Surgical trauma decreases glutathione synthetic capacity in human skeletal muscle tissue

JIA-LI LUO, FOLKE HAMMARQVIST, KERSTIN ANDERSSON, AND JAN WERNERMAN
Department of Anesthesiology and Intensive Care, Clinical Research Center, Huddinge University Hospital; Department of Surgery, St. Göran's Hospital; and Karolinska Institute, 141 86 Huddinge, Stockholm, Sweden

Luo, Jia-Li, Folke Hammarqvist, Kerstin Andersson, and Jan Wernerman. Surgical trauma decreases glutathione synthetic capacity in human skeletal muscle tissue. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E359–E365, 1998.—To gain insight into cellular metabolism underlying the glutathione (GSH) alterations induced by surgical trauma, we assessed postoperative skeletal muscle GSH metabolism and its redox status in 10 patients undergoing elective abdominal surgery. Muscle biopsy specimens were taken from the quadriceps femoris muscle before and at 24 and 72 h after surgery. GSH concentrations decreased by 40% at 24 h postoperatively compared with the paired preoperative values (P < 0.001) and remained low at 72 h (P < 0.01). The concentration of GSH disulfide (GSSG) did not significantly change throughout the study period, whereas the total GSH (as GSH equivalent) concentration decreased after surgery. Of the GSH constituent amino acids, the concentration of cysteine remained unchanged throughout the study period (from 28.2 ± 10.1 preoperatively to 29.4 ± 13.9 at 24 h postoperatively and to 28.3 ± 15.6 μmol/kg wet wt at 72 h postoperatively). Despite a reduction in glutamate concentration by 40% 24 h after surgery, no correlation was established between GSH and glutamate concentrations postoperatively. Activity of γ-glutamylcysteine synthetase did not change significantly after surgery, whereas GSSG synthetase activity decreased postoperatively (from 66.4 ± 19.1 preoperatively to 41.0 ± 10.5 24 h postoperatively, P < 0.01, and to 46.0 ± 11.7 μU/mg protein 72 h postoperatively, P < 0.05). The decrease of GSH was correlated to the reduced GSH synthetase activity seen at 24 h postoperatively. These results indicate that the skeletal muscle GSH pool is diminished in patients after surgical trauma. The depletion of the GSH pool is associated with a decreased activity of GSH synthetase, indicating a decreased GSH synthetic capacity in skeletal muscle tissue.

The reduced form of glutathione (GSH) is synthesized by virtually all cells and is maintained at millimolar concentrations under physiological conditions (28). Cellular GSH is a major nonprotein reductant that provides a reducing environment for the defense against free radicals and reactive oxygen species generated during normal oxidative metabolism and oxidative stress (20, 28). Several studies suggest that localized tissue GSH deficiency plays a role in the pathogenesis of diseases such as pulmonary fibrosis (5, 29), human immunodeficiency virus (HIV)-related disease (15, 33), and circulatory shock (7). Studies with mice and rats indicate that skeletal muscle tissue contains a major fraction of the total body GSH (26). Skeletal muscle has been recently recognized as a tissue in which GSH depletion may cause cellular damage reflected by muscle degeneration and mitochondrial damage (25). Because of the comparatively low levels of catalase and superoxide dismutase in skeletal muscle (10), one may expect to see GSH-dependent reactions for detoxification of reactive oxygen species. Patients resuscitated from cardiogenic shock show low skeletal muscle total GSH content in parallel with impaired mitochondrial function (7).

The metabolic response to surgical trauma is characterized by protein depletion in skeletal muscle (35) along with an increased oxidation and an efflux of the intracellular free amino acid pool (6, 12). Likewise, patients undergoing elective surgery show a marked decrease of the cellular GSH concentrations in skeletal muscle in the immediate postoperative period (23). This change is most profound at 24 h, and the muscle GSH levels remain low 48 h after surgery. The decrease of cellular glutathione levels is mainly due to a decrease in GSH, whereas a relatively stable GSH disulfide (GSSG) is also observed. Among the precursor amino acids, both glutamate and glutamine, which are characteristic markers of muscle protein catabolism and GSH constituent amino acid, are depleted by > 40% after surgical trauma (23). It has also been shown that GSH metabolism changes during critical illness when low levels of GSH and total GSH (as GSH equivalent) are found in skeletal muscle (16).

Cellular GSH homeostasis depends on a complex process of precursor amino acid uptake, synthetic enzymatic activity, redox status, and efflux of both GSSG and GSH from cells. The previous findings prompted us to try to clarify the mechanism by which surgical trauma alters the GSH synthetic process and oxidation of GSH in skeletal muscle of surgical patients. We extended the study period to the third postoperative day to gain more information concerning the temporal effect of surgical trauma on muscle GSH pools. The purpose of the present study was to characterize and quantify changes in GSH redox status, the enzymatic processes of GSH synthesis and oxidation, and changes in GSH precursor amino acid concentrations that occur in skeletal muscle postoperatively.

MATERIALS AND METHODS

Materials. GSH, cysteine (Cys), γ-glutamylcysteine (GSH), purity, dithiothreitol (DTT), N-ethylmaleimide (NEM), N-ethylmaleimide and amino acid standard solution were purchased from Sigma Chemical (St. Louis, MO). Monobromobimane (thiolyte monobromobimane) was obtained in >99% purity from Calbiochem (La Jolla, CA). Acetonitrile (HPLC grade) was purchased from Labscan (Dublin, Ireland). Acetic
acid, perchloric acid, and sulfosalicylic acid (SSA) were from Merck (Darmstadt, Germany).

Subjects and study protocol. Patients (n = 10) without either endocrine disorders or medication for any metabolic diseases who were undergoing elective abdominal surgery were studied. The characteristics of the patients and the surgical procedures are presented in Table 1. All patients were operated on under general anesthesia. A glucose-free electrolyte solution (sodium chloride; Baxter, Bromma, Sweden) was given during surgery, and during the postoperative period, 2.0 g glucose·kg body wt \(^{-1}\)·24 h \(^{-1}\) (10% glucose; Pharmacia, Stockholm, Sweden) were given on the first day and 3.0 g glucose·kg body wt \(^{-1}\)·24 h \(^{-1}\) on the following 2 days. A nutritionally normal glucose supply was chosen, as this is the routine management of nonmalnourished patients after elective abdominal surgery. Percutaneous muscle biopsies were taken immediately after induction of general anesthesia but before surgery and at 24 and 72 h after the start of the operations. Parallel blood samples were also taken at each time point. The purpose, procedure, and possible risks involved in the study were explained to the patients before their voluntary consent was obtained. The study protocol was approved by the Ethics Committee of the Karolinska Institute (Stockholm, Sweden).

Sample collection and preparation. At each sampling time point, percutaneous muscle biopsy specimens (100–150 mg wet wt) were obtained from the lateral portion of the quadriceps femoris muscle 15 cm above the knee using the percutaneous needle biopsy technique after local anesthesia of the skin. Each specimen of 20–50 mg wet weight was weighed and shock-frozen in liquid nitrogen within 2 min of sampling. Samples were stored at \(-80^\circ\)C for the analysis of GSH, free amino acids, and enzyme activity. Blood samples were collected by venipuncture into heparinized vacutubes and processed immediately.

Tissue samples were homogenized in 6.5% (wt/vol) SSA solution in a glass homogenizer on ice and then centrifuged at 12,000 \(g\) for 15 min at 4°C. The acid extracts were then split into portions for the measurements of thiols, disulfides, and total thiols (as thiol equivalent). For the analysis of muscle free amino acids, the frozen muscle biopsy specimen was homogenized in 4% (wt/vol) SSA containing norleucine as internal standard. The homogenate was centrifuged at 12,000 \(g\) for 15 min at 4°C, and the supernatant was stored at \(-80^\circ\)C, pending analysis of free amino acids.

For the measurement of thiols (GSH and Cys), the direct derivatization procedure was performed as described previously (24). Briefly, a portion of 100 \(\mu\)l SSA extracted sample was neutralized with NaHCO\(_3\) powder and mixed with 100 \(\mu\)l monobromobimane (20 \(\mu\)M in sodium N-ethylmorpholine, pH 8.0). The reaction was allowed to proceed for 10 min in the dark at room temperature and stopped by the addition of 10 \(\mu\)l of 90% (wt/vol) SSA.

For the measurement of disulfides (GSSG and cystine), a second portion of 100 \(\mu\)l SSA extracted sample was adjusted to neutral pH by NaHCO\(_3\) powder. NEM was added to the neutralized sample at a final concentration of 10 mM, and sample was incubated at room temperature for 5 min. Excess NEM was destroyed by a 3 M potassium phosphate (pH 13) buffer to adjust the pH for alkaline hydrolysis (final pH 11.5–11.8). After 25 min of incubation for the hydrolysis of NEM, the samples were neutralized by addition of 10 \(\mu\)l of 90% (wt/vol) SSA. The GSSG and cystine were then reduced with 10 \(\mu\)l of 50 mM DTT for 30 min and thereafter derivatized with 100 \(\mu\)l monobromobimane (20 \(\mu\)M) in the dark for 10 min. Reactions were stopped by acidifying with 10 \(\mu\)l of 90% (wt/vol) SSA.

Total glutathione and cysteine were measured in their thiol equivalents (e.g., total GSH and total Cys). A third portion of 100 \(\mu\)l neutralized sample was treated first with 10 \(\mu\)l DTT (50 mM) for 30 min, followed by a derivatization of 100 \(\mu\)l monobromobimane (20 \(\mu\)M) in the dark for 10 min. Reactions were stopped with 10 \(\mu\)l of 90% (wt/vol) SSA.

Plasma samples were prepared by centrifugation at 4°C, 3,000 rpm for 2 min, immediately after sampling. Whole blood was mixed with 3 vol Millipore water and underwent three freeze-thaw cycles to disrupt the membranes of the red blood cells. Separated portions of both samples were treated directly with monobromobimane for the measurement of thiols and with NEM and DTT for disulfides and total thiols, respectively. The standards of GSH, Cys, and \(\gamma\)-glutamylcysteine were derivatized as for the treatment of the thiols described above.

Measurement of GSH, Cys, and their disulfides. The HPLC separation of the thiol-bimane adducts was achieved on a reversed-phase Supercosil LC-18 octadeclaryl silica column (150 \(\times\) 4.6 mm ID, 3-\(\mu\)m particle size) and followed by fluorimetric detection at an excitation wavelength of 394 nm and an emission wavelength of 480 nm. The system consisted of a Waters 625 LC pump system, a Waters 470 scanning fluorescence detector, and a Waters 715 Ultra WISP sample processor (Millipore, Milford, MA). Elution solvent A was 7.0% (vol/vol) aqueous acetonitrile containing 0.25% (vol/vol) acetic acid and perchloric acid; the final pH was adjusted to 3.71 with sodium hydroxide. Elution solvent B was 75% (vol/vol) acetonitrile. The elution program consisted of 100% solvent A for 11 min, followed by 100% solvent B for 5 min to elute matrix interference, returning to solvent A for reequilibration for 9 min. The flow rate was 1.0 ml/min.

Table 1. Characteristics of the patients and the operative procedure

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gender</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>Operation Time, min</th>
<th>Blood Loss, ml</th>
<th>Operation Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>63</td>
<td>67</td>
<td>159</td>
<td>100</td>
<td>100</td>
<td>Sigmoid resection</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>64</td>
<td>87</td>
<td>183</td>
<td>170</td>
<td>1,200</td>
<td>Anterior resection</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>75</td>
<td>90</td>
<td>190</td>
<td>140</td>
<td>200</td>
<td>Gastric resection</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>75</td>
<td>48</td>
<td>150</td>
<td>90</td>
<td>300</td>
<td>Hemicolectomy dexter</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>70</td>
<td>71</td>
<td>173</td>
<td>140</td>
<td>450</td>
<td>Fundoplicatio</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>59</td>
<td>85</td>
<td>175</td>
<td>160</td>
<td>500</td>
<td>Resection of the small intestine</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>74</td>
<td>84</td>
<td>172</td>
<td>110</td>
<td>300</td>
<td>Fundoplicatio</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>72</td>
<td>64</td>
<td>167</td>
<td>100</td>
<td>300</td>
<td>Anterior resection</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>65</td>
<td>97</td>
<td>180</td>
<td>90</td>
<td>400</td>
<td>Fundoplicatio</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>78</td>
<td>74</td>
<td>173</td>
<td>130</td>
<td>1,200</td>
<td>Anterior resection</td>
</tr>
</tbody>
</table>

M, male; F, female.
The monobromobimane derivatives of GSH and Cys in each portion of derivatized samples were then separated by HPLC and quantified on the basis of peak areas and compared with the authentic GSH and other thiol standards.

Measurement of activities of GSH-related enzymes. The muscle biopsy specimens were homogenized in 50 mM Tris·HCl buffer (pH 8.0) containing 150 mM KCl, 0.1 mM EDTA, and 100 units of heparin/ml. The homogenates were centrifuged at 12,000 g for 15 min. The activities of γ-glutamylcysteine synthetase and GSH synthetase were assayed by measurement of their respective enzymatic products, γ-glutamylcysteine and GSH. The procedure and condition employed in the sample derivatization and HPLC separation for the measurement of GSH and γ-glutamylcysteine were the same as described above. The incubation mixture for the assay of γ-glutamylcysteine synthetase contained 1 M Tris·HCl (pH 8.2), 6 mM ATP, 50 mM KCl, 6 mM DTT, 20 mM MgCl2, 3 mM Cys, and 15 mM glutamic acid and was preincubated at 37°C for 15 min to ensure the complete reduction of thiols. The reaction was initiated by addition of the supernatant for muscle tissue. The same incubation mixture was used for the assay of GSH synthetase, with the exception that the Cys and the glutamic acid were substituted by 3 mM γ-glutamylcysteine and 30 mM glycine. After 30 min of incubation at 37°C, the reactions were stopped by adding 10 μl of 90% (wt/vol) SSA. After removal of protein, samples of 100 μl were taken from the protein-free supernatant for derivatization of the reaction products. For the time 0 sample, 10 μl of 90% (wt/vol) SSA were added to the incubation buffer before the addition of tissue samples. The resulting protein was removed by centrifugation, and 100 μl of supernatant were withdrawn for derivatization of the reaction products. The samples to be derivatized were added to an Eppendorf tube containing 100 μl of 20 mM monobromobimane (in 50 mM N-ethylmorpholine, pH 8.4). The mixtures were placed in the dark, at room temperature, and allowed to stand for 10 min. The reactions were stopped by the addition of 10 μl of 90% SSA. Samples were filtered before HPLC analysis. One unit of enzyme activity was defined as the amount that catalyzed formation of 1 μmol of product/min at 37°C. GSH peroxidase activity was determined as described by Flohé and Günzler (13) with H2O2 as substrate. One unit of GSH peroxidase activity was the amount of enzyme that oxidizes 1 μmol of GSH (disappearance of GSH)/min at 37°C.

Measurement of muscle free amino acids and protein content. The concentration of free amino acid in the supernatant was determined using an automated amino acid analyzer (Alpha Plus; LKB Pharmacia), as described previously (4). The amino acids were separated on an Ultropac 8 Lithium form ion-exchange resin using lithium citrate buffers (Pharmacia, Biochrom, Cambridge, UK). The amino acids were detected and quantified by postcolumn derivatization with o-phthalaldehyde and fluorescent detection at excitation wavelength of 350 nm and emission wavelength of 420 nm. Protein content was quantified by the method of Lowry et al. (22).

Data expression and statistical analysis. All data are given as means ± SD. Concentrations of free amino acids and GSH in muscle biopsy are expressed per kilogram wet weight specimen when the weighing was done. All enzymatic units are normalized with respect to milligram of protein and expressed as units per milligram protein. One-factor ANOVA for repeated measurements was used to assess the significance of changes. Scheffé’s post hoc test was performed as a multiple comparison test, and P < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Effect of surgical trauma on GSH in skeletal muscle. The effect of elective surgery on GSH concentration in muscle was evaluated before and at 24 and 72 h after surgery (Fig. 1 and Table 2). The concentration of GSH decreased by 40% at 24 h postoperatively compared with the paired preoperative values (P < 0.001) and remained low at 72 h postoperatively (P < 0.01). However, GSH concentrations did show signs of recovery at 72 h compared with the 24-h values (P < 0.05). The concentrations of GSSG remained unaltered throughout the study period (Table 2), whereas total GSH concentrations decreased after surgery. The GSH-to-GSSG ratio decreased significantly after surgical trauma (P < 0.05).

Effect of surgical trauma on GSH in blood. Table 3 shows a summary of plasma and whole blood concentrations of GSH and GSSG of the patients studied. Neither GSH nor GSSG changed significantly in either plasma or whole blood of the patients after surgery.

GSH-related amino acids in muscle tissue. Concentrations of Cys and γ-glutamylcysteine were measured simultaneously with GSH using the HPLC method. The concentrations of all other GSH-related amino acids in skeletal muscle were determined by ion-exchange chromatography. The concentrations of Cys and γ-glutamylcysteine remained unchanged throughout the study period (Table 4). The concentrations of glutamate decreased by 40% 24 h after surgery compared with the paired preoperative values (P < 0.001) and stayed low at 72 h (P < 0.001) with no tendency to recovery. The glutamine concentrations decreased by 20% at 24 h (P < 0.01) and by 40% at 72 h (P < 0.001).
postoperatively compared with the paired basal preoperative values. Glycine concentrations were elevated by 62% at 24 h postoperatively (P < 0.05) but returned to normal at 72 h postoperatively.

Effect of surgical trauma on activities of GSH-related enzymes in skeletal muscle. To investigate if changes in GSH synthetic enzyme activities might be responsible for the decrease in muscle GSH concentration, we measured enzyme activities in extracts of muscle biopsies before and after surgery. γ-Glutamylcysteine synthetase activity did not change significantly after surgery (Table 5), whereas GSH synthetase activity decreased postoperatively (Fig. 2 and Table 5). At 24 h after surgery, GSH synthetase activity was 38% lower than its preoperative values (P < 0.01), whereas the activity was still 31% lower than preoperative values (P < 0.05) at 72 h postoperatively. To see if the loss of cellular GSH was related to an increase in GSH oxidation after surgery, the activity of GSH peroxidase was assayed before and after surgery. Decreased GSH peroxidase activity was seen at both 24 h (7.0 ± 1.3 µU/mg protein, P < 0.05) and 72 h (8.0 ± 1.5 µU/mg protein, P < 0.05) postoperatively compared with the paired preoperative values (10.3 ± 2.4 µU/mg protein; Table 5).

**DISCUSSION**

We observed that surgical trauma induced a depletion of the skeletal muscle GSH pool by decreasing the capacity for GSH synthesis. This was associated with a decreased GSH synthetase activity that was most pronounced at 24 h after surgery and was correlated with the decreased muscle GSH levels. Several factors can potentially influence the cellular levels of GSH. These include 1) the availability of precursor amino acids, 2) the regulation of the enzymes involved in GSH synthesis, and 3) alterations in the cellular redox system. We set out to systematically examine each possibility to understand the mechanism causing the depletion of skeletal muscle GSH after surgical trauma.

In most tissues, the availability of the precursor amino acids is considered a regulatory factor for GSH synthesis (8). It is generally believed that Cys is a limiting precursor for GSH synthesis and that glutamate and glycine are not dependent factors, as their intracellular concentrations are high and both amino acids can be synthesized via other metabolic pathways. The enzyme γ-glutamylcysteine synthetase exhibits apparent Michaelis constant (Kₘ) values for glutamate and Cys of 1.8 and 0.3 mM, respectively (30). The available data suggest that it is regulated at a lower level than the apparent Kₘ value for Cys (34). In the present study, tissue concentrations of free Cys, glycine, glutamate, and glutamine were measured to determine the quantitative significance of the noted amino acid changes on the altered levels of GSH in skeletal muscle. After surgical trauma, the concentrations of free amino acids in skeletal muscle showed characteristic changes that reflect muscle protein metabolism. There was a 40% drop in glutamate and a
20% drop in glutamine concentrations in skeletal muscle 24 h after surgery (Table 4). Both Cys and \( \gamma \)-glutamylcysteine showed relatively low concentrations but remained unaltered postoperatively compared with the preoperative values. Although the change in glutamate concentration was concomitant with the change in GSH concentrations in muscle tissue after surgery, a correlation was not seen postoperatively between the changes of glutamate and GSH concentrations. With reference to a previous study (2), it was noted that fasting for 3 days also decreased the concentrations of glutamate and glutamine in skeletal muscle tissue to the extent seen in the present study but produced no significant change in GSH level. The data indicate that, under the catabolic conditions studied, muscle tissue is capable of maintaining its GSH level despite the reduction of glutamate concentration. It is therefore suggested that the significant decreases in muscle tissue glutamate are not related to the decreased GSH levels on surgical trauma patients. Other evidence that appears to support this conclusion is that the \( \gamma \)-glutamylcysteine level in skeletal muscle was not significantly affected by surgical trauma when a decrease of glutamate concentration occurred (Table 4). However, the effect of alteration on glutamate and glutamine levels seems to vary between tissues. For example, the availability of glutamate is shown to be favorable for the liver GSH synthesis on starvation and refeeding (34). Moreover, alterations of cellular glutamine concentration may affect GSH metabolism in tissues like liver and kidney (9, 17, 18, 32), and glutamine becomes rate limiting for GSH synthesis under oxidative stress (36). The reason for the variation is not clear, but a stable level of Cys in tissues may play a relevant role in maintaining the availability of Cys as the key rate limiting precursor of GSH synthesis.

In the present study, low but significant activities of \( \gamma \)-glutamylcysteine synthetase and GSH synthetase were found by in vitro assay in human skeletal muscle. The activity of \( \gamma \)-glutamylcysteine synthetase was unaffected by surgical trauma and showed no correlation to the decrease of GSH concentration in skeletal muscle. In contrast, surgical trauma altered the activity of GSH synthetase in skeletal muscle, giving decreases at 24 h, and it remained significantly low at 72 h after surgery compared with the preoperative values. The decrease of GSH synthetase activity was most pronounced at 24 h postoperatively, which is consistent temporally with the decrease of the muscle GSH level. The consistency was also shown when the mean values of GSH concentration were plotted in parallel with the means of GSH synthetase activity (Fig. 3). This finding indicates that there is a direct relationship between tissue GSH concentration and GSH synthetase activity and suggests a mechanism accounting for the decrease of muscle GSH level after elective surgery. Further ki-

![Fig. 2. Effect of elective abdominal surgery on activities of GSH synthetase in human skeletal muscle tissue. Activity of GSH synthetase decreased by 38% at 24 h (\( P < 0.01 \)) and 72 h (\( P < 0.05 \)) postoperatively compared with the preoperative values. Open circles represent individual values of each patient (n = 10), and error bars indicate means ± SD. Activity of GSH synthetase was measured in the crude homogenates in 50 mM Tris·HCl buffer (pH 8.0) as described in MATERIALS AND METHODS.](http://ajpendo.physiology.org/)

![Fig. 3. Association between means of GSH synthetase activity and GSH concentration in skeletal muscle of subject undergoing elective abdominal surgery (n = 10). Values are means ± SD. Decrease of GSH synthetase activity is most pronounced at 24 h after surgery, which is consistent temporally with the decrease of muscle GSH concentration.](http://ajpendo.physiology.org/)

E363MUSCLE GLUTATHIONE METABOLISM IN SURGICAL TRAUMA

http://ajpendo.physiology.org/ by 10.220.33.4 on August 14, 2017
nomic analysis of GSH synthetic enzymes is required to elucidate whether the GSH synthetase reduction is due to an RNA or a protein synthesis-dependent mechanism.

In contrast to the significant decrease in GSH concentration, surgical trauma was found to cause no accumulation of GSSG in skeletal muscle of the operated patients. Coincidentally, in vitro assay of muscle GSH peroxidase showed a significant decrease in the activity of this enzyme in response to surgical trauma (Table 5). These results indicated that, after surgical trauma, the loss of cellular GSH was not related to an increase in GSH oxidation. However, due to a significant decrease in GSH concentration, the GSH-to-GSSG ratio decreased consequently 24 h after surgery. This finding may imply an altered reductive potential and may reflect an oxidative condition in muscle tissue under the enhanced catabalism after surgical trauma. A previous study of skeletal muscle in critically ill patients showed similar results (16), with a significant decrease of GSH concentration and an apparent decrease in the ratio of GSH to total GSH (as GSH equivalent), which reflected a change in the redox status of GSH during critical illness. The cause of a decrease on GSH peroxidase activity is unclear. It is considered that surgical trauma might have affected the kinetic properties of muscle GSH peroxidase.

We have previously used a similar surgical trauma model of patients with elective abdominal surgery (23). In the present study, we used an extended study period to 72 h postoperatively to investigate the temporal effect of surgical trauma on the muscle GSH pool. The results confirmed a decrease of GSH concentration in skeletal muscle by 40% at 24 h postoperatively. In addition, a partial recovery of the GSH level at 72 h postoperatively was found. GSH deficiency, as determined by measurements in individual tissues and body fluids, has been documented in several studies (7, 15, 16, 23, 29). The impacts of GSH depletion are not clearly understood and have only been discussed in the context of an experimental depletion of the tissue GSH pool. In skeletal muscle of mice, there is tissue regeneration associated with mitochondrial damage after treatment with the irreversible inhibitor of γ-glutamylcysteine synthetase (25). Other studies in rats have shown that GSH depletion decreases the skeletal muscle citrate synthase activity (21) but increases the risk for oxidative damage with an increased production of reactive oxygen species (39).

To determine whether the changes in muscle amino acids and GSH seen postoperatively are caused by nutritional deprivation, rather than surgical trauma, we previously studied the short-term effects of fasting on muscle GSH levels (2). We found that fasting for 3 days had no significant effect on the concentrations of GSH in skeletal muscle, although glutamate and glutamine levels were affected during fasting. In the present study, the nutritional status of patients studied was reflected by their body mass index pre- and postoperatively. The patients were well nourished before and after surgery, and no nutritional disorder was observed. Thus we believe that the changes in amino acid concentration, and in GSH and GSH-dependent enzymes, may not simply be a kinetic response to the nutritional status of those patients studied. The impacts of general anesthesia and surgery on GSH, oxidative stress, and protein turnover have been also characterized in different studies. It is shown that tissue antioxidant enzyme activities were minimally affected by the use of anesthetics before euthanasia (14) and that general anesthetics have no effect on GSH in tissues except when fluroxene is used (31, 37). Previous work has shown that the immediate effects in muscle protein synthesis rate are not affected by general anesthesia but by surgery alone (11).

In conclusion, the skeletal muscle GSH pool was depleted in patients after surgical trauma. The decrease of the skeletal muscle GSH pool was associated with the decreased GSH synthetase activity. These data imply that patients undergoing elective surgery exhibit a diminished GSH synthetic capacity in skeletal muscle. The depletion of GSH concentrations may lead to an impaired intracellular antioxidant system in conjunction with an increased metabolic activity under catabolic conditions.

We thank Marianne Zander for excellent nursing, Liselott Thunblad for skillful technical assistance, and Dr. Richard F. Cowburn for revising the language of the manuscript. This work was, in part, supported by the Swedish Medical Research Council (Project 04210), the Stockholm County Council, Public Health and Medical Science, Department of Research Development and Teaching, the Trygg-Hansa Research Fund, and Åke Wiberg Research Fund.

Address for reprint requests: J.-L. Luo, Anesthesiology Metabolism Unit, Clinical Research Center, Novum, Huddinge Univ. Hospital, 141 86 Huddinge, Sweden.

Received 22 August 1997; accepted in final form 15 April 1998.

REFERENCES


