Early assessment of exercise induced skeletal muscle injury using plasma fatty acid binding protein

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Abstract

Objective—To test whether fatty acid binding protein (FABP) is a useful plasma marker for the early detection of exercise induced skeletal muscle injury in healthy subjects.

Methods—Plasma concentrations of FABP and myoglobin (Mb) were measured in six healthy physical education teacher trainees after 20 minutes of downhill running (16% incline; mean lactate 4 mmol/l; 70% VO_{2\text{MAX}}). Creatine kinase (CK) was measured for comparison.

Results—Significant increases were found in plasma FABP (mean peak level 50 µg/l), Mb (823 µg/l), and CK (491 U/l). Mb and FABP concentrations were already significantly elevated (p<0.05) at 30 minutes, but CK not until two hours after exercise. Whereas Mb and FABP decreased to normal levels within 24 hours, CK activity remained elevated until 48 hours. The Mb to FABP ratio in plasma after exercise induced muscle injury was 15.0 (1.3) (mean (SEM)) (range 7.4–31.1), which is within the range of ratios calculated for skeletal muscle tissue contents of Mb and FABP, but different from the reported plasma ratio after myocardial injury (4–6).

Conclusions—After eccentric exercise induced muscle injury, plasma FABP and Mb increase and decrease more rapidly than CK, indicating that both FABP and Mb are more useful than CK for the early detection of such injuries and the monitoring of injury during repeated exercise bouts. In addition, the Mb to FABP ratio in the plasma indicates the type of muscle injured.

Keywords: fatty acid binding protein; myoglobin; creatine kinase; exercise induced muscle injury; eccentric exercise
any specific training. The risks and benefits of the study were explained, and written informed consent was obtained from each participant. All subjects were instructed to refrain from unaccustomed exercise during the course of the study starting 48 hours before the exercise session.

Warm up protocol
The 15 minutes warm up consisted of five minutes running on a treadmill at a speed which corresponded to the individual aerobic threshold, followed by five minutes of stretching the leg muscles and finishing up with three series of eight knee bends.

Eccentric exercise regimen
The probands successfully performed a test of their maximum oxygen uptake (VO2MAX) on a treadmill ergometer. The exercise session consisted of 20 minutes of downhill treadmill running (16% incline). The exercise intensity (running speed) on the treadmill was heart rate controlled. The target heart rate during downhill running was that found at 70% of VO2MAX in the VO2MAX test performed two weeks before downhill running. Blood samples used for the determination of muscle proteins were taken immediately before exercise, and 30 minutes and two, six, 24, and 48 hours after exercise.

LABORATORY ANALYSES
Lactate
Capillary blood samples for the determination of lactate concentration were collected from a prewarmed ear lobe. Immediately 100 µl of the sample was mixed with 200 µl of a cold 8% perchloric acid solution and refrigerated until subsequent lactate determination by an enzymatic method (LACT; Boehringer-Mannheim, Mannheim, Germany).

Blood collection
Blood was collected from a superficial forearm vein in EDTA coated tubes (Sarstedt, Nümbrecht, Germany). CK activity was assayed without delay. Blood samples for FABP, cTnI, and Mb measurements were immediately centrifuged and the plasma subsequently frozen and stored at −20°C until assayed.

FABP
Blood samples from 62 apparently healthy blood donors, including 44 men and 18 women, with a mean (SD) age of 32 (10) years were used to establish the reference range for the FABP assay used.

FABP was determined in plasma using an enzyme linked immunosorbent assay of the antigen capture type (sandwich ELISA). Specific monoclonal antibodies directed to human muscle-type FABP were coated on 96-well microtitre plates in 0.1 mol/l carbonate buffer, pH 9.4, at 4°C overnight. After coating and five washes with phosphate buffer (pH 7.2; 0.1% BSA and 0.05% Tween 20; PBT), 50 µl of a secondary antibody directly conjugated with horseradish peroxidase and 50 µl of sample or standard were incubated at 37°C for 30 minutes in a humid environment with gentle shaking. After five washes with PBT, 100 µl tetramethyl benzidine substrate mixture was added to each well. The reaction was stopped after seven minutes with 50 µl 2 mol/l H2SO4, and the absorbance at 450 nm was measured with the use of a microplate reader. The detection limit of the assay was 0.6 µg/l (mean + 3SD of the zero standard; n = 35).

CK, cTnI, and Mb
These were measured by commercially available assays (CK: Granutest 15, Merck, Darmstadt, Germany; cTnI: Troponin-I Pasteur, Sanofi-Diagnostics-Pasteur, Marnes-la-Coquette, France; Mb: Access, Sanofi-Diagnostics-Pasteur).

CALCULATIONS AND STATISTICS
The ratio of Mb to FABP was calculated only for those samples in which both proteins were increased to at least twice their baseline value. For this reason, calculations were not performed for some time points. For the calculation, the individual basal levels of all three proteins were subtracted from the plasma levels measured after exercise. All variables were tested for normal distribution and equal variance. Mean and SEM were calculated to describe continuous variables. Student’s paired t test, linear regression, one way analysis of variance for repeated measurements, and post hoc comparisons by the Student-Newman-Keuls methods were used for intra- and inter-group comparison. p<0.05 was considered to indicate statistical significance.
Results

PLASMA REFERENCE VALUE
The mean (SD) FABP concentration in plasma from healthy blood donors was 1.9 (0.8) µg/l. The upper reference limit, calculated non-parametrically as the 97.5 percentile of FABP concentrations, was 3.4 µg/l. Plasma FABP in the six subjects under study before exercise was 3.0 (1.3) µg/l (mean (SEM)), which is not significantly different from that of the blood donors.

ECCENTRIC EXERCISE
During exercise, plasma lactic acid levels rose to about 4 mmol/l in all six downhill runners. cTnI could not be detected in any sample taken, but FABP, Mb, and CK all increased significantly after exercise. The time to reach peak concentration and return to normal was significantly shorter for FABP and Mb than for CK (fig 1). FABP and Mb were already significantly (p<0.05) increased, relative to pre-exercise values, at 30 minutes after exercise (mean increase for FABP 13.9-fold and for Mb 8.7-fold). Mean peak values were reached for FABP (50 (13) µg/l) and Mb (826 (230) µg/l) between two and six hours after exercise, while the values found at 24 and 48 hours were not significantly increased (fig 1). In contrast, the increase in plasma CK activity was not significant at 30 minutes after exercise (mean increase 2.1-fold), but became and remained significant (p<0.05) between two and 48 hours after exercise (fig 1). The peak value for CK was reached at six hours and amounted to 491 (121) U/l. Peak FABP concentrations in individual subjects correlated with peak CK levels (r = 0.74; p<0.05).

A positive correlation was found for the time courses of plasma FABP and Mb (r(x) = -7.85 + (15.4x); r = 0.93, p<0.0001). The calculated Mb to FABP ratio was constant after exercise without significant differences. For all blood samples taken after the end of eccentric exercise, the Mb to FABP ratio (calculated for 20 samples with significantly elevated Mb and FABP levels) was 15.0 (1.3) (mean (SEM)).

Discussion
Unaccustomed exercise bouts especially with eccentric muscle contractions frequently produce skeletal muscle damage, which results in a temporary loss of muscle function and coordination. Increased blood levels of CK and Mb are frequently used to diagnose exercise induced muscle damage. More recently, muscle-type FABP has been introduced as a plasma marker for the early detection of necrotic muscle injury in patients. The present study shows for the first time that plasma FABP increases after physical exercise by healthy subjects and that its pattern of release into and clearance from the blood is similar to that of Mb. For both FABP and Mb a significant increase was reached earlier (30 minutes) than for CK (two hours), indicating the usefulness of the former for the early detection of muscle injury. In addition, both FABP and Mb had returned to baseline values at 24 hours after exercise whereas CK remained elevated until (at least) 48 hours after exercise. The relatively short half-life of FABP and Mb in plasma relates to their rapid elimination from plasma mostly by renal clearance. Our findings suggest that FABP and Mb, in comparison with CK, are also more useful for the separate monitoring of muscle injury during repeated exercise bouts.

The calculated Mb to FABP ratio (mean value 15.0, range 7.4–31.1) is comparable with that found in skeletal muscle tissue (20–70 for six skeletal muscle types studied). cTnI was not detectable in plasma from the investigated athletes, indicating that the source of muscle protein release after downhill running is damaged skeletal muscle rather than heart muscle. Our present results extend the previous (preliminary) observation that plasma FABP and Mb increase after heavy exercise on a treadmill, with a calculated Mb to FABP ratio of 23 (mean for four volunteers), and are also in accordance with the finding that plasma FABP and Mb increase after abdominal aortic surgery, with a calculated Mb to FABP ratio varying from 35 to 50. However, the ratio of the plasma concentrations of Mb to FABP after physical exercise is different from the ratio of 5 (range 2–9) found after acute myocardial infarction.

In conclusion, FABP, like Mb, allows earlier assessment of exercise induced skeletal muscle injury than does CK, and because of its rapid plasma clearance it is also particularly suited to assessment of recurrent injury. A recently developed electrochemical immunosensor for the rapid (about 15 minutes) assay of FABP in plasma will be useful for this purpose. In addition, the different ratios of the plasma concentrations of Mb to FABP found after skeletal and myocardial tissue injury reflect the different ratios found in the affected tissue, and thus allow the differentiation whether Mb is released from skeletal muscle or myocardium. Therefore, we suggest that simultaneous measurement of FABP and Mb in plasma could be helpful for the early diagnosis of skeletal muscle damage, as the combination of these markers can give trainers and athletes early and specific information about the exercised skeletal muscles in training sessions and after competition. This information could help to avoid additional skeletal muscle damage or overtraining and could allow better control of specific training sessions.

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Commentary

Biochemical assessment of muscle damage, to follow the course of a disease, diagnose it or its carriers, or monitor (over)training, is based on measuring serum CK activity. Skeletal muscle has a high content, but CK occurs in virtually all cells. Therefore more specific proteins have been suggested. Some have clear (theoretical) advantages: Mb has been shown to enter the circulation before CK, 1 CK isoenzymes allow discrimination between heart and skeletal muscle, and carbonic anhydrase III is more muscle-specific. 2 Yet, CK is still the standard, partially because of its ease and accessibility. This article shows that FABP has the same characteristics as Mb, and therefore has no advantages as a marker. The FABP to Mb ratio may be more informative, but in order to prove this, it should be compared with other methods, involving—for example, CK isoenzymes and imaging to assess damage. The non-standard character of the FABP assay may hamper its general use.

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