Isolation and characterization of fatty acid binding proteins from mammary tissue of lactating rats

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A protein fraction with fatty acid binding activity has been isolated from mammary tissue from lactating rats by a process involving DEAE-cellulose ion-exchange chromatography, heat treatment, CM-cellulose ion-exchange chromatography and finally ammonium sulphate precipitation. The purified fraction migrated as a single band on SDS/polyacrylamide-gel electrophoresis with an apparent molecular mass of 14400. However, when this protein fraction was electrophoresed under non-dissociating conditions, two species were observed in a 4:1 ratio. The two components were separated using h.p.l.c. Both bind fatty acids and appear to have similar amino acid compositions although exhibiting different pI values of 4.8 and 4.9. The mammary fatty acid binding proteins appear to be very similar to the fatty acid binding protein isolated from rat heart based on the electrophoretic mobilities and amino acid composition. The major mammary form (pI 4.9) has been partially sequenced and the amino acid sequences obtained can be aligned with 67 residues of the revised rat heart amino acid sequence [Heuckeroth, Birkenmeier, Levin & Gordon (1987) *J. Biol. Chem.* **262**, 9709–9717]. Both mammary species also showed immunochemical identity to rat heart fatty acid binding protein when tested with an anti-serum raised against the heart protein. Anti-sera raised against the minor mammary form (pI 4.8) specifically precipitated this form under non-denaturing conditions but both forms after they had been denatured. Quantitative immunoassays using the anti-(heart fatty acid binding protein) serum showed that concentrations of the fatty acid binding proteins present in mammary cytosols increase during lactation and increase further after feeding a high-fat diet.

INTRODUCTION

Low molecular mass fatty acid binding proteins (FABPs) have been isolated from several rat tissues including intestine [1], liver [2–4], heart [2,5–9] and brain [10]. These proteins are characterized by their low molecular mass, relative abundance and affinity for hydrophobic ligands. At least three different molecular forms have been isolated and those from the liver, intestine and heart have each been cloned and sequenced [11–14]. However, their ability to bind ligands on the basis of hydrophobicity has resulted in many groups purifying the same protein under different names. For example, it is now believed that rat sterol carrier protein 1, amino azo dye-binding protein, band C and Z-protein are the same as the rat liver FABP [15,16].

There is evidence to suggest that these proteins may be involved in several aspects of lipid metabolism. A number of enzymes involved in triacylglycerol synthesis have been shown to be stimulated *in vitro* by FABP [17–19]. Factors that modulate lipid synthesis in the liver and/or intestine, e.g. sex steroid treatment [20], fat feeding [1], clofibrate treatment [21,22] and cholestyramine treatment [23,24], all increase the concentration of the FABP. Reagents which inhibit fatty acid uptake by everted jejunal sacs such as flavaspidic acid and α -bromopalmitate have been shown to be competitive inhibitors for fatty acid binding to the FABP [1]. These findings are all consistent with a role for the FABP in triacylglycerol synthesis. However, Fournier *et al.* [5,25]

have proposed that in heart, a heart muscle-specific FABP regulates the supply of fatty acids to the mitochondria for β -oxidation.

This paper describes the isolation and characterization of two FABPs found in the mammary glands of rats. The lactating mammary gland is active in fatty acid and triacylglycerol synthesis and it was thought that, if fatty acid binding proteins have a role in complex lipid synthesis, they should be abundant in mammary tissue. The mammary gland is also an inducible system and previous studies have shown that several lipogenic enzymes increase in activity and amount many-fold at the initiation of lactation [26–32]. Therefore, we have also measured the concentration of FABP in mammary homogenates prepared from rats at different stages of lactation and after feeding a high-fat diet which is known to reduce mammary fatty acid synthesis [32–34].

EXPERIMENTAL

Materials

Radiolabelled [1- 14 C]oleic acid was purchased from Amersham (Surrey Hills, Sydney, NSW, Australia). DEAE-cellulose (DE-52) was from Whatman (Maidstone, Kent, U.K.) and CM-cellulose, defatted bovine serum albumin, Tween 20, oleic acid and the proteases, trypsin [tosylphenylalanylchloromethane (TPCK) treated], chymotrypsin [tosyl-lysylalanylchloromethane (TLCK) treated], proteinase K, Pronase E and

Abbreviations used: FABP, fatty acid binding protein; PAGE, polyacrylamide gel electrophoresis.

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subtilisin were from Sigma (St. Louis, MO, U.S.A.). Centricon-10 concentrators were obtained from Amicon Corporation (Danvers, MA, U.S.A.), Thiomersal from B.D.H. (Poole, Dorset, U.K.), 4-chloro-1-naphthol from Bio-Rad (Richmond, CA, U.S.A.), nitrocellulose from Schleicher and Schull (Dassel, W. Germany), and goat anti-rabbit IgG conjugated to horseradish peroxidase (EC 1.11.1.7) from Tago (Burlingame, CA, U.S.A.). All other reagents were of the highest purity available.

Female rats of the Wistar strain were purchased from the University of Otago Animal Breeding Station and mated at between 3 and 4 months of age. Litter sizes were standardized at ten pups within 48 h of parturition. The rats were fed a commercial pelleted 'breeder' diet (A. E. Reeves, Dunedin, New Zealand) or a semi-synthetic 20% peanut oil diet described previously [34]. Rats were housed in a room at 20 °C with 12 h light/12 h dark cycles. Animals were killed at 10:00 h, midway through the dark period by anaesthetizing with ether followed by cervical dislocation. Tissues were removed and homogenized in 5 vol. of 0.23 M-sucrose/1 mM-EDTA/15 mM-2-mercaptoethanol/10 mM-Tris/HCl buffer, pH 7.4. Cytosolic fractions were obtained as supernatants after centrifugation at 100 000 *g* for 60 min.

Purification of mammary and heart FABPs

Mammary and heart cytosols were mixed with an equal volume of a slurry of DE-32 in water (0.1 g dry wt./ml) and the pH was adjusted to 7.4. This mixture was stirred on ice for 30 min and then centrifuged at 12 000 *g* for 10 min and the supernatant was discarded. To the pellet of DE-32 was added one volume of 10 mM-phosphate buffer (pH 7.4)/100 mM-KCl and the resuspended mixture was stirred on ice for 30 min before being centrifuged at 12 000 *g* for 10 min. The second supernatant was rapidly heated to 90 °C and maintained at this temperature for 6 min before being centrifuged at 40 000 *g* for 1 h to precipitate the denatured protein. The supernatant from the heat step was mixed with an equal volume of a slurry of CM-cellulose in water (0.1 g dry wt./ml), the pH was adjusted to 5.5, stirred on ice for 30 min and filtered to remove the CM-cellulose. The FABP fraction was isolated by ammonium sulphate precipitation between 60 and 80% saturation at pH 7.4.

This procedure yielded a protein fraction which bound fatty acids and appeared to be homogeneous on SDS/PAGE. However, when these fractions were subjected to non-dissociating PAGE, two distinct protein fractions were seen in both the heart and mammary preparations. These two components from the mammary preparations were separated by DEAE-h.p.l.c. on a TSK DEAE-5PW (Toyo Soda Manufacturing Co., Tokyo, Japan) column using a Waters/Millipore Liquid Chromatography system with a model U6K injector fitted with a 2 ml loading loop. Eluted material was monitored at 280 nm. Proteins were applied to the column in 10 mM- Na_2HPO_4 , pH 8.5, with which the column had previously been equilibrated. The column was then developed with a non-linear gradient of KCl from 0 to 500 mM (Fig. 3).

Electrophoresis

PAGE was performed in the presence of 0.1% SDS according to the method of Laemmli [35]. Mini-gels (9 cm × 6 cm with a thickness of 0.5 mm) were electrophoresed at a constant current of 15 mA. The buffer system, for non-dissociating gels, was identical except

that SDS was omitted. Gels were run as 7 cm-long tube gels, diameter 5 mm, at a constant current of 2 mA/gel, or as 9 cm × 6 cm mini-gels. Gels were stained for protein with Coomassie Blue.

Isoelectric focusing was performed on ultra-thin (0.5 mm) agarose gels with pH 4–6.5 Ampholines (LKB Producter AB, Bromma, Sweden) as described in the LKB Instruction Manual 1818-A.

Immunochemical studies

Rabbit antisera to rat heart FABP were prepared as previously described [9]. A rabbit anti-serum to the minor mammary FABP component (band II) was also prepared by three injections each of 250 µg in adjuvant. The IgG fraction was precipitated at 30% ammonium sulphate saturation and resuspended at 5 times the original concentration in 20 mM-phosphate buffer (pH 7.4)/0.9% (w/v) NaCl/0.01% (w/v) NaN_3 and stored in aliquots at –80 °C. Attempts to raise anti-sera to the band I protein have so far been unsuccessful.

For Western blot analysis, proteins were electrophoresed as described above and the gels soaked for 10 min in transfer buffer (192 mM-glycine/25 mM-Tris, pH 8.3). Methanol (20% v/v) was added for the non-dissociating gels. Transfer to nitrocellulose membranes was performed at 10 V/cm for 6 h, following which filters were blocked in a buffer containing 40 mM-Tris (pH 7.6), 150 mM-NaCl, 0.05% (w/v) Tween 20, 0.001% (w/v) thiomersal and 0.5% (w/w) Anchor non-fat dried milk (New Zealand Co-operative Dairy, Hamilton, New Zealand) at 37 °C for 1 h. They were then incubated with anti-FABP serum [anti-band II serum diluted 1:750 and anti-(heart FABP) serum diluted 1:75] in the blocking buffer for 1 h, washed in blocking buffer for 30 min with three changes of buffer and then incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase diluted 1:2000 for 1 h. The filters were then washed as before and developed in enzyme substrate containing 83 mM- Na_2HPO_4 , 40 mM-citric acid, 0.17 mg of 4-chloro-1-naphthol/ml and 0.012% (v/v) H_2O_2 .

Total FABP content of mammary cytosolic fractions was determined using quantitative radial immunodiffusion [36] using anti-serum raised to heart FABP [9].

Fatty acid binding assay

Fatty acid binding to protein samples was determined using an equilibrium partitioning assay based on that of Goodman [37,38] which involved the partitioning of radiolabelled fatty acids between a fatty acid solution in hexane and an aqueous protein solution. For the standard assay 25 µg of protein in 100 µl was overlaid with an equal volume of the fatty acid solution in hexane (2 mM-oleic acid, 1000 c.p.m./nmol). The mixtures were shaken for 2 min and centrifuged to separate the phases. Two portions (25 µl) of the aqueous phase were removed and counted for radioactivity. The degree to which the FABP protein enhanced oleic acid solubility in the aqueous phase over that measured in buffer-only blanks represented the fatty acid binding activity. Radioactivity was determined by liquid-scintillation counting using a scintillant containing 0.3% (w/v) 2,5-diphenyloxazole and 20% (v/v) Triton X-100 in toluene. Known amounts of labelled lipids were counted under the same conditions and activities were calculated as nmol of fatty acid bound/mg of protein.

Fig. 1. Purification of mammary FABP

Fractions were electrophoresed in a 10–25% gradient gel and stained for protein with Coomassie Blue. Samples were (b) mammary 100 000 g supernatant, (c) after DEAE elution, (d) after heat treatment, (e) after CM-cellulose treatment and (f) after ammonium sulphate precipitation. Lanes (a) and (g) contained molecular mass markers [bovine serum albumin (M_r 66 000), ovalbumin (M_r 45 000), glyceraldehyde 3-phosphate dehydrogenase (M_r 36 000), carbonic anhydrase (M_r 29 000), trypsinogen (M_r 24 000), soybean trypsin inhibitor (M_r 20 100) and α -lactalbumin (M_r 14 200)]. All lanes were loaded with 5 μ g of protein.

Amino acid analysis and sequencing

Amino acid analysis was performed on a Waters/Millipore h.p.l.c. amino acid analyser. Protein samples were extensively dialysed against distilled water and dried under vacuum at 80 °C before being hydrolysed for 24, 48 and 72 h in 6 M-HCl containing 0.1% (w/v) phenol. Peptide fragmentation patterns were determined on samples of both mammary proteins that had been treated with CNBr or the proteases; trypsin (TPCK treated), chymotrypsin (TLCK treated), proteinase K, Pronase E and subtilisin. Digests were analysed by reversed-phase h.p.l.c. using a Waters Radpack C₁₈ column and a non-linear gradient of acetonitrile. The amino acid sequences of selected peptides were determined by Edman degradation using a gas-phase sequencer (Applied Biosystems, model 470A with on-line detection for amino acid phenylthiohydantoin derivatives).

Miscellaneous analytical techniques

Densitometry of PAGE gels, isoelectric focusing gels and Western blots was performed on a LKB Ultrascan XL laser densitometer. Protein was determined by the method of Lowry as modified by Peterson [39] using bovine serum albumin as standard.

RESULTS

Purification of mammary and heart FABPs

Fatty acid binding fractions were prepared as described in the Experimental section from both mammary and heart cytosols with yields of 0.8 and 0.3% of the total soluble protein respectively. Fig. 1 shows the purification of the mammary FABP. Both preparations appeared

Fig. 2. Non-dissociating PAGE of rat mammary and heart FABPs

Samples were electrophoresed in 7 cm-tube 10% acrylamide gels and stained for protein with Coomassie Blue. Samples were (a) 125 μ g of mammary gland 100 000 g supernatant, (b) 60 μ g of heart 100 000 g supernatant, (c) 36 μ g of heart FABP, (d) 55 μ g of mammary gland FABP, (e) 25 μ g of mammary FABP and 25 μ g of heart FABP and (f) 50 μ g of mammary FABP and 50 μ g of heart FABP.

homogeneous when analysed by SDS/PAGE with an apparent molecular mass of 14.4 kDa. However, when either the mammary or the heart preparations were examined using non-dissociating PAGE, two distinct species were observed to be present. On the basis of their electrophoretic mobilities we have designated these species band I and band II, band I being the major and slower-migrating species and band II the minor and faster-migrating form. Band I and band II for the mammary and heart preparations co-migrated when electrophoresed together suggesting that each form was identical in both tissues (Fig. 2). Densitometry of similar gels showed that the band II species comprised 20% of the total mammary FABP.

The two proteins isolated from the mammary gland were separated by DEAE-h.p.l.c. (Fig. 3). Electrophoresis of the major peaks from the h.p.l.c. showed that each contained a single peptide (Fig. 3, inset). A third peak eluting at the end of the gradient contained salts and minor protein species. Analytical isoelectric focusing of the purified proteins showed band I to have a pI of 4.9 and band II a pI of 4.8. Preincubation in a 100-fold molar excess of oleic acid did not affect the pI values observed. Using the equilibrium partitioning assay both the h.p.l.c.-purified band I and band II bound oleic acid with similar capacities; 55 and 48 nmol/mg of protein (0.7 and 0.6 mol/mol) respectively (Fig. 4).

The amino acid compositions of the two mammary FABPs are listed in Table 1. No major differences were apparent between the two mammary FABPs and the two proteins are more like heart FABP than any of the other rat FABPs previously reported [4,8]. The peptide fragmentation patterns obtained after CNBr or protease

Fig. 3. H.p.l.c. purification of mammary FABPs

Mammary FABP after ammonium sulphate precipitation (1 mg) was loaded on to a TSK DEAE-5PW h.p.l.c. column in 10 mM- Na_2HPO_4 , pH 8.5, and eluted with a gradient of KCl as indicated in the same buffer. This trace is representative of approx. 30 separations. Fractions collected are denoted by the arrows. Inset: densitometer scans of non-dissociating PAGE performed using tube gels and stained with Coomassie Blue; they represent material as supplied to h.p.l.c. (L), peak A (A) and peak B (B); each 20 μg of protein.

Fig. 4. Binding of oleic acid to purified mammary FABPs

Fatty acid binding activity was assayed using the equilibrium partitioning assay described in the Experimental section with both purified band I and band II proteins. Buffer blanks were used to determine the 'zero protein' baseline.

treatment were also very similar for the two mammary FABPs and it was not possible to identify and collect any peptides unique to either protein. The amino acid sequences of ten of the peptides obtained from the digests of the major (band I) species were determined. The sequences obtained were aligned with the rat heart FABP sequence such that a total of 66 out of 67 residues were identical to those in the heart FABP sequence [13]. The difference was at residue 65 where the sequences obtained for two different mammary peptides suggested there was a deletion at this point when compared with the heart protein. However, a recent revision of the heart FABP sequence based on DNA sequencing [14] matches the mammary sequences we obtained for this region of the molecule (Fig. 5). Attempts to sequence either the intact mammary band I or band II proteins were unsuccessful indicating that the *N*-terminus may be blocked to Edman degradation.

Table 1. Amino acid composition of rat mammary FABPs

Abbreviation: nd, Not determined.

Amino acid	Band I (mol/10 ³ mol of amino acid recovered)	Band II (mol/10 ³ mol of amino acid recovered)
Asx	111	106
Thr	131	141
Ser	61	71
Glx	111	101
Pro	0	0
Gly	111	101
Ala	51	50
Val	91	96
Met	10	10
Ile	41	40
Leu	91	81
Tyr	10	10
Phe	41	40
His	10	10
Lys	111	112
Arg	31	31
Cys	nd	nd
Trp	nd	nd

Immunochemical studies

Anti-sera raised to the heart FABP showed a reaction of complete identity with the mixed mammary gland preparation, band I, band II and heart FABP when examined in a double diffusion assay [40] (results not shown). Neither of the purified mammary proteins showed any reaction to anti-sera raised to rat liver or rat intestinal FABP, although mammary cytosols showed a weak reaction to anti-sera raised to liver FABP (N. M. Bass, unpublished work). By contrast, anti-sera raised specifically to band II precipitated the band II but not band I protein in similar assays (results not shown).

Fig. 6 shows the results of Western blot analysis of the mammary gland FABPs using both anti-sera. In transfers from SDS gels each anti-serum reacted with both the purified bands I and II and showed only a single peak with mammary cytosols indicating that these anti-sera were specific for the FABPs (Figs. 6b and 6d). Both anti-sera also detected band I and band II after non-dissociating PAGE and transfer to nitrocellulose (Figs. 6a and 6c). This result was unexpected given the specificity detected for the anti-band II protein in the double diffusion assays and probably results from denaturation occurring during the transfer to the filters. These assays also showed the presence of other slower-migrating material which cross-reacted with the anti-sera. Given the observation that in the transfers from SDS gels only the 14000 kDa molecular mass species were detected, it is likely that these slower-migrating forms represent aggregates of the FABPs. Aggregation of the heart FABP has been observed previously in both electrophoretic and e.s.r. studies [5,41]. Further evidence for the aggregation of the mammary FABPs was obtained in experiments where either a mixed band I plus band II preparation or the purified band I were chromatographed on Sephadex G-50 in the presence of radioactive fatty acids. In each case two peaks of fatty acid binding

Fig. 5. Partial amino acid sequence comparison of mammary FABP and heart FABP

Peptides were sequenced as described in Experimental section. Sequences are aligned with the published, revised sequence of heart FABP [14]. Peptide nomenclature uses the initial of the proteinase (T = trypsin, S = subtilisin, P = Pronase E) with the elution time from the h.p.l.c. separation (e.g. peptide P20.4 was produced by pronase E digestion and eluted at 20.4 min on the h.p.l.c. gradient). All proteinase digests were carried out using a 1:50 (w/w) ratio of proteinase to pure band I protein. Trypsin peptides were prepared by the digestion of 200 μ g of band I protein for 21 h at 37 °C, Pronase E and subtilisin digestions were carried out using 100 μ g of band I for 2 h at 37 °C. Separation of peptides was performed on a Waters Radial Compression C₁₈ cartridge. Buffer A contained 0.1% (v/v) trifluoroacetic acid in water, pH 2.5. Buffer B contained 0.1% (v/v) trifluoroacetic acid in acetonitrile. The column was equilibrated with 90% buffer A/10% buffer B and after injection the column was eluted with 10% buffer B for a further 5 min. The concentration of buffer B was then raised linearly to 70% over 35 min. Sequencing of tryptic peptides eluting at 22.4 and 18.3 min each produced two clearly distinguishable sequences. '*' indicates unidentifiable amino acid; lower-case letters indicate where assignment was uncertain but represent the 'probable' amino acid; ':' indicates identity of amino acids.

Fig. 6. Western blot analysis of mammary FABPs

Western blotting were performed as described in the text using mini-gels for both SDS and non-dissociating gels. Filters were scanned densitometrically and absorbance is given on an arbitrary scale. (a) and (c) represent non-

occurred; one near the exclusion volume of the column and another with an apparent molecular mass of monomeric FABP. Electrophoresis showed the presence of FABP in each of these fractions and no evidence for any selectivity for bands I or II in either of these peaks could be detected (Fig. 7). It is likely then that the mammary FABPs form loose aggregates in solution that are only partially disrupted by non-denaturing PAGE.

Mammary concentrations of FABPs

The concentrations of the total FABP were determined in mammary cytosols prepared from rats at various stages of lactation and after feeding with a high-fat diet. Anti-serum to the heart FABP was used in a radial immunodiffusion assay. This anti-serum was chosen because it showed an identical reaction with both band I and band II species in the double diffusion assay and thus could be used to measure the total mammary FABP content. There was an apparent increase in the concentration of the FABP at the beginning of lactation which was maintained throughout lactation. Following weaning at day 20, the concentrations returned to those observed in late pregnancy (Fig. 8). The FABP concentrations were also significantly higher ($P < 0.01$, Student's *t*-test) in mammary cytosols of rats consuming

dissociating gels probed with anti-band II and anti-heart FABP, respectively, whereas (b) and (d) are SDS gels probed with anti-band II and anti-heart FABP, respectively. Samples on each gel were 0.25 μ g of band I (I), 0.25 μ g of band II (II) and 5 μ g of mammary cytosol (M).

Fig. 7. Aggregation of mammary FABPs in solution

Samples were prepared by incubating 200 μg of mixed mammary FABP (a) or 200 μg of purified band I (b) with 100 nmol of [$1\text{-}^{14}\text{C}$]oleic acid for 15 min at 37 °C and chromatographed on a column (50 cm $\times$ 1 cm) containing Sephadex G-50 (fine) eluted with 10 mM- Na_2HPO_4 (pH 7.4)/100 mM-KCl at a flow rate of 0.2 ml/min. Elution of fatty acids was monitored by liquid-scintillation counting. B.D. indicates the void volume of the column as determined by the leading edge of the elution peak for Blue Dextran (average M_r 2000000). Peaks containing fatty acids were pooled, concentrated using a Centricon 10 membrane and electrophoresed in non-denaturing gels (insets). L is 20 μg of the sample as loaded and A and B are total protein from peaks A and B, respectively.

a high-fat (20% peanut oil) diet than those from rats consuming the control pelleted diet, with values of $34.4 \pm 2.8 \mu\text{g}/\text{mg}$ of cytosolic protein and $27.1 \pm 2.8 \mu\text{g}/\text{mg}$ of cytosolic protein respectively (mean $\pm$ s.d. for five rats in each group). These results also demonstrate the abundance of FABPs in the mammary gland with levels between 2 and 4% of the total soluble protein.

DISCUSSION

Our studies have demonstrated the presence of two low molecular mass fatty acid binding proteins in mammary homogenates from lactating rats. Both species were also present in heart FABP preparations. Amino acid analyses and immunochemical studies have shown both forms to be very similar to each other. The difference in charge of the two forms is sufficient, however, to allow them to be separated by DEAE-h.p.l.c.

The possibility that the second minor form might be an artefact arising from the heat step used in purification

Fig. 8. Concentration of total FABP in mammary cytosols from rats at different stages of lactation

Total FABP was assayed by radial immunodiffusion using the anti-(heart FABP) serum and points represent values obtained in mammary cytosols from individual rats killed at the stage of lactation indicated. P represents parturition and W, weaning.

is considered unlikely. Repeated heat treatment of the purified bands I and II did not result in any modification that could be detected by electrophoresis (results not shown). Indeed, once purified, there was no evidence for the interconversion of the two species. In addition, heart FABP purified by quite a different method [9] also showed small quantities of the band II species (N. M. Bass, unpublished work). At present the exact difference between the two forms remains to be elucidated. It is not a consequence of fatty acid binding as saturation with oleic acid failed to modify the observed pI values. It is unlikely that either phosphorylation or glycosylation accounts for the difference as, to date, no evidence for either modification of FABPs has been reported. An alternative source of variation might be a difference in the amidation of the numerous glutamate and aspartate residues of the proteins. This possibility can only be confirmed by determining the complete primary sequence of each protein.

Partial sequence analysis of the major mammary form (Fig. 5) suggests that it is likely to be identical to the heart FABP. Immunochemical evidence for the presence of heart FABP in several other rat tissues has recently been reported [9] at concentrations ranging between 1 and 7.2% of that present in the heart ($51 \mu\text{g}/\text{mg}$ of cytosolic protein). The results of the present study show the heart-type FABP to be present in mammary gland at much higher concentrations, equivalent to between 50 and 60% of those found in the heart.

The role, if any, of the FABPs in lipid metabolism in mammary tissue also remains to be verified. Failure to detect a major change in the concentration of FABP during the lactation cycle does not negate such a role, since the protein is present in substantial abundance even before the onset of lactation. The increase in concentration of FABPs following feeding with the high-fat diet is of the same order as that reported previously for intestinal FABP following fat feeding [1] where it was argued that this protein functions in the intracellular transport of absorbed fatty acids. Whereas the heart FABP has been proposed to have a regulatory function with respect to β -oxidation in that tissue [5,24], the mammary gland is a highly lipogenic tissue and fatty acids are not likely to be a major fuel for its metabolism.

The mammary FABP may be important in the uptake and intracellular transport of fatty acids removed by the mammary gland from the blood. This possibility is supported by the report of Whetstone *et al.* [42] in which bovine mammary FABP was isolated and its fatty acid binding characteristics both *in vivo* and *in vitro* were determined. This protein bound only long-chain fatty acids and their CoA thioesters and not the short-chain fatty acids that are synthesized *de novo* in the mammary gland.

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