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# Skeletal Muscle Glutathione After Surgical Trauma

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## Objective
The authors investigate the effect of surgical trauma on skeletal muscle concentrations of glutathione in patients undergoing selective abdominal surgery.

## Summary Background Data
The posttraumatic state is accompanied by characteristic changes in the pattern of free amino acids and a decline of protein synthesis in human skeletal muscle. Glutathione has multiple metabolic functions that are involved in cellular homeostasis. It is unknown how surgical trauma affects the glutathione metabolism of skeletal muscle in surgical patients.

## Methods
Eight patients undergoing elective abdominal surgery were investigated. Percutaneous muscle biopsies and blood samples were taken before operation and at 6, 24, and 48 hours after operation. The concentrations of glutathione were determined in muscle tissue, plasma, and whole blood, as well as the concentrations of the related amino acids in muscle and plasma.

## Results
In skeletal muscle, the levels of both reduced and total glutathione decreased by 40% ($p < 0.01$) at 24 hours and remained low at 48 hours after operation compared with the preoperative values. The glutathione concentration in plasma was 20% lower after operation compared with the concentration before operation ($p < 0.05$). There were no changes at the whole blood levels of glutathione. Tissue glutamate and glutamine decreased significantly after operation ($p < 0.001$), whereas intracellular cysteine and glycine remained unchanged.

## Conclusions
Skeletal muscle glutathione deficiency occurs after surgical trauma. This may lead to an increase in the susceptibility to intracellular oxidative injury.

The posttraumatic state is accompanied by a series of metabolic changes in the skeletal muscle, which lead to alterations in the pattern of free amino acids and the rate of protein synthesis resulting in a negative whole body nitrogen balance. Animal studies suggest a correlation between the protein homeostasis and the redox status in muscle. The more oxidized conditions seen after fasting, trauma, or cortisol administration correlate with an accelerated proteolysis. Conversely, a more reduced state is associated with feeding, insulin, catecholamine, and leucine administration as well as protease inhibitors.[1,2] Reactive oxygen metabolites recently have been implicated as a major cause of tissue destruction in a variety of diseases. Mechanisms that protect tissue from oxidant stress and damage are particularly important during critical illness and after surgical trauma because the production of oxygen-free radicals is often accelerated under these conditions.[3]

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) is an endogenous-reducing agent and antioxidant that plays an important role in cellular metabolism. It acts as a scavenger that protects protein thiol groups from free-radical-induced oxidant injury, especially for maintaining cellular integrity.[4,5] Glutathione has been implicated in a variety of metabolic processes, including energy metabolism,[6] amino acid transport, and active-form enzyme maintenance.[7] In addition, glutathione also has been suggested to play a role in the maintenance of a reduced environment for protein synthesis.[8] Experimental and clinical evidences indicate that tissue glutathione store is affected during serious illnesses. Skeletal muscle glutathione is depleted by 60% in patients with cardiogenic circulatory shock.[9] In rats, a glutathione-deficient state is associated with multiple organ dysfunction and leads to marked mortality in rats after hemorrhagic shock.[10] Experimental glutathione depletion in mice leads to skeletal muscle degeneration accompanied by mitochondrial damage.[11]

Characteristic changes in the concentrations of intracellular amino acids occur in skeletal muscle after surgical trauma.[12] The decreases in the concentrations of glutamine and glutamate as well as an increase of branched chain amino acids and aromatic amino acids are the most consistent findings. Surgical trauma may affect the relationship between the levels of glutathione and its constituent amino acids. Although glutathione metabolism has been examined extensively *in vitro* and in animal studies, very little is known of its metabolism in human tissues during metabolic stress.

The aims of the present study were to examine whether surgical trauma induces alterations in the concentrations of thiol (*i.e.*, glutathione and cysteine) in skeletal muscle tissue during 48 hours after surgery. In addition, the muscle-free amino acids were determined to establish how surgery may influence the amino acids that are metabolically related to glutathione.

## PATIENTS AND METHODS

### Subjects and Study Protocol

Metabolically healthy patients (n = 8) undergoing elective abdominal surgery of variable severity participated in the study. The characteristics of the patients and the operative procedures are presented in Table 1. The operation took place the day after admission to the hospital. All patients were operated on under general anesthesia. A glucose-free electrolyte solution (Natriumklorid, Baxter, Bromma, Sweden) was given during the operation. In the postoperative period, 2.0 g glucose/kg body weight (Glukos 10%, Pharmacia, Stockholm, Sweden) was given on the first day, and on the following 2 days, 3.0 g glucose/kg body weight was given. A hypocaloric glucose supply was chosen, because this is routinely given to nonmalnourished patients after elective abdominal surgery. Percutaneous muscle biopsies were taken immediately after induction of general anesthesia before surgery, and at 6, 24, and 48 hours after operation. Blood samples for the determination of glutathione and amino acids concentrations also were taken at each examination. 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.

### Muscle Biopsy and Blood Sampling

Percutaneous muscle biopsy specimens for glutathione and amino acid analysis were obtained using the percutaneous needle biopsy technique from the lateral portion of the quadriceps femoris muscle above the knee after local anesthetization of the skin.[13] Specimens of 50 to 60 mg wet weight were used for glutathione determination and 20 to 30 mg wet weight for amino acid analysis. The specimens used for amino acid analysis were

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**Table 1. CHARACTERISTICS OF THE PATIENTS AND THE OPERATIVE PROCEDURE**

| Patient (n) | Sex | Age (yrs) | Weight (kg) | Height (cm) | Operation Time (cm) | Blood Loss (mL) | Operation Procedure |
|---|---|---|---|---|---|---|---|
| 1 | M | 70 | 55 | 171 | 280 | 2000 | Rectal amputation, R. hemicolectomy |
| 2 | M | 59 | 90 | 167 | 260 | 400 | R. hemicolectomy |
| 3 | F | 80 | 62 | 167 | 130 | 880 | Anterio resection of rectum |
| 4 | F | 55 | 60 | 170 | 130 | 350 | Anterio resection of rectum |
| 5 | F | 44 | 72 | 169 | 150 | 900 | Reconstruction after Hartmans operation |
| 6 | F | 78 | 63 | 156 | 130 | 800 | Resection of colon sigmiodeum |
| 7 | F | 63 | 82 | 168 | 120 | 500 | Resection of colon sigmiodeum |
| 8 | M | 68 | 67 | 170 | 80 | 500 | Fundoplicatio |
| Mean | 5/3 | 65 | 69 | 167 | 160 | 791 | |

weighed and frozen in liquid nitrogen within 3 minutes and stored at −80 C for further preparation before analysis. The sample for muscle glutathione determination was frozen in liquid nitrogen immediately after sampling and transported to the research laboratory for further sample preparation within 1 hour. Venous blood was taken in parallel to the muscle biopsies to determine glutathione and cysteine in plasma and whole blood.

## Sample Preparation

The method for determining the concentration of glutathione in skeletal muscle has been described in detail elsewhere.[14] Briefly, the frozen biopsy specimens were homogenized within 1 hour of sampling in 6.5% (w/v) sulfosalicylic acid (SSA) solution in a glass homogenizer on ice and then centrifuged at 3000 × g for 15 minutes at 4 C. The pH of the supernatant was adjusted to neutrality with excess sodium bicarbonate powder, and the sample was derivatized directly. For the determination of whole blood glutathione, whole blood samples were diluted with 3 volumes of 40% SSA and treated to 3 freeze–thaw cycles rapidly in liquid $N_2$ before centrifugation. Blood samples were centrifuged at 3000 × g at 4 C for 15 minutes, and the supernatant was treated as for muscle. Glutathione in plasma has a high turnover rate (within a few minutes). Reduced glutathione (GSH) is rapidly converted to the oxidized form, because accurate GSH measurement plasma derivatization needs to be carried out as rapidly as possible.

For the determination of free amino acids, the frozen muscle sample was homogenized on ice in 6.5% (w/v) SSA containing norleucine as internal standard and then centrifuged at 3000 × g for 15 minutes at 4 C. The pH of the supernatant was adjusted to 2.2 using 3 mol/L lithium hydroxide. The supernatant was frozen before analysis.

## Sample Analyses

The derivatization procedure was performed as described previously.[14] Briefly, samples of reduced glutathione or reduced cysteine (GSH/CySH) standards (Sigma, St. Louis, MO), plasma or SSA-soluble fraction from muscle biopsies, and whole blood (100 μL) were mixed with 100 μL 8 mmol/L monobromobimane (Calbiochem, La Jolla, CA) in sodium N-ethylmorpholine and allowed to react for 5 minutes in the dark before the reaction was stopped by the addition of 10 μL 100% SSA. Aliquots of the derivatized samples were filtered using 0.22 μm filter and applied to the high-performance liquid chromatography column for the determination of thiol bimane adducts.

Total glutathione (GSH + GSSG) and total cysteine (CySH + CySS) were derivatized by the same method after a reduction step of GSSG and CySS with dithiothreitol after protein precipitation. Briefly, 100 μL portion of plasma or neutralized muscle and whole blood supernatant were treated with 10 μL 50 mmol/L dithiothreitol, mixed and allowed to stand at room temperature for 30 minutes, and then derivatized with 100 μL 20 mmol/L monobromobimane in the dark for 5 minutes. The reaction was stopped by acidifying with 10 μL 100% SSA.

The high-performance liquid chromatography separation of glutathione and cysteine was achieved on a column (4.5 × 150 mm) packed with 3 μm octadecyl-silica reversed-phase resin (Supelco, Inc., Bellefonte, PA) followed by fluorescent detection at excitation 394 nm and emission 480 nm (Millipore Co., Milford, MA).

The free amino acids from skeletal muscle biopsies were separated by ion-exchange chromatography using an Ultropac 8 lithium form ion-exchange column (202 × 4.6 mm inside diameter, 8 μm particle size) in an automated amino acid analysis system (Alpha Plus, LKB Pharmacia Co., Stockholm, Sweden) using lithium citrate buffers (Pharmacial Co., Biochrom, Cambridge,

**Table 2. TISSUE CONCENTRATIONS OF REDUCED GLUTATHIONE (GSH) AND TOTAL GLUTATHIONE (GSH + GSSG) IN PATIENTS AFTER SURGICAL (N = 8)**

| Samples | Thiol | Preoperative | 6 Hrs Postoperative | 24 Hrs Postoperative | 48 Hrs Postoperative |
|---|---|---|---|---|---|
| Plasma | GSH | 5.4 ± 2.7 | 5.7 ± 2.5 | 4.1 ± 1.5* | 4.0 ± 1.7* |
| (μM) | GSH + GSSG | 6.4 ± 2.6 | 7.2 ± 3.0 | 5.7 ± 2.1 | 5.6 ± 1.7 |
| Ratio | GSH/GSH + GSSG | 0.80 ± 0.16 | 0.82 ± 0.14 | 0.75 ± 0.06 | 0.67 ± 0.13 |
| Whole blood | GSH | 561 ± 92 | 527 ± 175 | 470 ± 134 | 550 ± 141 |
| (μM) | GSH + GSSG | 775 ± 132 | 849 ± 223 | 804 ± 189 | 789 ± 183 |
| Ratio | GSH/GSH + GSSG | 0.73 ± 0.11 | 0.63 ± 0.12 | 0.59 ± 0.11* | 0.71 ± 0.13 |
| Skeletal muscle | GSH | 1.43 ± 0.17 | 1.32 ± 0.31 | 0.87 ± 0.25† | 0.96 ± 0.31† |
| (mmol/kg ww) | GSH + GSSG | 1.58 ± 0.23 | 1.49 ± 0.32 | 0.94 ± 0.25† | 1.06 ± 0.23† |
| Ratio | GSH/GSH + GSSG | 0.91 ± 0.04 | 0.89 ± 0.07 | 0.92 ± 0.05 | 0.89 ± 0.08 |

Values are expressed as mean ± SD.
* $p < 0.05$.
† $p < 0.01$ significantly different after surgery when compared with preoperative values.

United Kingdom). Orthopthaldehyde was used for post-column derivatization of the amino acids and fluorescent detection (Shimadzu RF-535, Shimadzu Corp., Kyoto, Japan) at excitation 350 nm and emission 420 nm. Cysteine was determined by derivatization with monobromobimane as described above rather than orthopthaldehyde.

## Statistical Analysis

The glutathione and cysteine concentrations and the free amino acid concentrations in muscle and in plasma are given as means with SD. The changes in the concentrations of glutathione and amino acids in muscle and blood after surgical trauma were assessed by one-factor analysis of variance for repeated measurements. Scheffé F test was performed as a multiple comparison test, and $p < 0.05$ was considered to indicate a statistically significant difference.

## RESULTS

Eight patients undergoing elective abdominal surgery were investigated before operation and at 6, 24, and 48 hours after operation. Percutaneous muscle biopsy specimens and blood samples were taken for the determination of levels of reduced and total glutathione and the amino acid concentrations.

## Glutathione in Muscle Tissue, Plasma, and Whole Blood

In muscle tissue and blood, the concentrations of the reduced (GSH) as well as the total form (GSH + GSSG) of glutathione were affected after surgical trauma (Table 2). In skeletal muscle, the levels of both GSH and GSH + GSSG were reduced by 40% ($p < 0.01$) at 24 hours and remained low at 48 hours after operation as compared with the preoperative values (Fig. 1). In addition, the GSH level of plasma was 20% lower after operation compared with before operation ($p < 0.05$). However, there were no changes in the whole blood levels in either form of glutathione after surgical trauma.

The ratio of reduced glutathione to total glutathione (GSH/GSH + GSSG) was calculated (Table 2). In skeletal muscle, the ratio amounted to 90% and in plasma to 80%. In these two compartments, the GSH/GSH + GSSG ratio was unchanged during the period of study. In whole blood, however, the ratio decreased from 70 to 60% at 24 hours after operation, but at 48 hours after surgery, the preoperative level was restituted.

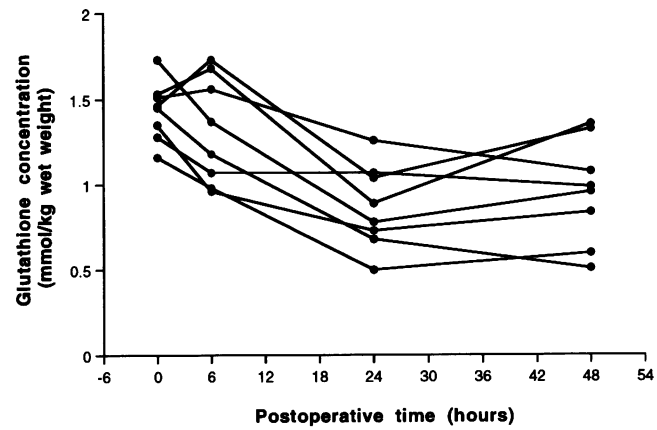


**Figure 1.** The reduced glutathione concentration in skeletal muscle after surgical trauma. The individual values of eight patients are given, illustrating the uniform temporal pattern regardless of a variation in the size of surgical trauma. The mean values decreased at 24 and 48 hours compared with the preoperative mean value at 0 hour ($p < 0.01$).

**Table 3. TISSUE CONCENTRATIONS OF REDUCED CYSTEINE (CYSH) AND TOTAL CYSTEINE (CYSH + CYSS) IN PATIENTS AFTER SURGICAL TRAUMA (N = 8)**

| Samples | Thiol | Preoperative | 6 Hrs Postoperative | 24 Hrs Postoperative | 48 Hrs Postoperative |
|---|---|---|---|---|---|
| Plasma | CySH | 6.8 ± 1.4 | 7.8 ± 1.3 | 8.8 ± 1.9 | 5.6 ± 1.7 |
| (μmol/L) | CySH + CySS | 220 ± 33 | 194 ± 19 | 168 ± 28 | 228 ± 30 |
| Whole blood | CySH | UD | UD | UD | UD |
| (μmol/L) | CySH + CySS | 94.6 ± 23.7 | 96.7 ± 27.4 | 81.7 ± 31.6 | 121.3 ± 27.6 |
| Skeletal muscle | CySH | 0.05 ± 0.02 | 0.04 ± 0.02 | 0.05 ± 0.03 | 0.04 ± 0.02 |
| (mmol/kg ww) | CySH + CySS | 0.07 ± 0.02 | 0.07 ± 0.02 | 0.08 ± 0.03 | 0.07 ± 0.02 |

Values are expressed as mean ± SD.
UD = undetectable.

## Constituent Amino Acids of Glutathione and Other Free Amino Acids in Muscle Tissue and Plasma

Cysteine was separated and determined by the high-performance liquid chromatography assay after reduction of SH group with dithiothreitol and derivatized with monobromobimane. Neither CySH nor total CySH was changed after surgical trauma in skeletal muscle, whole blood, or plasma (Table 3). The concentrations of all other glutathione-related amino acids of skeletal muscle and plasma were determined by ion-exchange chromatography. In skeletal muscle, the concentration of glutamate decreased by 40% at 6 hours after surgery compared with the preoperative value ($p < 0.05$) and stayed low at 24 and 48 hours ($p < 0.01$) without any tendency toward restitution. The glutamine concentration decreased by 20% at 24 hours and by 50% at 48 hours after operation compared with the basal preoperative values ($p < 0.01$). Muscle concentrations of taurine, serine, and methionine were not altered during the study period. The concentrations of alanine and branched chain amino acids in muscle increased after operation compared with the levels seen before surgery ($p < 0.01$).

## DISCUSSION

Humans have a relatively high concentration of glutathione in skeletal muscle,[14] but the levels are lower than those found in liver and gastric mucosa.[15,16] After elective surgical trauma, the concentrations of glutathione in skeletal muscle and plasma decreased, whereas they remained unaltered in whole blood. The regulation of the glutathione concentration in muscle tissue and its interorgan transport in blood of traumatized patients is not known, but alterations in the relative rate of synthesis, degradation, and transport, or an altered intracellular glutathione redox status in the muscle tissues may explain the present findings.

Glutathione is synthesized intracellularly from its three precursor amino acids glutamate, cysteine, and glycine *via* two sequential adenosine triphosphate-consuming reaction catalyzed by γ-glutamylcysteine synthetase and glutathione synthetase. The γ-glutamylcysteine synthetase is the rate-limiting enzyme in glutathione synthesis, and it is subject to feedback inhibition by GSH. The feedback regulation of γ-glutamylcysteine synthetase by GSH can be prevented by an excess of glutamate that blocks the regulatory site on the enzyme.[17] Among the substrates used for the synthesis of glutathione, glutamate is generally present in large excess compared with cysteine or glycine. After surgical trauma, the concentrations of free glutamate in skeletal muscle decreased by 40% and the glutamine concentration by 31%, whereas the concentrations of glycine and cysteine stayed unaltered (Table 4). Even so, the concentrations of glutamate and glutamine were maintained at the millimole level compared with the levels of cysteine and glycine. It is unlikely that the decline of glutamate and glutamine caused a shortage of substrates for the synthesis of glutathione under this condition. However, glutamate competes with GSH for binding at the regulatory site of γ-glutamylcysteine synthetase for the synthesis of GSH. During surgical trauma, the observed decrease in intracellular concentration of glutamate in muscle may weaken its competitive ability with GSH and lead to an increased inhibitory effect on the synthesis of GSH by GSH itself, therefore resulting in the decreased glutathione concentration. Another possible regulatory mechanism of GSH synthesis is the alteration of the activity of γ-glutamylcysteine synthetase. Human skeletal muscle has been shown to contain high levels of γ-glutamylcysteine synthetase RNA transcriptions.[18] However, this enzyme may be depressed under condition of metabolic

**Table 4. CONCENTRATIONS OF FREE AMINO ACIDS IN SKELETAL MUSCLE OF PATIENTS AFTER SURGICAL TRAUMA (N = 8)**

| Amino Acids* | Preoperation | 6 Hrs Postoperative | 24 Hrs Postoperative | 48 Hrs Postoperative |
|---|---|---|---|---|
| Glu | 1.79 ± 0.44 | 1.12 ± 0.45† | 0.97 ± 0.39‡ | 0.99 ± 1.19‡ |
| Gln | 9.26 ± 1.99 | 8.59 ± 1.86 | 7.31 ± 1.76‡ | 4.83 ± 1.19‡ |
| Gly | 0.79 ± 0.16 | 0.71 ± 0.15 | 0.88 ± 0.12 | 0.73 ± 0.17 |
| Cys§ | 0.07 ± 0.02 | 0.07 ± 0.02 | 0.08 ± 0.03 | 0.07 ± 0.02 |
| Tau | 7.96 ± 2.63 | 8.55 ± 2.79 | 7.91 ± 2.32 | 8.00 ± 3.16 |
| Ser | 0.34 ± 0.08 | 0.28 ± 0.07 | 0.31 ± 0.08 | 0.32 ± 0.09 |
| Met | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.05 ± 0.01 | 0.04 ± 0.01 |
| Ala | 1.81 ± 0.32 | 2.22 ± 0.32† | 2.18 ± 0.42‡ | 2.00 ± 0.67 |
| BCAA | 0.31 ± 0.05 | 0.33 ± 0.08 | 0.55 ± 0.09‡ | 0.39 ± 0.14 |

* Values of amino acids are expressed as mmol/kg wet weight tissue ± standard deviation.
† $p < 0.05$.
‡ $p < 0.01$.
§ Values are total CySH from HPLC-based assay with monobromobimane derivatization.
p values are significantly different after surgical trauma when compared with preoperative values.

stress, because its activity in erythrocytes is shown to be lower in diabetics compared with that of normal controls.[19] Therefore, if the decrease of glutathione concentration in muscle tissue after surgery is attributed to the decrease of GSH synthesis, a decrease of $\tau$-glutamylcysteine synthetase activity rather than a low concentration of substrates is a more likely explanation.

Intracellular GSH also may be consumed by reducing $H_2O_2$ or lipid peroxides *via* the glutathione peroxidase reaction to GSSG (oxidized form of glutathione).[20] This reaction is reversed using reduced nicotinamide-adenine dinucleotide phosphate (NADPH) to reduce GSSG *via* the glutathione reductase reaction. Under normal conditions, the equilibrium is far in the direction of maintaining cellular glutathione in its reduced state. However, in cases when rapid production of the GSSG occurs, it can accumulate. A surplus of GSSG is either transported out of the cell to be degraded or reacts with protein sulfhydryl, *via* a mixed disulfide reaction, potentially causing an impaired protein function.[1] The net result of an elevated GSSG formation may thus be a loss of intracellular GSH. In animal studies, fasting, trauma, or cortisol treatment leads to a more oxidized state in muscle when the lowered ratios of NADPH/NADP (oxidized form of NADP) and lactate/pyruvate are taken as indicators of cellular redox status.[2,21] The findings during the course of circulatory shock in patients also suggest the involvement of oxygen free-radical and the oxidative damage of mitochondria as reflected by the fall of superoxide dismutase activity and content as well as glutathione content in skeletal muscle.[9] The present study showed a deficiency in both GSH and total glutathione in the muscle tissue of patients after surgical trauma. This finding may have therapeutic implications on cellular protection against oxidant stress.

Liver is the major source of plasma glutathione, and the plasma glutathione concentration has traditionally been used to evaluate whole body glutathione metabolism.[22] However, whole blood glutathione concentrations provide a different and, possibly, more complete picture of the glutathione metabolic status. In rats, total net hepatic output of glutathione in whole blood is, on the average, 20-fold greater than the output in plasma.[23] We found that glutathione in plasma and in whole blood responded differently in patients undergoing surgical trauma. The concentrations of GSH in plasma decreased slightly, whereas the whole blood concentrations of GSH remained unaltered (Table 2). The mechanism whereby liver glutathione or its constituents are made available to red blood cells is not presently known. Its output into red blood cells across liver may involve the release of glutathione precursors, uptake of precursors into red blood cells, and *de novo* synthesis of glutathione within the red blood cells. The lowering of plasma GSH concentration found at 24 and 48 hours after operation may indicate a decreased output of liver GSH or an increased uptake of GSH by other organs or both.

In rats, it has been described that experimental liver damage induced by acetaminophen overdose causes a rapid depletion of the hepatic glutathione stores and is associated with a significant mortality.[10] The damage is markedly attenuated when a glutamine-enriched parenteral nutrition is given.[24] Furthermore, in glutathione-depleted rats, the administration of parenteral glutathione monoethyl ester, but not glutathione itself, restitutes liver glutathione stores.[25] Experimental depletion of glu-

tathione in animals is associated with oxidative damage in several tissues (*e.g.*, lung, kidney, liver, brain, lens). In particular, a marked depletion of muscle glutathione is associated with histologic muscle degeneration and mitochondrial damage.[11] Although glutathione deficiency in human muscle after surgical trauma has not been observed previously, the ability to prevent and restore the depletion of tissue anti-oxidant stores may be of value in patients who have low glutathione levels.

Cysteine is found in human plasma in one of three forms: 1) free cysteine, 2) free cystine (oxidized cysteine), or 3) protein-bound cysteine.[26,27] The cystine concentration is much higher than that of cysteine because of the efficient uptake of cysteine across the membrane of cells (cystine transport has a $K_m$ of 0.306 mmol/L, whereas cysteine transport has a $K_m$ of 0.0415 mmol/L, indicating a sevenfold greater affinity of the cysteine transport system for its substrate than of the cystine transport system). In the present study, we found that the cellular constitution of cysteine in human skeletal muscle is different from that in plasma (Table 3). In muscle, cysteine accounts for about 70% of total free cysteine, whereas cystine corresponded to less than 30% of total cysteine. After surgical trauma, muscle cysteine stayed unaltered compared with the preoperative values. Still, the regulation of intracellular cysteine in muscle is not fully understood. Plasma cystine can be taken up effectively by muscle tissues during fasting in humans.[28] In cultured cells, cystine is taken up by a special membrane transport system, in which cystine is transported into cells accompanied by export of glutamate.[29] Intracellularly, cystine is easily reduced to cysteine. This system can be induced by oxidative stress and the depletion of glutathione.[29-31] Cysteine is classified as a nonessential amino acid; humans can synthesize it from methionine. However, the formation of cysteine from methionine is only active in liver cells, but is not available to most cell types because of lack of cystathionine $\beta$-synthase.[32,33] Therefore, the intracellular level of cysteine in muscle may be regulated mainly by its membrane transport. Our results showed a depletion of muscle glutathione, which may suggest the existence of an oxidative stress in muscle tissue after surgical trauma; however, the tissue cysteine concentration was stable. This may be explained by the availability of cystine in plasma and probably the induction of the membrane transport system specific for cystine and glutamate in muscle cells. A maintained concentration of cysteine in muscle may be vital for protein synthesis as well as for intracellular glutathione homeostasis.

In summary, the effect of surgical trauma on glutathione status of human skeletal muscle has been evaluated. A substantial decrease in muscle glutathione concentration was observed at 24 hours after operation and it remained low at 48 hours. The findings are relevant to establish the role that glutathione deficiency plays in the pathogenesis of the metabolic alterations associated with trauma and oxidative damage, in particular the changes in protein metabolism. Factors that regulate the intracellular concentrations of glutathione are complex. Further studies are needed to elucidate the complexities of glutathione metabolism and its redox status in skeletal muscle of the surgical trauma patient.

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