Impact of surgical trauma on human skeletal muscle protein synthesis

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ABSTRACT

Muscle protein catabolism is a considerable clinical problem following surgery. However, the impact of surgical trauma on muscle protein synthesis is not well characterized. In this pilot study, we therefore investigated whether the severity of surgical trauma is related to a decrease in muscle protein synthesis rate in humans. Metabolically healthy patients (n = 28) were included in the study. Eight of the patients were day-care patients undergoing minor breast surgery (defined as minor surgery). The other 20 patients were subjected to major abdominal surgery and were therefore scheduled to stay overnight in the recovery room during the first postoperative night (defined as major surgery). Protein FSRs (fractional synthesis rates) in skeletal muscle were determined during a measurement period of 90 min before surgery and immediately after termination of surgery. FSR in skeletal muscle of the minor surgery patients was 1.72 ± 0.25 %/24 h before surgery and 1.67 ± 0.29 %/24 h after surgery (P = 0.68). In the major surgery group, FSR was 1.62 ± 0.30 %/24 h before surgery and 1.57 ± 0.40 %/24 h (P = 0.59) immediately following surgery. The observations made in this pilot study could not confirm a size-related decrease in muscle protein synthesis immediately following minor and major surgery. This finding is discussed in relation to confounders, postoperative course and to muscle protein degradation. The shortage of knowledge in this field is emphasized.

INTRODUCTION

Muscle protein catabolism is a considerable clinical problem following surgical trauma. This muscle-wasting is a result of an imbalance between muscle protein synthesis and breakdown. However, little is known about the precise imbalance in muscle protein turnover and the effect of the severity of surgical trauma on this. Whole-body protein degradation generally increases in response to surgical trauma [1], whereas whole-body protein synthesis is variable according to the severity of the insult [2–4]. Together, the changes in whole-body protein turnover result in a period of negative nitrogen balance. This whole-body nitrogen loss is proportional to the severity of trauma [5]. However, whole-body measurements reflect changes in all the tissues and, therefore, it might not reflect changes in skeletal muscle, despite muscle being the largest tissue.

It has been shown that the rate of muscle protein synthesis decreases by 30 % immediately after open cholecystectomy [6], a surgical procedure of medium severity, with a further decrease 3 days post-surgery to 50 % of the pre-surgery rate [7]. It is not known whether the magnitude of the decrease in muscle protein synthesis rate after surgery is related to the severity of the traumatic insult associated with the surgery. Several metabolic changes are related to the severity of surgery, such as the reduction in insulin sensitivity [8] and nitrogen balance [3]. In addition, the cytokine response of IL-6 (interleukin-6) as a marker of tissue damage correlates with

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Abbreviations: BMI, body mass index; FSR, fractional synthesis rate; MPE, molar percentage excess.
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the severity and duration of surgery [9]. However, the relationship between the magnitude of surgical trauma and the suppression of the rate of muscle protein synthesis is not well characterized. Therefore the aim of this pilot study was to determine the effect of minor and major surgery on muscle protein FSR (fractional synthesis rate) in human skeletal muscle in the immediate postoperative period. Minimal invasive breast surgery performed as day cases was defined as minor surgery. Major abdominal surgery scheduled for an overnight stay in the recovery room during the first postoperative night, according to the pre-operative evaluation procedure, was defined as major surgery.

MATERIALS AND METHODS

Materials

L-[ring-2H5]Phenylalanine (99 atom%; Cambridge Isotope Laboratory, Andover, MA, U.S.A.) was dissolved in sterile water together with unlabelled phenylalanine (Ajinomoto Company, Tokyo, Japan) to a concentration of 20 g/l and an enrichment of 7.5 and 15 MPE (molar percentage excess). The solutions were prepared, filter sterilized and stored in sterile containers by the hospital pharmacy. All solutions were tested for sterility and pyrogenity.

Subjects

Metabolically healthy patients planned for elective surgery participated in the study. All had normal routine blood chemical analyses for haemoglobin, leucocytes, glucose, liver enzymes and renal function tests at inclusion in the study. No interventions were done regarding diet before arrival to the hospital or prior to the surgical procedure. Eight female patients scheduled for minor breast surgery, mainly for removal of benign tumours as a day-care procedure, were included in the minor surgery group [mean age, 50.4 \(\pm\) 6.4 years; mean BMI (body mass index), 24.6 \(\pm\) 2.4 kg/m\(^2\)]. The major surgery group contained 20 patients undergoing planned major abdominal surgery. Initially, ten patients (Group A) were included in the major surgery group; however, to enable better identification of possible confounders in this rather heterogeneous group of patients, the Ethical Committee approved the addition of another ten patients (Group B) to the major surgery group. Characteristics of the patients are given in Table 1. The patients included in the major surgery group were admitted to the hospital the day before surgery and they were scheduled to stay in the recovery room during the first postoperative night. According to the standard clinical practice, all patients were fasted overnight before surgery.

The nature, purpose and potential risks of the experimental procedures were explained to the patients before
obtaining their voluntary consent. The Ethical Committee of the Karolinska Institutet, Stockholm, Sweden approved the research protocol.

**Study protocol**

The patients were taken to the operating theatre after an overnight fast. The first determination of protein synthesis was performed during a period of 90 min before surgery. An intravenous injection of L-[2H5]phenylalanine (45 mg/kg of body weight as a 2 % solution of 7.5 MPE) was given in the antecubital vein over 10 min. Blood samples were taken before and at 5, 10, 15, 30, 60 and 90 min after the phenylalanine injection for determination of the L-[2H5]phenylalanine enrichment in plasma. A muscle biopsy was taken 90 min after the start of isotope injection, for assessment of L-[2H5]phenylalanine enrichment in muscle protein. This first muscle biopsy was taken immediately after induction of anaesthesia but before the start of surgery.

The second determination of muscle protein synthesis rate was started immediately after termination of surgery, starting with an initial muscle biopsy at time zero. An intravenous injection of L-[2H5]phenylalanine (45 mg/kg of body weight as a 2 % solution of 15 MPE) was given and the assessment of protein synthesis was completed according to an identical protocol that applied for the pre-operative measurement.

**Anaesthesia and operation**

**Minor surgery**

Anaesthesia for minor surgery was standardized so that all patients received an intramuscular injection of oxicon-scopolamine as a premedication. Anaesthesia was induced with thiopental before the first muscle biopsy, and anaesthesia was then maintained with 70 % nitrous oxide in oxygen together with isoflurane. Minor surgery patients did not receive any muscle relaxants. Perioperatively the patients were given an intravenous infusion of saline (3 ml · kg⁻¹ of body weight · h⁻¹).

**Major surgery**

Anaesthesia for major surgery group was standardized within Groups A and B, although there was a difference between the two groups. Group A received an intramuscular injection of oxicon-scopolamine as a premedication. Anaesthesia was then induced with thiopental, fentanyl and diazepam, and anaesthesia was then maintained with 70 % nitrous oxide in oxygen together with isoflurane. Group B received a premedication of morphine intramuscularly. Anaesthesia was induced by thiopental, fentanyl and diazepam and maintained with isoflurane or sevorane. In both groups, repeated doses of analgesics and muscle relaxants, fentanyl and pancuronium, were given to provide stable anaesthesia and muscle relaxation. All patients in the major surgery groups were given an infusion of glucose [5 % (v/v); 1 ml · kg⁻¹ of body weight · h⁻¹]. Fluid balance in the major surgery group was individualized according to the patient’s clinical status by the anaesthesiologist in charge. In the major surgery group, 17 out of 20 patients received an indwelling epidural catheter before induction of anaesthesia, but the epidurals were not activated until after the final determination of muscle protein synthesis rate. Details regarding anaesthesia, surgery and operation are listed in Table 1.

**Muscle biopsy technique**

Percutaneous muscle biopsies of approx. 50 mg of wet weight were taken with the Bergström needle [10] from the lateral portion of the quadriceps femoris muscle, 10–20 cm above the knee. The initial biopsy was taken from one leg and the two following biopsies from the other leg approx. 10 cm apart. The biopsy material was washed in ice-cold 0.9 % saline before being carefully dissected to remove visible fat and connective tissues, and then divided into two parts of equal size. The specimens were frozen in liquid nitrogen within 60 s and stored at −80 °C.

For the final muscle biopsies in the conscious patients, local anaesthesia was given in the skin and to the muscle fascia.

**Measurement of protein synthesis**

The measurement of protein synthesis rate in muscle tissue by the flooding-dose technique has been described in detail previously [11]. In brief, the determination of L-[2H5]phenylalanine enrichment in plasma samples, as well as in samples of hydrolysed muscle protein, was done by GC–MS with electron-impact ionization and selective ion monitoring. The enrichment in plasma was measured by monitoring the ions at masses of m/z 336 and 341 of the tertiary butyldimethylsilyl derivate of phenylalanine. The enrichment of phenylalanine from protein hydrolysates was measured following enzymic decarboxylation to phenylethylamine and subsequent analysis of its heptafluorobutyryl derivative at masses of m/z 106 and 109 [12]. The determinations were made on a quadrupole mass spectrometer (VG 12-253; VG Biotech, Altrincham, U.K.).

Protein FSR (in percentage/24 h) was calculated from the formula:

\[
FSR = \left[ \frac{P(t) - P(0)}{A} \right] \times \frac{100}{A}
\]

where P(0) and P(t) are the enrichments of phenylalanine in muscle protein at the beginning and at the end of the incorporation period (MPE) respectively, and A is the area under the curve for plasma phenylalanine enrichment (MPE × time in days).

**Statistics and calculations**

The comparison of the rates of protein synthesis was made by Student’s t test for paired samples. Correlations
RESULTS

Minor surgery
Mean operating time was 34 ± 13 min, and blood losses were minor. All patients were awake during the second determination of FSR.

Major surgery
One patient did not survive the surgical procedure and was excluded from the study. The mean age and BMI of the remaining 19 patients was 58.1 ± 10.5 years and 23.4 ± 3.8 kg/m² respectively. Mean operating time was 211 ± 80 (range, 90–340) min, and mean blood loss was 1450 ± 1411 (range, 100–5200) ml. Postoperative body temperature at the beginning of the second measurement was 35.9 ± 1.2 °C (Table 1). Eleven of the patients were sedated and mechanically ventilated during the second determination of protein FSR.

Muscle protein synthesis
Muscle protein FSR was determined after an overnight fast before surgery and immediately after termination of elective surgery (Figure 1). Muscle protein FSR in the minor surgery patients was 1.72 ± 0.25%/24 h before surgery and 1.67 ± 0.29%/24 h after surgery (P = 0.68). In the major surgery group, FSR was 1.62 ± 0.30%/24 h before surgery and 1.57 ± 0.40%/24 h (P = 0.59) immediately following surgery.

DISCUSSION
The main hypothesis of the present study was that surgical trauma would decrease FSR of muscle proteins in relation to the severity of the trauma. The results from the study showed, as expected, that minor surgery did...
not have any detectable impact on muscle protein FSR. However, muscle protein FSRs were also shown to be unaltered following major surgery.

The lack of impact of major surgery on the muscle protein FSR was somewhat surprising. Surgery of medium severity, such as open cholecystectomy, is associated with a decrease in FSR by 30% at the end of the surgical procedure. Furthermore, a decrease of this magnitude is also reported during the initial postoperative days following laparoscopic cholecystectomy [13], hip replacement [14] and colonic resection [15]. The measured decrease in muscle protein FSR after cholecystectomy is also supported by a reduction in the concentration of polyribosomes 24 and 72 h postoperatively [16].

An inventory of possible confounding factors in the group of major surgery patients includes the type of surgical procedure, anaesthesia procedure, malignancy, the amount of bleeding, the need for inotropic drugs, the fluid balance, the duration of surgical procedure, the body temperature, age and gender, as well as Group A and B studied at different time points using slightly different anaesthesia techniques. When calculating correlation coefficients for these factors none of them could be identified as a confounder (Table 2).

First, two differences in experimental set-up between the present study and the earlier study [6] reporting a decrease in skeletal muscle protein synthesis immediately following open cholecystectomy must be considered. The general anaesthesia administered was different between the two studies, reflecting the general development in anaesthesia techniques over the last 10–15 years. The general anaesthesia reported to have no impact on muscle protein FSR was a modified neurolept anaesthesia [6]. In the present study, however, general anaesthesia was based on the volatile agents isoflurane and sevoflurane. This may be a confounder, but whole-body protein synthesis is not affected differently by intravenous anaesthesia (propofol/remifentanil) or desflurane/remifentanil [17]. Furthermore, the use of a continuous epidural blockade has been reported to attenuate the decrease in muscle protein synthesis 48 h postoperatively [15]. However, in the present study, the indwelling epidural catheters were not activated during the study period.

In addition, the amino acid label used to determine muscle protein synthesis was different between the two studies. For the open cholecystectomy patients [13C]leucine was used [6], whereas, in the present study, [13H3]phenylalanine was employed. In healthy volunteers, the absolute level of skeletal muscle FSR is slightly lower when phenylalanine is used [11,18]. However, there are no indications of a difference in the response to physiological stimuli measured with the two different amino acid tracers. Although these methodological confounders may contribute to a difference in results, this is not a likely explanation.

One possible explanation for the lack of an expected suppression in the rate of muscle protein synthesis following major surgery is that the time course following surgical trauma may be variable in relation to the severity of surgery. The previously reported decrease in muscle protein synthesis was in open cholecystectomy patients, which have no elevation in muscle protein degradation [19–22]. In the present study, muscle protein FSR was determined immediately after the end of surgery, but the effects on muscle protein FSR later in the postoperative course following major surgery have not been studied so far. The changes in ribosomal pattern seen after elective surgery are still not completely restored 1 month postoperatively [23]. On the other hand, the negative whole-body protein balance seen postoperatively [24] is primarily related to the increase in whole-body protein degradation being larger than whole-body protein synthesis.

A second possibility is that a larger variation in the magnitude of the postsurgical muscle protein synthesis rate prevented the detection of a suppression. This was also the rationale for extending the major surgery group to 20 individuals. However, the results obtained were very similar to the initial ten patients in the major surgery group. The level of scatter in the measurements in the present study, as illustrated in Figure 1, implies that we were only able to exclude a change in FSR > 15% in the major surgery group. For patients undergoing minor surgery, we were only able to exclude any change > 17%. Skeletal muscle protein synthesis rate in the major surgery patient group in the present study is comparable with that observed in intensive care patients [25,26]. Both major surgery and intensive care patients show a mean value of FSRs in skeletal muscle which is similar to that

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**Table 2** Major surgery and correlations between the change in FSR and different patient and perioperative parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r value</th>
<th>P value</th>
</tr>
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<tr>
<td>Gender</td>
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<td>0.55</td>
</tr>
<tr>
<td>Age</td>
<td>−0.32</td>
<td>0.18</td>
</tr>
<tr>
<td>Length</td>
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<td>0.36</td>
</tr>
<tr>
<td>Weight</td>
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<td>0.09</td>
</tr>
<tr>
<td>BMI</td>
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<td>0.09</td>
</tr>
<tr>
<td>FSR 1</td>
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<td>0.07</td>
</tr>
<tr>
<td>Operation time</td>
<td>−0.05</td>
<td>0.84</td>
</tr>
<tr>
<td>Inotropic support</td>
<td>−0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>Blood products</td>
<td>0.12</td>
<td>0.59</td>
</tr>
<tr>
<td>Blood loss</td>
<td>0.05</td>
<td>0.84</td>
</tr>
<tr>
<td>Colloids</td>
<td>0.02</td>
<td>0.92</td>
</tr>
<tr>
<td>Crystalloids</td>
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<td>0.10</td>
</tr>
<tr>
<td>Postoperative temp.</td>
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<td>0.62</td>
</tr>
<tr>
<td>Malignancy</td>
<td>−0.22</td>
<td>0.37</td>
</tr>
<tr>
<td>Group (A) and (B)</td>
<td>0.02</td>
<td>0.92</td>
</tr>
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</table>
observed in healthy individuals, but with a somewhat larger than normal S.D. What actually causes this large scatter in the two patient groups with severe, but also very heterogeneous metabolic stress, needs more investigation. One possible confounding factor may be the heterogeneity of muscle tissue. Muscle tissue consists of different fibre types. In addition, inflammatory cells, fat cells and connective tissue could infiltrate the muscle tissue. Muscle is a mixture of many different proteins that turnover at different rates. During severe trauma, these different cells or proteins might respond differently in different parts of the muscle causing the large scatter.

As muscle protein mass is regulated by the rates of both synthesis and degradation of protein, the results from the present study suggest that the muscle mass loss following major surgery is due to increases in muscle protein degradation rates. For protein synthesis, techniques are available which allow us to make reliable quantitative assessments by direct detection of the incorporation of a labelled amino acid. For degradation, on the other hand, only derived values using indirect measurements and mathematical modelling are available. Several of these semi-quantitative techniques indicate that muscle protein degradation is enhanced in patients in the intensive care unit [27–29]. Also, the efflux of 3-methylhistidine from the leg has been used to assess the level of degradation of contractile protein, which is reported not to be elevated following surgical trauma [30,31]. A new more sensitive technique employing isotopically labelled 3-methylhistidine is now available [32], which may help us measure the synthesis and degradation simultaneously with a higher precision in the future. As synthesis and degradation are likely to be regulated independently, it is highly advisable that such parallel measurements employ techniques to assess synthesis and degradation which rest on different underlying assumptions.

Surprisingly few studies are available assessing the effect of surgery on muscle protein synthesis. Therefore this relationship is rather poorly characterized. Also, the literature concerning the postoperative period following surgical trauma reveals only a small number of studies, and several of these studies are quite old employing surgical and anaesthesia techniques that are now obsolete.

In summary, the observations made in this pilot study could not confirm a decrease in muscle protein FSR immediately following surgical trauma related to the severity of surgery. The postoperative period is still insufficiently characterized in terms of muscle protein catabolism and, in particular, the extended time course of muscle protein metabolism. Good qualitative and quantitative methods to study both protein synthesis and protein degradation simultaneously, in order to fully understand the human response to surgical trauma in terms of protein metabolism, are needed.

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