Fatty acid metabolism in human breast cancer cells (MCF7) transfected with heart-type fatty acid binding protein*

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Abstract

The human breast cancer cell line MCF7 does not express heart-type fatty acid binding protein (H-FABP), a marker protein for differentiated mammary gland. MCF7 cells transfected with the bovine H-FABP cDNA expressed the corresponding protein and were characterized by growth inhibition and lower tumorgenicity in nude mice [22]. By enzyme linked immunoassay we now determined the amount of bovine H-FABP in these cells as 638 ± 80 ng/mg protein and used the transfected cells to study the role of H-FABP in fatty acid metabolism. Compared to control cells the uptake of radioactively labelled palmitic acid and oleic acid into MCF7 cells after 30 or 60 min was increased by 67% in H-FABP expressing transfectants, demonstrating a stimulatory role for this FABP-type in fatty acid metabolism. However, preferential targeting of [^{14}C]oleic acid into neutral or phospholipid classes was not observed by the criterion of high performance thin layer chromatography followed by autoradiography. A reason for the modest increase of fatty acid uptake in H-FABP transfected MCF7 cells may be the basal expression of epidermal-type FABP, which was detected for the first time in these cells. It appears that the small amount of E-FABP expressed in MCF7 cells fulfils the need of the cells for a cytosolic fatty acid carrier under culture conditions and that even high concentrations of another FABP do only slightly increase the uptake due to limitations of fatty acid transport through the plasma membrane or of metabolism. (Mol Cell Biochem **199**: 41–48, 1999)

Key words: fatty acid binding protein, mammary derived growth inhibitor, MCF7 cells, fatty acid uptake, transfected cells, lipid analysis

Introduction

Heart-type fatty acid binding protein (H-FABP) belongs to a family of intracellular hydrophobic ligand binding proteins, comprising 8 different types of fatty acid binding proteins (FABPs), the ileal lipid binding protein and 4 cellular retinoid binding proteins [1–3]. The *in vitro* binding of fatty acids by FABPs and their predominant expression in tissues with active lipid metabolism has led to the proposal that these proteins function in intracellular lipid transport, storage and metabolism [1–4]. Nevertheless, only limited direct evidence has been provided so far for such a role. Cell culture systems are particularly suited to study the effect of FABPs on fatty acid metabolism. Employing photoaffinity labelling and photobleaching experiments a direct interaction of intracellular FABPs with fatty acids and an increased diffusion of

fatty acids in the cell has been demonstrated [5, 6]. In some cell culture systems transfection with intestinal (I-), liver (L-) or adipocyte-type (A-)FABP stimulated uptake and esterification of fatty acids [7–11]. The abundant expression of H-FABP in differentiated mammary gland [12, 13] with the onset of milk lipid production [14] is in line with a role for this FABP-type in fatty acid transport and metabolism.

Interestingly, the mammary derived growth inhibitor (MDGI) originally identified by Böhmer *et al.* in lactating mammary gland [15] has recently been shown to be identical to H-FABP [16]. This reflects a second set of functions related to growth regulation [17, 18] and differentiation, the latter now attributed to H-FABP [17, 19], A-FABP [20], and to I-FABP [21]. In accordance with the growth inhibitory activity of H-FABP, transfection of the human breast cancer cell line MCF7 with the bovine H-FABP cDNA revealed also tumor suppressor activity in the nude mice model [22]. MCF7 cells, although equipped with several proteins characteristic for mammary epithelial cells, do not express H-FABP [22], in contrast to highly differentiated mammary epithelial cells [23]. Further studies demonstrated that hypermethylation of sites upstream of the first exon and within the first intron are responsible for the silencing of the H-FABP gene in human breast cancer cell lines and primary breast tumors [24].

In this study we took advantage of the already established MCF7 cell line transfected with bovine H-FABP cDNA [22] to ask the question, whether the ectopic expression of bovine H-FABP in MCF7 cells is correlated with an increased uptake of palmitic acid and oleic acid. It is known that at the time points studied by us (30 or 60 min) uptake of these fatty acids is strongly linked to metabolism [7, 25], whereas the 'initial fatty acid uptake' (up to 1 min) is generally studied when addressing the transport of fatty acids through the plasma membrane, e.g. in experiments revealing the role of membrane bound fatty acid transporters. To distinguish between an unspecific stimulation of fatty acid metabolism and specific targeting to certain metabolizing enzymes we studied the incorporation of [^{14}C]oleic acid into different lipid classes. Our results led us to search for the presence of other FABP types in these MCF7 cells.

Materials and methods

Materials

Dulbecco´s modified Eagle´s medium (DMEM) with 4.5 g/l glucose, L-glutamine and gentamycin were obtained from Biochrom (Berlin, Germany). Bovine insulin and fetal calf serum were from Boehringer Mannheim (Mannheim, Germany), geniticin from Gibco (Eggenstein, Germany) and plastic ware for cell culture from Nunc (Wiesbaden, Germany). The substrates for Western blot detection 4-nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) were from Roth (Karlsruhe, Germany) and Silica gel 60 plates for high performance thin layer chromatography (HPTLC) from Merck (Darmstadt, Germany). Microconcentrators Microcon-3 were obtained from Amicon (Witten, Germany), nitro-cellulose membrane from Schleicher and Schuell (Dassel, Germany) and [1-^{14}C]oleic acid (53 Ci/mol) and [9,10-^{3}H]palmitic acid (50 Ci/mmol) from Amersham (Braunschweig, Germany). CH-activated sepharose 4B for affinity purification of antibodies was from Pharmacia (Freiburg, Germany). Bovine serum albumin (BSA, fraction V, essentially fatty acid free), Tween 20, anti-rabbit IgG alkaline phosphatase conjugate and all other chemicals were supplied by Sigma (Deisenhofen, Germany).

Cell culture and tissue preparations

Human breast cancer cells (MCF7) were maintained in DMEM with 4.5 g/l glucose containing 10% fetal calf serum, 4 mM L-glutamine, 5 µg/ml bovine insulin at 10% CO_2. The generation of MCF7 cells stably transfected with the bovine H-FABP cDNA (clone 44) and of mock transfectants (clone 79) was described elsewhere [22]. These cells were cultured under selective conditions by supplementation with 0.8 g/l geniticin. Cell growth was measured by trypsination of cells and counting in a hemocytometer. Sections from bovine mammary gland and heart tissue, which had been freshly obtained from a local slaughterhouse, were washed in phosphate buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, pH 7.4), cut in small pieces and homogenised with a Potter Elvehjem homogeniser. Samples were centrifuged at 100,000 × g for 1 h at 4°C and stored at –70°C until analysis.

Antibody preparation and ELISA

Antibodies were generated in New Zealand White rabbits by injection of 500 µg of recombinant bovine H-FABP [26], human H-FABP [27], human A-FABP and human epidermal-type (E-)FABP (Hohoff, C, Börchers, T and Spener, F, unpublished results), respectively, followed by several booster injections. The antibodies against H- and E-FABP were affinity purified using Sepharose matrices to which the corresponding recombinant FABPs had been covalently linked according to instructions of the manufacturer. Since the A-FABP antiserum exhibited a high crossreactivity towards human E-FABP it was freed from crossreacting antibodies by passage over an affinity column with immobilized human E-FABP. For quantification of bovine H-

FABP, human H-FABP or human E-FABP enzyme linked immunosorbent assays (ELISA) of the sandwich type with biotinylated FABP antibodies as detector antibodies (2 µg/ml) and a streptavidin-peroxidase conjugate (1:5,000) were employed as described earlier [19, 28]. The linear range of the ELISAs was typically between 0.2 and 3.0 ng FABP per ml.

Western blotting

The supernatants of the cell lysates were concentrated using microconcentrators and protein concentrations were determined by the bicinchoninic acid assay [29]. Cell lysates were separated by sodiumdodecyl sulfate polyacrylamide gelelectrophoresis (SDS-PAGE, 15% C, 2.7% T) [30] together with different amounts of the respective recombinant FABP. Proteins were transferred (1 h, 1.5 mA/cm^2) to a nitrocellulose membrane in 25 mM Tris/HCl, 192 mM glycine (pH 8.3) containing 20% methanol. The membrane was blocked (30 min) with TBS (20 mM Tris/HCl, 154 mM NaCl, pH 7.4) containing 3% (w/v) BSA, and incubated first with antibody (1 h, 2.5 µg/ml) in TBS, 0.05% (w/v) Tween 20 (TBST) containing 1.5% (w/v) BSA and then with anti-rabbit IgG alkaline phosphatase conjugate (1 h, 1:10,000) in the same buffer. Washes between these steps (2 × 3 min) were done with TBST. After equilibration (3 min) in 0.1 M Tris/HCl, 0.1 M NaCl, 5 mM $MgCl_2$, pH 9.5 bands were visualised by incubation with a solution of NBT (0.33 µg/ml) and BCIP (0.165 µg/ml) in this buffer.

To distinguish closely related FABP types immunochemically, the crossreactivities of the antibodies with the respective FABP types were addressed. Whereas anti E-FABP did not recognize H-FABP (not shown) a pronounced crossreactivity towards both, H-FABP and E-FABP, was found for the antiserum directed against human A-FABP (not shown). After passing over a column with human E-FABP the serum still sensitively recognized human A-FABP, whereas no signal was observed with human E-FABP (Fig. 4B).

Fatty acid uptake

For uptake studies MCF7 cells were cultured in 25 cm^2 plastic dishes until near confluence was reached. Cell monolayers were washed twice with DMEM (37°C) and incubated at 37°C with 100 µM fatty acids, bound to 33 µM BSA in DMEM containing 12.5 mg/l gentamycin. Prior to incubation [1-^{14}C]oleic acid and [9,10-^{3}H]palmitic acid, respectively, were diluted with cold fatty acids to give final specific activities of 1–1.5 Ci/mol. Incubation times were 30 or 60 min, both in the linear range of the fatty acid uptake into MCF7 cells (data not shown). For termination of fatty acid uptake the incubation medium was aspirated and cells were washed 3 times with ice-cold PBS containing 0.2 mM phloretin as a nonselective inhibitor of membrane transport [25]. After the third washing step no residual radioactive fatty acid could be detected in the wash solution. Cells were scraped off and lysed in distilled water by passing them 10 times through a syringe needle. The lysate was subjected to scintillation counting and protein determination [29].

Lipid extraction and analysis

Total lipids from cell lysate samples after fatty acid incubation were extracted according to Bligh and Dyer [31], concentrated under N_2 and dissolved in chloroform. Different lipid classes were separated by high performance thin-layer chromatography (HPTLC) as described elsewhere [32]. Briefly, HPTLC Silica gel 60 plates were first developed with chloroform-methanol-acetic acid-formic acid-water 35:15:6:2:1 (v/v), followed by hexane-diisopropyl ether-acetic acid 65:35:2 (v/v). Following chromatography lipids were sprayed with 3% cupric acetate and 8% phosphoric acid and then charred at 120°C for 15 min [33]. Incorporation of radioactive label was analysed by densitometry of X-ray films (Hyperfilm ECL, Amersham) after 7–9 days exposure to the HPTLC plates at –70°C.

Statistical analysis

Data are given as mean ± S.E.M. if not indicated otherwise. Statistical analysis was carried out using the standard unpaired Student´s *t*-test. $P < 0.05$ was indicative of a significant statistical difference between groups.

Results

Characterization of MCF7 cells transfected with bovine H-FABP cDNA

Upon transfection of MCF7 cells with the bovine H-FABP cDNA Huynh *et al.* [22] observed a remarkable reduction of the proliferation rate, in accordance with the now established identity of H-FABP and MDGI [16]. This observation could be confirmed in the present experiments, even after several passages of the cells (Fig. 1). In the previous work the content of bovine H-FABP in transfected MCF7 cells was qualitatively assessed by Western blot analysis [22], we now employed a sandwich ELISA with affinity purified polyclonal antibodies for quantification of bovine H-FABP (Table 1). For comparison the content of bovine H-FABP in bovine heart, lactating mammary gland and milk was measured as well. Although the H-FABP content of tissues was 15–50 times

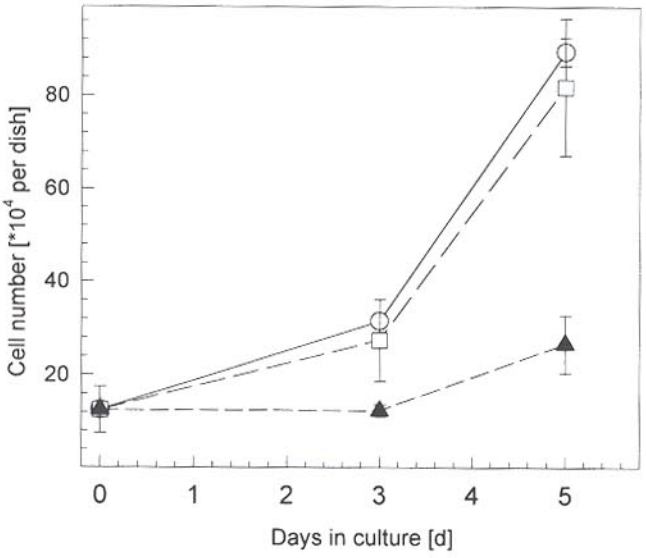

Fig. 1. Growth inhibition of MCF7 cells transfected with bovine H-FABP cDNA. Non transfected (–○–), mock transfected (–□–) and H-FABP transfected (–▲–) cells were seeded at 5000 cells/cm² in six well (9.6 cm²) plastic dishes. At each of the indicated days cells from three dishes were trypsinised and counted.

higher than the 638 ± 80 ng/mg found in transfected MCF7 cells, the latter was still significant as even terminally differentiated C2C12 muscle cells contained only 420 ng H-FABP per mg protein [19]. A sandwich ELISA for human H-FABP with similar sensitivity, and without significant crossreactivity towards bovine H-FABP, revealed that no human H-FABP was present in normal and mock transfected MCF7 cells. Moreover, up-regulation of endogenous human H-FABP due to ectopic bovine H-FABP expression in the transfected MCF7 cells was not recognized. Such up-regulation could be expected from data of Yang *et al.* [19], who observed an increase of the H-FABP mRNA level upon addition of bovine H-FABP to a mammary gland organ culture.

Table 1. Quantification of bovine H-FABP in MCF7 cells and tissues

Source	Protein[1] mg/ml	Bovine H-FABP µg/mg
MCF7	0.89 ± 0.10	n.d.[2]
MCF7, mock transfected	0.73 ± 0.08	n.d.
MCF7, bovine H-FABP transfected	0.87 ± 0.07	0.64 ± 0.08
Bovine heart	3.00 ± 0.12	10.3 ± 1.0[3]
Bovine mammary gland	0.51 ± 0.08	33.5 ± 5.8
Bovine milk, 6 day *post partum*	10.5 ± 2.1	0.4 ± 0.07

Data are given as mean ± S.D. (n =5). [1]15,000 × g supernatant of MCF7 cells and 100,000 × g supernatant of tissues and milk; [2]nd, not detectable. Limit of detection 0.001 µg/mg; [3]corresponding to 308.8 µg/g wet wt.

Effect of H-FABP expression on fatty acid uptake

This set of MCF7 cells differing in their H-FABP content enabled us to study a possible stimulatory role of H-FABP in fatty acid metabolism. As a parameter for the metabolism of fatty acids their uptake after 30 or 60 min was chosen, since at that time only a very small portion of the fatty acids taken up can still be recovered in non-esterified form from the cell lysate [7, 25]. Non-transfected, mock-transfected and bovine H-FABP transfected MCF7 cells were cultured in monolayers near confluence and fatty acids were applied in complex with BSA. No significant difference between uptake of oleic and palmitic acid was observed and in the various experiments performed the uptake rate for these fatty acids into MCF7 cells was 0.68 ± 0.14 nmol · min^{-1} · mg^{-1} protein (n = 11). The bovine H-FABP expressing MCF7 cells showed a modest but significant increase (67%) in the uptake rates of both, oleic acid (Fig. 2A) and palmitic acid (Fig. 2B), as compared to the control cells. The small increase in uptake rate of palmitic acid for mock-transfected cells in comparison to non-transfected cells was not significant (Fig. 2B).

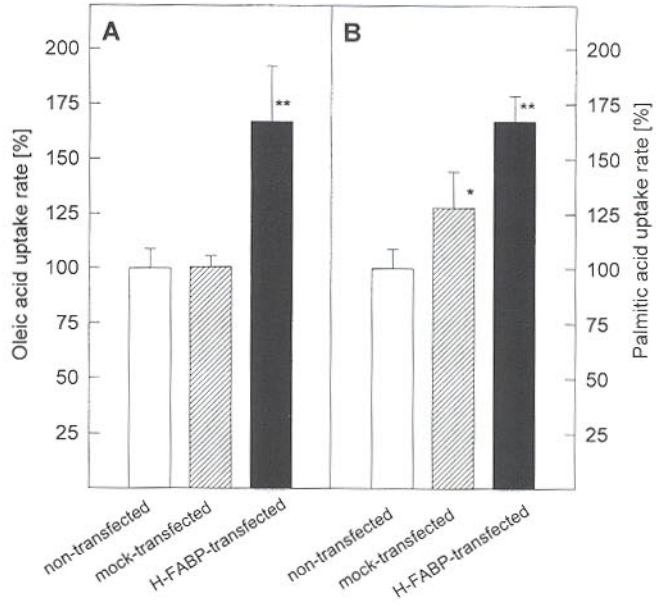

Fig. 2. Increased fatty acid uptake rate of MCF7 cells expressing bovine H-FABP. Cells were incubated with 100 µM oleic acid (panel A) or palmitic acid (panel B) in the presence of BSA as described in materials and methods. Data for 30 min (n = 4 for A, n = 3 for B) and 60 min (n = 2 for A, n = 2 for B) were combined, since preliminary experiments revealed that uptake rates were not different at these time points. Each experiment consisted of 3 to 4 cell culture dishes. Values represent the mean ± S.E.M (*$p > 0.1$; **$p < 0.05$, compared to non-transfected cells).

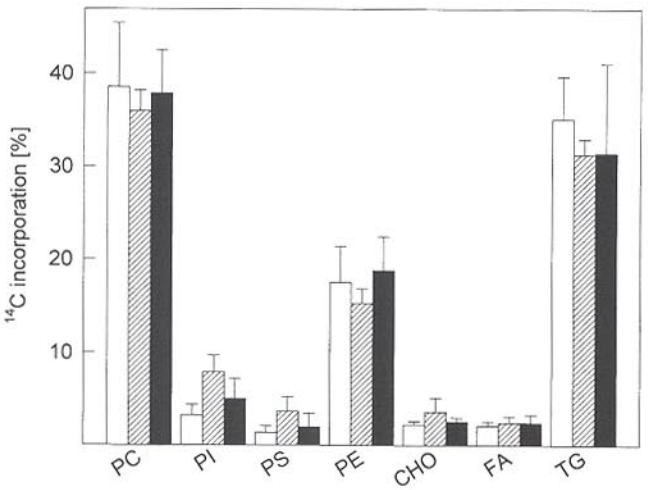

Fig. 3. Incorporation of ^{14}C-oleic acid into different lipids. MCF7 cells were incubated with 100 µM ^{14}C-oleic acid for 60 min, harvested and applied to high performance thin layer chromatography for separation of phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), cholesterol (CHO), free fatty acids (FA) and triacylglycerols (TG) as described in materials and methods. The incorporated radioactivity is expressed as percentage of that found in all lipid classes analysed for MCF7 cells (open bars), mock-transfected (grey bars) and H-FABP transfected MCF7 cells (black bars). Values represent mean ± S.E.M. (n = 4).

Effect of H-FABP on fatty acid distribution

We next investigated the incorporation of the radioactively labelled oleic acid into individual lipid classes. For this, cholesterol, free fatty acids, triacylglycerols and 4 different phospholipid species were separated by high performance thin layer chromatography and radioactive label was detected by densitometric analysis of autoradiograms. Most of the ^{14}C-oleic acid taken up during 60 min was incorporated into phosphatidylcholine and triacylglycerols, whereas only a small amount of the label was found in the free fatty acid pool. The distribution of the radioactivity revealed no significant differences between the three cell lines investigated (Fig. 3).

Occurrence of other FABP-types

To understand the limited effect of the ectopically expressed bovine H-FABP on MCF7 fatty acid metabolism we speculated that other FABP-types perhaps may be present in this cell line. Candidate FABPs besides H-FABP for expression in cells from the mammary gland are the recently discovered E-FABP, which has also been identified in mammary gland tissue by Northern blot analysis [34], and A-FABP, which was not only present in adipocytes of the mammary gland but also in myoepithelial cells around the alveolar epithelium [16]. Western blot analysis of mock transfected MCF7 cells indeed revealed the presence of E-FABP (Fig. 4 A) whereas A-FABP was absent from these cells (Fig. 4 B). Similar results were found in Western blot analysis of non-transfected and bovine H-FABP transfected MCF7 cells. In order to detect a possible alteration in the E-FABP content of MCF7 cells caused by the ectopic expression of bovine H-FABP, again a sandwich-type ELISA was employed, this time using affinity purified polyclonal antibodies against recombinant human E-FABP. With this assay an E-FABP level of 42.8 ± 19.8 ng/mg protein was found in control cells (normal and mock transfected MCF7 cells) compared to 31.8 ± 6.8 ng/mg in the H-FABP

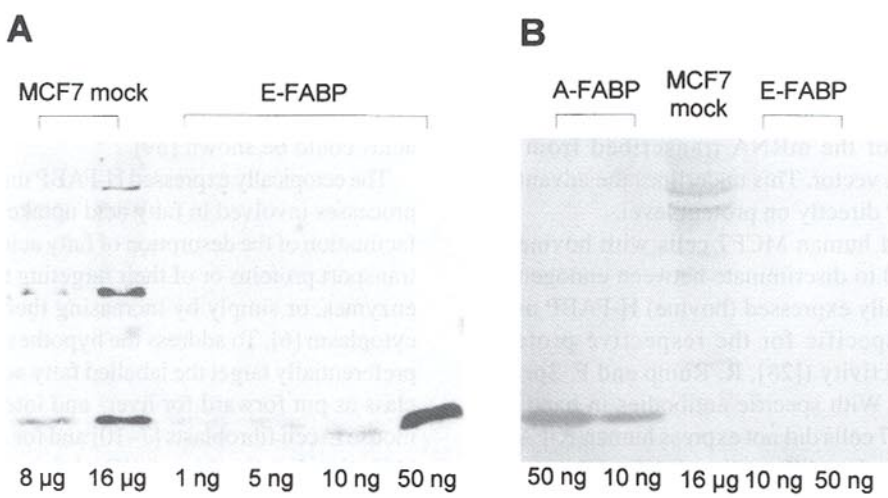

Fig. 4. Detection of endogenous E-FABP in MCF7 cells. A: Western blot analysis of lysates from mock-transfected MCF7 cells and E-FABP standards with polyclonal antibodies against human E-FABP. Higher molecular weight bands in Western blot analysis as seen in the first two lanes of panel A are sometimes also observed with recombinant E-FABP (not shown), indicating a tendency of E-FABP to aggregate; B: Western blot analysis of lysates from mock-transfected MCF7 cells, A-FABP standards and E-FABP as control for possible crossreactivity with antiserum against human A-FABP.

transfected cells. This small decrease in the E-FABP level, however, did not reach statistical significance ($p > 0.05$).

Discussion

The experiments reported by Huynh *et al.* [22] were inspired by the growth inhibitory activity reported for H-FABP [15, 18] which was recently shown to be identical to the earlier described MDGI [16]. Transfection of MCF7 cells with bovine H-FABP cDNA indeed revealed anti-proliferative effects, both on plastic and soft agar, and furthermore a significant reduction of tumorgenicity when injected into mammary fat pads of nude mice [22]. In contrast to the earlier experiments on growth inhibition by exogenously added H-FABP [15, 18], these effects were exerted intracellularly by ectopically expressed H-FABP. On the one hand the H-FABP level in transfected MCF7 cells (638 ng/mg protein) was relatively low compared to that of H-FABP rich tissues like heart and lactating mammary gland (Table 1). H-FABP concentrations in bovine (33.5 µg/mg) and rat lactating bovine mammary gland (59 ± 19 µg/mg [13]) were comparable and even higher than those found in heart. On the other hand the H-FABP level observed in transfected MCF7 cells was even slightly higher than that of mouse H-FABP in the fully differentiated C2C12 muscle cell line (420 ng/mg) [19] and much higher than that of H-FABP in endothelial cells cultured from bovine aorta (90 ng/mg) [35]. For comparison, levels of I-FABP and L-FABP reported for respective transfected L-cells were 3500 and 4000 ng/mg cytosolic protein [9, 10], whereas the A-FABP level in transfected CHO cells was as low as 100 ng/mg soluble protein [11]. In contrast to our findings Huynh *et al.* [22] reported comparable mRNA levels in transfected MCF7 cells and lactating rat breasts. The discrepancy may be due to different hybridization efficiency of the probe to rat and bovine H-FABP mRNA, respectively, or to unknown regulatory effects in the MCF7 cells like a decreased stability of the mRNA transcribed from the eucaryotic expression vector. This underlines the advantage of analysing H-FABP directly on protein level.

The transfection of human MCF7 cells with bovine H-FABP cDNA allowed to discriminate between endogenous (human) and ectopically expressed (bovine) H-FABP using sandwich ELISAs specific for the respective proteins without any crossreactivity ([28], R. Rump and F. Spener, unpublished results). With specific antibodies in hand, we could show that MCF7 cells did not express human H-FABP, which is in line with the less differentiated state of this cancer cell line in comparison to the mammary epithelial cell line HH2a. The latter expressed H-FABP when grown in released collagen gels and in presence of lactogenic hormones, conditions leading to a highly differentiated state as documented morphologically by formation of duct like outgrowths and spherical clusters [23]. The intracellular bovine H-FABP in the transfected MCF7 cells did not stimulate the expression of endogenous human H-FABP, in contrast to the differentiation promoting effects shown for exogenously added bovine H-FABP in embryonic stem cells [36] as well as in organ cultures of mammary gland [19]. In the latter case a more differentiated phenotype, induced expression of β-casein and autostimulation of H-FABP mRNA expression was observed.

As epidemiological studies suggested a causal relationship between dietary fatty acids and breast cancer [37], we were interested whether the role in fatty acid metabolism, traditionally attributed to FABPs, could be investigated with these cells as well and perhaps even related with the observed tumor suppressor activity. The initial step of fatty acid uptake, presumably mediated by several membrane fatty acid transport proteins [38], can only be studied by using non-metabolizable fatty acids like *cis*-parinaric acid [7, 10] or by using natural fatty acids at very short incubation times, since it has been shown for mouse L-cells [7] and rat myocytes [25] that already after 3 min most of the fatty acids taken up are metabolized. Thus, in our study fatty acid uptake after incubations for 30–60 min was employed as a general parameter to reveal a possible stimulation by H-FABP of intracellular fatty acid transport or delivery to metabolizing enzymes. The modest but significant increase of 67% in uptake rates of ^{14}C-oleic acid and ^{3}H-palmitic acid in H-FABP transfected MCF7 cells is similar to the effect observed for uptake of oleic acid into CHO cells transfected with A-FABP [11] and into L-cell fibroblasts transfected with L-FABP [10] and indicative for a stimulatory role of H-FABP in fatty acid metabolism. Interestingly, in recent studies of Prows *et al.* [7, 9] I-FABP did neither stimulate initial uptake of *cis*-parinaric acid nor uptake of oleic acid after 30 min. This is the first report about a stimulatory role for the heart-type FABP, in earlier experiments employing stable transfection of 3T3-L1 preadipocytes and transient transfection of COS-cells with rat H-FABP cDNA no increase in uptake of long chain fatty acids could be shown [39].

The ectopically expressed H-FABP may interact with several processes involved in fatty acid uptake and metabolism, like facilitation of the desorption of fatty acids from the membrane transport proteins or of their targeting to specific esterifying enzymes, or simply by increasing their diffusion rate in the cytoplasm [6]. To address the hypothesis that H-FABP might preferentially target the labelled fatty acid into a specific lipid class as put forward for liver- and intestinal-type FABPs in mouse L-cell fibroblasts [7–10] and for A-FABP in CHO cells [11], the distribution of ^{14}C-oleic acid into various lipid classes was studied in more detail. Unlike L-FABP and I-FABP which appear to target oleic acid taken up into L-cell fibroblasts to the phospholipid and triacylglycerol pool, respectively [7], and A-FABP which in CHO cells increased the label in the phospholipid/acyl-CoA pool [11], H-FABP

in MCF7 cells did not significantly increase the incorporation of label into any of the lipid classes studied. This renders it also unlikely that H-FABP exerts its tumor suppressor activity via distinct changes in the activity and regulation of pathways involved in phosholipid metabolism and breakdown as suggested by Ting *et al.* [40] as a possible mechanism for malignant transformations of mammary epithelial cells. However, a more detailed analysis of phospholipids and their metabolites may be worthwhile.

It should be noted that the approach of transfection with sense cDNA is restricted to cells that do not normally express the FABP studied, these may thus be characterized by a low lipid metabolism or may be adapted to a situation with a very low FABP content. This would explain the low increase of the fatty acid uptake rate in the transfection experiments. It has been argued that in tissues where FABPs constitute as much as 1–5% of the soluble proteins the rate of fatty acid uptake and trafficking may be enhanced to a greater extent [11]. It is possible that the basal expression of E-FABP which we detected by Western blot analysis and which is comparable to that in cultured endothelial cells [41] is responsible for the observed fatty acid uptake rate of the non-transfected MCF7 cells. Under the conditions of cell culture the low expression level of this FABP may not be limiting for fatty acid metabolism. Expression of high amounts of another FABP in the transfected cells would not lead to a concomitant increase of the fatty acid uptake rate when other processes become rate limiting, like entrance of fatty acids into the cells or metabolic activity. The presence and activity of membrane associated fatty acid transporters which are believed to be important for the first step of cellular fatty acid uptake [38] has not yet been studied in MCF7 cells. Interestingly, the amount of E-FABP was not significantly altered upon expression of the additional FABP as measured by ELISA. A compensation effect had been observed for E-FABP, as this was upregulated in A-FABP deficient mice [42] and for L-FABP which became down-regulated in Caco-2 cells transfected with I-FABP cDNA [43].

A good alternative for the sense transfection approach may be transfection of cell lines which express a particular FABP with anti-sense cDNA and the correlation of residual FABP with fatty acid uptake (C. Wolfrum, C. Buhlmann, B. Rolf, T. Börchers and F. Spener, Biochim Biophys Acta, in press). In future, mice deficient for an FABP-type [42] may serve as a source for primary cell cultures or immortalized cell lines.

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