Fibrinogen Synthesis Is Elevated in Fasting Cancer Patients with an Acute Phase Response

Tom Preston, Christine Slater, Donald C. McMillan, J. Stuart Falconer, Alan Shenkin and Kenneth C. H. Fearon

Isotope Biochemistry Laboratory, SURRC, East Kilbride, Glasgow G75 0QF, UK; *University Department of Surgery, Royal Infirmary, Glasgow G31 2ER, UK; †University Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW, UK; **Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool L69 3GA, UK

ABSTRACT The unusual amino acid composition of acute phase proteins may be relevant to our understanding of the mechanism of tissue wasting in chronic inflammatory disease. During periods in which demand for amino acids outstrips dietary supply, skeletal muscle protein may be mobilized to meet this demand. An imbalance in the amino acid composition of these proteins may thus be detrimental to the body's nitrogen economy. To address this problem, we have measured the synthetic rate of fibrinogen (perhaps the major acute phase protein) and plasma amino acid profiles in a group of patients with adenocarcinoma of the pancreas and an ongoing inflammatory response. Fibrinogen synthesis was measured after an overnight fast, using a flooding dose of $^{2H_5}$-phenylalanine. The fractional rate of fibrinogen synthesis was significantly elevated in the cancer group compared with healthy controls [39.3 (20.0-49.9) and 21.9 (13.2-37.7) %/d, respectively; median (range), P < 0.05]. The absolute rate of fibrinogen synthesis was also elevated [84 (33-143) and 26 (15-43) mg/(kg·d), respectively; median (range), P < 0.01]. We calculated that, in cancer patients with anorexia-cachexia (i.e., documented ongoing weight loss in the absence of any other obvious infective or inflammatory cause), the absolute rate of fibrinogen synthesis from skeletal muscle reserves may be wasted to synthesise 1 g fibrinogen. Interpretation of the observation that circulating free tryptophan concentrations were significantly reduced in the cancer patients will have to await flux measurements. The metabolic changes accompanying the inflammatory response suggest that down-regulation of this process may be beneficial.


KEY WORDS: amino acids, fibrinogen,$^{2H_5}$-phenylalanine, pancreatic cancer, protein synthesis rates, humans

Restoration of lean body mass in patients with gastrointestinal malignancies has proven extremely difficult despite extensive investigations over the last two decades (Fearon 1992, Heys et al. 1992). Chronic inflammation, evidenced by an ongoing acute phase response, has been associated with the prevention of such restoration (McMillan et al. 1994b and 1994c). The unusual amino acid composition of acute phase proteins may constitute part of the mechanism. Grimble (1990) suggested that acute phase protein synthesis may introduce an additional demand for the metabolically interrelated amino acids glycine, serine, methionine and cysteine. Body composition studies (Preston et al. 1987) have shown that skeletal muscle protein is the most likely reserve of amino acids that are mobilized in the anorectic-cachectic patient. Reeds et al. (1994) argued that, because acute phase proteins are not rich in sulfur amino acids, it is the supply of aromatic amino acids (tryptophan, phenylalanine and tyrosine) from skeletal muscle that most likely limits acute phase protein production.

There have been a number of studies that have compared circulating amino acid concentrations in healthy subjects and cancer patients (Bennegard et al. 1984, Clarke et al. 1978, Levin et al. 1983, Norton et al. 1985). However, such studies have yielded conflicting results and comparison of findings is difficult because there has been no apparent attempt to relate these to the presence of an acute phase response. Furthermore,
the potential effect of acute phase protein synthesis on skeletal muscle protein reserves in cancer patients is unclear because data on the magnitude of acute phase protein synthesis are scarce. Fibrinogen is one of the most important acute phase proteins, accounting for ~10% of liver export protein synthesis in normal fasting subjects (McMillan et al. 1996), a figure that appears to be significantly greater in cancer patients (Stein et al. 1978b).

A number of procedures for measuring protein synthesis in the clinical situation have been described. The flooding dose protocol (Garlick et al. 1989) has a number of practical advantages, including being well accepted by patients and clinical staff. Despite this, its use in humans has been limited. In particular, there are few data to establish protocols for repeat measurements or data on the reproducibility of the measured rates. With respect to the measurement of fibrinogen synthesis, no such data have been reported.

The aim of this study was to examine the fibrinogen synthesis rates and amino acid concentrations in normal subjects and in cancer patients with an acute phase response. Experience of the use of the flooding dose technique for measuring the fibrinogen synthetic rate is also reported. The importance of these findings is discussed in relation to the body's nitrogen economy.

SUBJECTS AND METHODS

Patients. Six patients with histologically proven adenocarcinoma of the pancreas were entered into the study. None of these patients had undergone surgery in the preceding 2 mo, but all had an ongoing inflammatory response and had a weight loss of >10% of their pre-illness weight. Serum C-reactive protein (CRP) was >10 mg/L; other obvious infective or inflammatory causes were not apparent. Seven patients, who had been admitted to hospital for minor procedures (e.g., inguinal hernia repair), were also studied as normal subjects. These subjects were weight stable, had normal biochemical liver function tests and had no evidence of an acute phase response (CRP <5 mg/L) or any other metabolic or endocrine disorder. None of the patients were jaundiced, pyrexial or had clinical or radiological evidence of infection or were severely anemic. The study was approved by the local ethical committee. All patients were informed of the purpose and procedure of the study and all gave written informed consent.

Experimental design. After an overnight fast, a venous catheter was inserted into each antecubital fossa. One catheter was used for the administration of a bolus solution of H\textsubscript{3}-phenylalanine, over 10 min (3.5 g of phenylalanine, 10 mol % excess phenyl-H\textsubscript{3}-l-phenylalanine, 2 g/L in saline, Tracer Technologies, Somerville, MA). The catheter in the opposite arm was used to sample 10 mL blood before and 10, 20, 40, 60, 80 and 120 min after receiving the H\textsubscript{3}-phenylalanine dose, for preparation and storage as plasma and serum (−20°C). In one normal volunteer, the protocol was repeated with additional blood samples taken at 6, 12 and 24 h and 2, 3, 4, 7, 10 and 22 d. The blood sample collected before the “flooding” dose was analyzed for baseline phenylalanine enrichment and albumin, fibrinogen, C-reactive protein and amino acid concentrations. Blood samples taken at the time points after the flooding dose were analyzed for H\textsubscript{3}-phenylalanine enrichment in fibrinogen and in the plasma free phenylalanine pool. Plasma volume was predicted from body weight and height (Retzlaff et al. 1969). Because the protocol was to be performed in fasting subjects, no dietary history or nutrient intake was recorded for the period before the investigation.

Analytical methods. Albumin concentrations were measured using the bromocresol green method (Doumas et al. 1971) on a Technicon RA-1000 automated analyzer (Technicon Instruments, Tarrytown, NY).

C-Reactive protein was measured by Fluorescence Polarization Immunoassay by using an Abbott TDX analyzer and Abbott reagents (Abbott Laboratories, Abbott Park, IL). The limit of detection of this assay is a C-reactive protein concentration of 5 mg/L. Within-assay variability was <5%. Between assay variability was <5%. The mid-range of the assay was 50–150 mg/L.

Fibrinogen concentration in plasma sampled in an EDTA-treated tube was measured with a turbidometric assay of carefully controlled fibrinogen precipitation using ammonium sulfate (Macart et al. 1989). Within-assay variability was <5%. Between assay variability was <10%. The mid-range of the assay was 2.5–4.5 g/L. The limit of detection was 1.0 g/L.

The concentration of free amino acids in the plasma were determined by HPLC, using an ASTED dialysis system for deproteinization (Anachem, Luton, UK) and orthophthalaldehyde for quantitation using a fluorescence detector (Peek et al. 1988).

Sample preparation and isotope analysis. The study protocol involved the measurement of H\textsubscript{3}-phenylalanine enrichment in the plasma free phenylalanine pool and in plasma fibrinogen. For free phenylalanine analysis, 1.5 mL plasma was diluted with 5 mL distilled deionized water and cycloleucine (250 nmol) was added as an internal standard. Samples were then deproteinized by ultrafiltration (25,000 MW cut-off Centrifree cone, Amicon, Gloucestershire, UK), acidified and the amino acids purified by cation exchange. H\textsubscript{3}-Phenylalanine enrichment was measured by gas chromatography-mass spectrometry as its tert-butyldimethylsilyl derivative (Slater et al. 1995). For fibrinogen analysis, the sample in the ultrafiltration cone was then washed three times with 5 mL distilled deionized water to remove traces of free phenylalanine. Fibrinogen was then extracted by diluting the plasma proteins to 20 mL with saline and 0.5 mL calcium chloride (0.5 mol/L), adding 15 units of human (albumin-free) thrombin (Sigma-Aldrich, Poole, UK); after 10 min, the fibrin was collected on a glass rod. The fibrin was then hydrolyzed under vacuum at 145°C for 4 h with 6 mol/L HCl, and its H\textsubscript{3}-phenylalanine enrichment was measured as previously described (Slater et al. 1995). The remaining serum sample could then be used for analysis of the synthetic rates of other plasma proteins (McMillan et al. 1996).

Calculations. Fractional synthesis rates of fibrinogen were calculated by dividing the rate of change of H\textsubscript{3}-phenylalanine enrichment of fibrinogen by the area under the curve of precursor enrichment vs. time as described by Ballmer et al. (1990). Similarly, the secretion times for fibrinogen were calculated as described by Ballmer et al. (1990).

Statistics. Data are presented as medians and ranges. Where appropriate, data were tested for statistical significance using the Mann-Whitney U-test (Minitab, State College, PA). Correlations between appropriate variables were tested using Spearman’s Rank Correlation (Minitab).

RESULTS

The clinical characteristics of the control and cancer patients are shown in Table 1. The normal subject and cancer patient groups did not differ in terms of age and body mass index. There were significantly lower circulating albumin concentrations and significantly greater fibrinogen and C-reactive protein concentrations in the cancer patients compared with the control group (P < 0.01).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal subjects</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
</tr>
<tr>
<td>Age, y</td>
<td>60 (44–69)</td>
</tr>
<tr>
<td>M:F</td>
<td>5:2</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>23 (21–29)</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>43 (40–46)</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.0 (2.4–3.4)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

\(^1\) Values are medians (ranges); BMI, body mass index; ** P < 0.01.
**TABLE 2**

**Fibrinogen synthesis in fasting healthy subjects and cancer patients**

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><strong>FSR, %/d</strong></td>
<td>21.9 (13.2–37.7)</td>
<td>39.3 (20.0–49.9)*</td>
</tr>
<tr>
<td><strong>ST, m</strong></td>
<td>32 (18–43)</td>
<td>32 (23–40)</td>
</tr>
<tr>
<td><strong>PV, mL</strong></td>
<td>2876 (2116–3510)</td>
<td>2602 (1824–2759)</td>
</tr>
<tr>
<td><strong>IFM, g</strong></td>
<td>8.0 (6.6–12.5)</td>
<td>11.4 (9.7–15.5)*</td>
</tr>
<tr>
<td><strong>ASR, mg/(kg·d)</strong></td>
<td>26 (15–43)</td>
<td>84 (33–143)**</td>
</tr>
<tr>
<td><strong>TSR, g/d</strong></td>
<td>2.3 (0.9–2.5)</td>
<td>4.7 (1.9–7.7)*</td>
</tr>
</tbody>
</table>

1 Values are medians (ranges).
2 FSR, fractional synthetic rate; ST, secretion time; PV, plasma volume; IFM, intravascular fibrinogen mass; ASR, absolute synthetic rate; TSR, total synthetic rate; *P < 0.01, *P < 0.05.

Total, fractional and absolute fibrinogen synthesis rates were significantly greater in the cancer group compared with the control group (Table 2; P < 0.05). In contrast, there was no significant difference in the fibrinogen secretion times of the two groups. The intravascular fibrinogen mass (the product of its concentration and plasma volume) was significantly greater in the cancer group compared with the healthy controls (P < 0.05). When both normal subjects and cancer patients were combined, there were significant rank correlations between circulating fibrinogen concentration and fibrinogen fractional synthetic rate (r = 0.80, P < 0.01), absolute synthetic rate (r = 0.90, P < 0.001) and fibrinogen total synthetic rate (r = 0.91, P < 0.001). Furthermore, there was significant rank correlation between C-reactive protein concentration and fibrinogen absolute synthetic rate (r = 0.87; P < 0.01).

The serum amino acid concentrations of the control and cancer patients, measured before the start of the flooding phenylalanine dose, are given in Table 3. In the cancer patients, there were significantly lower circulating concentrations of tryptophan (P < 0.01) and higher concentrations of citrulline (P < 0.01), cysteine and glycine (P < 0.05) than in the control group. The observation that circulating tryptophan concentrations were significantly reduced in the cancer patients has since been confirmed by an independent stable-isotope-dilution approach (Preston, T., unpublished results). Circulating concentrations of phenylalanine were marginally elevated (P = 0.09), and those of tyrosine, glutamate, arginine, ornithine, methionine, serine and other amino acids were not significantly different between the two groups. When both normal subjects and cancer patients (n = 12) were combined, there were significant rank correlations between the absolute synthetic rates of fibrinogen and circulating amino acid concentrations as follows: cysteine (r = 0.79, P < 0.01), citrulline (r = 0.68, P < 0.05), tryptophan (r = −0.60, P < 0.05) and glycine (r = 0.53, P < 0.05).

The relationship between phenylalanine enrichment in the plasma and fibrinogen samples was followed in a healthy volunteer for 22 d and is shown in Figure 1. There was no significant difference between phenylalanine enrichment in the plasma free pool and that of fibrinogen-bound phenylalanine from 7 h onwards (mean bound enrichment as a percentage of that of the plasma free pool was 101.3 ± 3.3%, SEM; n = 7, from 7 h to 22 d). The intersubject variability (CV within the group of cancer patients) was 27% for fibrinogen fractional synthetic rate. Repeatability of measuring $^{15}$N-glycine as tracer has been reported previously by Stein et al. (1978b). These authors reported the fractional synthetic rate of fibrinogen to be ~15%/d in healthy subjects and 26%/d in cancer patients. Although these values are similar to those from this study (21.9%/d and 39.3%/d, respectively), they are consistently lower. The earlier study had a number of design and methodological limitations. A

**DISCUSSION**

It has been assumed that elevated fibrinogen concentrations frequently observed in cancer patients (Dvorak 1987) that are associated with an ongoing inflammatory response (Preston et al. 1987 and 1995) are the result of increased synthetic rate, rather than reduced breakdown or reduced pool size. However, confirmation of this hypothesis and determination of the magnitude of any increase have only once been attempted previously (Stein et al. 1978a). In this study, we have determined the increase in fibrinogen fractional and absolute synthetic rate to be ~79 and 220%, respectively, compared with healthy subjects. Furthermore, we have demonstrated that there are significant correlations between the fibrinogen kinetic parameters (i.e., fractional and absolute synthetic rate) and its circulating concentration. This would support the hypothesis that increased fibrinogen synthesis largely determines the observed increase in circulating concentrations. Cytokines, especially interleukin-6 (IL-6), are thought to be responsible for the mediation of enhanced acute phase protein synthesis by the hepatocytes (Heinrich et al. 1990). Indeed, we have previously reported the association of elevated circulating IL-6 with the acute phase response in patients with cancer (Fearon et al. 1991, Scott et al. 1996).

The direct measurement of fibrinogen synthesis in cancer patients using $^{15}$N-glycine as tracer has been reported previously by Stein et al. (1978b). These authors reported the fractional synthetic rate of fibrinogen to be ~15%/d in healthy subjects and 26%/d in cancer patients. Although these values are similar to those from this study (21.9%/d and 39.3%/d, respectively), they are consistently lower. The earlier study had a number of design and methodological limitations. A

**TABLE 3**

**Serum free amino acid concentrations in fasting healthy subjects and cancer patients**

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><strong>Aspartate</strong></td>
<td>16.8 (10.9–21.8)</td>
<td>19.7 (14.9–26.5)</td>
</tr>
<tr>
<td>Glutamate</td>
<td>343.2 (100.4–473.9)</td>
<td>409.1 (289.9–477.0)</td>
</tr>
<tr>
<td>Cysteine</td>
<td>138.2 (107.5–162.1)</td>
<td>193.4 (137.2–266.1)*</td>
</tr>
<tr>
<td>Serine</td>
<td>127.1 (96.5–169.7)</td>
<td>118.1 (91.1–164.0)</td>
</tr>
<tr>
<td>Histidine</td>
<td>71.5 (40.6–90.9)</td>
<td>73.9 (58.8–113.2)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>410.5 (215.0–560.3)</td>
<td>412.5 (146.0–506.0)</td>
</tr>
<tr>
<td>Glycine</td>
<td>227.5 (185.0–329.7)</td>
<td>382.1 (251.4–440.2)*</td>
</tr>
<tr>
<td>Threonine</td>
<td>119.6 (87.0–160.0)</td>
<td>141.0 (99.0–182.3)</td>
</tr>
<tr>
<td>Citrulline</td>
<td>38.2 (28.8–58.8)</td>
<td>133.0 (73.9–213.8)**</td>
</tr>
<tr>
<td>Arginine</td>
<td>102.9 (56.0–142.4)</td>
<td>148.7 (50.3–216.8)</td>
</tr>
<tr>
<td>Alanine</td>
<td>338.3 (234.4–405.9)</td>
<td>334.2 (279.8–401.1)</td>
</tr>
<tr>
<td>Taurine</td>
<td>110.2 (46.6–169.7)</td>
<td>108.4 (94.8–164.7)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>67.3 (53.0–102.0)</td>
<td>59.9 (33.1–63.7)</td>
</tr>
<tr>
<td>Valine</td>
<td>213.5 (142.0–253.2)</td>
<td>195.5 (137.4–276.1)</td>
</tr>
<tr>
<td>Methionine</td>
<td>28.9 (23.1–38.0)</td>
<td>27.9 (23.8–46.8)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>90.0 (62.5–98.4)</td>
<td>44.1 (33.4–63.2)**</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>65.5 (52.6–80.1)</td>
<td>77.3 (65.4–90.1)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>81.4 (44.8–103.3)</td>
<td>82.6 (54.0–116.1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>139.1 (81.4–169.6)</td>
<td>136.5 (78.8–171.6)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>78.9 (52.2–101.8)</td>
<td>78.4 (57.2–100.6)</td>
</tr>
<tr>
<td>Lysine</td>
<td>153.4 (84.8–177.5)</td>
<td>141.8 (89.7–183.3)</td>
</tr>
</tbody>
</table>

1 Values are medians (ranges); *P < 0.05, **P < 0.01.
heterogeneous group of cancer patients was studied and the
presence of an acute phase response was not established. Use
of plasma total α-amino acid $^{15}$N enrichment as the precursor
after administration of $^{15}$N-glycine, although measuring fib-
ринogen-bound α-amino acid $^{15}$N enrichment, ignores any
differences in $^{15}$N enrichment between plasma and the intralo-
hepatic free amino acid pool and also ignores differences in amino
acid composition between the plasma precursor pool and fibrino-
gen. Although debate continues concerning the site of the precursor pool for liver export protein synthesis (Connell et al. 1997), the earlier approach may have overestimated precursor amino acid enrichment and have led to an underesti-
mate of fibrinogen synthetic rate. The flooding dose protocol
used in this study gives greater confidence in the assumption
that the free amino acid pool at the site of protein synthesis is in isotopic equilibrium with that measured in the plasma.

Over the past five years, use of the flooding dose protocol
to measure rates of protein synthesis has increased. In our
experience, the short protocol has proven convenient in the
clinical setting. The flooding dose was introduced to overcome
uncertainty in the enrichment of the precursor pool. However,
considerable debate remains concerning whether the large
dose of an essential amino acid used in the flooding protocol
can stimulate protein synthesis. This is especially true in the
case of skeletal muscle protein (Rennie et al. 1994), which
may incorporate more tracer in response to a transient increase in
insulin concentrations or to elevated intracellular concentra-
tions of essential amino acids. To our knowledge, there is
no evidence to suggest that this may be the case with acute
phase proteins whose synthesis seems to be regulated largely
by cytokines (IL-6, Heinrich et al. 1990). Furthermore, this
study was designed as a comparative study. When measuring
liver export protein synthesis, plasma free tracer enrichment
is assumed to reflect precursor enrichment. In this study, plasma free phenylalanine came into isotopic equilibrium with
fibrinogen-bound phenylalanine within 7 h and remained so
for 22 d (mean fibrinogen-bound $^{3}$H$_{5}$-phenylalanine enrich-
ment was 101% of that in the plasma free pool between 7 h
and 22 d; see Fig. 1), indirectly validating the equivalence of
the plasma and the precursor pool within the flooding dose
protocol.

Grimble (1990) suggested that there may be increased
demand for the amino acids glycine, serine, methionine and
cysteine in fasting subjects with an acute phase protein re-
response. In this study, an increase in fibrinogen synthesis in the
cancer group of ~2.4 g/d (the mean change in fibrinogen total
synthetic rate) was associated with a significant increase in
circulating concentrations of glycine and cysteine with no
change in serine or methionine concentrations. As noted by
Reeds et al. (1994), fibrinogen is not particularly rich in sulfur
amino acids; however, it is relatively rich in glycine and serine.
De novo synthesis of these amino acids via glutamate transami-
nation must compensate, but failure to meet this extra demand
may lead to conditional essentiality of these amino acids
(Slater et al. 1996). With all subjects included, there were
significant rank correlations between the concentrations of
glycine and cysteine and the absolute fibrinogen synthetic
rates. It can be estimated (assuming that albumin and fibrino-
gen together account for 80–90% of liver export protein syn-
thesis) that there was an overall increase of ~35% in the
liver's requirement for these interrelated amino acids. It is
therefore of interest that despite a likely increase in demand,
the circulating concentrations of these amino acids were main-
tained or increased and would suggest that there was a consid-
erable increase in the flux of these amino acids and in particu-
lar, glycine and cysteine. It is not known whether this could be
related to increased degradation of albumin, a protein rich in
cysteine whose synthesis is maintained at normal levels while
its concentration is significantly lowered (Fearon et al. 1998).
A large increase in glycine flux may compromise whole-body
protein turnover measurements when using $^{15}$N-glycine as
tracer in cancer patients with an acute phase protein response,
although this tracer may be a sensitive indicator of the phe-
nomenon. Indeed, we have previously questioned the accuracy
of such measurements (McMillan et al. 1994a, Preston et al.
1995). The finding that circulating C-reactive protein concen-
trations rank correlate significantly with fibrinogen absolute
synthetic rate parallels our previous observation of the correla-

**FIGURE 1** Plasma free phenylalanine and fibrinogen-bound phenylalanine over a period of 22 d, after a flooding dose of $^{3}$H$_{5}$-phenylalanine in
a healthy volunteer.
of C-reactive protein concentration and whole-body protein synthesis (Preston et al. 1995). These findings support our assertion that rates of whole-body protein synthesis, measured using \(^{15}\)N-glycine as tracer, tend to be overestimated in subjects with an acute phase protein response.

Reeds et al. (1994) argued that increased demands for amino acids from immune system proliferation and glutathione synthesis, in addition to the acute phase protein response, are also likely to contribute to muscle protein breakdown. Park et al. (1994) studied peripheral blood lymphocyte protein synthesis in patients with colorectal cancer, using a flooding dose of \(^{13}\)C-leucine, and concluded that although lymphocyte protein fractional synthetic rate may be \(9%/d\), posing a similar amino acid demand to that of constitutive liver proteins, lymphocyte protein FSR of cancer patients was significantly reduced. Glutathione is formed by condensation of glutamate, cysteine and glycine. Cysteine can be supplied from the essential amino acid methionine, provided this is available. In this study, circulating concentrations of cysteine and glycine were significantly greater in the cancer patients, whereas those of glutamate and methionine were unaffected, providing little evidence for a failure of their supply. However, interpretation of changes in circulating amino acid concentration is problematic, and the current observations would have to be supported by flux measurements before any conclusions can be drawn.

It has been postulated that the negative nitrogen balance that occurs as part of the acute phase response is due to the difference between the amino acid composition of acute phase proteins and skeletal muscle protein, because amino acids that are surplus to requirements for protein synthesis are oxidized (Reeds et al. 1994). Such calculations highlight the limited supply of aromatic amino acids, especially phenylalanine, in such conditions. This calculation was based on the increase of a number of acute phase proteins after acute injury (surgery), whereas fibrinogen appears to be the major acute phase protein in chronic inflammation (cancer). By using acute phase protein changes immediately after surgery, Reeds et al. (1994) calculated that C-reactive protein, \(\alpha_1\)-antitrypsin and fibrinogen contributed 30, 24 and 24%, respectively, to total acute phase protein synthesis. In contrast, when analyzing longitudinal concentration changes of acute phase proteins after intervention with anti-inflammatory agents in cancer patients (Preston et al. 1995), we have calculated that these three proteins contribute 1, 25 and 59%, respectively, to total acute phase protein synthesis. Fibrinogen thus may be the most important acute phase protein in chronic inflammation, possibly contributing 15% of total liver export protein. With the use of our fibrinogen synthesis data, we have calculated that in fasting subjects, \(~7.9\ g\) of skeletal muscle protein (36 g skeletal muscle tissue) would be required daily to support the synthesis of an extra 3 g of fibrinogen (Slater et al. 1996). This would be a major contribution to tissue wasting if it continued unabated. This calculation showed that tryptophan (followed by serine and then tyrosine) was the most limiting amino acid. It is of interest that a negative correlation was observed between circulating concentrations of tryptophan and the absolute synthetic rate of fibrinogen and that tryptophan concentrations were reduced the most compared with the control group. This would appear to support the observation that tryptophan can be a limiting amino acid in cancer patients with an acute phase protein response.

To represent a continued drain on scarce amino acid reserves, synthesis of fibrinogen and other acute phase reactants would have to be inefficiently recycled. If there are few quantitative data on acute phase protein synthesis, there is even less information on their breakdown rate. This is due to the greater difficulty in establishing potential sites and measuring the extent of the latter process. Indirect evidence for increased turnover, such as elevated circulating concentrations of fibrin degradation products, has been observed (Dvorak 1987, McMillan et al. 1994a), although there has been no measure of the rate of amino acid return from fibrinolysis. Should recycling be incomplete, or separated temporarily or spatially from synthesis, then acute phase protein synthesis could promote tissue wasting and continued negative nitrogen balance in the anorexia-cachexia patient.

Waterlow (1991) analyzed the data of Fleck et al. (1985) and argued that at the peak of the catabolic response to infection, acute phase protein production could amount to 30% of the total body protein synthesis. Acute phase protein synthesis is unlikely to be of this magnitude in chronic inflammation, but our estimates show that it may contribute 15–25% to liver protein synthesis or 3–5% to whole-body protein synthesis. The realization that an imbalance in the amino acid composition between the supply (skeletal muscle) and demand (acute phase proteins) can exacerbate the negative nitrogen balance may have a profound effect on the nitrogen economy of the cachectic cancer patient with an ongoing acute phase protein response. Clearly, however, for this to be the case the patient would have to be profoundly anorectic.

We have for the first time quantified fibrinogen synthesis in fasting cancer patients with an acute phase response and related this to circulating amino acid concentrations. Although interpretation of the relevance of changes in circulating amino acid concentrations is less obvious than that of direct flux measurements, this study supports the hypothesis that aromatic amino acids, especially tryptophan, can be limiting in such patients. Targeted nutritional support, to spare skeletal muscle protein reserves, may prove most effective when combined with anti-inflammatory intervention to reduce the demand for these amino acids.

ACKNOWLEDGMENTS

We thank C.S. McArdle, University Department of Surgery, Royal Infirmary, Glasgow and D.C. Carter, University Department of Surgery, Royal Infirmary, Edinburgh for their support throughout this work. We thank J.A. Ross, University Department of Surgery, Royal Infirmary, Edinburgh for his help and advice on biochemical assays and C. Lane, Department of Clinical Chemistry, University of Liverpool for his help with amino acid analysis.

LITERATURE CITED


