Liver export protein synthetic rates are increased by oral meal feeding in weight-losing cancer patients

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1Department of Surgery, Royal Infirmary of Edinburgh, Edinburgh EH3 9YW; 2Department of Surgery, Glasgow Royal Infirmary, Glasgow G31 2ER; and 3Isotope Biochemistry Laboratory, Scottish Universities Research and Reactor Centre, East Kilbride, United Kingdom G75 0QF

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Barber, Matthew D., Kenneth C. H. Fearon, Donald C. McMillan, Christine Slater, James A. Ross, and Tom Preston. Liver export protein synthetic rates are increased by oral meal feeding in weight-losing cancer patients. Am J Physiol Endocrinol Metab 279: E707–E714, 2000.—We have demonstrated previously that, in the fasting state, whereas albumin synthesis is similar in cachectic cancer patients compared with controls, fibrinogen synthesis is increased. Whether synthesis of these proteins is altered after an oral meal was examined in eight weight-losing pancreatic cancer patients and six healthy controls by use of an intravenous flooding dose of [2H5]- or [ 2H8]phenylalanine. Cancer patients had a median weight loss of 19%, a significantly lower serum albumin concentration, and a significantly higher plasma fibrinogen concentration than controls (P < 0.005). Fasting albumin synthesis rates were similar between cancer patients and controls (median total synthesis rate 11.3 vs. 13.9 g/day, respectively) and rose on feeding by a similar degree (median 29 and 24%). The fasting fibrinogen total synthesis rate was significantly higher in cancer patients than in controls (median 3.3 vs. 1.0 g/day, P = 0.0019). In cancer patients in the fed state, fibrinogen synthetic rate rose by a median of 38% (P = 0.012), whereas in controls there was no significant change. These findings demonstrate significant upregulation by feeding of acute-phase protein synthesis in cachectic cancer patients.

Albunin; fibrinogen; acute-phase protein response; pancreatic cancer

WEIGHT LOSS IS A MAJOR CAUSE of morbidity and mortality in advanced cancer (14, 32, 43). This weight loss has proven difficult to reverse, and it has been suggested that the metabolic response to cancer prevents the efficient use of food (29). Part of this metabolic response is the hepatic acute-phase protein response. The acute-phase protein response has been shown to be associated with hypermetabolism, accelerated weight loss (17, 38, 45), and poor survival in patients with advanced cancer (7, 18). The acute-phase protein response is characterized by elevated circulating concentration of so-called positive acute-phase proteins, such as C-reactive protein and fibrinogen, and reduced concentrations of negative acute-phase proteins, such as albumin (6). The circulating concentrations of these proteins depend on their rates of synthesis (largely in the liver), degradation, and transcapillary escape. The potential importance of the acute-phase response is that, in patients with inadequate nutritional intake, the net increase in hepatic protein synthesis may provide an increased drain on the protein store of the body, the skeletal muscle, and thus accelerate loss of lean tissue (34).

It has previously been shown that, in the fasting state, the total albumin synthesis rate is unchanged in patients with advanced cancer compared with controls despite much lower albumin concentrations (19). By contrast, the total synthesis rate of fibrinogen is significantly increased in the fasting state in cancer patients, accompanied by higher circulating concentrations compared with controls (33). This would suggest that, in the fasting state for albumin at least, a reduced synthesis rate does not explain the hypoalbuminemia observed. In contrast, plasma fibrinogen concentrations reflect, at least in part, an increased rate of synthesis.

The synthesis of albumin is thought to be stimulated by feeding in healthy subjects (13, 24); however, in contrast, fibrinogen synthesis remains unchanged (10, 13). If there were a reduced albumin synthetic response to feeding in cancer, this might contribute to hypoalbuminemia. Alternatively, if there were increased fibrinogen synthesis in response to feeding in cancer (and this process affected other positive acute-phase proteins), then this might contribute to the metabolic demands on the nitrogen economy of such patients and might ultimately contribute to loss of lean tissue, particularly if dietary protein intake were restricted.

In an attempt to explore these hypotheses, the aim of the present study was to assess whether weight-losing patients with advanced pancreatic cancer have an abnormal albumin synthetic response in the fed state, and whether synthesis of the positive acute-phase

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protein fibrinogen is altered with feeding in such patients.

METHODS

Subjects. Eight patients with an unequivocal diagnosis of pancreatic cancer, who were losing weight with no clinical evidence of ascites or peripheral edema, were examined in the study. Six weight-stable healthy individuals served as controls. None of the patients received chemotherapy or radiotherapy, and none had undergone surgery in the preceding 4 wk. No patients had clinical or radiological evidence of infection, were jaundiced or severely anemic, or were receiving steroids. The study was approved by the local ethical committee, and all subjects gave written informed consent.

Study protocol. The study protocol is presented diagrammatically in Fig. 1. Subjects attended at 8 AM on two consecutive days. On the 1st day, after an overnight fast, a venous catheter was inserted into the subject's antecubital fossa. The patient rested in a supine position for ≥30 min and underwent measurement of resting energy expenditure by means of a ventilated hood technique (Deltatrac, Datex, Helsinki, Finland), as previously described (17). At 10 AM, subjects received an intravenous bolus of [2H5]- or [2H8]-phenylalanine (3.5 g/10 mol% labeled L-phenylalanine, 2% in saline; CK Gas Products, Finchampstead, UK) over ~5 min. The tracer was prepared under sterile conditions, tested for sterility and absence of pyrogens, and administered via a 0.22-μm filter. With care taken to flush the catheter to avoid tracer contamination, 10-ml blood samples were obtained before and 20, 40, 60, 80, 100, and 120 min after the start of the tracer infusion. The blood sample taken before the "flooding" dose was analyzed for baseline albumin and fibrinogen concentrations and for [2H5]- or [2H8]-phenylalanine enrichment in plasma albumin and fibrinogen and in the plasma-free phenylalanine pool. Subsequent samples were analyzed for [2H5]- or [2H8]-phenylalanine enrichment in plasma albumin and fibrinogen and in the plasma-free phenylalanine pool. Subjects remained fasting throughout this period. On the 2nd day, again after an overnight fast, a venous catheter was inserted into the subject’s antecubital fossa, and subjects received a meal in the form of a balanced whole protein liquid diet under measurement of energy expenditure × 1.4) (24). The supplement provided 13% of energy from protein, 48% from carbohydrate, and 39% from fat, similar to a "typical" British diet. Subjects received such a feed on a hourly basis until the end of the study period 4 h later. At 10 AM (after two hourly feeds), a similar flooding dose of phenylalanine was given, as described above, with the alternative label and blood samples collected similarly. Plasma volume was predicted from body weight and height (35).

Sample preparation and isotope analysis. The method of sample preparation has previously been described (27). Briefly, the study protocol required the measurement of labeled phenylalanine enrichment in the plasma-free phenylalanine pool and in plasma albumin and fibrinogen. For free phenylalanine analysis, 1.5-ml plasma samples were diluted with 5 ml of deionized distilled water, with 250 nmol cycloleucine added as an internal standard. Diluted samples were then deproteinized by ultrafiltration (25,000 molecular weight cut-off Centrifree cone, Amicon, Gloucestershire, UK) and acidified, and the amino acids were purified by cation exchange. [2H5]- or [2H8]-phenylalanine enrichment was measured by gas chromatography-mass spectrometry (GC-MS) as its tert-butyldimethylsilyl derivative (37). [2H8]phenylalanine was found to form [2H7]-phenylalanine over time (circulating free phenylalanine comprised ~50% [2H2]-phenylalanine some 100 min after the flooding dose of [2H8]phenylalanine). This phenomenon occurred in vivo and not in tracer standards or during sample processing. To overcome this problem, both isotopomers were measured in all samples, and their sum was used in all calculations.

Albumin was extracted from 1 ml of serum by differential solubility in absolute ethanol from 10% (wt/wt) trichloroacetic acid-precipitated protein. To remove traces of free phenylalanine, the ethanolic albumin solution was washed three times with 5 ml of deionized distilled water by ultrafiltration. Purified albumin was then hydrolyzed, and labeled phenylalanine enrichment was measured by GC-MS (37).

After washing of the plasma three times in an ultrafiltration cone, as described above, fibrinogen was removed as a fibrin clot. Clotting was performed by diluting 1.5 ml of plasma to 20 ml with saline and 0.5 ml calcium chloride (0.5 mol/l). Fifteen units of human albumin-free thrombin (Sigma, Poole, UK) were then added, and after 10 min the fibrin was collected on an etched glass rod. The fibrin was then hydrolyzed under vacuum at 145°C for 4 h with 6 mol/l HCl, and its labeled phenylalanine enrichment was measured as described above.

Labeled phenylalanine enrichment of the plasma-free pool, albumin, and fibrinogen in the fasting and fed states is shown in Fig. 2.
Protein assays. Serum albumin concentrations were measured using the bromocresol green method on a Technicon RA-1000 automated analyzer (Technicon Instruments, Tarrytown, NY). Plasma fibrinogen concentrations were measured by assessing clotting time in the presence of a high thrombin concentration on a KC4A Coagulometer (Baxter Healthcare, Thetford, UK).

Calculations. Fractional synthesis rates of albumin and fibrinogen were calculated by dividing the rate of change of labeled phenylalanine enrichment of albumin or fibrinogen by the area under the curve of precursor enrichment vs. time (2).

Mediator measurement. Samples for the measurement of insulin, cortisol, and interleukin-6 concentrations were taken at 8 AM after an overnight fast. Serum concentrations of interleukin-6 were measured by ELISA (Quantikine, R&D Systems, Abingdon, UK). The limit of detection was 0.5 pg/ml. The coefficient of variation was <8.8% across the concentration range studied. Insulin and cortisol concentrations were analyzed by radioimmunoassay. The limit of detection of insulin was 0.7 mU/l. The coefficient of variation was <10%.

Statistics. Unless otherwise noted, data are presented as medians and ranges. Data were tested for significance using the Mann-Whitney U-test, χ² test, or the Wilcoxon signed-rank test as appropriate (Statview, Abacus Concepts, Berkeley, CA). A P value of <0.05 was taken to denote significance.

RESULTS

Subject characteristics are shown in Table 1. Cancer patients weighed substantially less than controls, having lost ~20% of their preillness stable weight. Cancer patients had significantly lower serum albumin concentrations and significantly higher plasma fibrinogen concentrations compared with controls. Plasma concentrations of insulin were significantly lower in cancer patients, and interleukin-6 concentrations were significantly higher, compared with controls.

Albumin synthesis rates are shown in Table 2. Changes in albumin synthesis between the fasted and fed states are presented in Fig. 3. There was no difference in albumin synthesis rates between weight-losing...
cancer patients and healthy controls in the fasted [median 14.2 vs. 15.7 g/day (P = 0.30)] or fed [total synthetic rate median 11.3 vs. 13.9 g/day (P = 0.70)] state. Cancer patients and controls showed a similar pattern of significant stimulation of albumin synthesis in the fed state, of medians 29 and 24%, respectively.

Fibrinogen synthesis rates are shown in Table 3. Changes in fibrinogen synthesis between the fed and fasted states are presented in Fig. 4. Cancer patients had substantially and significantly elevated rates of fibrinogen synthesis in both the fasting [median 3.3 vs. 1.1 g/day (P = 0.0019)] and fed [median 4.5 vs. 1.3 g/day (P = 0.0019)] states compared with controls. There was no significant change in fibrinogen synthesis with feeding in control patients (median 14%, P = 0.12). By contrast, there was a statistically significant stimulation of fibrinogen synthesis with feeding in the cancer patients (median 38%, P = 0.012). Although the percent stimulation of fibrinogen synthesis was not significantly different between control and cancer patients, there was a substantial and significant absolute difference in synthesis rate between the two groups [median increase in fibrinogen total synthetic rate in cancer patients 1.24 g/day (range 0.12–6.91) vs. 0.17 g/day (20.16 to 0.57) in controls, P = 0.014].

**DISCUSSION**

The present study has shown that, despite significantly lower circulating albumin concentrations,
weight-losing patients with pancreatic cancer undergo stimulation of albumin synthesis with feeding, and the degree to which this occurs is similar to that of healthy controls. Circulating fibrinogen concentrations are significantly elevated in cancer patients, and in the fasted state, fibrinogen synthesis is elevated significantly above control values. Although healthy individuals show no significant stimulation of fibrinogen synthesis in the fed state, there is a significant increase with feeding in cancer patients.

With use of hepatocytes cultured ex vivo, previous studies have suggested that, during an acute-phase response, the characteristic change in the circulating concentration of positive (increased) and negative (decreased) acute-phase proteins can be ascribed mainly to the effects of proinflammatory cytokines causing an increase or decrease in the synthetic rate of the relevant proteins (9). Although increased synthetic rates in vivo may be the dominant factor influencing the elevated circulating concentrations of positive acute-phase proteins (e.g., fibrinogen, see above), it is unclear whether a reciprocal reduction in the synthesis rate of negative acute-phase reactants (e.g., albumin) can be said to account for their reduced concentration in the circulation. In support of the concept that during an acute-phase response the synthesis rates of negative reactants is reduced, a decrease in hepatocyte albumin mRNA has been noted in the liver of animals given either interleukin-1 or turpentine (a potent stimulus for proinflammatory cytokine production) (1). However, in the present study, despite evidence of an ongoing acute-phase response, albumin synthesis rates were not reduced in either the fasting or fed state. These findings are in keeping with other studies showing that, in the fasting state, albumin synthesis is not reduced in cancer (19) after surgery (5, 42), after vaccination (12), or after stress hormone infusion (28). Moreover, in the fed state, it has been shown that albumin synthesis rates are, in fact, higher than in control subjects after acute head injury (25). The synthesis rate of acute-phase proteins is known to be influenced not only by proinflammatory cytokines but also by factors such as insulin and the counterregulatory hormones (30), both of which can be altered in cancer (20) and other clinical states associated with systemic inflammation. Indeed, an elevated interleukin-6 concentration and reduced insulin concentration were observed in the present study. Moreover, the circulating concentration of plasma proteins is determined not only by synthesis, but also by degradation and redistribution between intravascular and extravascular compartments. The findings of the present study, particularly in relation to albumin, suggest a complex interplay between mediators and pathways rather than the more straightforward view provided by studies on hepatocytes cultured ex vivo.

Albumin synthesis has been shown to be stimulated significantly by feeding in healthy individuals (13, 24), and the administration of protein alone appears to provide a significantly greater stimulus to albumin synthesis than a protein-free feed (10, 11). These findings suggest that, under normal conditions, dietary amino acids can provide a significant stimulus to the synthesis of hepatic export proteins. However, different methods of measurement of protein synthesis and different routes of feeding have been used in the above studies. With use of a primed constant infusion method and intraduodenal feeding, an increase of ~90% in albumin synthesis in the fed state has been documented (13), whereas a flooding dose protocol and oral feeding have suggested an increase of ~20% (24). The present study utilized the flooding dose protocol for measurement of protein synthesis (2, 22, 23) and confirmed the stimulatory effects on albumin synthesis of feeding a mixed diet to healthy subjects, as described by Hunter et al. (24). The flooding dose technique is convenient for both staff and experimental subjects because it allows performance on an outpatient basis. It also allows more certainty of enrichment of the appropriate precursor pool for protein synthesis, as demonstrated previously with a phenylalanine flood (33). However, which method gives the more accurate quantification of the stimulus to hepatic protein synthesis provided by oral feeding cannot be ascertained at present.

Albumin synthesis has not previously been studied in the fed state in cancer patients, but the present study, which uses a flooding dose method and oral feeding, suggests that synthesis is stimulated to a similar degree (~30%) compared with control subjects. Thus an abnormally low albumin synthetic response to feeding is not likely to explain the lower circulating albumin concentrations in these patients. As with previous studies (24), in the present study, the size of the feeding stimulus was determined by measured resting energy expenditure. Because the cancer patients had a significantly lower body weight than controls with a similar resting energy expenditure, the feeding stimulus was larger per unit body weight in the cancer patients [cancer patients median 3.15 kcal/kg (range 2.73–3.37) vs. controls 2.36 kcal/kg (1.92–2.71), \(P = 0.0019\)]. It is possible that this relatively larger meal size may have contributed to the significant stimulation of both albumin and fibrinogen synthesis in the cancer patients, whereas only albumin but not fibrinogen synthesis was significantly stimulated by meal feeding in the healthy controls.

As in the current study, the synthesis of fibrinogen has previously been shown to be elevated up to twofold in the fasting state in cancer patients when compared with controls (33, 34). Moreover, a similar elevation of fibrinogen synthesis has been demonstrated in trauma and burns patients (41), and this is consistent with the concept that, during an acute-phase response, the synthesis rate of positive reactants is generally increased. In healthy subjects, feeding has been shown to have either no effect (10, 13) or a small (20%) stimulatory effect on fibrinogen synthesis (8). This is consistent with the nonsignificant change observed in the healthy volunteers in the current study. In contrast, feeding caused a marked further increase in the fibrinogen synthesis rates of the cancer patients. A similar obser-
vation has been made in head-injured patients (25), and this suggests that, once an acute-phase response has been initiated by factors such as proinflammatory cytokines, the synthesis of positive reactants (e.g., fibrinogen) may be stimulated further by dietary intake.

In the present study, for individual subjects there was no obvious relationship between circulating protein concentrations and rates of synthesis of the relevant protein. In addition, there was no significant change in the concentration of albumin or fibrinogen over the 4-h study period. However, with a fasting fibrinogen fractional synthesis rate of $\sim$1%/h, a fed rate of $\sim$1.25%/h, and a fractional breakdown rate of $\geq$1%, one would not expect an increase in fibrinogen concentration within the precision of the assay. With its larger pool and slower turnover, any change in albumin concentration would be even smaller.

Patients with advanced cancer exhibit marked loss of weight, a large component of which is skeletal muscle (20). This weight loss is particularly marked in the presence of an acute-phase protein response (17, 38, 45), which appears to contribute to worsened survival (7, 18). If the synthesis of negative acute-phase proteins such as albumin is not suppressed during the acute-phase response, and the synthesis of positive acute-phase proteins is markedly elevated (when patients are in the fasted or fed state), then the demand for amino acids to manufacture these proteins may accelerate the breakdown of labile reserves (e.g., skeletal muscle), especially if nutritional intake is inadequate. It has been suggested that, during an acute-phase response, the differences in the amino acid composition of acute-phase proteins and skeletal muscle may further increase the need for muscle breakdown (34). To represent a continuing drain on amino acid resources, recycling of amino acids from acute-phase proteins would have to be inefficient. At present, however, it is difficult to estimate such an effect, because there have been no measures of amino acid return from acute-phase protein breakdown in humans. Independent of these arguments, the synthesis and recycling of protein require energy, and this may contribute to the hypermetabolism observed along with the acute-phase response (17, 38, 45) in cancer patients. This might explain, in part, the association between the acute-phase response and accelerated wasting in these individuals.

Albumin may account for 5–7% of whole body protein synthesis in the fasted state in healthy subjects (13). This may increase to $\geq$10% in the fed state (11, 13). It has been estimated that synthesis of all acute-phase proteins may account for as much as 30% of total protein synthesis during infection in the fasted state (44). Although this total may be less in a chronic inflammatory state such as cancer, with the stimulation of both positive and negative acute-phase protein synthesis by feeding, acute-phase protein synthesis may represent a substantial proportion of total body protein synthesis. Controlled studies of advanced cancer patients have failed to show any nutritional benefit from the consumption of apparently sufficient nutrients compared with a reduced borderline intake (16, 31). The present study suggests that a proportion of dietary amino acids may be used for accelerated acute-phase protein synthesis and thus may not be available for peripheral tissue anabolism. In addition, dietary amino acids delivered first to the liver via the portal system and used for acute-phase protein production will result in a particular pattern of consumption of essential amino acids. The remaining amino acid mixture may thus be unbalanced, thereby further inhibiting efficient skeletal muscle anabolism. Taken together, these findings suggest that downregulation of the acute-phase response may be of clinical benefit when nutritional support of the wasted cancer patient is attempted.

Protein synthesis rates are only one factor in determining circulating concentrations, but at present there is limited information on acute-phase protein breakdown and transcapillary escape. There is indirect evidence of increased fibrinogen breakdown in cancer, with elevated concentrations of fibrin degradation products (15, 26); however, the role of fibrinogen in coagulation complicates the relevance of this finding. Transcapillary escape has been shown to occur at an elevated rate in weight-losing cancer patients and has been suggested to contribute to the hypoalbuminemia of the acute-phase response (21). However, lymphatic return must be similarly increased, because there is no relationship between transcapillary escape and albumin concentration (1) and no change in the intravascular albumin pool with elevated transcapillary escape (4). Limited work has suggested a moderate increase in albumin breakdown in cancer patients, although it is not clear whether the patients studied were losing weight (36). It has also been suggested that the tumor itself may consume albumin (39), although the significance of this phenomenon in human disease remains unclear. Further study of acute-phase protein breakdown in weight-losing cancer patients is required.

In summary, the present study has demonstrated that the increase in albumin synthesis with feeding in cancer patients is identical to that in controls and that cancer patients exhibit a substantial stimulation of already elevated fibrinogen synthesis in the fed state. These findings suggest that acute-phase protein synthesis may represent a significant drain on the protein reserves of the body and may therefore provide a mechanism whereby the acute-phase protein response is associated with acceleration of lean tissue loss and worsened survival in cancer patients.

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REFERENCES


