Synthesis rates of total liver protein and albumin are both increased in patients with an acute inflammatory response

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ABSTRACT

The general perception that catabolism and inflammation are associated with a high synthesis rate of total liver protein and a low albumin synthesis rate has been challenged in recent years by several studies in man, indicating that the synthesis rate of albumin in response to a catabolic insult is increased rather than decreased. Thus changes in liver protein synthesis rates in conjunction with catabolism and acute inflammation in man need to be characterized better. The aim of the present study was to measure protein synthesis rates of total liver protein and albumin during a state of acute inflammation. Patients (n = 10) undergoing acute laparoscopic cholecystectomy due to acute cholecystitis were investigated. FSRs (fractional synthesis rates) of total liver protein (liver biopsy specimens) and albumin (plasma samples) were investigated as early as possible during the surgical procedure, using a flooding dose of L-[2H5]phenylalanine. The results were compared with a reference group of patients without cholecystitis undergoing elective laparoscopic cholecystectomy (n = 17). FSR of total liver protein was 60% higher (P < 0.001) and the FSR of albumin was 45% higher (P < 0.01) in the cholecystitis patients compared with the control group.

In conclusion, the synthesis rates of total liver protein and albumin are both increased in patients with an acute general inflammatory reaction undergoing laparoscopic cholecystectomy.

INTRODUCTION

Catabolic states are characterized by specific changes in protein metabolism. As the liver plays an important role in protein metabolism, this organ is vital for the response to a catabolic insult. Based primarily on results from studies in animals, the concept of increased total liver protein synthesis (including the so-called acute-phase response) and a corresponding decrease in albumin synthesis in these conditions has generally been accepted as being relatively uniform and reproducible [1–6]. However, as discussed below, studies from our group and others indicate that these results may not be immediately applicable to human conditions.

By acquiring liver tissue specimens during laparoscopic cholecystectomy it has become possible to elucidate further the specific role of liver in human protein metabolism. The synthesis rates of total liver protein and albumin can be measured simultaneously [7,8]. Surgical trauma of moderate severity (laparoscopic

Key words: cholecystitis, inflammation, liver protein, MS, stable isotope, synthesis rate.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ASR, absolute synthesis rate; CRP, C-reactive protein; FSR, fractional synthesis rate; γ-GT, γ-glutamyltransferase; MPE, mole percent excess; WBC, white blood cell.

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cholecystectomy) is associated with immediate decreases in the synthesis rates of both total liver protein and albumin [8,9]. On the other hand, increased synthesis rates of total liver protein have been observed in conjunction with other kinds of catabolic states, such as inflammatory bowel disease and sepsis [10,11]. Also, albumin synthesis in man has been shown to increase after stress hormone infusion [12], after endotoxin administration [13], in dialysis-treated renal failure [14], in cancer [15] as well as in critical illness [16–18]. It may therefore be speculated that the characteristics of the changes in synthesis rates of different populations of liver proteins are related to the type of insult (both in terms of quantity and quality) and possibly also the time factor (i.e., when in relation to the insult the measurement is performed). Thus the changes observed in response to various catabolic stimuli, such as trauma and critical illness, in humans appear to be more complex and variable than previously thought.

The aim of the present study was to characterize further the synthesis rates of total liver protein and albumin in response to a generalized inflammatory process. Patients suffering from acute cholecystitis, being subjected to acute laparoscopic cholecystectomy, were investigated and compared with a reference group without cholecystitis investigated under similar conditions.

MATERIALS AND METHODS

Materials
$L-[^{2}H_{5}]$Phenylalanine (99 atom percent; Cambridge Isotope Laboratories) was dissolved in sterile water, together with unlabelled phenylalanine (Ajinomoto Company), to a concentration of 20 g/l [7.5 MPE (mole percent excess)].

Subjects
A total of 10 patients undergoing acute laparoscopic cholecystectomy due to acute cholecystitis, but otherwise healthy, participated in the study (Table 1). Patients were included if they were scheduled for acute laparoscopic surgery, had a fever of $>38^\circ$C, had a CRP (C-reactive protein) plasma concentration of $>100$ mg/l and had cholecystitis as verified by abdominal ultrasound (wall thickness of the gall bladder $>4$ mm and cholecystolithiasis). Patients with evidence of obstructive jaundice were excluded. For practical reasons it was not possible to standardize periods of fasting among patients. All patients in the cholecystitis group were operated upon within 2–4 days after initiation of symptoms. Patients undergoing elective laparoscopic cholecystectomy investigated previously under identical conditions, but without concurrent cholecystitis, served as controls ($n = 17$) [7,8]. The nature, purposes and potential risks of the experimental procedures were explained to the patients before obtaining their voluntary consent. The research protocol was approved by the Ethical Committee as well as the Isotope Committee (plasma volume measurements) of Karolinska Institutet, Stockholm, Sweden.

Experimental protocol
An identical protocol for sampling has been used in several previous studies (e.g. [7,8]). The patients were investigated in the fasted state. Before surgery, antecubital venous lines were inserted bilaterally, one of which was used for blood sampling, the other for administration of labelled phenylalanine. At the time of the induction of anaesthesia, $L-[^{2}H_{5}]$phenylalanine (45 mg/kg of body weight; 7.5 MPE) was given intravenously over 10 min. The patients were anaesthetized in a standardized manner with propofol (1.5–2.5 mg/kg of body weight), fentanyl (2.5–5.0 μg/kg of body weight) and atracurium (0.5 mg/kg of body weight) intravenously. Anaesthesia was maintained by sevoflurane in a mixture of oxygen/air, with intermittent doses of fentanyl and atracurium when needed. The patients were given Ringer’s solution intravenously peri-operatively (100–400 ml/h). CO$_{2}$ was insufflated into the abdomen, resulting in a pressure of 10–13 mmHg, which was maintained throughout the procedure. Ventilation was adjusted to maintain a normal end-tidal CO$_{2}$ level. After the insertion of four trocars through the abdominal wall, but before the cholecystectomy was initiated, a liver biopsy specimen was acquired as soon as possible using laparoscopic scissors, at a standardized location (the most ventral and medial part of the right liver lobe). No complications due to bleeding were observed.

Blood sampling protocol
Venous blood samples were drawn at the following intervals: 0, 5, 10, 15, 30, 50, 70 and 90 min after the injection of phenylalanine for the determination of isotopic enrichment of free and protein-bound $L-[^{2}H_{5}]$phenylalanine in plasma and albumin respectively. Blood samples drawn at 0 min were also used to determine the plasma concentrations of albumin and CRP, WBC (white blood cell) count and the serum concentrations of liver function indices (ALT (alanine aminotransferase), ALP

Table 1  Characteristics of patients and controls

<table>
<thead>
<tr>
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<th>Patients with cholecystitis</th>
<th>Controls</th>
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<tbody>
<tr>
<td>$n$</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>8/2</td>
<td>6/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.8 ± 10.6</td>
<td>46.5 ± 9.0$^*$</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 ± 0.09</td>
<td>1.70 ± 0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.5 ± 13.8</td>
<td>73.9 ± 12.9</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.5 ± 3.5</td>
<td>25.5 ± 3.1</td>
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(alkaline phosphatase), γ-GT (γ-glutamyltransferase) and bilirubin. The samples were stored at −80 °C prior to analysis. All the blood samples were centrifuged at 981 g for 20 min and stored in a −80 °C freezer pending analysis. Plasma volume was measured, beginning 30 min after the injection of phenylalanine, with 131I-labelled albumin (100 kBq; Institutt for Energetikk, Kjeller, Norway). Blood samples were taken at 0, 20, 30, 40 and 45 min to assess isotope dilution.

**Sample preparation**

The details of sample preparation and analysis have been extensively described elsewhere (e.g. [7,8]). Briefly, liver tissue specimens (n = 8) were homogenized in sulphosalicylic acid. The protein precipitates were washed repeatedly to remove traces of free phenylalanine, followed by hydrolysis in 6 M HCl for 24 h at 110 °C. After trichloroacetic acid/ethanol extraction of albumin in plasma, these samples were prepared in the same way as the tissue samples. After enzymatic conversion into phenylethylamine, the enrichment of [2H5]phenylalanine from tissue and albumin hydrolysates was determined by monitoring the ions at m/z 180 and 183 of the t-butyl-dimethylsilyl derivative of phenylethylamine on an Agilent 5973 mass spectrometer (Agilent Technologies). Analysis of plasma free phenylalanine enrichment was performed after acid precipitation and cation-exchange chromatography by monitoring the ions at m/z 336 and 341 of the t-butyl-dimethylsilyl derivative. By employing 70 eV instead of 45 eV for the MS analysis, a different distribution was obtained for the most prominent ion fragments compared with the study where this derivative was first introduced [19].

**Other analytical procedures**

Concentrations of albumin and CRP in plasma, and liver function tests in serum, were analysed by routine clinical laboratory methods.

**Calculation and statistics**

FSR (fractional synthesis rate) of total liver protein was expressed as percentage/day, i.e. the daily amount of protein synthesized by the liver, both endogenous and secreted proteins, expressed as a percentage of the total protein content of the liver, and was calculated according to the formula described previously [7]:

$$\text{FSR} = \frac{P \times 100}{\text{AUC}}$$

where $P$ is the isotopic enrichment of phenylalanine (MPE) in total liver protein of the liver biopsy specimen, and AUC is the area under the curve for plasma free phenylalanine enrichment (MPE) over time (days).

FSR of albumin, i.e. the fraction of the intravascular albumin pool that is synthesized every day, was calculated using the formula described previously [20]:

$$\text{FSR} = \left[ P(2) - P(1) \right] \times \frac{100}{\text{AUC}}$$

where FSR is expressed as %/day and $P(1)$ and $P(2)$ represent enrichment of phenylalanine in albumin at two time points, $t(1)$ and $t(2)$, after the curve of enrichment becomes linear, based on three samples (samples at 50, 70 and 90 min). AUC is the area under the curve for enrichment of plasma free phenylalanine between time points $t(1)$ and $t(2)$ adjusted for the secretion time ($T_s$), i.e. the temporal lag period before the appearance of labelled albumin in plasma. The secretion time was assessed by plotting the each individual’s regression line for the linear part of the albumin enrichment curve and extrapolating to the baseline enrichment [20]. In order to facilitate the assessment of the relationship between the duration of surgery and FSR of albumin among patients, the measurement interval between samples at 50 and 90 min for albumin enrichment was approximated with one point in time (70 min). ASRs (absolute synthesis rates) of albumin were calculated as the product of FSR of albumin and the intravascular albumin mass, calculated from the plasma albumin concentration and the measured plasma volume.

Data are presented as means and S.D. Differences between patients suffering from acute cholecystitis and controls were assessed using Student’s $t$ test for unpaired samples. Correlations were assessed using ‘simple regression’ ($r$ = Pearson’s coefficient of correlation).

**RESULTS**

FSR of total liver proteins was 38.8 ± 7.8 %/day in the cholecystitis group and 24.4 ± 3.8 %/day in the control group ($P < 0.001$; Figure 1). FSR of albumin was 8.8 ± 2.4 %/day in the cholecystitis group and 6.1 ± 1.2 %/day in the control group ($P < 0.01$; Figure 2). ASR of albumin was 136 ± 38 mg·day⁻¹·kg⁻¹ of body weight in the cholecystitis group and 104 ± 20 mg·day⁻¹·kg⁻¹ of body weight in the control group ($P < 0.01$; Figure 3). In the cholecystitis group, the liver biopsy specimen was acquired 15.9 ± 6.4 min after the initiation of surgery, whereas the corresponding value in the control group was

![Figure 1](image1.png)  
**Figure 1** FSR of total liver protein in patients with cholecystitis ($n = 8$) and controls ($n = 17$) during laparoscopic surgery  
Circles represent individual values, and bold lines indicate means.
FSR of albumin in patients with cholecystitis (n = 10) and controls (n = 17) during laparoscopic surgery
Circles represent individual values, and bold lines indicate means.

Figure 3 ASR of albumin in patients with cholecystitis (n = 10) and controls (n = 17) during laparoscopic surgery
Circles represent individual values, and bold lines indicate means.

16.4 ± 5.2 min (P value was not significant). The measurements of albumin synthesis were also performed after similar durations of surgery (18.6 ± 5.4 min in the cholecystitis group and 17.9 ± 5.6 min in the control group; P value was not significant).

The patients in the cholecystitis group had a longer period of fasting before the procedure compared with controls (Table 2). Also, temperature, WBC and CRP were significantly higher in cholecystitis patients, as was serum bilirubin concentration (Table 2).

Plasma albumin concentration was lower in the cholecystitis group compared with the control group, whereas plasma volume was higher. As a consequence, intravascular albumin mass was similar in the two groups (Table 3). The albumin secretion time (i.e. the time that elapsed before isotopically labelled albumin started to appear in peripheral blood) was similar in the two groups (Table 3).

A positive correlation was observed between FSRs of total liver protein and albumin (r² = 0.57, P < 0.001) as well as between FSR of total liver protein and ASR of albumin (r² = 0.38, P < 0.01). This was true for the combined group (cholecystitis + controls) as well as for the cholecystitis group only (r² = 0.63 and r² = 0.64, respectively; P < 0.05), but not for the controls only.
importance (i.e. controlled surgical trauma compared with generalized inflammation), also implying that the point in time when measurements are made must be taken into consideration. Based on the results from the present study, it is not possible to discern exactly how the acute-phase response develops over time, especially not in the early phase after the insult, or how the degree and type of insult affect this development. These issues certainly warrant further study but, given the difficulties in studying intracellular processes in human liver (e.g. non-secreted proteins, protein degradation and mRNAs) and the consistency of the changes in total liver protein synthesis in response to a catabolic insult studied in animals and man, animal models may be considered appropriate for studying this time pattern [6].

The reduction in plasma albumin concentration that was observed among cholecystitis patients in the present study is a characteristic feature of acute catabolic and inflammatory states [21] (Table 3). In principle, plasma albumin concentration is the result of three processes, namely synthesis, distribution and degradation. Whereas there is little information on albumin degradation at present, previous studies have indicated that the decrease in plasma albumin concentration observed during catabolic states is strongly related to an increase in albumin efflux, i.e. redistribution of albumin to the extravascular space, as well as to changes in the size of the intravascular fluid space [22–24]. Also, based primarily on studies in small animals, the common assumption regarding albumin synthesis has been a decrease in these situations, thus contributing to hypoalbuminaemia [1,3, 5,6]. However, in recent years, several studies in man have shown an increased synthesis rate of albumin in various kinds of catabolic states [12–18], which was also the case in the present study (Figure 2). Of note is the fact that ASR of albumin was also high among cholecystitis patients (Figure 3). Thus, in man, in contrast with findings in animal studies, we and others have shown that suppression of albumin synthesis cannot explain the low plasma albumin concentration observed in catabolic states. On the contrary, an increased albumin synthesis is observed, potentially striving to maintain the intravascular albumin mass (Table 3).

Furthermore, for albumin synthesis, despite comparable time frames (up to 2 h), quite opposite effects are observed in conjunction with laparoscopic surgery (decrease) [9] and after endotoxin administration (increase) [13]. Therefore the high albumin synthesis rate also observed in the critically ill [16–18] and in the present study in cholecystitis patients, as well as the increase in synthesis rate observed after endotoxin administration, suggest that the underlying triggering mechanisms may be similar. On the other hand, different underlying mechanisms may be responsible for the decrease during elective laparoscopic surgery (Figures 2 and 3). Thus the degree and/or type of catabolic insult seem to be important.

The question remains of how the laparoscopic procedure itself may have affected the results of the present study. We have shown previously that total liver protein synthesis decreases in a continuous manner during the first hour of laparoscopic surgery, whereas albumin synthesis decreases promptly very early during the procedure, an alteration which seems to be non-progressive [8,9]. Taken together, these results suggest that different populations of liver proteins react differently during a laparoscopic procedure, which in itself may have implications for other types of catabolic insults, as shown previously in animals [25,26]. Regarding the results of the present study, it is possible that albumin synthesis rates were somewhat lower than they would have been had the patients not been subjected to laparoscopic surgery. This may also apply to the rate of total liver protein synthesis. However, by performing the synthesis measurements, including the acquisition of the liver biopsy specimen, as soon as possible during the procedure, the effects of anaesthesia and surgery were minimized [8,9]. Also, since these effects are probably seen in both groups of patients, it should still be possible to make comparisons.

The patients in the present study were fasted, and the patients in the cholecystitis group were fasted for a longer time period than the controls (Table 2). Previous studies show that synthesis rates of liver proteins are responsive to nutrition, via the oral route or after several days of parenteral infusion [27,28], whereas fasting is associated with decreased synthesis rates of liver proteins, at least in animals [29]. These effects are to some extent related to variations in insulin levels [30]. In a previous study [31], it was shown that a flooding dose of [3H]-phenylalanine was associated with a small increase in insulin concentration. Although no effect on muscle protein synthesis was observed in that study, the potential effect of insulin must be taken into consideration when interpreting the present results, as insulin levels were not measured here. However, regarding the possible effect of fasting, it would be reasonable to say that the synthesis rates would decrease to a greater extent in the cholecystitis patients (because of the longer fasting period). Thus the present results are not invalidated by the potential effect of fasting.

When comparing the two groups investigated in the present study it was observed that the patients in the cholecystitis group were slightly older and included a relatively larger proportion of males than the control group (Table 1). Whereas increasing age is known to be associated with a loss of fat-free mass in man, the effects on the synthesis rates of liver proteins are unknown [32]. No gender differences have been observed, at least not where albumin synthesis is concerned [33]. However, the differences between the groups should be borne in mind when interpreting the results of the present study.

In order to attain the correct assessment of protein synthesis rates for specific proteins such as albumin,
the isolation technique is of vital importance, i.e. contamination by other proteins must be avoided. In the present study the acidified ethanol extraction technique was employed for this purpose. Although partially questioned in a recent study [34], this technique has been shown previously to produce pure fractions of albumin in health and disease [7,12,16,17].

In conclusion, the synthesis rates of total liver protein and albumin in patients suffering from acute cholecystitis is higher than in comparable controls. These results provide further insight into the characteristics of the dynamic changes in the synthesis rates of different populations of liver proteins in conjunction with catabolic and inflammatory insults in man.

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REFERENCES