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CLINICAL ARTICLE

Vaginal delivery and serum markers of ischemia/reperfusion injury

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Abstract

Objective: Vaginal deliveries have been associated with pelvic organ prolapse and incontinence. The objective was to show whether markers of ischemia/reperfusion injury are dependent upon the mode of delivery and length of labor. *Method:* Complete venipuncture sets were obtained on 62 subjects. All samples collected were analyzed for serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). Lipid peroxidation was analyzed, using thiobarbituric acid reactive substances (TBARS), on a subset of 37 patients. *Results:* There was a significant increase in CPK from admission to 1 h postpartum and postpartum day 1 in vaginal delivery versus cesarean delivery. Longer second stages were associated with significant increases in CPK. There were no significant changes in either LDH or TBARS from admission to any other time point regardless of mode of delivery. *Conclusion:* Vaginal delivery and longer second stages were associated with a much greater increase in one of these injury markers.

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1. Introduction

The levator ani muscle group is the largest component of the pelvic floor. This collection of muscles is composed of the iliococcygeus, pubococcygeus, and puborectalis muscles. The levator ani has two important functions. The first is to maintain a constant basal tone that keeps the urogenital hiatus closed and prevents descent or prolapse of the pelvic viscera. The second is to contract reflexively in response to an increase in abdominal pressure (cough, laugh, sneeze) and assist in maintaining continence during such events [1]. Childbirth exposes the pelvic floor to direct compression by the fetal head and to pressure generated by maternal expulsive efforts. There is a strong association between childbirth and the development of both incontinence and pelvic organ prolapse. Indeed, MRI studies have clearly shown damage to the levator ani muscles in women who have undergone vaginal deliveries, as opposed to nulliparous women, and the majority of women with levator ani abnormalities in these studies had stress incontinence [2]. However, the underlying mechanism leading to these injuries is unclear. One theory has been that ischemia/reperfusion (I/R) injury is responsible for damage to the muscle.

Ischemia activates the complement system and increases the expression of adhesin molecules on endothelial surfaces, which result in the recruitment of neutrophils into the muscle. During the phase of reperfusion, oxygen becomes available. As a result, the numerous neutrophils found in the muscle produce oxygen radicals, and membrane lipid peroxidation ensues, leading to considerable structural and functional damage [3].

Because I/R reactions are difficult to measure, in vivo serum markers can be measured instead [4]. Creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) are nonspecific markers of tissue and muscle injury, whereas thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) are more specific markers of lipid peroxidation. Importantly, the elevation of these markers has been correlated with an increase in both structural (e.g., mitochondrial damage, interstitial edema, and dissolution of myofibrils) [3,5] and functional (reduced resting membrane potential and reduced tetanic tension) [6] damage to skeletal muscle.

Because of the direct compression of the pelvic floor by the fetal head, childbirth may provide an ideal model for I/R injury, with the ischemic period represented by the second stage of labor. The aim of this study is to determine if the mode of delivery and length of the second stage affect markers of skeletal muscle damage and specific markers of lipid peroxidation.

2. Materials and methods

This prospective study was approved by the NYU Hospitals School of Medicine Investigational Board of Research Associates (IRBA #11282). Nulliparous women at term ($\geq$37 weeks gestation) who chose to deliver at NYU Tisch Hospital during the period of study (November 2003—December 2004) were approached upon their presentation to labor and delivery for participation in the study. Written informed consent was obtained from each subject. Patients who were non-English speaking or had multiple gestations, myopathies, or recent history of aspirin or nonsteroidal anti-inflammatory use were excluded. Demographic information, including mother's age, height, weight, race/ethnicity, past medical history, and medication usage, was obtained. In addition, information on indication for admission, cervical dilation/station at multiple time points (admission, full dilation, and/or time of decision to undergo cesarean delivery), and fetal weight was recorded. The course of the patients' labor and delivery was not altered by their participation in the study. The intent was to evaluate 5 groups among the patients enrolled in the study: (1) spontaneous vaginal delivery, (2) operative vaginal delivery, (3) elective/scheduled cesarean delivery, (4) cesarean delivery secondary to arrest of dilation, and (5) cesarean delivery secondary to arrest of descent. Women who had emergent cesarean sections for fetal indications were also excluded from study as their natural labor curve was truncated and time did not allow for the venipunctures as per protocol.

The protocol specified that participants have venipuncture upon admission to the hospital, 1 h postpartum, and 1 day postpartum. In addition, those patients attempting vaginal delivery had an additional blood draw upon reaching full cervical dilation or at the time of the decision to perform a cesarean section (whichever time point was reached first). The samples were coded without any patient-identifying markers and analyzed for creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and lipid peroxidation.

Serum CPK and LDH were quantitatively measured in units/liter (u/l) on a Vitros 950 automated analyzer (Ortho-Clinical Diagnostics, Inc; Rochester, New York) shortly after the receipt of the specimen. The samples that were to be analyzed for lipid peroxidation were centrifuged (3000 *g* for 7 min at

20 °C) and stored at−80 °C until assayed. The following thiobarbituric acid reactive substances (TBARS) protocol was used: 100 μl of plasma was mixed with 500 μl of 0.4% thiobarbituric acid in 10% acetic acid, pH 5.0, and heated to 90 °C for 1 h. After being cooled under cold tapwater, an equal volume (600 μl) of butanol was added and the mixture was vigorously shaken. After centrifugation at 7500 rpm for 10 min, the fluorescence intensities of TBARS in the butanol phase were measured in arbitrary units (a.u.), using a Gemini fluorescence—chemiluminescence microplate reader (Molecular Devices, Sunnyvale, California) at an excitation wavelength of 515 nm (bandwidth 4.5 nm) and an emission wavelength of 553 nm (bandwidth 9.0 nm) [7].

Subject demographics were compared among modes of delivery, using the Fisher exact test when the variables were categorical (e.g., race) and the ANOVA when the variables were continuous (e.g., age, EGA, body mass index, and fetal weight). Within each mode of delivery, differences between time points were compared by using the Student-*t* tests. The ANOVA test was used in comparing the exact current value or change from admission in a marker at specific time points across the various modes of delivery. The Student-*t* test was used to compare the values of serum markers for those with second stage ≤ 1 h versus > 1 h, and linear regression was performed by using the length of the second stage as a continuous variable. All statistical analyses were performed with S-PLUS 6.2 for Windows (Insightful Corporation, Seattle, WA).

3. Results

One hundred and eleven subjects were enrolled. One subject was excluded because she was enrolled at a gestation age of 36 2/7 weeks and therefore did not meet inclusion criteria. Two subjects withdrew from participation after initially consenting. Four subjects had emergent cesarean deliveries and were therefore excluded from the study. Of the remaining 104 subjects, 42 patients had incomplete venipuncture sets, with the 24 h postpartum blood draw being the sample most often missed. Complete venipuncture sets were obtained on 62 subjects. This resulted in analysis of 32 spontaneous vaginal deliveries, 9 operative vaginal deliveries (6 forceps and 3 vacuums), 10 elective cesareans, 11 cesareans for arrest of dilation, and 0 cesareans for arrest of descent.

All samples collected were analyzed for serum CPK and LDH. TBARS was initially analyzed on a subset of 37 patients (24 vaginal and 13 cesarean deliveries). Interim analysis revealed that changes in levels of TBARS from admission to any other time point were not significant regardless of mode of delivery. It appeared that the levels of lipid peroxidation in our samples were below the detection limit for this assay, and therefore the remaining samples were not analyzed.

The demographic characteristics of the group of 62 patients with complete venipuncture sets are presented in Table 1. Age, body mass index, and race were not significantly different among the 4 modes of deliveries. There was, however, a significant difference in EGA and fetal weight between the delivery modes. Regression analysis revealed that EGA was significantly higher in both operative deliveries ($p<.02$) and cesareans for arrest of dilation ($p<.01$) than in elective cesareans and that the weights of babies born after elective section ($p<.03$) or operative delivery ($p<.01$) were less than those born after cesarean for arrest of dilation.

Changes in levels of LDH from admission to any other time point were not significant regardless of mode of delivery. However, there was a significant increase ($p\leq.01$) in CPK from admission to 1 h

Table 1 Demographic information by mode of delivery

	Spontaneous vaginal deliveries	Elective cesareans	Arrest of dilation cesareans	Operative vaginal deliveries
Number	32	10	11	9
Age (years)	31.6	32.6	33.9	29.8
EGA (weeks)	39 4/7	38 6/7*†	40 2/7†	40 1/7*
BMI (kg/m^2)	29.1	27.1	29	29.6
Fetal weight (g)	3458	3314‡	3773‡§	3181§
Race (%)				
Caucasian	22 (69%)	8 (80%)	8 (73%)	8 (89%)
Asian	4 (12.5%)	1 (10%)	1 (9%)	0 (0%)
Hispanic	4 (12.5%)	1 (10%)	1 (9%)	1 (11%)
African—American	1 (3%)	0 (0%)	1 (9%)	0 (0%)
Other	1 (3%)	0 (0%)	0 (0%)	0 (0%)

[*$p<0.02$; †$p<0.01$; ‡$p<0.03$; §$p<0.01$].

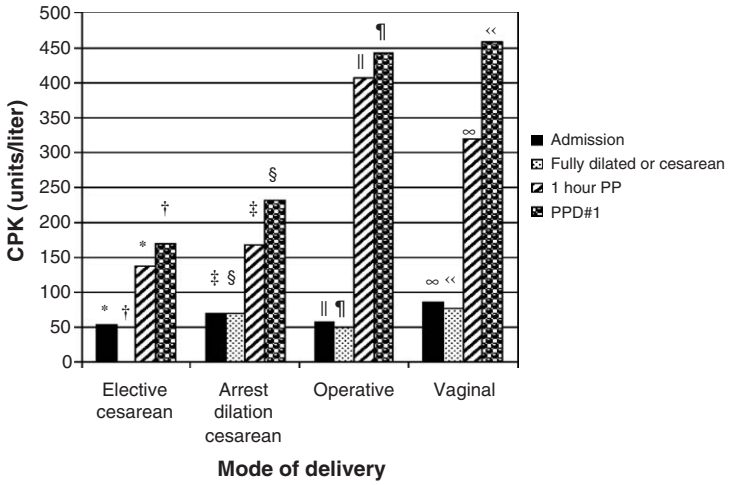

Figure 1 CPK values at each time point for each mode of delivery. [*$p<.01$; †$p<.001$; ‡$p<.01$; §$p<.001$; ||$p<.01$; ¶$p<.001$; ∞$p<.01$; ‹‹$p<.001$].

postpartum and from admission to postpartum day 1 ($p \leq .001$) for all modes of delivery (Fig. 1). In addition, from admission to full dilation (or the time that the cesarean was called), there was no significant change in CPK levels among the modes of delivery.

Using the admission time as the baseline, Fig. 2 illustrates the change in CPK for each mode of delivery. From admission to the 1 h postpartum time point, there was a significant increase in CPK in the spontaneous vaginal group when compared to either the elective cesarean or the cesarean for arrest of dilation group (233 u/l vs. 83 u/l and 97 u/l, respectively; $p \leq .02$). There was also a statistically significant increase in CPK in the operative vaginal group when compared to either the elective cesarean or cesarean for arrest of dilation groups (348 u/l vs. 83 u/l and 97 u/l, respectively; $p \leq .001$). The significant changes in CPK from admission to postpartum day 1 between the various modes of delivery mirrored those from admission to 1 h postpartum, with a significant increase in CPK in both the spontaneous vaginal group and the operative vaginal group when compared to either the elective cesarean or the cesarean for arrest of dilation groups (372 u/l and 384 u/l vs. 115 u/l and 163 u/l, respectively; $p < .04$). However, there was no significant difference in CPK between the two vaginal modes or between the two cesarean modes of delivery when either 1 h postpartum or postpartum day 1 was compared to baseline.

Because it was theorized that compression of the pelvic floor by the fetal head represented ischemic injury time, the length of the second stage was analyzed, separating the vaginal delivery group into those with a second stage >60 min ($n = 18$) and ≤60 min ($n = 14$). The change in CPK from baseline to 1 h postpartum was significantly greater in the group with the longer second stage ($p < .02$). As demonstrated in Fig. 3, regression analysis estimated that for every increase of 1 min in the second stage of labor, CPK increased by 2.15 u/l in

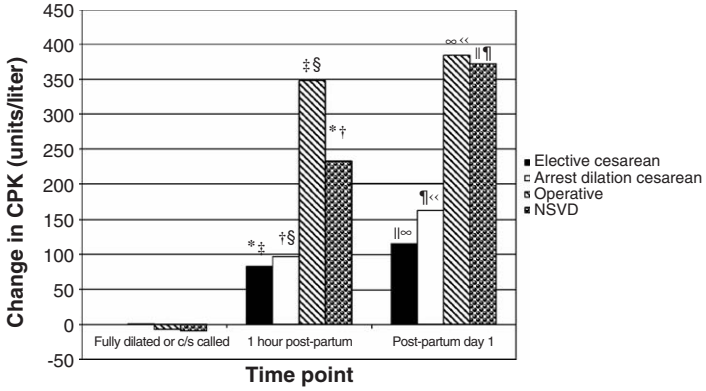

Figure 2 Changes in CPK from admission (baseline) to each time point. [*$p<.02$; †$p<.02$; ‡$p<.001$; §$p<.001$; ||$p<.004$; ¶$p<.01$; ∞$p<.02$; ‹‹$p<.04$].

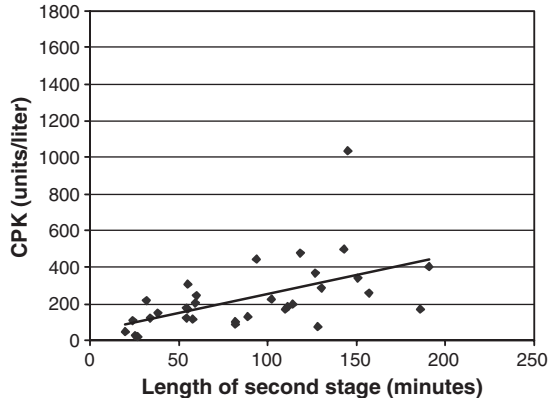

Figure 3 Change in CPK from admission to 1 h postpartum in spontaneous vaginal deliveries.

the reperfusion phase ($p = .001$). This difference was even more pronounced by postpartum day 1. For every increase of 1 min in the second stage, CPK increased by 2.35 u/l (Fig. 4) by postpartum day 1 ($p < .03$). These changes were not significant in the operative vaginal delivery group ($n = 9$). There was no significant difference in this change when comparing those patients who had a spontaneous vaginal delivery with a second stage greater than 1 h and patients who had an operative vaginal delivery with a second stage less than 1 h.

4. Discussion

I/R injury in various skeletal muscles has been previously studied both in animal and in human models. Many of these studies have been done in the context of introducing free radical scavengers or other antioxidants, such as vitamin E, in an attempt to mitigate the damage that occurs. The addition of these substances has been shown to suppress the increase of nonspecific markers of muscle damage such as CPK and LDH, as well as specific markers of lipid peroxidation (e.g., MDA), and ameliorate functional and structural damage to these muscles [3,4,8,9]. However, I/R injury has never been studied or documented in the pelvic floor, as demonstrated by a search of Medline (1966—May 2005; search terms: "reperfusion injury" combined with "pelvic floor or pelvis or levator ani").

Even in other skeletal muscles of the body, the time course of I/R injury has not been completely elucidated. However, the recent development of a cytochrome c-based biosensor has shed some light on this process by allowing the measurement of superoxide radicals in vivo. This body of work has shown that during the ischemic episode, superoxide radicals are not generated. These radicals are produced only after the ischemic event, taking on average 60—90 s for the blood flow to reach the muscle, reoxygenate the cells, and result in their production. Moreover, both maximum superoxide concentration and the total time of superoxide production are strongly dependent on the period of ischemia [10].

In this study, the changes in CPK showed marked increases during the reperfusion phase (e.g., from delivery to 1 h postpartum). In addition, an increase in the length of the ischemic phase (e.g., an increase in the length of the second stage of labor) caused a significant increase in the level of CPK during the reperfusion phase. Although the exact timing of post-partum day 1 blood draws were variable (always done the morning after delivery, making it between 6 and 30 h after childbirth), their continued increase as compared to 1 h postpartum suggests that the reperfusion injury changes to the pelvic floor were still ongoing at this time.

Because of the logistical difficulties with drawing blood samples at the time of delivery, it was not included as a time point. It was felt that knowing exactly when in the course of childbirth the ischemia and the elevation in markers of I/R injury occurred was not as clinically important as a comparison between the various modes of delivery. In this study both spontaneous and operative vaginal delivery were associated with an increase in a marker of I/R injury compared to both elective cesarean and cesarean for arrest of dilation. In addition, the first stage of labor does not appear to contribute significantly to markers of I/R injury, as the change in CPK from baseline to full dilation or the time at which a cesarean was called did not differ significantly between the modes of delivery.

Although the cesarean modes did not differ from one another, perhaps the significant changes in CPK

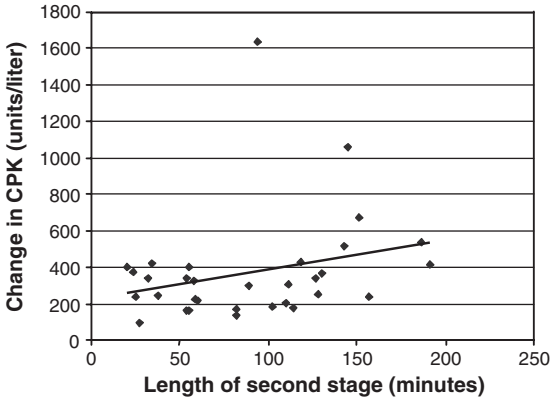

Figure 4 Change in CPK from admission to 1 day postpartum in spontaneous vaginal deliveries.

from baseline to the postpartum time points within each group represent tissue damage that is inherent in undergoing surgery. Unfortunately, none of the women who had complete venipuncture sets had a cesarean for arrest of descent, as this delivery group would represent an injury group combining both a complete ischemic phase (second stage of labor) and the muscular trauma of surgery.

Furthermore, although not a goal of our study, it is interesting to note that the level of CPK in the reperfusion period following operative delivery with second stages ≤60 min was not significantly different from that found following spontaneous delivery with a second stage >60 min. This suggests that pelvic floor protection is not necessarily gained by using forceps to shorten the second stage of labor [11]. Perhaps more interesting is that operative vaginal delivery was not associated with a significant elevation of CPK compared to spontaneous vaginal delivery, suggesting that although the former may lead to clinical damage of the perineum and vaginal epithelium, it does not necessarily augment the inherent pelvic floor damage that occurs with vaginal delivery, contrary to popular theory [1,12].

Some statistically significant differences were found in the demographic variables between delivery groups. The elective cesarean group had a significantly lower EGA, probably because elective sections are traditionally performed at 39 weeks. In addition, as cephalopelvic disproportion is often the presumed etiology, it follows that fetal weights might be greater in cesareans for arrest of dilation than in elective sections or vaginal deliveries.

In this study LDH was not a marker that reflected I/R injury in the pelvic floor. Although in many studies LDH results mirror those of CPK and TBARS, in a study by Koksal et al., LDH was the only marker that failed to show a significant difference between the control and treatment group [13]. This may occur because LDH is an enzyme present in the cytosol of all human cells, whereas CPK is found mainly in skeletal and cardiac muscle. The measurement of LDH may not have been sensitive enough to detect damage specific to the skeletal muscles of the pelvic floor.

The assay used to measure TBARS also did not reflect I/R injury. Further investigation of this assay revealed a significant flaw in its utility for this study because the amount of lipid peroxidation detected in the collected samples was consistently below the level of detection for this particular assay. In addition, because this assay has been typically performed on tissue cell culture (rather than plasma, as in this study), the appropriate concentrations of the reagents for plasma could potentially be different, and this also contributes to its poor reliability for this study. Future study using the more expensive methods needed to detect malondialdehyde (MDA), a stable intermediate of lipid peroxidation that is widely accepted as a marker of reactive oxygen species mediated lipid peroxidation [3—6,8,14], could provide a better, more specific assay for I/R injury.

While the elevations seen in the non-specific marker (CPK) were not paralleled by elevations in the marker that is more specific for lipid peroxidation (TBARS) in this study, it should be noted that previous studies have documented such a relationship. These studies made use of the more expensive methods of detecting lipid peroxidation (MDA) that were mentioned above. When such methods were used to investigate I/R injury in skeletal muscles other than the pelvic floor, the significant changes in plasma CPK did in fact mirror those of MDA [5,8]. Moreover, the changes in both types of markers (specific and non-specific) correlated with evidence of histological damage [5].

In summary, vaginal deliveries, both spontaneous and operative, resulted in higher levels of markers of muscle cell damage than did cesarean deliveries. The first stage of labor did not appear to significantly affect these markers while a longer second stage of labor was associated with higher levels of these injury markers. This study provides preliminary data to suggest that evidence of nonspecific muscle cell damage correlates with I/R injury of the pelvic floor.

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