DURING THE CLASSIC "flow phase" after trauma, critically ill patients exhibit an important loss of body proteins due to an imbalance between proteolysis, which increases (19), and protein synthesis. Changes of protein synthesis were extensively studied at the tissue level in numerous animal models of acute injury such as trauma, sepsis, or pure inflammation (e.g., turpentine). Although dependent in part on the type of injury, the most typical changes included a depressed muscle protein synthesis (5, 10) and an increased synthesis of total liver proteins (5, 11, 24, 31) and of positive acute-phase proteins (18, 27, 31). Synthesis of negative acute-phase proteins (e.g., albumin) decreases in some (4, 26, 27) if not all (18, 31) studies, and its gene expression is depressed (21, 22). Finally, modification of protein synthesis in other tissues, such as gut, is more controversial (24, 11).

However, the clinical situations differ from animal models with respect to their duration. Whereas intensive care patients frequently exhibit severe inflammation and protein losses for days or weeks, animal studies are performed 1–5 days (at the most) after injury and thus might not be representative of what occurs in humans. Furthermore, few data exist for patients; although some studies have examined whole body protein synthesis (e.g., Ref. 14) and, more occasionally, muscle (13) and fibrinogen or fibronectin synthesis (29), there is no comprehensive study of these modifications in the same patients. In particular, data on albumin synthesis are extremely scarce (22). On the basis of animal studies, albumin synthesis is widely believed to decrease (1).

Therefore, the aim of this study was to assess in critically ill patients the modifications of protein synthesis, with simultaneous measurements at the whole body level and in muscle- and liver-derived proteins. For liver-derived proteins, we measured the synthesis of both a positive (fibrinogen) and negative (albumin) acute-phase protein. We chose to study severe head-injured patients for the following reasons: 1) they are known to experience a severe muscle loss, 2) they constitute a rather homogeneous group receiving a standardized treatment, and 3) they have a well-characterized and severe inflammatory response (20) even in the absence of sepsis. These patients were compared with a group of matched healthy subjects receiving identical nutritional intakes. The data on the proteolytic systems expression in the muscle of these patients have been published previously (19).

MATERIALS, SUBJECTS, AND STUDY PROTOCOL

Materials, subjects, and protocol were previously described in detail (19). Briefly, six patients with exclusive (no sepsis) and severe head traumas were compared with five healthy volunteers matched for age and body mass index. Patients were studied on day 8 after admission, while they were receiving continuous enteral nutrition for at least 3 days (Nutrison E+, Nutricia, The Netherlands, 2.0 kcal·kg⁻¹·day⁻¹ and 1.49 ± 0.09 g protein·kg⁻¹·day⁻¹, means ± SE). A primed continuous (0.17 μmol·kg⁻¹·min⁻¹) infusion of L-[¹³C]leucine (Mass Trace, Somerville, MA) was given through a central catheter for 10 h. The pool of bicarbonate was primed with 5 mg of NaH¹³CO₃. Blood samples were taken at regular intervals, and muscle biopsies were taken before and at the end of the infusion. Indirect calorimetry was performed throughout the study using a Deltrac (Datex, Geneva, Switzerland) connected to the respirator. Expired gas samples were taken in a Douglas bag at the exhaust of the respirator during 5 min at the same times as the blood samples. Breath samples were then transferred into Vacutainers (Becton-Dickinson, Grenoble, France). In addition, blood samples were also taken to
measure plasma albumin, fibrinogen, orosomucoid, C-reactive protein, haptoglobin, and cytokines at days 4 and 8. Daily 24-h urinary excretion of cortisol (day 8) and nitrogen (from day 5 to day 7) were measured.

The isotopic study was performed in the control subjects in a similar manner. The enteral diet (34 ± 2 kcal·kg⁻¹·day⁻¹ and 1.40 ± 0.09 g protein·kg⁻¹·day⁻¹) was given only during the 10 h of the tracer infusion. The infusion rate of [¹³C]-leucine was 0.10 ± 0.01 µmol·kg⁻¹·min⁻¹. Indirect calorimetry was performed with the same device with the use of a canopy.

Analytic methods. Plasma [¹³C] enrichments of leucine and ketosaccharate (KIC) were determined as previously described (19). [¹³C] enrichments of CO₂ were measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS, Microgas, Fisons Instruments, Middlewich, UK). In two additional control subjects, it was verified that the diet used in the controls (which was made with potato maltodextrin) did not modify the natural [¹³C] abundance in the breath (data not shown). In the patients who received maltodextrins made from corn, the basal [¹³C] abundance was higher but was stable throughout the study, since this diet had been continuously administered for at least 3 days before the isotopic study.

Incorporation of [¹³C]leucine into the muscle proteins was measured by GC-combustion-IRMS (Isosochrom, Fisons Instruments) according to Yarasheski et al. (32) with slight modifications. Briefly, 10–15 mg of muscle were powdered in liquid nitrogen and homogenized in trichloroacetic acid (TCA). The protein pellet was washed with TCA, hydrolyzed, filtered, and dried. N-acetyl propyl derivatives of leucine were separated from other amino acids onto a nonpolar GC column, and the leucine peak was directed through the furnace, where combustion in CO₂ occurred. CO₂ was then directed toward the IRMS in alternation with pulses of reference CO₂ gas. Each sample was injected five to six times, and the mean coefficient of variation (CV) of replicates was 8% (range 4–11%). Standard curves covering a range of similar enrichments (0–0.005 atom percent excess, r² ≥ 0.99) were run simultaneously. [¹³C] leucine enrichments in albumin and fibrinogen were also determined by GC-combustion-IRMS, as previously described (9). Purity of the isolated fractions was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in both controls and patients (data not shown). Plasma protein (except fibrinogen) concentrations were measured by immunonephelometry. Fibrinogen concentration was determined by a turbidimetric assay (Biodirect, Les Ulis, France).

Calculations. Whole body leucine flux and oxidation were calculated at steady state, using the plasma [¹³C]KIC enrichment as the precursor pool. CO₂ recovery factors of 0.82 in the controls and 0.90 (30) in the patients were used. Nonoxidative leucine disposal, an index of whole body protein synthesis, was the difference between flux and oxidation. Leucine disposal, an index of whole body protein synthesis, was calculated as the ratio between the [¹³C]leucine incorporation in muscle (i.e., enrichment at time 10 h minus natural abundance at time 0) and the plasma [¹³C]KIC enrichment at plateau, multiplied by 2.4. The [¹³C]leucine incorporation rate into albumin and fibrinogen was calculated between time 6 and 10 h by least square regression analysis. FSIs were then obtained by dividing the slopes of incorporation (corrected for 24 h) by the plasma [¹³C]KIC enrichment at plateau. Absolute synthesis rates (ASR, in mg protein·kg⁻¹·day⁻¹) were then calculated as FSI (%/day) times plasma albumin or fibrinogen concentrations (mg/ml) times plasma volume (ml/kg). The latter was calculated from the estimated blood volume (69 ml/kg in men, 65 ml/kg in women) (17) and the measured hematocrit.

Statistical analysis. All values are expressed as means ± SE. Between groups comparisons were made by two-tailed unpaired t-tests. Data in Table 1 (controls and trauma patients at day 4 and day 8) were compared by analysis of variance, followed by a Scheffé test.

RESULTS

All the patients were in negative nitrogen balance (19). Their energy expenditure was similar to that of the controls (31.9 ± 3.6 and 26.8 ± 1.2 kcal·kg⁻¹·day⁻¹, not significant), and their respiratory quotient was higher (0.95 ± 0.04 and 0.84 ± 0.01, P < 0.05). As shown in Table 1, patients had a severe biological inflammatory response increasing from day 4 to day 8, as assessed by high plasma levels of C-reactive protein, orosomucoid, haptoglobin, and fibrinogen. By contrast, plasma albumin concentrations decreased in the patients, the decrease being significant only at day 8. Plasma levels of interleukin-1β (IL-1β) and interleukin-6 (IL-6), but not of tumor necrosis factor (TNF), were elevated, and so were cortisoluria (19). Hematocrit at day 8 was lower in the patients (31 ± 2%) than in the controls (46 ± 1%, P < 0.001), and the calculated plasma volume was therefore higher in the patients than in the controls (46 ± 1 vs. 37 ± 1 ml/kg, respectively, P < 0.001).

Plateaus of leucine and KIC enrichments were obtained in all subjects after 2 h of infusion. Whole body leucine flux was increased by 50% in the patients (P < 0.001), as previously reported (19). Leucine oxidation was doubled (P < 0.01), and nonoxidative leucine disposal increased by 28% in patients (P < 0.02; Table 2). Leucine balance was positive during feeding in the controls, whereas it remained negative in the patients (P < 0.01). Similar qualitative results were obtained when leucine enrichments were used instead of KIC enrichments (data not shown).

Muscle FSR was sharply decreased in the patients compared with the controls (1.94 ± 0.23 vs. 0.86 ± 0.21%/day, P < 0.01). The incorporation of the tracer into albumin and fibrinogen is displayed on Fig. 1 for both the control subjects and the patients. The slopes of incorporation were all linear (mean r² = 0.98 ± 0.01, range 0.91–0.99). They were greater in the patients than in the controls, whereas the plateaus of plasma KIC enrichments were similar in both groups. Therefore, the FSR of fibrinogen and albumin were higher in

Table 1. Plasma protein concentrations in control subjects and in head-injured patients at days 4 and 8 after admission

<table>
<thead>
<tr>
<th>Protein</th>
<th>Controls Day 4</th>
<th>Patients Day 4</th>
<th>Patients Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, g/l</td>
<td>1.50 ± 0.14</td>
<td>6.64 ± 0.80†</td>
<td>5.94 ± 1.12‡</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td>[1&lt;]</td>
<td>1.14 ± 14</td>
<td>151 ± 26</td>
</tr>
<tr>
<td>Orosomucoid, g/l</td>
<td>0.42 ± 0.04‡</td>
<td>1.43 ± 0.06†</td>
<td>1.91 ± 0.11†</td>
</tr>
<tr>
<td>Haptoglobin, g/l</td>
<td>0.74 ± 0.12</td>
<td>2.05 ± 0.23‡</td>
<td>2.93 ± 0.43‡</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>33.7 ± 1.2‡</td>
<td>30.5 ± 3.7</td>
<td>25.2 ± 1.2‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 controls and 6 patients. *P < 0.02, †P < 0.01, and ‡P < 0.001 vs. controls.
Table 2. Whole body leucine kinetics measured by isotopic dilution during a 10-h constant infusion of L-[1-13C]leucine in enterally fed control subjects and head-injured patients 8 days after admission

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine flux</td>
<td>2.36 ± 0.15</td>
<td>3.52 ± 0.10†</td>
</tr>
<tr>
<td>Leucine oxidation</td>
<td>0.80 ± 0.07</td>
<td>1.54 ± 0.16†</td>
</tr>
<tr>
<td>Nonoxidative leucine disposal</td>
<td>1.55 ± 0.11</td>
<td>1.99 ± 0.12*</td>
</tr>
<tr>
<td>Leucine balance</td>
<td>+0.18 ± 0.07</td>
<td>-0.52 ± 0.15†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 controls and 6 patients. All fluxes are in µmol leucine·kg⁻¹·min⁻¹. 13C enrichment of plasma ketoisocaproate was used as precursor pool. Leucine flux is an index of whole body protein turnover. Nonoxidative leucine disposal is an index of whole body protein synthesis. *P < 0.02, †P < 0.01, and ‡P < 0.001 vs. controls.

Fig. 1. Rates of incorporation of L-[1-13C]leucine into albumin (A) and fibrinogen (B) in head trauma patients (●) and in control subjects (○). [13C]leucine enrichments into proteins were measured by isotope ratio mass spectrometry during a 10-h constant intravenous infusion of L-[1-13C]leucine. C: plateau of 13C enrichment into plasma ketoisocaproate (KIC), representative of intracellular free leucine enrichment. All data are means ± SE. APE, atom percent excess.

Fig. 2. Plasma concentrations, fractional synthesis rates (FSRs), and absolute synthesis rates (ASRs) of albumin (A) and fibrinogen (B) in head trauma patients (filled bars) and in control subjects (open bars). FSRs were determined by incorporation of [13C]leucine into proteins during a constant 10-h infusion of L-[1-13C]leucine, using plasma KIC enrichment as precursor pool. ASRs were calculated as FSR × plasma concentration × plasma volume. All data are means ± SE; *P < 0.02, †P < 0.01, ‡P < 0.001.

DISCUSSION

The patients in this study were head trauma patients, and they might not be representative of all the trauma situations, due to the unique locus of injury, but, as far as acute inflammation is concerned, their inflammatory profiles are very similar to those observed in other traumas or sepsis. It is possible that factors other than the head trauma itself contributed to the observed changes. For example, prolonged immobilization reduces muscle protein synthesis (16). Also, central catheters can induce inflammation in rats (11). However, for ethical reasons it was not possible to fit the control subjects with such a catheter, and, furthermore, a full surgical procedure is needed in rats, whereas catheters are implanted percutaneously in a few minutes in humans.

Whole body protein metabolism was assessed with the well-established [13C]leucine method, and our data confirm a recent study showing an increased whole body synthesis 48 h after head injury (14). For leucine oxidation calculations, we used a recovery factor of 0.9, which was measured in patients, including those with head trauma, with an artificial ventilation very similar to that of the patients in our study (30). In any case, using any factor between 0.82 and 1.0 does not affect our conclusions, given the large difference of leucine oxidation between the patients and controls.

Whole body protein synthesis reflects the sum of protein synthesis rates of all tissues and, in particular, of muscle and liver, which together account for the major part of the whole body protein synthesis rate. In muscle, plasma KIC enrichments slightly overestimate both leucyl tRNA and intracellular free leucine enrichments (7), and our FSR is thus likely to be slightly underestimated. Although this problem could have been overcome by using the flooding dose method, this
approach does not allow simultaneous measurements of whole body protein synthesis and could possibly modify protein synthesis in itself (25). Also, the large difference of muscle FSR between the controls and the patients was possibly overestimated because of the higher muscle proteolysis in the patients (19), probably leading to a higher dilution of the label in the precursor pool. However, the leucyl tRNA enrichment would have to be as low as 36% of the KIC enrichment to fully compensate for this difference, which is highly unlikely. Furthermore, our observation is consistent with animal and human studies performed, with the flooding dose method showing that sepsis or inflammation in rats (5, 10) or surgery in humans (13) all decrease muscle FSR. Cytokines and glucocorticoids were probably responsible for this decrease. IL-1β depresses muscle protein synthesis (5), TNF inhibits amino acid uptake [this effect being mediated in part by glucocorticoids (33)], and finally, IL-1 receptor antagonist prevents the sepsis-induced inhibition of synthesis (10). Our patients did exhibit hypercortisoluria and high plasma levels of IL-1β, but we failed to show increased TNF levels, which are very transient and which probably would have occurred early after admission.

The dramatic increase of fibrinogen FSR and ASR was expected, fibrinogen being taken as a paradigm for the positive acute-phase proteins, and Thompson et al. (29) reported similar increases in three traumatized patients. This is also consistent with the increased expression of the fibrinogen gene in rat liver after surgery (21). IL-6 is considered as the cytokine predominantly involved in the increased fibrinogen synthesis (8). Although the rate of synthesis of other positive acute-phase proteins was not measured, the dramatic increase of their concentrations suggests that their synthesis was stimulated as well.

The issue of albumin synthesis is more complicated, since we observed increased FSR (×1.6) and ASR (×1.5) despite hypoalbuminemia. Albumin concentration always decreases in severe inflammatory, septic, or injured patients and animals; for example, it was shown to reach a nadir 7 days after injury in a large series of head-injured patients (20). Hypoalbuminemia might be due to a reduced synthesis or an increased degradation or an alteration of the transcapillary escape rate. The former hypothesis is the most widely accepted (1) on the basis of numerous animal studies showing a decreased albumin gene expression, occurring rapidly after various aggressions (e.g., Refs. 21 and 22). However, actual measurements of albumin synthesis are less consistent, showing either a decrease (4, 22, 26, 27) or an increase (18, 31). A reduced dietary intake also participates in the depressed albumin synthesis (2). Data in humans are extremely scarce. Moshage et al. (22) reported, in four normally fed patients with various diseases and profound hypoalbuminemia, a 25% decrease of albumin ASR but an unchanged FSR.

Thus there is little doubt that an acute inflammation initially depresses albumin gene expression and synthesis, which might well have occurred in our patients and been responsible for the initiation of hypoalbuminemia. However, the hypoalbuminemia observed later on is actually associated with an increased albumin synthesis. This suggests a biphasic pattern (decrease, then increase) for albumin synthesis. Such a response was demonstrated in a prolonged animal model of sepsis in which albumin synthesis was measured 16 (26) and 96 h (31) after injury. Glucocorticoids are known to increase albumin synthesis in vivo (12) and to upregulate albumin gene expression in vitro (3). In our patients, cytokine levels (IL-6, IL-1β) were stable between days 4 and 8, whereas cortisoluria doubled over the same period (19). Therefore, we could speculate that glucocorticoids mediate the secondary increase of albumin synthesis. Alternatively, plasma amino acids and particularly leucine concentrations were possibly elevated in the patients due to their higher proteolysis (and to the slightly higher rate of tracer infusion). They could stimulate albumin synthesis, as demonstrated in cancer patients fed with a branched-chain amino acid formula (28). The persistent hypoalbuminemia could be explained by an escape of plasma albumin in the extravascular space. In this respect, the albumin transcapillary escape rate was found to be increased by 50–300% in critically ill (15) and in infected patients (6). Also, IL-1β was reported to dramatically increase albumin transfer across endothelial cells (20). However, the positive correlation between albumin levels and its transcapillary escape rate reported by Ballmer et al. (6) suggests that other mechanisms, such as an increased degradation rate, could also contribute to hypoalbuminemia in the acute-phase response.

In conclusion, we demonstrated that, in continuously fed humans with severe inflammation, there are opposite changes in the synthesis of muscle and acute-phase hepatic protein, including albumin. This could be interpreted as a necessity for the liver to be supplied with large amounts of amino acids for sustaining the acute-phase hepatic response, the amino acids being derived from increased breakdown and decreased synthesis in muscle. This is different from what is observed in rats with moderate inflammation, in which liver synthesis increases while muscle is unaffected (24). Also, it was recently shown that the reversal of muscle loss by cytokine antagonists does not affect hepatic synthesis in septic rats (11). Therefore, further studies are needed both in humans and animal models to establish whether or not there is a causal relationship between changes in muscle and liver protein synthesis during severe inflammation.

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