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Minireview

Unbound Free Fatty Acids and Heart-Type Fatty Acid–Binding Protein: Diagnostic Assays and Clinical Applications

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Background: **A biomarker that reliably detects myocardial ischemia in the absence of necrosis would be useful for initial identification of unstable angina patients and for differentiating patients with chest pain of an etiology other than coronary ischemia, and could provide clinical utility complementary to that of cardiac troponins, the established markers of necrosis. Unbound free fatty acids (FFA_u) and their intracellular binding protein, heart-type fatty acid–binding protein (H-FABP), have been suggested to have clinical utility as indicators of cardiac ischemia and necrosis, respectively.**
Methods: **We examined results of clinical assessments of FFA_u and H-FABP as biomarkers of cardiac ischemia and necrosis. Data published on FFA_u and H-FABP over the past 30 years were used as the basis for this review.**
Results: **Although little clinical work has been done on FFA_u since the initial reports, recent studies documented an association between increased serum FFAs and ventricular dysrhythmias and death in patients with acute myocardial infarction (AMI). Recent data suggest that serum FFA_u concentrations increase well before markers of cardiac necrosis and are sensitive indicators of ischemia in AMI. H-FABP is abundant in cardiac muscle and is presumed to be involved in myocardial lipid homeostasis. Similar to myoglobin, plasma H-FABP increases within 3 h after AMI and returns to reference values within 12–24 h.**
Conclusions: **FFA_u may have a potential role in identifying patients with cardiac ischemia. H-FABP is useful for detecting cardiac injury in acute coronary syndromes and predicting recurrent cardiac events in acute coronary syndromes and in congestive heart failure patients. Assays are available for both markers that could facilitate further clinical investigations to assess their possible roles as markers of cardiac ischemia and/or necrosis.**

Myocardial ischemia, a major cause of myocardial injury and necrosis, is initiated whenever the coronary arterial flow cannot supply sufficient oxygen to the myocardium. Within seconds of myocardial ischemia, several changes occur within myocytes, such as termination of aerobic metabolism, onset of anaerobic glycolysis, potassium ion leakage, and cessation of contraction to reduce the energy demands, causing wall motion abnormalities that can be detected by echocardiography. Within minutes, other changes follow, including leakage of metabolites, a decrease in pH, and increased intracellular calcium concentrations and osmotic load. Early ultrastructural changes include swollen mitochondria, edema, and cytoplasmic blebbing. The general cause of irreversible changes, within hours of ischemia, is progressive and prolonged ATP depletion. The hallmarks of this stage, which represents the "point of no return", are sarcolemmal disruption and leakage of cardiac macromolecules such as cardiac troponins I and T (cTnI[4] and cTnT) and creatine kinase-MB (CK-MB). The pathophysiologic changes and

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[4] Nonstandard abbreviations: cTnI and cTnT, cardiac troponin I and T, respectively; CK-MB, creatine kinase-MB; H-FABP, heart-type fatty acid–binding protein; FFA_u, unbound free fatty acids; AMI, acute myocardial infarction; ADIFAB, acrylodated intestinal fatty acid–binding protein; URL, upper reference limit; PCTA, percutaneous transluminal coronary angioplasty; ECG, electrocardiography; IHD, ischemic heart disease; tPA, tissue plasminogen activator; CHF, congestive heart failure; and ACS, acute coronary syndrome(s).

metabolic progression of ischemia to necrosis have been described in detail in a recent text *(1)*.

The release of cardiac biomarkers is influenced by a variety of factors:

- Cytosolic enzymes: An increase in intracellular calcium activates a variety of enzymes, including phospholipases and the protease calpase. Calpase contributes to the early degradation, dissociation, and release of myofibrillar proteins (such as cTnI and cTnT) after myocardial damage. pH-dependent dissociation of structural proteins could also affect release of such markers. On the other hand, lysosomes are stable within the first 3–4 h after onset of ischemia and do not affect the breakdown of subcellular structures.
- Subcellular location: Soluble cytosolic molecules, such as fatty acid–binding proteins (FABPs), are released more rapidly than structurally bound molecules.
- Molecular mass: Within a particular intracellular localization, smaller molecules such as myoglobin and FABPs may enter the vascular system to a large extent directly via the microvascular endothelium.
- Plasma clearance: Smaller molecules such as FABPs (also myoglobin) pass through the glomerular membranes and are reabsorbed and metabolized in tubular epithelial cells *(2)*. Falsely increased plasma concentrations (caused by impaired clearance attributable to renal failure) or falsely decreased concentrations (in patients with hypermetabolic states) for both markers may therefore be observed.
- Concentration gradients: Concentration gradients between cardiomyocytes and interstitial spaces as well as local blood and lymphatic flow may also affect the appearance of markers in the general circulation.

Whether the release of biomarkers from the injured myocardium indicates irreversible damage and cardiac necrosis remains an issue of debate. The classic hypothesis suggests that release of biomarkers from the cardiomyocyte is possible only from irreversibly injured myocytes and is based on the hypothesis that plasma membranes are physiologically impermeable to macromolecules *(3)*. An alternative hypothesis proposes that release is a metabolically controlled property of cell membranes and that small extracellular increases in cardiac biomarkers may be caused by reversible disturbance of cell metabolism *(4)*. Recent evidence suggests that under moderate ischemic stress, myocardial tissue can release small amounts of macromolecules from the cytosol by mechanical mechanisms other than persistent membrane perforation *(4)*. The prevention of membrane leaks is an energy-dependent process in which myocardial plasma membranes become permeable to intracellular macromolecules under conditions of energy shortage. However, the appearance of mitochondrial enzymes and prolonged increases in cardiac proteins in serum are generally accepted as indicators of myocardial necrosis.

Testing the specificity of novel biomarkers of ischemia is challenged by the absence of a "gold standard" for myocardial ischemia. Comparison with troponin concentrations will not be valid because the ischemia marker would be expected to increase in unstable angina patients, who should not have any detectable increases in cardiac troponins. Achieving clinical acceptance of the proposed biomarker as the new gold standard will require extensive laboratory and translational research.

In this review, we discuss the physiology and pathophysiology of unbound free fatty acids (FFA_u) and heart-type FABP (H-FABP) and their proposed clinical applications as new biomarkers of cardiac ischemia and necrosis, respectively.

Unbound FFAs

PHYSIOLOGY AND PATHOPHYSIOLOGY

FFAs play several essential roles in physiologic homeostasis. Under aerobic conditions, nonesterified long-chain FFAs represent the primary metabolic sources for the myocardium, accounting for almost two-thirds of the ATP generated, with glucose metabolism generating the remaining one-third of myocardial oxygen demand *(5)*. Plasma long-chain fatty acids are either esterified to glycerol or nonesterified (or FFAs), most of which are bound to albumin. The mechanism for uptake of FFAs into myocytes remains unclear but involves passive diffusion and/or active carrier-mediated transport *(6)*. In the cytoplasm, long-chain FFAs are bound to FABP, which presumably facilitates their transport to the outer mitochondrial membrane where they become esterified/activated by long-chain acyl-CoA synthetase (Fig. 1). Once activated, acyl-CoA esters are directed mainly to β-oxidation, but some may be stored as triglycerides or converted into membrane phospholipids.

During hypoxia and ischemia, nonesterified fatty acids/FFAs have damaging effects on heart tissue and have been associated with an increased incidence of ventricular dysrhythmias and death in patients with acute myocardial infarction (AMI) *(7, 8)*. Proposed mechanisms for the damaging effects of FFAs during ischemia include accumulation of toxic intermediates of fatty acid metabolism, suppression of glucose utilization, and uncoupling of oxidative metabolism from electron transfer *(5)*. Inhibitors of FFA metabolism have been shown experimentally to reduce the infarct size and decrease the postischemic cardiac dysfunction in animal models *(9)*. Shown in Table 1 is a comparison of FFA_u and H-FABP concentrations in circulation and in myocytes under physiologic and ischemic conditions.

FFA_u ASSAYS

Although most of the FFAs in serum are bound to albumin, a small amount is unbound; this is frequently referred to as the "free" fraction. Serum FFA_u concentrations are determined from the ratio of total serum FFAs to total serum albumin *(10)*. A method for estimating serum FFA concentrations is based on the breakdown of pyro-

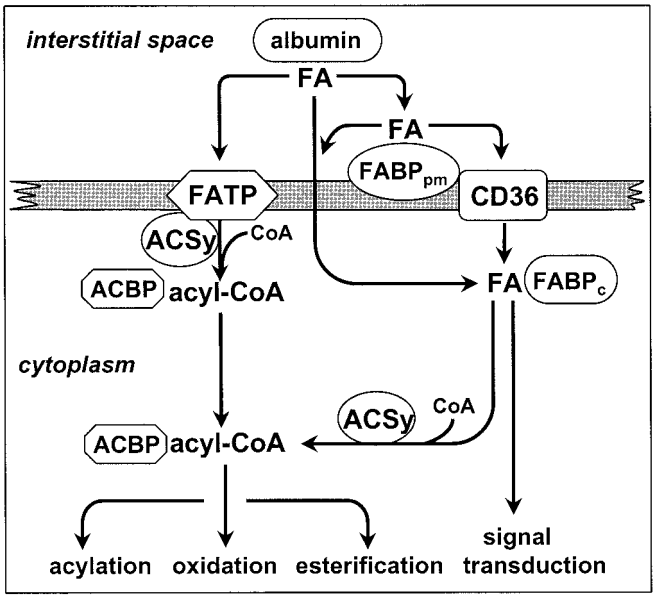

Fig. 1. Schematic overview of the molecular mechanism of cellular uptake and use of long-chain fatty acids (*FA*).

After dissociation from plasma albumin, fatty acids are translocated through the lipid bilayer (*gray*) via passive diffusion, membrane-associated proteins, or a combination of both (*right side* of *schematic*). The membrane-associated fatty acid transporters fatty acid-binding protein ($FABP_{pm}$), fatty acid translocase (*CD36*), and fatty acid transport protein (*FATP*) are involved. Intracellular fatty acids are bound to cytoplasmic H-FABP ($FABP_c$) and, after activation to fatty acyl-CoA, to acyl-CoA–binding protein (*ACBP*). *ACSy*, acyl-CoA synthetase.

phosphate, which is formed by thioesterification of FFAs with ATP and CoA in the presence of acyl-CoA synthetase, to inorganic phosphate, which is measured by reaction with molybdate *(11)*.

A recently developed method for measurement of serum FFA_u uses a fluorescent probe of FFA_u, termed acrylodated intestinal fatty acid–binding protein (ADIFAB), which is prepared by derivatizing a recombinant intestinal FABP with the fluorescent molecule acrylodan *(12)*. Binding of a single FFA_u molecule to ADIFAB, which does not interact with other serum molecules, displaces the fluorescent tag, producing a spectral shift from 432 nm to 505 nm that can be measured with a fluorometer. Human serum contains a mixture of 6 FFAs: palmitate (25%), stearate (10%), oleate (38%), linoleate (22%), arachidonate (3%), and linolenate (2%) *(13)*. Richieri and Kleinfeld *(13)* reported ADIFAB dissociation constants, determined at 37 °C and at concentrations below the critical micelle concentrations, of 0.31, 0.08, 0.28, 0.97, 1.63, and 2.50 μmol/L for palmitate, stearate, oleate, linoleate, arachidonate, and linolenate, respectively. Variations in dissociation constants are highly correlated with the solubility of the specific fatty acid in water, suggesting that all of these fatty acids bind to intestine FABP with a similar conformation.

A second-generation version of the assay uses a handheld reader and 15 μL of plasma and provides turnaround times <1 min *(14)*. The assay shows improved low-end sensitivity and is not affected by hemoglobin. The CV for duplicate measurements is 7%. The FFA_u upper reference limit (URL), determined at the 97.5th percentile of value distribution, is 2.7 nmol/L [mean (SD), 1.5 (0.6) nmol/L; range, 0.6–4.5 nmol/L] *(14)*.

Reports have suggested that heparin may cause FFA increases in vivo and perhaps in vitro as well. This is because heparin is known to stimulate the activity of lipoprotein lipase, which releases FFAs from triglycerides associated with blood lipoproteins. Blood collected into heparin-containing flasks or tubes or from patients receiving therapeutic heparin may therefore not be suitable for the FFA_u test. There has been controversy regarding this issue, however. Thus, assays considered for clinical use must be evaluated in appropriate studies addressing the potential of heparin interference.

CLINICAL APPLICATIONS

The mean (SD) serum FFA_u concentration, measured with the ADIFAB assay, in 283 samples from healthy donors was 7.5 (2.5) nmol/L *(13)*, and the distribution of FFA_u values was independent of donor age and sex. Mean FFA_u values increased significantly (by 1.5 nmol/L; $P < 0.001$) after overnight fasting. The clinical uses of FFA_u concentrations are summarized in Table 2.

Using the fluorescent probe ADIFAB, Kleinfeld et al. *(15)* measured serum FFA_u concentrations in 22 patients 5 min before and 30 min after percutaneous transluminal coronary angioplasty (PCTA). Post-PCTA concentrations were higher than baseline values in all patients, with the mean FFA_u concentration increasing 5-fold compared with the mean value [7.5 (2.5) nmol/L] observed in healthy patients. Ischemic ST changes monitored by electrocardiography (ECG) were observed in only 50% of

Table 1. Serum and intracellular concentrations of H-FABP and FFA_u under physiologic and pathophysiologic conditions.

	Physiologic conditions	Ischemia	Necrosis
Plasma concentrations			
FABP	0–6 μg/L	6–20 μg/L *(72)*	6–2000 μg/L *(47)*
FFA_u	7.5 (2.5) nmol/L *(13)*	High	High
Intracellular concentrations			
FABP	500 μg/g *(22)*	Low	Very low
FFA_u	Normal	High	Low
Main source of myocytic ATP	Anaerobic metabolism (β-oxidation of fatty acids)	Aerobic metabolism (accumulation of lactate)	

Table 2. Clinical use of FFA_u measurements.

Clinical use	Study description	Findings	Conclusions/Comments	Reference
Marker of ischemia	FFA_u measured in 22 patients before and 30 min after coronary angioplasty	FFA_u increased 14-fold over baseline concentrations; highest concentrations were observed in patients with ischemic ST-segment changes	Abnormal FFA_u concentrations may be a sensitive marker of cardiac ischemia	*(15)*
Marker of ischemia	458 patients enrolled in TIMI II trial; blood was collected at presentation and 50 min, 5 h, and 8 h after tPA	Sensitivity of FFA_u was 91% at admission and 98% at 50 min after tPA (cutoff, 5 nmol/L); specificity was 93% for noncardiovascular patients and healthy persons; higher FFA_u concentrations correlated with mortality (4-fold higher rate of death)	Increased FFA_u may be a sensitive marker of cardiac ischemia	*(20)*
Prognostic value	5250 men, followed for 22 years; FFA_u concentrations were measured	FFA_u were found to be an independent risk factor for sudden death (OR[a] = 1.70; 95% CI, 1.21–2.13)	FFA_u may have an arrhythmogenic role and contribute to a higher frequency of premature ventricular complexes and may therefore contribute to death	*(17)*

[a] OR, odds ratio; CI, confidence interval.

patients. In addition, FFA_u concentrations were significantly higher in the ECG-positive group than the ECG-negative group. An increase in FFA_u concentrations was suggested as an early marker of ischemia caused by PCTA *(15)*.

In a different study, 9 MI patients had increased FFA_u concentrations, whereas only 2 of the 9 had increased cTnI *(14)*. In addition, 93% and 30% of other chest pain patients had increased FFA_u and cTnI concentrations, respectively. FFA_u concentrations were increased in every instance that cTnI was increased. In addition, there was a positive correlation between peak FFA_u and cTnI concentration. At presentation, all of the MI patients had increased FFA_u, whereas only 22% had increased cTnI. Some of these patients had additional diagnoses (such as cocaine abuse, sepsis, and cardiac contusion) that can cause myocardial ischemia and injury. The authors therefore proposed that FFA_u concentrations may increase in the presence of acute myocardial injury independent of plaque rupture *(14)*.

Circulating FFA concentrations have also been suggested as putative for ventricular arrhythmias and sudden death after MI *(16)*. In the Paris Prospective Study I, plasma FFA concentrations were measured in a cohort of 5250 middle-aged men free of known ischemic cardiac disease *(17)*. After 22 years of follow-up, increased FFA concentrations were found to be an independent risk factor for sudden death (relative risk, 1.70; 95% confidence interval, 1.21–2.13) but not for fatal MI.

Pirro et al. *(18)* examined the relationship between circulating FFA concentrations and risk of ischemic heart disease (IHD) in 2130 men with insulin resistance syndrome who were without IHD at enrollment. During a 5-year follow-up, 114 of these individuals developed IHD. After adjustment for nonlipid risk factors, increased circulating FFA concentrations conferred a 2-fold increase in the risk of IHD (odds ratio, 2.1; $P = 0.05$) compared with lower plasma FFA concentrations. However, after adjustment for triglyceride concentrations, HDL-cholesterol, small, dense LDL, apolipoprotein B, and fasting plasma insulin, the relationship between plasma FFA concentrations and IHD did not achieve statistical significance.

In another study, FFA_u concentrations were measured in serum samples from 458 AMI patients (75 females and 383 males), enrolled in the TIMI II trial *(19)*, who were treated with tissue plasminogen activator (tPA) *(20)*. FFA_u concentrations were measured with the ADIFAB2 assay in blood samples drawn on admission and 50 min, 5 h, and 8 h after initiation of tPA treatment. Relative to the control population, results of this study indicated an ~4-fold increase in serum FFA_u concentrations at enrollment, a further 2-fold increase after tPA administration, and then a gradual decrease within 5 h of tPA administration. At cutoff a 5 nmol/L, the predicted sensitivity was 98% based on the results for admission and 50-min samples. The specificity, based on comparison with healthy individuals and patients with noncardiovascular disease, was 93%. Although interesting, these data must be considered exploratory for indicating the potential clinical performance of FFA_u. This is because the TIMI II trial included only well-characterized AMI patients, who were compared with patients with known noncardiovascular disease and with healthy individuals, which will not be the way the test will be used in practice. FFAs also correlated well with mortality at 30 days in the TIMI II cohort *(20)*.

SUMMARY

Current data, although limited, suggest that monitoring of FFA_u concentrations in patients presenting with ischemic symptoms may provide an early indication of cardiac ischemia. Cohort trials that enroll a broad spectrum of suspected myocardial ischemia patients need to be

performed to fully evaluate the true potential of this biomarker. ROC curves need to be plotted for relevant populations.

H-FABP

PHYSIOLOGY

FABPs bind long-chain fatty acids reversibly and noncovalently. FABPs are relatively small (15 kDa) intracellular proteins that are abundantly produced in tissues having active fatty acid metabolism, including the heart, liver, and intestine *(21)*. FABPs each contain 126–137 amino acids, and their tertiary structure resembles a clam shell in which the ligand is bound between the 2 halves of the clam by interaction with specific amino acid residues within the binding pocket, the so-called β-barrel *(22)*. Currently, 9 distinct FABP types have been identified, with each type showing a characteristic pattern of tissue distribution and a stable intracellular half-life of 2–3 days *(21)*. H-FABP was first shown to be released from injured myocardium in 1988, after which several studies investigated its application as a biochemical marker of myocardial injury. The H-FABP isoform is produced not only in cardiomyocytes but also, to a lesser extent, in skeletal muscle *(23)*, distal tubular cells of the kidney *(24)*, specific parts of the brain *(25)*, lactating mammary glands, and placenta *(23)*. Human H-FABP contains 132 amino acid residues and is an acidic protein (pI 5) *(26)*.

The primary biological function of FABPs is to facilitate intracellular translocation of long-chain fatty acids (see Fig. 1), which is usually hampered by the very low solubility of these compounds in aqueous solutions *(21)*. H-FABP can therefore be regarded as the cytoplasmic counterpart of plasma albumin. H-FABP knock-out mice have a markedly lower (~50%) fatty acid uptake rate and oxidation *(24)*. Other functions of H-FABP include participation in signal transduction pathways, such as regulation of gene expression by mediating fatty acid signal translocation to peroxisome proliferator–activated receptors *(27)*, and putative protection of cardiac myocytes against the detergent-like effects of locally high concentrations of long-chain fatty acids, particularly during ischemia *(21, 28)*.

The cellular production of FABPs is regulated primarily at the transcriptional level. In experimental animals, FABP was increased by endurance training *(29)* and diabetes *(30)*.

IMMUNOLOGIC ASSAYS FOR H-FABP

The characteristics of several assays for human H-FABP are shown in Table 3. H-FABP is a stable protein; both plasma samples and recombinant protein solutions can be subjected to at least 8 freeze–thaw cycles without loss of immunoreactivity *(31)*. Samples can be stored for at least 2 years at −80 °C *(32)*. Recombinant H-FABP is immunochemically equivalent to the tissue-derived protein and generally serves as a calibration material in immunoassays *(26, 31)*.

ENZYME-LINKED IMMUNOASSAYS

Tanaka et al. *(2)* developed a competitive enzyme immunoassay for H-FABP in plasma and urine samples. However, the assay required a long assay time and was not suitable for clinical application. Wodzig et al. *(31)* developed a one-step ELISA with a total performance time of 45 min; this assay has sensitivity and specificity comparable to the two-step ELISA developed by Okhura et al. *(33)*. Both assays are commercially available and are used in clinical research.

Automated immunoassays. Several automated assays have been developed, including an enzyme immunoassay, an automated sandwich immunoassay *(34)*, and a fully automated microparticle-enhanced immunoassay (COBAS® MIRA Plus analyzer; Hoffmann-La Roche). These assays use carboxylated latex particles coated with 3 monoclonal anti-human H-FABP antibodies *(35)* and are not commercially available at present. Very recently, a new concept of precipitation ellipsometry has been reported *(36)*, with a rapid assay time of 10 min, but this assay is still in prototype form.

Lateral-flow assays. Qualitative H-FABP lateral-flow assays have also been developed, and 2 whole-blood tests are commercially available *(37–39)*. These qualitative tests have a 15-min analysis time, and the cutoff for normal vs

Table 3. Characteristics of immunoassays for human H-FABP.

Assay	Assay time, min	Sample	Calibration range, μg/L	Detection limit, μg/L	URL, μg/L	Year (Reference)
ELISA	45	Serum; plasma	0–60	0.3	6	1997 *(31)*
IFMA[a]	50	Serum; plasma	0–300	0.1	6	1997 *(73)*
EIA		Serum; plasma	0–100	1	7	1997 *(35)*
Immunosensor	20	Plasma	0–350	5	10	1997 *(42)*
Latex	10	Serum; plasma	0–150	1.1	14	1998 *(74)*
Lateral flow	15	Whole blood		6.2	6.2	2001 *(39)*
Immunosensor	50	Whole blood	0–250	4		2002 *(44)*
Lateral flow	15	Whole blood	0–125	2.8	7	2003 *(37)*

[a] IFMA, immunofluorometric assay; EIA, enzyme immunoassay.

high H-FABP concentrations is 6 μg/L. Drawbacks of these lateral-flow assays include substantial interobserver differences in interpretation of color development and the inability to differentiate between moderate and high H-FABP concentrations.

Immunosensors. Siegmann-Thoss et al. *(40)* developed a sandwich immunoassay that uses glucose oxidase–labeled detection antibodies. In the current format, this system requires sample predilution and is susceptible to plasma matrix effects. Real-time optical immunosensors have been developed *(41)*, but these require large sample volumes, have high detection limits, and are susceptible to interference from plasma lipids. As part of the EUROCARDI project, Schreiber et al. *(42)* and Key et al. *(43)* developed the first amperometric immunosensor for plasma H-FABP measurement. This rapid 20-min semiautomated analyzer gave results comparable to those obtained with the ELISA developed by Wodzig et al. *(31)*, but did not exhibit sufficient sensitivity in the low-normal concentration range of 5–15 μg/L. In 2002, O'Reagan et al. *(44)* described an H-FABP immunosensor that used whole blood and had an assay time of 50 min.

Recently, a prototype of an online immunodisplacement sensor has been developed for continuous monitoring of H-FABP *(45)*. In this immunosensor, blood samples are obtained via a microdialysis probe or via continuous ultrafiltration of venous blood.

CLINICAL INTERPRETATION OF PLASMA H-FABP CONCENTRATIONS

Under nonpathologic conditions, H-FABP is not present in plasma or interstitial fluid, and cytoplasmic concentrations of this protein are 2×10^5-fold higher than its vascular concentrations *(46)*. The plasma H-FABP concentration measured in apparently healthy individuals (<5 μg/L) is suggested to result from continuous release from damaged skeletal muscle cells.

The biological variation attributable to age, sex, and circadian rhythm significantly influences H-FABP reference values *(47)*. Probably because of their larger muscle mass, men have higher plasma H-FABP concentrations than women. Because H-FABP is eliminated from the circulation predominantly by renal clearance *(2)* and renal function decreases with age, plasma H-FABP concentrations increase during aging. In addition, H-FABP release from skeletal muscle may increase with age or exercise, as has been described for myoglobin *(48)*. A URL of 6 μg/L has been proposed independently by several groups *(47, 49)*. Selected studies indicating key clinical uses of H-FABP are summarized in Table 4.

CLINICAL APPLICATIONS OF H-FABP

Early marker of AMI. H-FABP was initially reported to be rapidly released from injured myocardium *(50)*. Because of the recent redefinition of MI *(51)*, biochemical markers have become even more important for assessment of suspected cardiac ischemia patients with non–ST-segment elevation. Because the plasma release characteristics of H-FABP after myocardial injury closely resemble those of myoglobin *(52)*, the application of H-FABP as a sensitive early marker for myocardial injury has been investigated by several groups *(53)*. In general, H-FABP was found to perform better than or similar to myoglobin *(53)*. The areas under the ROC curves for these comparisons, which used the admission blood samples from all patients, were significantly larger for H-FABP than for myoglobin, indicating better performance of H-FABP within 6 h after onset of symptoms. Furthermore, subgroup analysis of patients presenting <6 h after onset of symptoms showed better performance for H-FABP compared with myoglobin *(54)*. The observed higher sensitivity of H-FABP may be related to the higher cardiac tissue content of H-FABP compared with myoglobin. In addition, the reference values for H-FABP in plasma are far lower than those for myoglobin. Therefore, after myocardial injury, H-FABP increases to above the URL more rapidly than does myoglobin or troponin *(54–56)*. This rapid increase to above the URL can also be used to further improve the diagnostic value of the marker (i.e., rule-out power) by use of sequential plasma H-FABP measurements. When they evaluated plasma H-FABP values at admission and 1–2 h after admission, Haastrup et al. *(57)* reported an increased probability of detecting an AMI.

Okamoto et al. *(54)* measured concentrations of H-FABP, myoglobin, and CK-MB in 140 AMI patients, 49 non-AMI chest pain patients, and 75 healthy volunteers. The area under the ROC curve for H-FABP was significantly higher (0.921) than those of myoglobin (0.843) and CK-MB (0.654). In another study, H-FABP, cTnI, and creatine phosphokinase concentrations were measured in 218 patients with chest pain and suspected MI; 94 of these patients were eventually diagnosed with MI *(55)*. H-FABP showed 100% sensitivity and negative predictive value at 1 h after admission *(55)*. The areas under the ROC curves for H-FABP, creatine phosphokinase, and cTnI calculated at admission and 1 h after admission were 0.871 and 0.995, 0.711 and 0.856, and 0.677 and 0.845, respectively *(55)*. Measurement of H-FABP in serum or plasma was suggested to allow the earliest exclusion of non-AMI patients.

Seino et al. *(56)* compared the diagnostic efficacy of a newly developed whole blood rapid test for H-FABP with that of a rapid cTnT test in 129 consecutive patients with suspected cardiac ischemia, 31 of whom had a diagnosis of AMI. The respective temporal sensitivities of H-FABP and cTnT tests were 100% vs 50% at 3 h and 100% vs 100% at >12 h after onset of symptoms. The respective specificities were 63% vs 96.3% at 3 h and 75% vs 87.5% at >12 h. The negative predictive values were 100% vs 86.7% at 3 h and 100% vs 100% at >12 h. The rapid H-FABP assay was suggested to effectively exclude non-AMI patients within 3 h of onset *(56)*.

Table 4. Key clinical uses of H-FABP.

Clinical use	Study description	Findings	Conclusions/Comments	Reference
Early marker of MI	H-FABP was measured in samples from 22 AMI patients	In 18 of 22 AMI patients, H-FABP concentrations were at or above threshold in samples taken 3.5 h after first onset of symptoms	Within 0.5–3.5 h after symptom onset, H-FABP had >80% sensitivity for AMI diagnosis [In a different study, sensitivity of CK-MB, CK mass, or CK activity and troponins within 0–6 h of symptom onset was reported to be <65% *(75)*]	*(76)*
Urinary marker of AMI	Serum and urinary concentrations of H-FABP were determined in serial samples obtained from 11 AMI patients	H-FABP was significantly increased in serum and urine samples obtained 5–10 h after symptoms developed and decreased sharply afterward	H-FABP is a urinary marker of myocardial injury; H-FABP is eliminated from the circulation by the kidneys, but the exact mechanism is unknown; the only other urinary cardiac marker tested was myoglobin	*(2)*
Marker of myocardial injury after cardiac surgery	H-FABP, CK-MB, and TnT concentrations were measured in blood samples serially collected from 10 patients undergoing CABG[a]	The time to peak after aortic declamping was shorter for H-FABP [1.4 (0.5) h] than for CK-MB [2.5 (0.5) h] and TnT [6.6 (1.3) h]	H-FABP may be an early marker of myocardial injury in patients undergoing cardiac surgery	*(66)*
Detection of coronary reperfusion	H-FABP and myoglobin concentrations were measured in serum samples from 45 patients with AMI treated with intracoronary thrombolysis or direct PCTA	The predictive accuracies for H-FABP ratios >1.8 for detection of reperfusion within 15, 30, and 60 min of initiation of treatment were 93%, 98%, and 100%, respectively	H-FABP and myoglobin ratios had similar predictive accuracies for early detection of successful coronary reperfusion	*(63)*
Infarct sizing	H-FABP and myoglobin concentrations, CK-MB activity, and HBDH were assayed serially in plasma samples obtained from 20 AMI patients	In 15 AMI patients with normal renal function, agreement was good between infarct size estimated from H-FABP or myoglobin curves and that estimated with CK-MB or HBDH	Serial plasma H-FABP or myoglobin concentrations may be used for infarct sizing within the first 24 h after symptom onset only in AMI patients with normal renal function	*(77)*

[a] CABG, coronary artery bypass grafting; HBDH, hydroxybutyrate dehydrogenase.

Differentiation of cardiac and skeletal muscle injury. H-FABP is produced mainly in the heart, but to a lesser extent, it is also produced in skeletal muscle *(58)*. When patients suffered skeletal muscle injury as a result of cardioversion, multiorgan failure, postoperative states, or vigorous exercise such as running *(59)* or rowing *(60)*, H-FABP was released into the blood. The myoglobin/H-FABP ratio has been used to differentiate between heart muscle (ratio = 2–10) and skeletal muscles (ratio = 20–70), depending on the type of muscle *(58)*. In patients with AMI, the plasma myoglobin/H-FABP ratio was ~5 during the entire period of increased plasma concentrations, whereas for patients with aortic surgery (causing no-flow ischemia of the lower extremities), the plasma myoglobin/H-FABP ratio was 45 *(58)*. During defibrillation after AMI, the plasma myoglobin/H-FABP ratio increased from 8 to 60 in the 24 h after AMI as a result of injury of the intercostal pectoralis muscles *(58)*. In cases in which a second increase in plasma concentrations of marker proteins occurs, this ratio can be used to differentiate a recurrent infarction (ratio remains at 2–10) from additional skeletal muscle injury (ratio increases to 20–70).

Infarct size, reperfusion, and coronary bypass grafting. To evaluate the effect of thrombolytic therapy, the size of a myocardial infarct can be estimated by measuring the cumulative release of H-FABP. In patients treated with standard thrombolytic therapy after AMI, plasma concentrations of H-FABP and myoglobin peaked at ~4 h after first symptoms, whereas creatine kinase (creatine phosphokinase or CK-MB) and lactate dehydrogenase peaked at ~12 and 20 h, respectively *(52)*. Because H-FABP and myoglobin rapidly return to their respective URLs (within 24 h after AMI) as a result of renal clearance *(23, 52)*, both proteins can be used to assess a recurrent infarction within 10 h after first AMI *(58)*, which might be missed by CK-MB, cTnT, and cTnI because plasma concentrations of these markers return much more slowly to reference

values *(61, 62)*. If no thrombolytic therapy is administered, the H-FABP concentration in plasma peaks at 8 h and returns to within reference values after only 36 h, comparable to myoglobin *(62)*. These differences in release kinetics do not impact the measurement of cardiac proteins in plasma, however *(32, 58, 62)*. Because H-FABP is cleared by the kidneys, renal insufficiency could potentially impact its clinical utility; however, data from de Groot et al. *(32)* indicate that individually estimated clearance rates can be applied successfully for infarct size estimation.

The rapid release of H-FABP can also be used for the detection of successful coronary reperfusion in patients with AMI *(63–65)*. Both plasma H-FABP and myoglobin were found to increase sharply after successful reperfusion, but in patients with failed reperfusion, both proteins increased more slowly. The relatively low sensitivity and specificity of ~70% could be improved to 80% by normalization to infarct size.

The characteristics of rapid release and ability to differentiate between skeletal or cardiac muscle injury can be useful for early detection of postoperative myocardial tissue loss in patients undergoing coronary bypass surgery *(66)*.

Clinical assessment of congestive heart failure (CHF). Preliminary studies in patients with CHF indicate that increased plasma concentrations of H-FABP and cTnT are associated with progressive deterioration of ventricular function and a worse prognosis *(67)*. H-FABP concentrations were related not only to CHF severity (New York Heart Association classes 3 and 4) and serum cTnT concentrations *(67)*, but also to the occurrence of recurrent cardiac events *(68, 69)*. Knowledge regarding the significance of H-FABP as a marker of myocardial injury in CHF is continuing to evolve and needs further study.

Prognostic value. In the early hours of acute coronary syndrome (ACS), selection of patients who are at high risk for cardiac events is an important factor for determining the appropriate treatment strategy. The use of plasma H-FABP concentrations for early prediction of adverse clinical outcomes in patients with suspected ACS has only recently been the subject of investigation but shows promising results; increased plasma H-FABP concentrations significantly correlated with increased cardiac event rates and cardiac mortality *(70, 71)*. Pelsers et al. *(68)* showed that when plasma H-FABP was <6 μg/L, the negative predictive value for a recurrent event in CHF patients within 90 days was 81%, whereas cTnT <0.02 μg/L had a negative predictive value of 57%. This difference is most likely explained by insufficient sensitivity of the cTnT assay. Although for cTnT a cutoff value of 0.1 μg/L for indication of myocardial injury is commonly used, cutoff values of 0.05 and 0.02 μg/L are now being evaluated for more sensitive immunoassays.

H-FABP as a potential marker of cardiac ischemia

Although H-FABP is generally regarded as a marker of necrosis, one recent study has indicated its additional potential utility as a marker of ischemia *(72)*. H-FABP concentrations measured in pericardial fluid samples collected immediately after median sternotomy were significantly increased in 17 patients with unstable angina who had anginal symptoms and/or ST changes compared with 17 other patients who did not have these symptoms [mean (SD) values were 16.3 (2.0) vs 9.6 (1.0) μg/L; $P = 0.0046$]. H-FABP secretion into the interstitial space may be mediated by increased permeability of the myocardial cell membrane associated with severe ischemia.

The main advantage of H-FABP is its ability to exclude non-AMI patients very early after onset of symptoms. The fact that H-FABP may be present in the circulation in the absence of AMI makes it difficult to distinguish between patients with an AMI or unstable angina and warrants more investigation to definitively establish the diagnostic cutoff for H-FABP. In addition, only a few reports *(68, 70, 71)* have shown the prognostic value of H-FABP measurements in ACS patients. Further investigation of the prognostic value of H-FABP measurements is needed.

In combination with cardiac troponins, H-FABP may be useful to cover the complete diagnostic window of patients presenting with ACS in the emergency department, along with the electrocardiographic and clinical symptoms. Widespread availability on automated analyzers is necessary for routine applicability of H-FABP.

Conclusions

Preliminary data suggest that FFA_u concentrations have potential in identifying patients with cardiac ischemia. More work is needed, however, to clinically validate this marker and to meet quality specifications.

H-FABP is a useful biomarker for detection of cardiac injury in ACS within 6 h of symptoms onset. Limitations include a lack of complete cardiac specificity, a relatively small diagnostic window of 24–30 h after the acute event, and the probability of falsely increased values in patients with renal insufficiency. Although a relatively small number of clinical studies have been performed (12 studies involving a total of 2130 patients), all of these studies showed better or similar performance of H-FABP compared with myoglobin for the early diagnosis of AMI. H-FABP also has prognostic value to predict recurrent cardiac events in patients with ACS or CHF. The use of H-FABP in ruling out MI in patients with ACS is promising but needs further study.

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