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Citation for published version (APA):

Pelsters, M. M. A. L., Hanhoff, T., van der Voort, D., Arts, B., Peters, M., Ponds, R. W. H. M., Honig, A., Rudzinski, W., Spener, F., de Kruijk, J. R., Twijnstra, A., Hermens, W. T., Menheere, P. P. C. A., & Glatz, J. F. (2004). Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. *Clinical Chemistry*, *50*, 1568-1575. <https://doi.org/10.1373/clinchem.2003.030361>

Document status and date:

Published: 01/01/2004

DOI:

[10.1373/clinchem.2003.030361](https://doi.org/10.1373/clinchem.2003.030361)

Document Version:

Publisher's PDF, also known as Version of record

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Brain- and Heart-Type Fatty Acid-Binding Proteins in the Brain: Tissue Distribution and Clinical Utility

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Background: Detection of brain injury by serum markers is not a standard procedure in clinical practice, although several proteins, such as S100B, neuron-specific enolase (NSE), myelin basic protein, and glial fibrillary acidic protein, show promising results. We investigated the tissue distribution of brain- and heart-type fatty acid-binding proteins (B-FABP and H-FABP) in segments of the human brain and the potential of either protein to serve as plasma marker for diagnosis of brain injury.

Methods: B-FABP and H-FABP were measured immunochemically in autopsy samples of the brain (n = 6) and in serum samples from (a) patients with mild traumatic brain injury (MTBI; n = 130) and (b) depressed patients undergoing bilateral electroconvulsive therapy (ECT; n = 14). The protein markers S100B and NSE were measured for comparison. Reference values of B-FABP and H-FABP were established in healthy individuals (n = 92).

Results: The frontal, temporal, and occipital lobes, the striatum, the pons, and the cerebellum had different tissue concentrations of B-FABP and of H-FABP. B-FABP ranged from 0.8 $\mu\text{g/g}$ wet weight in striatum tissue to 3.1 $\mu\text{g/g}$ in frontal lobe. H-FABP was markedly

higher, ranging from 16.2 $\mu\text{g/g}$ wet weight in cerebellum tissue to 39.5 $\mu\text{g/g}$ in pons. No B-FABP was detected in serum from healthy donors. H-FABP serum reference value was 6 $\mu\text{g/L}$. In the MTBI study, serum B-FABP was increased in 68% and H-FABP in 70% of patients compared with S100B (increased in 45%) and NSE (increased in 51% of patients). In ECT, serum B-FABP was increased in 6% of all samples (2 of 14 patients), whereas H-FABP was above its upper reference limit (6 $\mu\text{g/L}$) in 17% of all samples (8 of 14 patients), and S100B was above its upper reference limit (0.3 $\mu\text{g/L}$) in 0.4% of all samples.

Conclusions: B-FABP and H-FABP patterns differ among brain tissues, with the highest concentrations in the frontal lobe and pons, respectively. However, in each part of the brain, the H-FABP concentration was at least 10 times higher than that of B-FABP. Patient studies indicate that B-FABP and H-FABP are more sensitive markers for minor brain injury than the currently used markers S100B and NSE.

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Rapid detection of tissue injury is very important for stratifying patients for treatment and for improved clinical outcome. Therefore, research in the field of brain-specific proteins as biochemical plasma markers for neurologic disorders or brain injury is expanding. The proteins S100B, neuron-specific enolase (NSE),⁸ myelin basic protein, and the recently introduced glial fibrillary acidic protein are currently being evaluated as protein

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Received December 10, 2003; accepted June 1, 2004.

Previously published online at DOI: 10.1373/clinchem.2003.030361

⁸ Nonstandard abbreviations: NSE, neuron-specific enolase; B-FABP and H-FABP, brain- and heart-type fatty acid-binding protein, respectively; MTBI, mild traumatic brain injury; ECT, electroconvulsive therapy; and PBS, phosphate-buffered saline.

markers in cerebrospinal fluid and/or blood for detection of brain injury in neurologic patients (1–3) and patients with cerebrovascular accidents (4), traumatic brain injury (5), stroke (6), global cerebral ischemia attributable to cardiac arrest or cardiopulmonary bypass surgery (7, 8), tumor cerebri (9), or dementia (10), with the aim to eventually locate the site of injury (neuron, glia, or myelin). Although S100B is the most promising marker, conflicting results for increased serum S100B concentrations after cardiac injury (11, 12) indicate that the specificity of this protein is limited.

Similar to the use of heart-type fatty acid-binding protein (H-FABP) as a plasma marker for the rapid detection of cardiac injury (13, 14), brain-type FABP (B-FABP) and H-FABP (15) may be suitable markers for the detection of brain injury. B-FABP and H-FABP are members of a family of nine distinct FABP types, each named after the tissue in which it was first detected (16). FABPs are 15-kDa cytoplasmic, nonenzymatic proteins involved in the intracellular buffering and transport of long-chain fatty acids. FABPs are released rapidly from damaged cells into the circulation and are cleared from the circulation by the kidney with a plasma half-life of 20 min (14). B-FABP was first identified in the brains of rodents and showed diverse tissue production during development (15, 17). In adult-stage mice, B-FABP is produced in very low concentrations and is detected only in glial cells (presumptive astrocytes) of the white matter (17–19). Unlike B-FABP, H-FABP is detected in the neurons of the gray matter (neuronal cell bodies) in mice and rats and constitutes 0.01% of total brain cytosolic protein (15, 20).

The aim of our study was to investigate the tissue distribution and concentrations of B-FABP and H-FABP in human brain and to study the potential of these proteins as serum markers for the detection of brain injury in patients. Two groups of patients were studied: (a) patients with mild traumatic brain injury (MTBI); and (b) a controlled study population of depressed patients undergoing bilateral electroconvulsive therapy (ECT). To establish an upper reference limit for B-FABP, we measured plasma concentrations in healthy individuals of various ages and genders.

Materials and Methods

HUMAN BRAIN TISSUE

Human brain tissue samples were obtained during autopsy of six individuals [five females and one male; age range, 51–71 years; Medical Academy (Bialystok, Poland), and Mental Hospital (Choroszcz, Poland)]; divided into frontal lobe, temporal lobe, occipital lobe, striatum, pons, and cerebellum; and directly frozen in liquid nitrogen. Subsequent sample preparations were performed at 4 °C. The study was approved by the local Medical Ethical Committee, and samples were obtained after informed consent of the relatives.

Before analyses, tissues were homogenized (50–160 g/L) in a sucrose buffer (0.25 mol/L sucrose, 2 mmol/L

EDTA, 10 mmol/L Tris, pH 7.4) by use of an Ultra-Turrax homogenizer (IKA Werke); tissues were homogenized by three 15-s bursts at 24 000 rpm, with 15-s intervals of cooling on ice. After sonication (three 15-s bursts in a MSE ultrasonic disintegrator, with 15-s intervals of cooling on ice), samples were centrifuged (10 min at 1000g), and the supernatant containing cytoplasmic proteins was stored at –80 °C until analyses.

PLASMA AND SERUM SAMPLES

Healthy controls. To determine the biological variation for circulating B-FABP and to establish a reference interval, we obtained plasma samples (EDTA) from 80 healthy individuals (40 males and 40 females; age ranges, 21–30, 31–40, 41–50, 51–60, and 61–70 years; n = 8 for each group) visiting the blood bank of Liège to study the influence of age and gender, and from another 12 healthy individuals (citrate plasma; 6 males and 6 females; age range, 19–27 years) from the Maastricht University student population to study the influence of circadian rhythm (21). Previously, we had observed no differences between plasma or serum (21).

Patients with MTBI. Patients (n = 130) were included in this study when all of the following criteria were met: (a) a blunt blow to the head leading to posttraumatic amnesia of <1 h; (b) initial loss of consciousness of <15 min; (c) Glasgow Coma Scale score >13 on presentation at the Emergency Department; and (d) absence of focal neurologic signs.

Approval from the local Medical Ethical Committee review board was obtained before the study. We examined all patients presenting with MTBI at the Emergency Department of the University Hospital Maastricht over a period of 2 years. Duration of posttraumatic amnesia and loss of consciousness were estimated from information obtained from patients and witnesses. If patients arrived at the Emergency Department within 6 h after the trauma and met the criteria for MTBI listed above, informed consent was obtained and blood samples for biochemical marker measurements were taken. Causes of trauma included traffic accidents, accidents in and around the house, accidents at work, sports-related accidents, and battering. Patients with alcohol abuse were excluded.

After clotting, samples were centrifuged at 4000g for 20 min at 4 °C, and after separation from the clot, serum was stored at –20 °C.

Patients undergoing ECT. Serum samples (n = 234) were obtained after informed consent (University Hospital Maastricht) from 14 depressed patients undergoing bilateral ECT (on average, 10 successive treatment sessions two times a week). Blood samples were taken at different time points (before ECT and 1 h and 3 h after ECT) during these sessions so that a total of 9–28 samples were collected from each patient.

After clotting, samples were centrifuged, and serum was stored at -20°C .

DEVELOPMENT OF IMMUNOASSAY FOR B-FABP

Production of recombinant B-FABP and antibodies against B-FABP. Human B-FABP was produced and purified as described previously (22). For antibody production, rabbits (New Zealand white) were immunized with $300\ \mu\text{g}$ of purified recombinant B-FABP and boosted every 4 weeks with $200\ \mu\text{g}$ of B-FABP. Polyclonal antibodies were purified by affinity chromatography using CH-activated Sepharose 4B (Pharmacia) to which purified B-FABP had been coupled. Specifically bound antibodies were eluted with $10\ \text{mmol/L}$ sodium citrate– $20\ \text{mmol/L}$ sodium phosphate, pH 2.8, and immediately neutralized with $0.5\ \text{mol/L}$ phosphate buffer, pH 7.4. The antibodies then were dialyzed against phosphate-buffered saline (PBS) containing $0.2\ \text{g/L}$ sodium azide and stored at 4°C .

Specific detection by Western blotting. To test the specificity and cross-reactivity of the antibodies used in the ELISA, we subjected samples containing B- and H-FABP (both $1\ \mu\text{g}$ and $10\ \mu\text{g}$ in each case) to 13.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, blotted the gels on a HyBond nitrocellulose membrane (Amersham Biosciences), and detected the FABPs with purified polyclonal antibodies against B-FABP and with monoclonal antibodies against H-FABP (67D3 and 66E2 as capture and detector antibodies, respectively, in the H-FABP ELISA; HyCult Biotechnology) (23, 24). Secondary antibodies, conjugated to horseradish peroxidase, were used in a 1:10 000 dilution. For visualization, ECL Western Blotting Detection Reagents (Amersham Biosciences) were used.

Immunoassay development. Purified polyclonal antibodies were used as either the capture (primary) or detector antibody (secondary). The latter was biotinylated by use of D-biotinoyl-6-amidohexanoic acid *N*-hydroxysuccinimide ester (Roche) according to the manufacturer's instructions. An ELISA of the antibody capture type (sandwich ELISA) was developed and performed in MaxiSorb microtiter plates (Nunc). All incubations were done at 37°C for 1 h with a total volume of $100\ \mu\text{L/well}$. Primary antibody ($2.5\ \text{mg/L}$) in $0.1\ \text{mol/L}$ sodium carbonate (pH 9.6) was used to first coat the wells. After washing with PBS (pH 7.4), the wells were blocked with $5\ \text{g/L}$ bovine serum albumin in PBS. After the wells were washed with PBS, the B-FABP calibrator and two- to fivefold-diluted samples in sample buffer (PBS containing $5\ \text{g/L}$ bovine serum albumin and $0.5\ \text{mL/L}$ Tween 20) were added. After incubation with the secondary biotinylated antibody ($2.5\ \text{mg/L}$) and with streptavidin–peroxidase diluted 1:10 000 in sample buffer, *o*-phenylenediamine ($1\ \text{g/L}$ in $50\ \text{mmol/L}$ sodium citrate, pH 5.0, supplemented with $1.5\ \text{mL/L}$ H_2O_2) was added as the peroxidase substrate (5–10 min incubation at room temperature). The

reaction was stopped by addition of $1\ \text{mol/L}$ H_2SO_4 , and the absorbance at $492\ \text{nm}$ was measured on a Microplate Reader MPP 4008 (Mikrotek Laborsysteme) with $405\ \text{nm}$ as reference. In addition, specificity testing was performed with epidermis-type FABP, liver-type FABP, and intestine-type FABP.

IMMUNOASSAYS FOR H-FABP, MYOGLOBIN, S100B, AND NSE

Tissue and plasma concentrations of H-FABP were measured with a sandwich-type ELISA obtained from HyCult Biotechnology (HK 403) exactly as described previously (21, 23). The intra- and interassay CVs were $<12\%$. In healthy individuals, the median plasma H-FABP concentration is $1.5\ \mu\text{g/L}$, whereas $6\ \mu\text{g/L}$ is considered the clinical cutoff value (21).

Myoglobin was also measured because the ratio of the plasma concentration of myoglobin to that of H-FABP is indicative of skeletal muscle injury (25, 26). Myoglobin was measured with an electrochemiluminescence immunoassay (Elecsys[®]; Roche Diagnostics). The intra- and interassay CVs were $<10\%$. The median plasma myoglobin concentration in healthy individuals is $27\ \mu\text{g/L}$, whereas $60\ \mu\text{g/L}$ is considered the clinical cutoff value (21).

The S100B concentration was measured by a commercially available immunoluminometric assay (Lia-mat Sangtec 100; Sangtec Medical). According to the manufacturer's instructions, this assay differentiates between the A1- and B-subunits of S100 protein and measures the B-subunit as detected by three monoclonal antibodies: SMST 12, SMSK 25, and SMSK 28. The B-subunit of the S100 protein is known to be the predominant form (80–96%) in human brain (27, 28). Each measurement was performed in duplicate according to the manufacturer's recommendations. As indicated by the manufacturer, the limit of detection of the assay ($B_0 + 3\ \text{SD}$) was $0.02\ \mu\text{g/L}$, and the within- and between-assay imprecisions (CV) were 5.5% and 10%, respectively, for concentrations of 0.28 – $4.17\ \mu\text{g/L}$; $0.3\ \mu\text{g/L}$ was considered the clinical cutoff value (29).

NSE was measured by a commercially available RIA (Pharmacia NSE RIA; Pharmacia & Upjohn) according to the manufacturer's instructions. The intra- and interassay CVs were $<7.5\%$ at $6\ \mu\text{g/L}$, $<4.7\%$ at $19\ \mu\text{g/L}$, and $<8.4\%$ at $90\ \mu\text{g/L}$; $10\ \mu\text{g/L}$ was considered the clinical cutoff value (30).

STATISTICS

The Wilcoxon signed-ranks test for unpaired values was used to evaluate significant differences among the tissue sections of the brain. The Student *t*-test was used to evaluate significant differences between marker concentrations in MTBI and ECT. $P < 0.05$ was regarded as significantly different. All data are reported as the mean (SD).

Results

B-FABP ELISA

Western blot analysis showed that the monoclonal anti-H-FABP antibody 67D3, used as capture antibody in the ELISA, cross-reacts slightly with B-FABP (Fig. 1A). However, no cross-reactivity with B-FABP was seen with the monoclonal anti-H-FABP antibody 66E2 used as detector in the ELISA (Fig. 1B). In both blots, recombinant H-FABP was added as positive control (Fig. 1, A and B, right-hand lanes). B-FABP (1000 $\mu\text{g/L}$) was also tested in the H-FABP ELISA and was not detectable. The polyclonal anti-B-FABP antibodies showed no cross-reactivity with human H-FABP (Fig. 1C). In this blot, recombinant B-FABP was added as positive control (Fig. 1C, left-hand lanes). H-FABP (1000 $\mu\text{g/L}$) was also tested in the B-FABP ELISA and was not detectable. In both ELISAs, no cross-reactivity was detectable with liver-, intestine-, or epidermis-type FABP. Therefore, the sandwich-type ELISAs specifically detected either B-FABP or H-FABP. The B-FABP ELISA had a detection limit of 5.0 $\mu\text{g/L}$ (blank + 2 SD; $n = 10$), and the calibration curve was linear between 10 and 100 $\mu\text{g/L}$. For the 20 and 100 $\mu\text{g/L}$ calibrators, the intra- and interassay CVs, calculated from 10 repetitive measurements, were <6% and <15%, respectively.

B-FABP AND H-FABP CONCENTRATIONS IN BRAIN TISSUE

In each part of the human brain studied, the tissue concentration of H-FABP was found to be >10-fold higher than that of B-FABP (Fig. 2). The H-FABP/B-FABP ratio was significantly different ($P < 0.05$) only for striatum compared with the frontal lobe and cerebellum (Fig. 2).

PLASMA B-FABP AND H-FABP REFERENCE CONCENTRATIONS

Plasma B-FABP concentrations in healthy individuals were <5 $\mu\text{g/L}$, the detection limit of the assay. Therefore, the influence of age, gender, and circadian rhythm could not be assessed. The mean (SD) H-FABP concentration in plasma of healthy individuals was 1.5 (2.2) $\mu\text{g/L}$, as described previously (21).

RESULTS FOR MTBI PATIENTS

B-FABP serum concentrations were increased (>5 $\mu\text{g/L}$) in 88 of 130 patients with MTBI, whereas H-FABP (>6 $\mu\text{g/L}$) was increased in 91 patients (Table 1). Serum S100-B protein concentrations were increased (>0.3 $\mu\text{g/L}$) in 59 patients, and serum NSE was increased (>10 $\mu\text{g/L}$) in 66 patients. The median (2.5–97.5 percentiles) H-FABP/B-FABP ratio in serum was 0.58 (0.1–8.4). B-FABP and H-FABP were both increased in 45% of all serum samples, whereas 23% of serum samples had only increased H-FABP and 16% had only increased B-FABP. Both proteins were negative in the remaining 16% of serum samples.

RESULTS FOR ECT PATIENTS

In all but 1 of 234 serum samples from patients undergoing ECT, S100B concentrations were below the reference value of 0.3 $\mu\text{g/L}$ (a single sample had an increased concentration of 0.35 $\mu\text{g/L}$; Table 2). Serum B-FABP was detectable (>5 $\mu\text{g/L}$) in only 15 samples, of which 13 were from a single patient. Serum H-FABP was above the reference value of 6 $\mu\text{g/L}$ in 39 samples (8 of 14 patients), and B-FABP was also increased in 8 samples.

In the 39 serum samples with increased H-FABP, mean

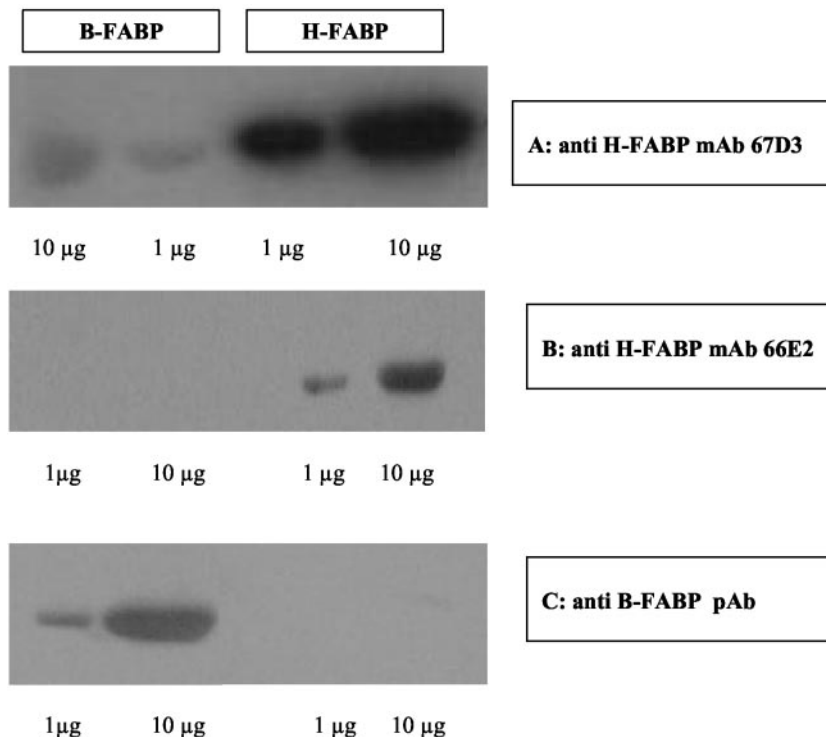


Fig. 1. Western blot of monoclonal anti-H-FABP antibody 67D3 (A), monoclonal anti-H-FABP 66E2 antibody (B), and polyclonal anti-B-FABP antibody with recombinant B-FABP and H-FABP (C).

mAb, monoclonal antibody; pAb, polyclonal antibody.

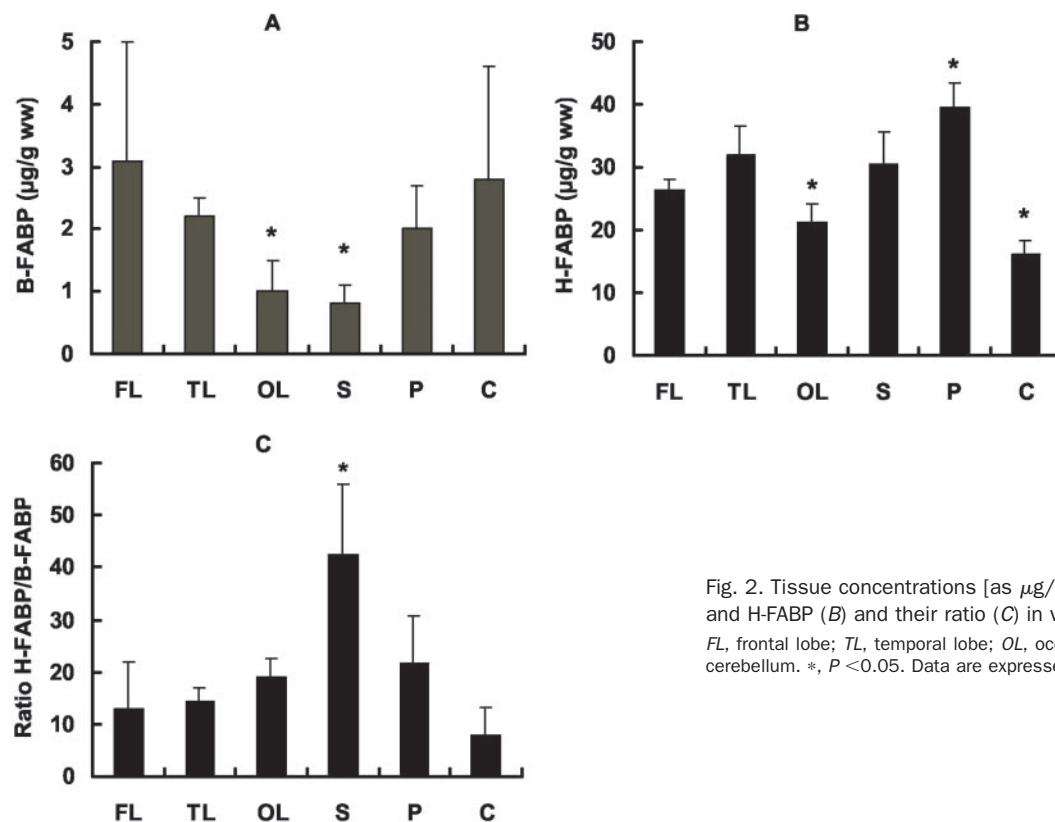


Fig. 2. Tissue concentrations [as $\mu\text{g/g}$ wet weight (ww)] of B-FABP (A) and H-FABP (B) and their ratio (C) in various brain tissues.

FL, frontal lobe; TL, temporal lobe; OL, occipital lobe; S, striatum; P, pons; C, cerebellum. *, $P < 0.05$. Data are expressed as the mean and SD (error bars).

(SD) plasma myoglobin was 63 (32) $\mu\text{g/L}$, and 15 of these samples were above the cutoff (>60 $\mu\text{g/L}$). The mean (SD) myoglobin/H-FABP ratio in these samples was 7.7 (2.9), which is markedly lower than that found in skeletal muscle tissue (ratio, 20 – 70) (25), indicating the contribution of H-FABP released from tissues other than skeletal muscle, i.e., the brain.

Pre- and post-ECT H-FABP values were assessed in patients with increased serum H-FABP ($n = 8$) and showed significantly ($P < 0.05$) increased concentrations after treatment, ranging from 5.1 (1.9) $\mu\text{g/L}$ before ECT to 8.0 (3.5) $\mu\text{g/L}$ and 6.9 (3.9) $\mu\text{g/L}$ 1 and 3 h, respectively, after ECT.

Table 1. Concentrations of different marker proteins in serum samples from patients ($n = 130$) with MTBI.

Marker protein	CCV, ^a $\mu\text{g/L}$	Serum samples above CCV		Range above CCV, $\mu\text{g/L}$
		n	%	
B-FABP	5	88	68	5.2–127.8
H-FABP	6 (21)	91	70	6.5–181.8
S100B	0.3 (29)	59	45	0.31–10.5
NSE	10 (30)	66	51	10.1–100.0

^a CCV, clinical cutoff value. Clinical cutoff value of B-FABP was calculated as mean value for blank + 2 SD ($n = 10$), that of H-FABP as mean value for healthy individuals + 2 SD, and those of S100B and NSE were obtained from De Kruijk et al. (29) and Skogseid et al. (30), respectively.

Discussion

This study documents the tissue concentrations of B-FABP and H-FABP in various parts of the adult human brain and shows that, in selected patients with minor brain injury, one or both proteins are present in blood serum in increased concentrations. These findings indicate the possible use of B-FABP and H-FABP as biochemical markers of brain injury.

Although several studies have described the occurrence of B-FABP and H-FABP in specific cells in the brain (31, 32), because of a lack of quantitative data the total tissue concentrations of these proteins were unknown, as was their possible clinical utility. We therefore developed a B-FABP ELISA that measures the concentrations of both B-FABP and H-FABP in the brain and investigated whether brain injury in MTBI and in ECT can be detected

Table 2. Concentrations of different marker proteins in serum samples ($n = 234$) from patients ($n = 14$) undergoing ECT.

Marker protein	CCV, ^a $\mu\text{g/L}$	Serum samples above CCV		Range above CCV, $\mu\text{g/L}$
		n	%	
B-FABP	5	15	6.4	7.2–50.5
H-FABP	6	39	17.0	6.2–20.3
S100B	0.3	1	0.4	0.35

^a CCV, clinical cutoff value.

by the release of these proteins into the blood serum. The tissue concentration of B-FABP was highest in the frontal lobe, whereas H-FABP was highest in the pons. Because it has been shown that the production of both FABPs alters during development in rodents (19, 20), it is likely that in humans the concentrations in tissue, as well as their ratio, may alter from postnatal to adult development. This aspect needs further investigation. An important finding was that the H-FABP/B-FABP ratio differs among the various parts of the human brain studied, indicating that this ratio in plasma or serum may be used to locate the site of brain injury. In the MTBI study, the H-FABP/B-FABP ratio was assessed in serum and found to be 0.58, indicating a relatively higher release of B-FABP compared with H-FABP. However, no data (e.g., positron emission tomography scan or magnetic resonance imaging) were available to correlate the localization of brain injury with the serum values. In addition, as stated above, potential drawbacks to use of the H-FABP/B-FABP ratio are the developmental and age-related changes in tissue production of both proteins. Future studies need to address these issues to establish the potential utility of the serum H-FABP/B-FABP ratio.

Although Guillaume et al. (33) recently proposed the possible use of H-FABP as a plasma marker for detection of Creutzfeldt–Jakob disease, no quantitative data for B-FABP and H-FABP as markers for brain injury have been published. To properly investigate the clinical utility of B-FABP and H-FABP as potential markers for brain injury, we attempted to establish a serum reference interval, using plasma samples from a small reference population of 80 healthy individuals for age and gender variation and from another 12 healthy individuals for circadian rhythm variation. Unfortunately, all samples had a B-FABP concentration below the detection limit of the assay ($<5 \mu\text{g/L}$), so that possible effects on plasma B-FABP could not be documented. The plasma H-FABP value for healthy individuals has been studied before and was found to increase during aging, was significantly higher in men than in women, and exhibited a circadian rhythm, with values highest during the night concomitant with a decrease in the glomerular filtration rate (21).

Because traumatic brain injury is a major cause of morbidity and mortality, we investigated 130 MTBI patients. Although current knowledge about the pathophysiology of MTBI is limited, traumatically induced axonal damage is thought to be the pathophysiologic mechanism in MTBI (34), as demonstrated by increased concentrations of S100B and NSE (30, 35). In our MTBI group, both B-FABP and H-FABP were increased in significantly ($P < 0.05$) more cases (68% and 70%, respectively) than were S100B (45%) and NSE (51%), suggesting a difference in sensitivity (Table 1). However, we found no significant correlations among serum concentrations of each of the biomarkers: only 45% of the samples had increases in both B-FABP and H-FABP, suggesting either different release kinetics or injury in different areas of the brain. The latter

seems more likely because the release kinetics are not expected to differ among types of FABP (14, 36). The FABPs, as well as myoglobin and S100B, are cytosolic proteins and, therefore, are released simultaneously from injured cells. In addition, the release of cerebrovascular proteins into blood plasma is dependent on disruption of the blood–brain barrier [reviewed recently by Marchi et al. (37)]. Because these proteins are of similar size (FABP, 15 kDa; myoglobin, 17 kDa; S100B, 22 kDa), they will not differentially pass through the blood–brain barrier. The similarity in the sizes of these molecules also implies that the elimination of these proteins from plasma occurs by renal clearance and at equal rates. B- and H-FABP (36) and S100B (38) have a plasma half-life of 20–25 min, indicating that the so-called diagnostic time window is limited but similar for these FABPs and S100B. The use of B- and H-FABP as biomarkers for early identification and treatment stratification of MTBI patients presenting with headache, dizziness, and nausea in the emergency room may improve patient care and outcome. It is known that in patients with acute ischemic injury, rapid initiation of treatment will decrease the amount of neuronal cell death (37).

ECT is an effective method for treatment of mental illness. Although this method is still subject to debate, several studies have reported that no structural brain damage occurs during such therapy (39). Thus, ECT, especially bilateral ECT, appears to be an effective short-term treatment for severe depression (40). Nevertheless, in our study we found 2 patients (of 14) with increased B-FABP during therapy, indicating minor brain injury. Clinical data indicated no other reasons for brain injury in these patients. In addition, H-FABP was randomly increased in 39 samples (8 of 14 patients; Table 2). Because one of the side effects of ECT is muscle stiffness and because H-FABP can also be released from cardiac or skeletal muscle (14), we measured myoglobin/H-FABP ratio in these samples because the plasma myoglobin/H-FABP ratio is indicative of the tissue that is injured (ratio, 20–70 for skeletal muscle, and 3–5 for cardiac muscle) (25, 26). In two of these samples the myoglobin/H-FABP ratios were 47 and 36, reflecting a dominant contribution from skeletal muscle injury in these cases. However, the mean (SD) ratio was 7.7 (2.9), which suggests, assuming no cardiac muscle injury, that release of H-FABP from the brain was more important than its release from skeletal muscle. Taken together, these findings indicate that ECT not only causes some skeletal muscle injury, but in selected cases may also cause minor brain injury, as evidenced from the release of H-FABP. Because S100B was increased in only 1 of 234 serum samples, the ECT study also shows that H-FABP is a more sensitive marker for minor brain injury than the currently used S100B.

In the present study, we have shown in two distinct patient groups that B-FABP and H-FABP may be useful markers for minor brain injury because they display a higher sensitivity than S100B. Correlations between injury

and function will depend on the localization of the injury. Future studies could attempt to confirm these observations. Now that the concentrations of FABP in brain tissues are known, and assuming that the release of FABPs into the circulation is the result of release from damaged brain cells rather than changes in permeability of the blood–brain barrier (37), the extent of brain injury may be estimated from the cumulative release of FABP into plasma, provided that (a) frequent blood samples are taken, (b) an exact location of the infarct is confirmed by magnetic resonance imaging, and (c) the changes in tissue production of the FABPs during aging are known.

Although B-FABP and H-FABP appear to be rapidly released into the circulation, early detection of acute brain injury such as in stroke would allow the early initiation of therapy only when rapid detection systems are available. For H-FABP, rapid assays are already available, such as a microparticle-enhanced immunoassay (41), an amperometric enzyme immunosensor (42), and lateral-flow assays (43). In addition, new developments in online measurement of FABP (44) could enable rapid detection of brain injury and/or changes in permeability of the blood–brain barrier attributable to reduced blood flow, for example, during cardiac by-pass surgery (7). However, renal impairment, complement activation, and lowered body temperature can influence the plasma protein pattern and will have to be taken into account.

Now that thrombolytic therapy after acute ischemic stroke is being used as an important new step to reduce the severity of brain injury (45), rapid detection of brain injury and monitoring of the effects of thrombolytic treatment are likely to receive increased attention in the future.

We thank Prof. J. Górski, Department of Physiology, Medical Academy (Białystok, Poland), for stimulating discussions, and Prof. W. Buurman and G. Francot, Hy-Cult Biotechnology (Uden, The Netherlands), for providing H-FABP immunoassays. This study was supported in part by the Dutch Technology Foundation (Grant GGN4680). Jan F.C. Glatz is a Netherlands Heart Foundation Professor of Cardiac Metabolism.

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