Biochemical Measurement of Muscle Injury Created by Lumbar Surgery

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Abstract

Purposes: 1. To determine whether lumbar disc surgery (LS) provides a sufficiently detectable rise in serum creatine kinase (CK) concentration to serve as a model to study biochemical measurement of muscle injury, and 2. To use the model to examine the consistency of the time course of CK concentration changes.

Method: The study used a repeated measures design. Six women and six men scheduled for LS were recruited. Blood samples were taken in the pre-operative waiting areas, immediately after surgery, at 6 hour intervals until discharge, and at 2, 4, and 6 to 7 days following surgery. Total serum CK was quantified using the Roche Modular to detect enzyme concentration.

Results: Following LS, mean Total CK increased from a baseline 50 U/L (SD = 53) to a peak 114 U/L (SD = 32) in women ($P < .001$) and from 183 U/L (SD = 69) to a peak 454 U/L (SD = 173) in men ($P < .05$). Baseline to peak changes in CK exceeded subjects' own baseline fluctuations in all 6 women and all 6 men, and amounted to a mean 6 fold (SD = 4) increase in women and 16 fold (SD = 31) increase in men. While CK concentrations returned to

baseline over the observation period in all subjects, time to peak ranged between 9 to 47 hours.

Conclusions: The LS model produced a consistently detectable CK response in both genders. Time to peak is variable indicating a need for multiple serial measures to capture this biochemical injury response.

Keywords: Biochemical markers, muscle injury, lumbar disc surgery, creatine kinase

Studying injury of musculoskeletal structures (i.e. muscle, tendon, ligament) has been limited by the lack of suitable objective measures.¹⁻³ Biochemical markers are being tested for application in various disease states⁴⁻¹⁵ to quantify changes in muscle, bone, lung and vascular connective tissue, and cartilage. In muscle injury, the proteins receiving the most attention to date are creatine kinase (CK), which is contained in the cytoplasm and is involved in energy production through the formation of adenosine triphosphate, and troponin, which helps form the contractile apparatus.⁸ Other proteins studied include myoglobin^{16,17}, heavy

chain myosin^{18,19}, and fatty acid binding protein.²⁰ There is an extensive literature on application of biochemistry for cardiac muscle injury and there has been a strong clinical impact.²¹⁻²⁷ A large literature also exists in exercise physiology on changes in circulating levels of all of these tissue proteins in response to heavy exercise.^{16,17,28-32} That line of research may provide a model of muscle injury related to exertion.² In contrast to the extensive research in those areas, only a small number of studies have focused on circulating tissue proteins following injuries in clinical settings.³³⁻³⁷

Minor tissue injuries associated with musculoskeletal surgeries may provide heuristics to study and develop a biochemical measurement approach. Surgery models allow pre-injury baselines, and enable within-subject, rather than between-subject, designs. These features could increase the signal to noise ratio relevant to quantifying injury responses. Second, the hospital stay and post-surgery medical contact provide opportunities to obtain some serial samples. Since the concentrations of muscle proteins in body fluids change rapidly following injuries, quantifying the biochemical injury response requires numerous within-subject measures, or a predictable enough time to peak to estimate response magnitude. Serial measures will help to identify the sensitive temporal window for detecting injury, and individual differences in peak magnitude and timing. In cardiology, serial measures of muscle proteins were important in the development of clinical guidelines specifying the number and timing of samples, as well as cut-off values for the presence and absence of infarct.³⁸⁻⁴⁰

Onuoha and coworkers³³ used open reduction and internal fixation of leg fractures as a model. Subjects entered with leg fractures and associated soft tissue injuries. A comparison against controls provided biochemical evidence of recent muscle injury based on serum concentrations of total CK, myoglobin, which is also contained in muscle cytoplasm, and for myosin heavy chain, a protein that works in conjunction with troponin to form the contractile mechanism. By using the first data point as a baseline, the study also allowed examination of within subject changes in response to surgery. At 24-hours following the opera-

tions, increases were evident in serum levels of the 3 tissue proteins.

In another study, serum proteins were measured in 20 subjects who had low back surgery and 7 who had pelvic osteotomies.³⁴ The purpose was to establish whether the hemodilution component of these surgical procedures damaged heart muscle, not to study the biochemical measurement of skeletal muscle injury. Data on the 2 surgical groups were pooled. Serial measures were data points at 3 hours post-surgery and then 1 and 3 days later. Serum concentration of cardiac troponin I did not increase. However, mean concentrations were elevated for total CK.

Research that goes beyond such initial studies could include surgery models with protocols that permit more detailed within subject (rather than between subject) measurement of the injury response with pre-injury baselines and multiple serial measures to capture the biochemical response curve. In lumbar disc surgery (LS), cutting of muscle is avoided. However, injury may be produced in many patients when muscle is stripped from the vertebral laminae, retracted away from the spine, and held with surgical clamps. Contributions might also be made by inadvertent muscle tearing, and pressure at retractor contact points. Accordingly, this study was conducted to determine whether detectable serum CK elevations are obtained in each gender following LS, and to use the model to examine the time course of this biochemical injury response.

Methods

Patients

Twelve adult patients (6 women, 6 men) were recruited from the population awaiting back surgery at St. Josephs Health Care, Hamilton, Ontario, Canada. The study received approval by the hospital ethics review board and all patients provided informed written consent.

Design and Analyses

A repeated measures design was used to study changes in serum total CK concentration over time.

Between subjects t tests were used to compare women and men on CK peak, and baseline to peak changes in serum. Within subject baseline-to-baseline fluctuation was measured between the pre-surgery and each subject's final data point. The injury response was defined as the pre-surgery to peak (highest CK obtained) change. The within subjects t-test was used to determine statistical significance of baseline to peak CK changes.

Protocol

Subjects were approached during the pre-operative education session, which formed part of the usual surgical preparation. Each patient signed an informed consent form according to the requirements of the ethical review committee. Those providing written consent completed a short history questionnaire to characterize the subject sample, and to screen for confounds known to affect circulating CK.

During surgery, the patient was placed in the supine position on a Relton frame. The operative site was land-marked. An adequate skin incision was undertaken based upon each patient's size. The lumbosacral fascia was released unilaterally on the operative side. Small tendons were cut from the spinous processes, as required, to expose the vertebrae. The lumbar erectors were isolated and retracted subperiosteally. The retractor size was constant over subjects. Muscle was stripped from the vertebral laminae but not resected in any cases. Hemostasis was attained and expansile retractors were placed to maintain the surgical field. After the decompression, the retractors were released. Hemostasis was attained and the wound was irrigated with bacitracin solution. Interrupted sutures were used to close the fascia, and then the subcutaneous tissue. The skin was closed with surgical staples.

Ward nurses drew the blood samples during the hospital stay according to instructions provided on a standardized doctor's order sheet. Instructions were for blood to be drawn at the following time points: 1) while waiting in the pre-operative area, 2) immediately post surgery, 3) 6 hours post-surgery, 4) 12 hours post-surgery, 5) at discharge the morning following

surgery, 6) in the home 2 days post-surgery, 7) in the home 4 days post-surgery, and 8) in the hospital blood lab on the day surgical staples were removed (6 to 7 days post-operation).

Blood was drawn from an indwelling catheter previously inserted in a superficial forearm vein of the non-dominant arm as part of the usual surgical preparation. At each time point, whole blood was collected into two, 4ml plain tubes and one, 4ml heparin coated tube. The extra plain sample allowed for repeat testing of questionable values. The heparin sample was taken for other research focusing on plasma analyses. To minimize a potential dilution factor, the IV was locked for 1 hour prior to each sample. The first 3 ml of blood were discarded. Following discharge from hospital, a venipuncturist traveled to the home to draw samples.

Samples were allowed to clot at room temperature and then centrifuged. Aliquots of serum were stored at -80°C. Total creatine kinase was measured using the Roche Modular system to detect enzyme changes. The method has a lower limit of sensitivity of 3 U/L and good precision; within-run (0.6%) and between-run (1.4%).⁴¹

A senior anesthesiologist reviewed all medications used in surgery as a check for drugs that might produce muscle injury or affect circulating CK.

Results

Review of history data led to 2 willing subjects, both women, being excluded. One had polymyalgia rheumatica and the other was taking thyroid replacement. Of the 12 included subjects, none had the following exclusion characteristics known or suspected to affect circulating CK levels: surgery in past 6 months, injuries in the past 6 months, musculoskeletal diseases, confounding medications, or exercise with weights. No depolarizing muscle relaxants were used. Anesthetics and other drugs used in varying doses and combinations for surgery included, 1. narcotics- fentanyl, sufenta, morphine; 2) volatile anesthetics- desflurane, sevoflurane, propofol; 3). a benzodiazepine-versed, 4). serotonin anatagonists- ondansetron, zofran; and 5) a non-depolarizing muscle relaxant-

TABLE 1. Subject Demographic and Clinical Characteristics

Sex	Age (yr)	Weight (kg)	Body Mass Index (BMI)	Active Hours/Week
Total (n = 12)	43, 31 - 69	81, 49 - 139	26, 20 - 36	10, 0 - 18
Women (n = 6)	38, 32 - 47	66, 49 - 84	23, 20 - 27	10, 5 - 14
Men (n = 6)	48, 31 - 69	98, 65 - 139	29, 22 - 36	10, 0 - 18
P	.22	.02	.05	1.0

Data are group means followed by range. Active hours are sum of housework and exercise.

rocuronium. Drugs used to reverse muscle relaxation included: 6) an anticholinesterase- neostigmine and 7) a muscarinic anticholinergic – glycopyrrolate. None had known susceptibility to malignant hyperthermia or symptoms of this condition during the surgical observation period.

Three men and 3 women attributed their disc problem to an accident. Four subjects, all women, were working for income up to the week of surgery. Two men, and 1 woman were bedridden at the time of surgery. Continuous variables collected on demographics and clinical characteristics are shown in Table 1. Women and men did not differ in mean age, or activity levels in the week before surgery. The men had higher mean body weights ($P < .05$) and higher mean body mass index ($P < .05$) than the women.

Individual subject serial data points are shown in Figures 1 and 2, separated by sex. Data are plotted according to time from the start of the surgery. Therefore, pre-surgery baselines are shown at a negative time point occurring 1 to 2 hours before the operation.

Baseline CK was higher in men (mean = 183, range = 58 to 235 U/L) than women (mean = 50, range = 37 to 85 U/L), $P < .001$. Peak CK was also higher in men (mean = 454, range = 251 to 694 I/U) than women (mean = 113, range = 82 to 162 U/L), $P < .001$. However, there was no difference between sexes when the injury response was expressed as a percentage change from each subject's baseline ($P > 0.5$).

Peak CK values were higher than baseline in women ($P < .01$) and men ($P < .05$). Total serum CK appeared to have returned to baseline in all 12 patients within the 6 to 7-day sampling period. The range for highest CK concentration obtained was 9 to 47 hours.

Figure 2 illustrates the degree to which injury responses were detectable over baseline fluctuations. Data are individual subject changes in CK concentration between the pre-surgery baseline and peak, and between pre-surgery and the last data point (follow-up baseline). The pre-injury baseline values tended to be higher than follow-up. This created negative baseline change scores for some subjects. Therefore, baseline scores were made positive to provide a second, more conservative comparison between the injury response and baseline fluctuations. Using the former scores, baseline and surgery related changes were separate distributions. Using the conservative baseline fluctuation values, 4 of the 6 men, and 5 of the 6 women had post-surgery CK increases that were non-overlapping with gender group baseline fluctuations. Within subjects, the baseline to peak change in CK exceeded subjects' own baseline fluctuations in all 6 women and all 6 men, and amounted to a mean 6 fold (SD = 4) increase in women and 16 fold (SD = 31) increase in men.

Discussion

A biochemical injury response to lumbar surgery was detectable in each gender using total CK concentration in blood indicating sensitivity of the model to in-

FIGURE 1. Effect of Lumbar Surgery on Serum Total Creatine Kinase in Women (Left) and Men (Right)

FIGURE 2. Comparison Between Injury Response and Baseline Fluctuations in Women and Men
Data are individual subject change scores (women left, men right) between pre-surgery baseline and highest CK obtained (Pre-Peak), and between baselines estimated by the pre-surgery and last follow-up data points (Pre-Fol). Negative changes were made positive (Low - High) for conservative comparison to the injury response.

jury. The baseline to peak change in CK exceeded subjects' own baseline fluctuations in all 6 women and all 6 men, which distinguished the biochemical injury response from "noise" to allow good specificity for the injured state. Increases of 40 U/L for women and 140 U/L for men distinguished the post-surgery CK values from baseline fluctuation in this surgery model. Concentrations returned to baseline within the 6 to 7 day sampling period in all subjects. However, the time to peak CK level was variable across subjects, with the highest CK obtained between 9 to 47 hours.

The sensitivity of serum CK to muscle injury is quite consistent across studies using different injury models.^{17,28,29,33-36} The individual variation in baseline CK, and peak magnitude in response to LS is also consistent with animal injury models⁴², and exercise induced injury.¹⁷⁻²⁰ Similar individual variation is characteristic of circulating troponins and myosin heavy chain.^{28,29,31,32} A number of non-injury related factors have been found to affect baseline CK values including sex, race, body mass, and activity levels.⁴³⁻⁴⁵ CK remained elevated for many hours before and after the peak. Similar findings have occurred in some studies on extreme exertion that provided serial sampling.^{1,17,19,29,46,47} Therefore, detecting the injury response may not necessarily require sampling precisely at the peak and protein elevation is detectable within a broad time frame. This concept has contributed to general recommendations for the timing of blood sampling within a sensitive time window in cardiac assessment.^{26,27} The variability in peak timing with LS in this study cannot be compared to other reports because presentation of serial measures on individual subjects is not customary in the literature. In fact, analyses of individual differences in time to peak need to be conducted for other injury models.

Previous research on skeletal muscle injury has focused largely on intensive exercise as a form of exertion related trauma. The surgery model differs from the exercise model in the production of injury. In the former, repetitive movements damage muscle. In back surgery, the muscle is strained in a constant manner over about 90 minutes to 2 hours. Muscle injuries presenting clinically often occur with single movements of short durations. The relevance of the

surgery and exercise models to clinically presenting injuries is not yet known.

The study did suggest some limitations that may reflect drawbacks of the protocol, the LS model, or the biochemical approach in general. One issue is the trend towards higher pre-surgery baselines than those observed at follow-up and, in some patients CK levels immediately after surgery were lower. This might be related to fluid restriction in the fasting protocol. A pre-surgery baseline prior to the day of surgery could correct this. A second issue is that none of the women and only 3 men had peak CK values that exceeded normal baseline ranges.⁴⁸ Therefore, LS would have low sensitivity in studies that use normal values to form injury cut-offs. The findings do demonstrate the value of the model in providing pre-injury baseline to enable within subject analysis to detect smaller CK responses. A third point is that the study included no control group. In fact, it is difficult to conceive of a surgical control that would avoid muscle trauma while maintaining other variables constant (e.g. patient characteristics, anesthetics, surgery duration). Therefore, it is not possible to know whether the CK elevations are influenced by factors other than muscle strain. More generally, the construct validity of circulating CK as a measuring of the amount of actual muscle damage cannot be determined from this study. While this question was not the focus, the absence of an understanding of the validity of CK for muscle injury leaves some uncertainty about how to interpret findings from this study and others. The design did allow the CK response to be compared to baseline fluctuations in the same subjects and this allowed for some control to rule out alternative explanations for the CK rise based on non-injury related factors. Variability in time to peak presents a fourth problem. This indicates that estimating peak based on the average peak timing across subjects could create significant error. While measurement error in the protocol might make a contribution to individual differences in time to peak, it could also mean that the concentration by time curve following muscle injury is not a constant. An unpredictable curve shape would preclude estimation of the biochemical injury response from a small number of measures in research and clinical applica-

tions. Alternatively, if the variability in time to peak is peculiar to LS, then the model is limited in this regard.

These limitations, as well as some of the study findings, suggest questions for future research necessary for clinical applications. The variability in CK magnitude needs to be explained. Ideally, this variability is largely related to the amount of surgically produced injury, with non-injury related individual factors contributing little to the peak. Studies examining the correlation between CK and other measures of injury are needed. It would be quite possible to incorporate measures of muscle injury for such investigation. The measures could include: duration of muscle retraction time, force required to achieve retraction, and surface area of the muscle isolated and retracted. Variability in time to peak also needs to be studied further. This could be investigated using LS in a larger sample to determine the degree of consistency or inconsistency in peak time and the related shape of the biochemical injury curve. Comparison with other injury models could also be instructive.

In conclusion, the findings indicate that LS produced a consistently detectable rise in circulating CK and therefore, may provide a model to study biochemical measurement of muscle injury. Further research is needed to explain individual differences in peak magnitude and the time course of protein concentrations, with emphasis on clarification of injury related versus non-injury related determinants.

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