Effects of oral meal feeding on whole body protein breakdown and protein synthesis in cachectic pancreatic cancer patients

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Abstract

Background Pancreatic cancer is often accompanied by cachexia, a syndrome of severe weight loss and muscle wasting. A suboptimal response to nutritional support may further aggravate cachexia, yet the influence of nutrition on protein kinetics in cachectic patients is poorly understood.

Methods Eight cachectic pancreatic cancer patients and seven control patients received a primed continuous intravenous infusion of L-[ring-2H5]phenylalanine and L-[3,3-2H2]tyrosine for 8 h and ingested sips of water with L-[1-13C]phenylalanine every 30 min. After 4 h, oral feeding was started. Whole body protein breakdown, protein synthesis, and net protein balance were calculated. Results are given as median with interquartile range.

Results Baseline protein breakdown and protein synthesis were higher in cachectic patients compared with the controls (breakdown: 67.1 (48.1–79.6) vs. 45.8 (42.6–46.3) μmol/kg lean body mass/h, P = 0.049; and synthesis: 63.0 (44.3–75.6) vs. 41.8 (37.6–42.5) μmol/kg lean body mass/h, P = 0.021). During feeding, protein breakdown decreased significantly to 45.5 (26.9–51.1) μmol/kg lean body mass/h (P = 0.012) in the cachexia group and to 33.7 (17.4–37.1) μmol/kg lean body mass/h (P = 0.018) in the control group. Protein synthesis was not affected by feeding in cachectic patients: 58.4 (46.5–76.1) μmol/kg lean body mass/h, but was stimulated in controls: 47.9 (41.8–56.7) μmol/kg lean body mass/h (P = 0.018). Both groups showed a comparable positive net protein balance during feeding: cachexia: 19.7 (13.1–23.7) and control: 16.3 (13.6–25.4) μmol/kg lean body mass/h (P = 0.908).

Conclusion Cachectic pancreatic cancer patients have a higher basal protein turnover. Both cachectic patients and controls show a comparable protein anabolism during feeding, albeit through a different pattern of protein kinetics. In cachectic patients, this is primarily related to reduced protein breakdown, whereas in controls, both protein breakdown and protein synthesis alterations are involved.

Keywords Cancer cachexia; Pancreatic cancer; Nutrition; Protein synthesis; Protein breakdown; Anabolic resistance

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Background Pancreatic cancer is the eighth leading cause of cancer deaths in men and the ninth in women with 138 100 and 127 900 annual deaths worldwide, respectively.1 Cancer cachexia, a complex syndrome characterized by weight loss and muscle wasting, is a major problem of pancreatic cancer and greatly decreases survival and quality of life.2,3 It is responsible for more than 80% of pancreatic cancer related deaths.4 The exact pathogenesis of cancer cachexia is not fully understood, but systemic
inflammation, a negative energy balance, malabsorption, and anorexia play an important role. Metabolic changes are most profound in protein metabolism, peripheral tissues furnishing nitrogenous and carbon substrate to sustain the acute phase response, and tumour growth. This leads to net negative peripheral protein metabolism, presenting as muscle wasting.

A key feature of cancer cachexia is that it cannot be fully reversed by regularly used nutritional support. This lack of response indicates that nutrient handling and especially protein metabolism are altered in cachectic patients. This might be due to a failing anabolic response (anabolic resistance), increased breakdown, or a reprioritisation of nitrogen economy away from peripheral tissues (muscle) towards increased hepatic production of acute phase proteins. The latter theory was studied previously by our group in cachectic pancreatic cancer patients with an acute phase response. We found normal albumin previously by our group in cachectic pancreatic cancer patients by using established methods of primed continuous feeding on whole body protein turnover, protein synthesis, and protein breakdown in cachectic cancer patients. Moreover, feeding in such patients was associated with a marked increase in fibrogen synthesis, supporting the theory of reprioritisation. However, the mechanisms involved remain unclear, and studies providing information on whole body protein synthesis and breakdown and their response on feeding in cachectic cancer patients are lacking.

This study aimed to investigate the effect of protein meal feeding on whole body protein turnover, protein synthesis, and protein breakdown in cachectic pancreatic cancer patients compared with non-oncologic surgical control patients by using established methods of primed continuous infusions of stable isotope-labelled amino acids.

Materials and methods

Subjects

To investigate the effect of enteral feeding on protein metabolism in cancer cachexia, cachectic patients with pancreatic head cancer were studied. Inclusion criteria were unresectable biopsy-proven, primary or recurrent pancreatic cancer, and cachexia. Cancer cachexia was defined as weight loss of >5% in 6 months, according to international consensus. Age-matched and sex-matched weight-stable patients admitted for surgery for benign disease (cholecystectomy or inguinal hernia repair) were included as a control group. Exclusion criteria for both groups were radiotherapy or chemotherapy, surgery in the month preceding the study, endocrine or metabolic disorders, fever, anaemia, and steroid use. Height, bodyweight, bodyweight loss, triceps skinfold thickness, mid-arm muscle circumference, and handgrip strength were measured on admission. Body weight loss was estimated by subtracting current measured body weight from reported normal body weight 6 months ago. For handgrip strength, the highest grip strength was recorded after measuring three times for both hands. Laboratory measurements that were taken at admission as part of standard care were recorded. Body composition was assessed by multiple frequency bioelectrical impedance measurements at 5 and 200 kHz with either a four-terminal RJL BIA 101 (RJL Systems, Detroit, USA) or the Bodystat Dualscan 2005 (Bodystat Ltd., UK). Since the study protocol is demanding, especially for severely ill patients, a sample size of eight cachectic patients and seven control patients was chosen. This sample size was proven by our research group to provide adequate (patho) physiological data in tracer studies in the past.

Study protocol

Patients and healthy subjects were studied after an overnight fast. Starting at 8 a.m., two cannulas were inserted: one into the antecubital vein for stable isotope infusion and one into the dorsal hand vein in the contralateral arm for arterialized blood sampling using a custom made heated box (50°C, J. Cambell and Dr. H. Brash, Department of Medical Physics, Royal Infirmary of Edinburgh). After a baseline blood sample had been taken for measurement of background isotope enrichment and C-Reactive-Protein (CRP), patients received a priming dose of L-[1-13C]phenylalanine (2.3 μmol/kg), L-[ring-2H5]phenylalanine (2.2 μmol/kg), L-[3,3-2H2]tyrosine (0.9 μmol/kg), and L-[ring-2H4]tyrosine (0.3 μmol/kg) followed by a continuous intravenous infusion of L-[ring-2H4]phenylalanine (3.5 μmol/kg/h) and L-[3,3-2H2]tyrosine (1.3 μmol/kg/h). Patients also received a primed continuous infusion of L-[2H2]phenylalanine for a separate research question not discussed in this paper. Subsequent arterialized blood samples were drawn hourly. For the first 4 h of the study, subjects drank 60 ml of water mixed with L-[1-13C]phenylalanine every 30 min to obtain a steady ‘infusion rate’ of 3.5 μmol/kg/h. Thereafter, subjects started drinking a commercially available sip feeding (Fortisip, Nutricia Ltd, Wiltshire, UK) mixed with L-[1-13C]phenylalanine at a rate of 60 ml per 30 min for another 4 h. Macronutrient composition of the sip feed was protein 50 g/L (casein), carbohydrate 180g/L, and fat 65g/L. The amino acid composition was alanine 1.7 g/L, arginine 2.0 g/L, aspartic acid 3.9 g/L, cysteine 0.15 g/L, glutamine 12.5 g/L, glycine 1.0 g/L, histidine 1.6 g/L, isoleucine 2.85 g/L, leucine 5.25 g/L, lysine 5.1 g/L, methionine 1.65 g/L, phenylalanine 2.8 g/L, proline 5.0 g/L, serine 3.3 g/L, threonine 2.45 g/L, tryptophan 0.7 g/L, tyrosine 3.05 g/L, and valine 3.6 g/L. The protocol was approved by the Lothian Research Ethical Committee of the Local Health Board in Edinburgh. Written informed consent was obtained from all patients.

Plasma analyses

For serum CRP, blood was collected in serum-gel clotting tubes and centrifuged for 5 min at 2500 rpm at 5°C. Aliquots

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of supernatant were frozen in liquid nitrogen and stored at
–70°C until analysis. CRP was measured by fluorescent polariza-
tion immunoassay using an Abbott TDX analyzer and
Abbott reagents (Abbott Laboratories, Maidenhead, UK) with
a limit of detection of 1 mg/L. Samples for plasma amino acid
measurements were collected in pre-chilled lithium-heparin
tubes, transported to the laboratory on ice, and centrifuged
for 5 min at 2500 rpm at 5°C. Plasma proteins were precipi-
tated using 5% sulfosalicylic acid. The samples were then
frozen in liquid nitrogen and stored at
70°C until analysis. Plasma amino acids were measured by routine high-
performance liquid chromatography techniques as described
previously.16 Plasma enrichments of L-[1-13C]phenylalanine,
tyrosine were determined by liquid chromatography–mass
spectrometry (LC–MS).17 During transamination in vivo, L-[2H5]phenylalanine exchanges the hydrogen atom at C2 position
with body water, forming L-[2H3]phenylalanine.18 During ioniza-
tion in the LC–MS, L-[2H5]phenylalanine exchanges with unlabelled phenylalanine, giving rise to some L-[2H3]phenylala-
nine. This interfered with the analysis of L-[1-13C]phenylalanine,
causing overestimation of the M + 1/M ratio, and the used
LC–MS method was not able to distinguish the contribution of the 1-13C and the 2H5. However, because of the isotopic
distribution of both tracers, L-[1-13C]phenylalanine will also
generate a sufficient increase of the M + 2 mass, while the
2H5 will only generate a very small amount. We therefore
used the M + 2/M ratio to back calculate the M + 1/M ratio,
caused by L-[1-13C]phenylalanine. Alanine transaminase,
gamma-glutamyl transpeptidase, alkaline phosphatase, and
bilirubin levels were analysed as part of standard hospital
care in the Department of Clinical Chemistry of the Royal
Infirmary of Edinburgh, Scotland, according to hospital
protocols.

Calculations

All data concerning amino acid metabolism were corrected
for lean body mass. In the post-absorptive state, protein
breakdown is the only source of essential amino acids. The
plasma rate of appearance of essential amino acids, such as
phenylalanine, can therefore be used as an index of protein
breakdown. Phenylalanine and tyrosine kinetics were calcu-
lated according to the approach of Tessari et al.19 The rate
of appearance of phenylalanine (RaPHE) was calculated from

\[ \text{RaPHE} = \frac{\text{IR}_{2H5-PHE}}{\text{TTR}_{2H5-PHE}} \] (1)

Phenylalanine hydroxylation (PH) was calculated from
the tyrosine plasma rate of appearance calculated from
L-[3,3-2H2]tyrosine TTR (TTR_{2H2-TYR}) and L-[3,3-2H2]tyrosine
infusion rate (IR_{2H2-TYR}) according to Equation 1 and from
the ratio between TTR_{2H5-PHE} and the plasma TTR of its deriv-
ative L-[ring-2H5]tyrosine (TTR_{2H4-TYR}):

\[ \text{PH} = \frac{\text{Ra}_{2H2-TYR} \times \text{TTR}_{2H4-TYR}}{\text{TTR}_{2H5-PHE}} \] (2)

The rate of non-hydroxylative phenylalanine disposal
(NHPD) is an index of incorporation of phenylalanine in protein
and, therefore, of protein synthesis. In steady state con-
ditions, the sum of NHPD and PH equals RaPHE. NHPD could thus be calculated from the results of Equations 1 and 2:

\[ \text{NHPD} = \text{RaPHE} - \text{PH} \] (3)

Splanchnic extraction (SPEPHE), the fraction of phenylala-
nine that is taken up by the gut and/or liver during its first-
pass, was calculated as the ratio of RaPHE calculated with the
intravenous (RaPHE-IV) and enteral phenylalanine tracer
(RaPHE-ENT):

\[ \text{SPEPHE} = \frac{(1 - \text{RaPHE-IV}/\text{RaPHE-ENT}) \times 100}{\%} \] (4)

Endogenous RaPHE was used as a measure of endogenous
protein breakdown (endoPB). This was calculated as the difference
between the total rate of appearance of phenylala-
nine and the exogenous, enteral phenylalanine ingestion
rate (EIRPHE), corrected for SPEPHE:

\[ \text{Endogenous Protein breakdown (endoPB)} = \text{RaPHE(endo)} = \text{RaPHE} - (\text{EIRPHE} \times (1 - \text{SPEPHE}/100)) \] (5)

The phenylalanine net balance, an index of protein bal-
ance, could be calculated from protein synthesis (NHPD,
Equation 3) and protein breakdown (endogenous RaPHE,
Equation 5):

\[ \text{Net protein balance (NB)} = \text{PS-endoPB} \] (6)

Statistics

All data are presented as median and interquartile range
(IQR). Tracer fluxes are expressed in \( \mu \text{mol/kg lean body}
\text{mass/h} \). Data were analysed using IBM SPSS 20 for Microsoft
Windows®. Due to the sample size and high likelihood of a
non-normal distribution, non-parametric tests were used.
Differences between groups were analysed using the
Mann–Whitney U test or Fisher’s exact test, where appropri-
ate. To analyse differences within groups at different time
points, the Wilcoxon signed-rank test and Friedman test
were used. For correlations, Spearman’s ranked correlation
coefficient \( r_s \) was used. A P-value of < 0.05 was considered
significant.

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Results

Patient characteristics

Eight cachectic pancreatic cancer patients and seven control patients (six with gallstones and one with bilateral inguinal hernia) were included in this study. Patient characteristics are displayed in Table 1. Two pancreatic cancer patients had proven liver metastasis. Groups were comparable with respect to age, sex, height, lean body mass, and male/female ratio. Weight, BMI, triceps skinfold thickness, and mid-arm muscle circumference were significantly lower in the cachexia group. Patients did not show signs of ascites or oedema. The percentage weight loss in the cachexia group was very high with almost all ($n = 7$) cachectic patients having more than 10% weight loss. All pancreatic cancer patients had some degree of functional status loss but were able to come to the hospital themselves using public transportation or a taxi. Patients were mildly to moderately anorexic but tolerated the protocol well. During the last 2 h of the protocol, some cancer patients consumed less liquid feed due to anorexia, resulting in a small non-significant difference in sip feed intake between the cachexia group and control group: 8.1 (IQR: 5.6–10.0) vs. 10.3 (IQR: 8.5–11.6) ml/kg lean body mass protein drink corresponding with 34.2 (IQR: 23.7–42.6) vs. 43.7 (IQR: 36.1–49.3) μmol/kg lean body mass/h phenylalanine ($P = 0.105$).

Inflammation and clinical chemistry

Serum CRP levels were significantly higher in cachectic cancer patients compared with control patients (67.1 IQR: 48.1–79.6 vs. 45.8 IQR: 42.6–46.3 μmol/kg lean body mass/h; $P = 0.049$, Figure 2A). This was positively correlated with serum CRP levels ($r_s = 0.658$, $P = 0.008$). During ingestion of the study feed, endo PB decreased significantly to 45.5 (IQR: 26.9–51.1) μmol/kg lean body mass/h ($P = 0.012$) in cancer patients and 33.7 (IQR: 17.4–37.1) μmol/kg lean body mass/h ($P = 0.018$) in control subjects (Figure 2B). The magnitude of this decrease was not significantly different between both groups ($P = 0.132$). Splanchnic extraction was similar between both groups during feeding ($P = 0.418$, Figure 2C).

Protein synthesis (non-hydroxylative phenylalanine disposal)

Protein synthesis was higher in weight-losing cancer patients than in controls at baseline (63.0 IQR: 44.3–75.6 vs. 41.8 IQR: 37.6–42.5 μmol/kg lean body mass/h; $P = 0.021$, Figure 3A). In cachectic patients, protein synthesis did not respond to sip feeding (58.4 IQR: 46.5–76.1 μmol/kg lean body mass/h; $P = 1.000$), whereas protein synthesis in control patients increased significantly (47.9 IQR: 41.8–56.7 μmol/kg lean body mass/h; $P = 0.018$). Accordingly, the response of protein

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Sex (n)</th>
<th>Cachectic patients (n = 8)</th>
<th>Control patients (n = 7)</th>
<th>P-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (5)</td>
<td>Male (3)</td>
<td>Female (4)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>71 (20)</td>
<td>67 (16)</td>
<td>66 (12)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>47.6 (25.3)</td>
<td>53.7 (16.6)</td>
<td>72.3 (22.4)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>153 (13)</td>
<td>175 (16)</td>
<td>158 (5)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>20.3 (7.7)</td>
<td>19.4 (3.75)</td>
<td>29.0 (7.2)</td>
</tr>
<tr>
<td>Weight loss$^a$ (kg)</td>
<td>7.7 (13.4)</td>
<td>19.2 (19.8)</td>
<td>—</td>
</tr>
<tr>
<td>Weight loss$^a$ (%)</td>
<td>16.6 (28.3)</td>
<td>37.2 (40.9)</td>
<td>—</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>33.7 (23.9)</td>
<td>46.6 (9.6)</td>
<td>42.8 (8.8)</td>
</tr>
<tr>
<td>Triceps skin fold (mm)</td>
<td>14.0 (3.0)</td>
<td>11.0 (7.0)</td>
<td>27.5 (11.0)</td>
</tr>
<tr>
<td>Mid-arm circumference (cm)</td>
<td>23.3 (7.0)</td>
<td>22.5 (3.0)</td>
<td>31.8 (4.0)</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>6.0 (13.0)</td>
<td>24.0 (1.0)</td>
<td>17.0 (6.0)</td>
</tr>
</tbody>
</table>

Data represent median and range

$^a$In 6 months

$^b$Cachexia group compared with control group
Feeding and protein metabolism in pancreatic cancer cachexia

Table 2 Laboratory results and amino acid concentrations

<table>
<thead>
<tr>
<th></th>
<th>Cachectic patients (n = 8)</th>
<th>Control Patients (n = 7)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>8.3 (4.2–31.3)</td>
<td>0 (0–1.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>WBC (*10^9/L)</td>
<td>8.4 (4.6–10.5)</td>
<td>7.1 (4.9–8.3)</td>
<td>0.431</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>10.0 (8.0–18.0)</td>
<td>10.5 (7.8–18.5)</td>
<td>0.943</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>36.0 (34.0–67.0)</td>
<td>20.5 (14.5–30.8)</td>
<td>0.015</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>93.0 (47.0–168.0)</td>
<td>21.0 (15.8–30.5)</td>
<td>0.010</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>214.0 (149.0–231.0)</td>
<td>67.5 (60.3–101.5)</td>
<td>0.007</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>4.9 (3.5–6.3)</td>
<td>5.2 (5.0–7.7)</td>
<td>0.391</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>81.0 (74.0–101.0)</td>
<td>92.0 (82.5–106.3)</td>
<td>0.199</td>
</tr>
<tr>
<td>Phenylalanine (fasted, μmol/L)</td>
<td>44.5 (41.3–60.5)</td>
<td>67.0 (59.0–71.0)</td>
<td>0.024</td>
</tr>
<tr>
<td>Phenylalanine (fed, μmol/L)</td>
<td>62.5 (56.3–69.5)</td>
<td>84.0 (70.0–96.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>Tyrosine (fasted, μmol/L)</td>
<td>50.0 (41.8–65.0)</td>
<td>53.0 (45.0–59.0)</td>
<td>0.817</td>
</tr>
<tr>
<td>Tyrosine (fed, μmol/L)</td>
<td>64.0 (45.0–75.0)</td>
<td>74.0 (55.0–90.0)</td>
<td>0.246</td>
</tr>
<tr>
<td>Valine (fasted, μmol/L)</td>
<td>124.5 (109.3–154.5)</td>
<td>204.0 (179.0–220.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Valine (fed, μmol/L)</td>
<td>162.0 (143.3–176.0)</td>
<td>252.0 (223.0–281.0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Laboratory results and amino acid concentrations are presented as median and interquartile range. Amino acid concentrations are given as average concentrations in the fasted (1–4 h) and fed state (5–8 h). All amino acid concentrations increased significantly (P < 0.05) during feeding except for Tyrosine in the cachexia group (P = 0.062).

ALP, alkaline phosphatase; ALT, alanine transaminase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; WBC, white blood cells

Figure 1 Mean isotope enrichments and standard deviations of phenylalanine and tyrosine over time. Feeding was started after 4 h (arrow). Steady states of all tracers were significantly different during the fasted state compared with the fed state (cachexia: P = 0.019; control: P < 0.001; Friedman test). Steady states of all tracers did not differ significantly between groups in both fasted (1–4 h) and fed (5–8 h) states (Mann–Whitney U test).

synthesis on feeding (ΔNHPD) was significantly lower in cachectic patients (−0.6 IQR: −3.8 to 6.5 μmol/kg lean body mass/h) compared with control patients (9.9 IQR: 5.3–16.0 μmol/kg lean body mass/h; P = 0.021). Phenylalanine hydroxylation rates increased during feeding in the control group (4.5 IQR: 3.1–5.8 to 6.3 IQR: 4.2–7.4 μmol/kg lean body mass/h; P = 0.018) but not in the cachexia group (4.1 IQR: 2.6–5.0 to 3.6 IQR: 2.8–5.7 μmol/kg lean body mass/h; P = 0.779, Figure 3B).
Protein balance

Both cachectic patients and control patients were able to achieve a positive and comparable net protein balance during feeding as shown in Figure 4. Net protein balance in the cachexia group increased from $-4.1$ (IQR: $-5.0$ to $-2.6$) to $19.7$ (IQR: $13.1$–$23.7$) μmol/kg lean body mass/h ($P = 0.012$), while in the control group, it increased from $-4.5$ (IQR: $-5.8$ to $-3.1$) to $16.3$ (IQR: $13.6$–$25.4$) μmol/kg lean body mass/h ($P = 0.018$).

Figure 3 Protein synthesis and phenylalanine hydroxylation. Dots represent individual patients. (A) Protein synthesis expressed as non-hydroxylative phenylalanine disposal. (B) Phenylalanine hydroxylation rates. *$P < 0.05$. 

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Feeding and protein metabolism in pancreatic cancer cachexia

Discussion

This study has analysed the effect of sip feeding on whole body protein turnover in cachectic pancreatic cancer patients. It demonstrated that whole body protein turnover is higher in cancer patients compared with that in control patients, possibly due to inflammation. Although both patient groups were able to generate a similar anabolic response to feeding, cachectic patients were only able to achieve this by reducing protein breakdown, whereas controls were also able to increase protein synthesis.

Previous studies investigating protein metabolism in cancer cachexia (gastrointestinal and hepatocellular carcinoma) found a higher whole body protein turnover compared with healthy controls, in line with our study. In cachexia associated with benign disease, the results are variable: whole body protein turnover was increased in patients with heart failure but not in tuberculosis (TBC). Cachectic patients with chronic obstructive pulmonary disease (COPD) may have an increased whole body protein turnover, but this is not always the case. These mixed results may indicate a different pathogenesis for non-cancer cachexia compared with cancer cachexia. Federica et al. did not find an increased whole body protein turnover in cachectic gastric cancer patients. This could be due to the fact that they included a small group (n = 4 cancer patients) and did not correct for lean body mass. This is important since body composition of cachectic patients differs greatly from that of healthy individuals. Enteral feeding is preferred to parenteral feeding, since this mimics the normal situation better and generates a higher anabolic response. Previous studies have mainly investigated the effects of total parenteral nutrition (TPN) or parenteral amino acids and found mixed results. Winter et al. found a normal anabolic response of whole body protein turnover following hyperaminoacidemia using intravenous amino acid infusions in moderately cachectic non-small cell lung carcinoma patients. Bozetti et al. studied the effect of TPN on protein synthesis and breakdown in cachectic gastric cancer patients. Unlike our study, they found an increase in protein synthesis in response to TPN and no change in breakdown. However, the number of patients was very low (n = 3) and did not include a control group.

The study by Williams et al., which analysed the fractional synthetic rate (FSR, protein synthesis) in the vastus lateralis muscle of colorectal cancer patients, found that FSR did not increase in cancer patients after the administration of a mix of parenteral amino acids, whereas FSR increased in matched healthy controls. Although FSR and whole body protein synthesis are difficult to compare, their general message was in line with our results. They also found a trend towards increased leg muscle protein breakdown in cachectic patients that did not change after feeding. Interestingly, after surgical removal of the tumour, the FSR increased as a result of feeding in these patients. Only two studies have assessed the effect of enteral feeding on protein kinetics in cachectic patients with benign disease, but apart from the present study, no data on the response of protein kinetics on enteral feeding in cancer-cachexia are at hand. First, Macallan et al. (1998) studied the effect of hourly feeding in cachectic TBC patients compared with that in non-cachectic malnourished and healthy controls. They found that whole body protein synthesis did not increase during feeding in cachectic TBC patients, whereas both malnourished and healthy controls showed a significant increase, which is in line with our results. Second, studies of Engelen et al. and Jonker et al. studied the effect of respectively sip feeding and bolus feeding on protein kinetics in cachectic COPD patients. In contrast with our results, both studies found a significant increase in protein synthesis in COPD patients, which was similar to the response in young healthy individuals in the study of Engelen et al. There are several reasons which may explain this difference in anabolic response. Firstly, our study population suffered from very severe weight loss (>10%), which could mean that their cachetic state was more advanced than in the COPD patients resulting in a worse anabolic response. Secondly, the COPD patients consumed a drink with higher whey protein content than our study drink. Whey protein is known to stimulate muscle protein synthesis more than other milk proteins (e.g. casein) in elderly patients. Finally, as mentioned earlier, the more chronic pathogenesis of COPD-related cachexia might be different from the relatively acute pathogenesis of pancreatic cancer cachexia, which could influence the way patients respond on feeding.

Another reason for the different protein kinetics found in the studies referred to above is that of all solid cancers, pancreatic cancers are strongly related to a pro-inflammatory state. In recent years, it has become evident that any rapidly proliferating tumour is associated with severe

Figure 4 Net protein balance expressed as net phenylalanine balance. Dots represent individual patients *P < 0.05.
Inflammatory activity in the tumour itself, in surrounding tissues, and systemically, which stimulates cancer cell proliferation. This leads to acute phase protein synthesis, proliferation of leukocytes, cancer cells, and synthesis of extracellular matrix which all contribute to increasing protein and DNA synthesis.\textsuperscript{9,11–13} This may partly explain the increase in basal protein synthesis in the present study.

The fact that net protein balance during feeding was similar in both groups despite the lack of increase in protein synthesis in cachectic patients indicates that protein conservation is regulated differently in cachexia. While control patients respond with both a decrease in protein breakdown and an increase in protein synthesis, cachectic patients seem only to decrease protein breakdown to preserve a positive net balance. An explanation for the lack of protein synthesis is that cachectic patients might have a higher anabolic threshold. This is also seen in the healthy elderly. Ingestion of a large bolus (35g) of whey protein generates a higher whole body anabolic response and muscle protein synthesis than a smaller bolus (10 or 20g).\textsuperscript{34} Thus, a single protein bolus might be more effective than sip feeding (which is comparable to continuous feeding). Moreover, studies using TPN indicate that a high infusion rate or a high content of branched chain amino acids stimulates whole body protein synthesis in cachectic cancer patients while standard TPN infusion does not.\textsuperscript{35,36} Clinical application will be difficult, however. Satiety effects of protein might be different in cachectic cancer patients, most patients suffer from anorexia,\textsuperscript{5,7,37} and dietary patterns and food preference vary greatly among cachectic cancer patients.\textsuperscript{38} Therefore, individualized dietary care might be needed for adequate nutritional support in cancer cachexia.

The current study has some limitations. We used bioelectrical impedance measurements for assessment of body composition. This method can underestimate the fat-free mass compared with computed tomography or dual-energy X-ray absorptiometry analyses in surgical and oncologic patients because of fluid shifts.\textsuperscript{39,40} However, since our patients did not show any signs of oedema, ascites, or dehydration (see urea levels in Table 2), underestimation is likely to be a minor issue. Another issue of the current study is that some patients in the cachexia groups consumed less sip feed than the control patients during the last 2 h of the study protocol. This might result in an underestimation of the phenylalanine rate of appearance and protein breakdown measurements. However, the difference was small (and non-significant) and did not influence the amino acid enrichments (see Figure 1). We are therefore confident that even though there may be some underestimation, the effect of the lower sip feed intake is too small to change the conclusions of this study.

In conclusion, this study shows that cachectic pancreatic cancer patients have a higher basal protein turnover, which is positively correlated with CRP levels. Both cachectic patients and controls are able to achieve a comparable positive net balance during feeding, suggesting that anabolic resistance may be less of an issue in cachectic patients than previously thought. However, in cachectic patients, this is only achieved by reduced protein breakdown, whereas in controls, both protein breakdown and protein synthesis are modulated. Impaired nutritional stimulation of protein synthesis in pancreatic cancer cachexia should be a topic in future studies to develop more effective nutritional interventions.

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Conflict of Interest

None declared.
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