Variation of Traditional Biomarkers of Liver Injury After an Ultramarathon at Altitude

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Background: Significant elevations of traditional biomarkers of liver injury can occur as a result of running an ultramarathon.

Hypothesis: Traditional serum biomarker levels of liver injury will significantly increase as the result of participating in this 161-km race at altitude.

Study Design: Prospective cross-sectional study.

Level of Evidence: Level 3.

Methods: A total of 64 (before) and 83 (after) volunteer runners participated in a prospective observational field-based study at the Leadville 100 ultramarathon race. Changes in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), and bilirubin levels were measured.

Results: Of 669 athletes who started the race, 352 successfully completed the race within the 30-hour cutoff (53%). Of 36 runners who had pre- and postrace blood samples taken, the mean ALT, AST, and bilirubin levels were increased from 23 ± 10 U/L, 23 ± 5 U/L, and 0.60 ± 0.29 mg/dL to 117 ± 106 U/L, 485 ± 500 U/L, and 1.60 ± 0.61 mg/dL, respectively (all $P < 0.001$). There was no change in the mean ALP level ($P = 0.11$). There were no significant correlations between postrace ALT, AST, ALP, lactate dehydrogenase (LDH), and bilirubin levels and athletes' age, sex, body mass index, or finishing time. Significant positive linear correlations between AST, ALT, and LDH with CK were seen. Athletes in this study did not seek medical attention after the race based on an electronic survey (92% response rate).

Conclusion: Significant elevations of traditional biomarkers of liver injury occurred as a result of running an ultramarathon at altitude. These correlated with CK, a marker of muscle injury.

Clinical Relevance: When reviewing laboratory studies of traditional biomarkers of liver injury in athletes after an ultramarathon, significant elevations may be seen from baseline but are likely to be of no clinical consequence.

Keywords: liver enzymes; running; ultramarathon; muscle injury

Participation in ultramarathons has been increasing over the past few decades.^{6,9,14,27} As a result, the understanding of physiologic changes from this physically demanding activity is increasing as more studies occur.^{20,24} Many biomarkers, including those traditionally indicative of liver injury, increase after an endurance event, with results returning to normal values within days of the event.^{13,19,21,28} These increases have not been associated with immediate or long-term deleterious effects on the athletes.^{9,22}

There also has been debate as to the muscle or liver origin of increased traditional biomarkers of liver injury after endurance

events.² A previous study found increases in laboratory values beyond the known process of hemolysis, suggesting that there may be a component of liver injury seen after endurance events.⁷ These may reflect similar increases seen in skeletal muscle markers after endurance events.^{9,22}

The Leadville 100 is a high-altitude, 161-km ultramarathon in Leadville, Colorado. The course ranges in altitude from 2800 to 3840 m, and the majority of the course is located on forest trails with some mountain roads.¹⁶ The purpose of our investigation was to determine specific variations in traditional liver biomarkers seen during an endurance event at altitude.¹⁵ We

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Table 1. Demographic information of participants

Participant Characteristics	Participants With Both Pre- and Postrace Samples (n = 36)	All Runners in the Race (N = 669)
Age, y, mean $\pm$ SD (range)	43 $\pm$ 10 (26-68)	40 $\pm$ 9 (19-74)
Male sex, n (%)	28 (78)	560 (84)
BMI, kg/m ² , mean $\pm$ SD (range)	22.7 $\pm$ 2.4 (19-29)	23.4 $\pm$ 2.3 (17-31)
Race completion status, n (%)		
Finished	27 (75)	352 (53)
Did not finish	9 (25)	317 (47)

BMI, body mass index.

also aimed to examine enzymes of muscle origin and compare them with traditional liver enzymes to determine correlations. The study hypothesis was that traditional serum biomarker levels of liver injury would significantly increase after participating in this race.

METHODS

The Colorado Multiple Institutional Review Board and the Leadville Race Series approved this study. The study was conducted during the Leadville 100 ultramarathon in Leadville, Colorado, in August 2014. Data from weather stations near the course were used to determine the meteorological conditions during the race. Race temperature at the start (4:00 AM) on August 16, 2014, was 2°C with a light wind. The temperature rose to approximately 22°C, and the wind reached 16 km/h by late afternoon. The race day was dry with no precipitation and a mean humidity of 57% (range, 22%-92%). To successfully complete the race, runners had to pass the finish line before 10:00 AM on the next day (30-hour cutoff). The weather was very similar on August 17, 2014.

Participants were recruited during the prerace medical check-in meeting and at the postrace finish line. The study was open to all runners who were interested in participating. There were no exclusion criteria. There was no compensation for participating in the study, but athletes were informed of their test results. Runners consented before participating in the study.

Blood samples were collected on the day before the race and at the finish line whether the runners finished or dropped out of the race. Blood samples were collected within 30 minutes after the completion of the race or when they dropped out at the 50-mile aid station. The 50-mile aid station was the major drop out location for this race; therefore, we were able to collect samples from those who only made it this far. For the few runners who dropped out in the second half of the race and participated in the study, blood collection occurred within 2 hours of their dropout at the finish line, along with those runners who finished the race. A total of 3 mL of blood was drawn while seated through an antecubital vein into heparinized tubes. Samples were refrigerated

and transported to the University of Colorado Hospital laboratory within 24 hours. Comprehensive metabolic panel and creatine kinase (CK) levels were measured. All prerace blood samples (n = 65) were within the normal limits. Runners' characteristics, medical histories, and race performances were available through online prerace/postrace questionnaires and other studies associated with the race.

Statistical Analysis

Statistical analyses were conducted using SPSS statistical software (version 23.0; IBM Inc). Descriptive statistics were used to examine the frequency, mean, median, and range for all variables. A Wilcoxon matched-pairs test for paired samples was used to assess the impact of the race on the data obtained in the 2 predetermined moments (pre- and postrace). As the postrace biomarkers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenase [LDH], CK, bilirubin) were not normally distributed (single-sample Kolmogorov-Smirnov test, $P < 0.001$), a nonparametric (samples sign test) analysis was performed, which showed the same results. A linear multiple regression model was used to determine the correlation between traditional liver injury biomarker level and runners' sex, age, body mass index (BMI), and race finish time. A P value of <0.05 was considered statistically significant. A linear regression model was used to determine the correlation between postrace traditional liver injury biomarkers, serum CK, and finish time for those who completed the race.

RESULTS

Of 669 athletes who started the ultramarathon, 352 (53%) successfully completed the race by the 30-hour cutoff (Table 1). Twenty-seven of the 36 runners who provided pre- and postrace samples were able to finish the race (75%). For these 36 runners, mean ALT, AST, and bilirubin levels were increased but there was no change in the mean alkaline phosphatase (ALP) level (Table 2). Overall, a total of 112 runners volunteered

Table 2. Changes in traditional liver biomarker levels of 36 runners before and after the race

Laboratory (RR)	Prerace, Mean $\pm$ SD (Range)	Postrate, Mean $\pm$ SD (Range)	P Value (Wilcoxon)
ALT (7-52 U/L)	23 $\pm$ 10 (11-56)	117 $\pm$ 106 (24-485)	0.000
AST (12-39 U/L)	23 $\pm$ 5 (15-43)	485 $\pm$ 500 (57-2077)	0.000
ALP (39-117 U/L)	57 $\pm$ 11 (36-80)	55 $\pm$ 9 (37-80)	0.121
Total protein (6.4-8.9 g/dL)	7.6 $\pm$ 0.4 (6.5-8.7)	7.2 $\pm$ 0.4 (6.6-8.1)	0.000 ^a
Albumin (3.5-5.7 g/dL)	4.6 $\pm$ 0.2 (4.2-5.0)	4.4 $\pm$ 0.2 (4.0-4.9)	0.000 ^a
Total bilirubin (0.1-1.3 mg/dL)	0.7 $\pm$ 0.3 (0.2-1.3)	1.6 $\pm$ 0.6 (0.6-2.9)	0.000

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; RR, reference range.

^aStatistically significant but clinically nonsignificant changes.

Table 3. Traditional liver biomarker levels of 83 runners after the race

Laboratory (RR)	Mean $\pm$ SD	Range	No. of Runners With Abnormal Levels (%)
ALT (7-52 U/L)	105 $\pm$ 98	21-498	58 above the RR (70)
AST (12-39 U/L)	431 $\pm$ 476	40-2489	83 above the RR (100)
ALP (39-117 U/L)	56 $\pm$ 11	26-87	2 below the RR (2)
Total protein (6.4-8.9 g/dL)	7.3 $\pm$ 0.5	6.3-8.6	1 below the RR (1)
Albumin (3.5-5.7 g/dL)	4.5 $\pm$ 0.3	3.6-5.3	0 (0)
Total bilirubin (0.1-1.3 mg/dL)	1.6 $\pm$ 0.8	0.6-5.2	75 above the RR (90)
LDH (124-271 U/L)	663 $\pm$ 406	270-2324	82 above the RR (99)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; RR, reference range.

to participate in the study. Of the total blood samples collected, 64 were collected prerace and 83 were collected postrate. No runners had elevated liver function tests at the beginning of the race. Of the 83 runners with collected blood samples after the race, 65 (79%) successfully finished the race.

Postrate samples demonstrated that the majority of biomarkers were above normal range after the race (Table 3). LDH was analyzed on postrate samples. Runners who were able to successfully complete the race demonstrated higher levels of ALT, AST, LDH, and bilirubin levels compared with those who were not able to complete the race (Table 4). Using a linear multiple regression model, there were no statistically significant correlations between postrate ALT, AST, ALP, LDH, and bilirubin levels and athletes' age, sex, BMI, or finishing time. Postrate bilirubin, total protein, and ALP were not correlated with postrate CK levels (Figure 1). There also was no linear correlation between finish times for those who completed the race and postrate ALT, AST, ALP, LDH, bilirubin, and total protein serum levels.

DISCUSSION

Ultramarathons can lead to increased serum biomarkers, including those traditionally for liver injury.⁵⁻⁷ While LDH and CK are common biomarkers of skeletal muscle damage,^{2,4,10,12} AST and ALT are also in muscle cells and are released during breakdown.^{10,11} There are many hilly sections in the Leadville ultramarathon with changes in altitude. The eccentric contractions seen in downhill running contribute to more muscle breakdown than running on a flat surface.^{2,8,10,26}

Debate has ensued as to the degree to which AST and ALT reflect liver versus muscle damage when measured in the clinical context of an endurance activity. Research has examined γ -glutamyltransferase (GGT) levels, a more specific test of liver damage. While 1 study demonstrated an increase in postrate GGT,⁷ this was not seen in other studies.^{1,3} The current study was preliminary with a limited budget, and we were not able to measure GGT. The relative increase of AST to ALT suggests a more muscular origin, as ALT is more specific to the liver.^{16,17}

Figure 1. Postrace (a) alanine aminotransferase (ALT), (b) aspartate aminotransferase (AST), and (c) lactate dehydrogenase (LDH) versus creatine kinase (CK).

The high significance of the linear correlation of CK with AST, ALT, and LDH also suggests a muscular origin.

Previous studies have measured changes in biomarkers reflecting hemolysis in endurance activities.^{5,9} Increases in bilirubin can occur and may be secondary to red blood cell breakdown and hemolysis.⁷ There was a trivial increase in bilirubin level that was nonsignificant compared with baseline. Overall, this study supports a greater contribution of muscle injury over liver injury to the increase in liver function tests, meaning that most of the elevation of the traditional biomarkers of liver injury likely do not reflect liver damage because of the direct correlation with muscle markers. As participation in endurance events continues to increase, it is imperative to be able to understand normal

physiological changes in the body. Importantly, none of the respondents of the postrace survey sought medical attention, demonstrating that these increased biomarker levels were physically tolerated without short-term harm.

A major limitation of this study is the lack of absolute causality of the increased biomarker levels. Biomarkers such as AST and ALT come from multiple sources, including liver, muscle, and plasma.^{11,12,18,23,25,26} Previous studies have not shown a change in GGT after endurance events, therefore demonstrating that the majority of the increase in these levels may be muscular in origin rather than liver.^{3,13,21,28} Another limitation of this study is the relatively small sample size and the lack of a sample size estimate to determine power, which marginalizes the results.

Table 4. Postrace traditional liver biomarker levels of finishers versus nonfinishers

Laboratory (RR)	Finishers, Mean $\pm$ SD (n = 65)	Nonfinishers, Mean $\pm$ SD (n = 18)	P Value (Wilcoxon)
ALT (7-52 U/L)	122 $\pm$ 103	44 $\pm$ 32	0.000
AST (12-39 U/L)	515 $\pm$ 502	130 $\pm$ 142	0.000
ALP (39-117 U/L)	56 $\pm$ 11	60 $\pm$ 11	0.177
Total protein (6.4-8.9 g/dL)	7.2 $\pm$ 0.4	7.5 $\pm$ 0.5	0.004 ^a
Albumin (3.5-5.7 g/dL)	4.4 $\pm$ 0.3	4.6 $\pm$ 0.3	0.036 ^a
Total bilirubin (0.1-1.3 mg/dL)	1.7 $\pm$ 0.8	1.5 $\pm$ 0.7	0.300
LDH (124-271 U/L)	734 $\pm$ 427	406 $\pm$ 133	0.000

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; RR, reference range.

^aStatistically significant but clinically nonsignificant differences.

CONCLUSION

Ultramarathon running causes significant increases in traditional liver and muscular serum biomarkers. It seems likely that these biomarkers are due to muscle enzyme release rather than that of the liver.

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