced by more than 80%. The difference in intake of food during the study was certainly not great enough to cause a significant difference in the circulating concentration of insulin (table II), the hormone that is believed to mediate the effect of food intake on protein synthesis in muscle.[15] Moreover, our previous experiments with mice showed that chronic restriction of food by up to 50% did not depress protein synthesis in muscle as much as did the presence of a tumour, which suppressed intake of food by only 15%.[11] Thus cancer probably has a specific effect on muscle protein metabolism in addition to the effect of the anorexia that it causes. The way in which this effect is exerted remains unknown at present but is likely to be mediated either by a disturbance of the normal balance of substrates and hormones or by an unidentified humoral substance secreted by the tumour.

The protocol we used allowed us to calculate the components of whole body protein metabolism as well as the specific rate of synthesis of muscle protein.[16] Leucine flux, calculated by dividing the rate of infusion of labelled leucine by the plateau labelling of α ketoisocaproate,[3] in the patients was not significantly different from that in the controls (table II). Leucine oxidation was calculated from the measured rate of production of carbon dioxide and the plateau enrichment of carbon-13 dioxide in expired air, assuming that 81% of production of carbon dioxide is recovered in expired air.[16] There was no significant difference in rate of leucine oxidation between the two groups (table II). The rates of protein synthesis (S) and degradation (D) in the whole body were then calculated from the flux (Q), rate of oxidation (O), and dietary input (I) of leucine using the equation $Q=S+O=D+I$.[16] Rates of whole body synthesis and degradation of protein were not significantly different in the cancer patients from those in the controls (table II).

Because muscle mass can be calculated from creatinine excretion[17] we could then calculate that the total amount of muscle protein synthesised was 1·1 g/hour in the patients compared with 10·5 g/hour in the controls. Muscle protein synthesis thus represented only 8% of whole body protein synthesis in the cachectic patients with cancer compared with 53% in the normal men in the fed state assuming that tissue proteins contain 8% leucine.[16] In contrast, the rate of protein synthesis in tissues other than skeletal muscle was 0·21 g/kg/hour in the patients compared with 0·12 g/kg/hour in the controls. This increased rate of protein synthesis in tissues other than muscle may have been partly due to protein synthesis in the tumour and probably also to increases in the rates of turnover of some fractions of liver protein, including the synthesis of acute phase proteins, as has been suggested from work with experimental animals.[18,19] It should be noted, however, that in conditions in which skeletal muscle has been extensively lost, with relative preservation of the visceral tissues in which there is a faster turnover of protein, whole body rates of protein synthesis expressed per unit body weight will appear to be higher than normal even when the rates of protein synthesis in individual tissues have not changed.

Heber *et al* reported that the rate of whole body synthesis of protein is greater in patients with cancer than in healthy controls when measured in the fasting state.[20] Our data show that this difference is not present in the fed state, suggesting that the rate of protein synthesis increases in response to feeding by a smaller amount in patients with cancer than in normal people. The normal increase in whole body protein synthesis, which occurs in response to feeding, is known to be due mainly to an increase in the rate of protein synthesis in skeletal muscle,[3] so the muscle of patients with cancer appears to be unable to respond to feeding by increasing its rate of protein synthesis. This may be partly due to insulin resistance, which has been observed in patients with a variety of cancers.[21]

In conclusion, we have shown that the rate of protein synthesis in skeletal muscle is specifically reduced in patients with cancerous cachexia. We suggest that stimulating muscle protein synthesis, perhaps with pharmacological anabolic agents together with appropriately timed nutritional support, may prevent the onset of muscle wasting and cachexia and so permit a better response to anti-tumour treatment and reduce the morbidity and mortality due to infections and respiratory failure. Moreover, the measurement of the rate of muscle protein synthesis could be used to monitor directly the effectiveness of such adjuvant treatment.

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