Adipose lipolysis is mediated, in part, via interaction of fatty acid-binding protein (FABP) with hormone-sensitive lipase (HSL). Mice with reduced FABP content in fat (adipocyte FABP null) exhibit diminished fat cell lipolysis, whereas transgenic mice with increased FABP content in fat (epithelial FABP transgenic) exhibit enhanced lipolysis. To examine the relationship between the binding of FABP to HSL and activation of catalytic activity, isothermal titration microcalorimetry as well as kinetic analysis using a variety of FABP isoforms have been employed. In the absence of fatty acids, no FABP-HSL association could be demonstrated for any FABP form. However, in the presence of 10 μM oleate, A-FABP and E-FABP each bound to HSL with high affinity (K_d of 0.5 and 3 nM, respectively) in a 1:1 molar stoichiometry, whereas liver FABP and intestinal FABP did not exhibit any association. To compare binding to catalysis, each FABP isoform was incubated with HSL in vitro, and enzymatic activity was assessed. Importantly, each FABP form stimulated HSL activity 2-fold using cholesteryl oleate as substrate but exhibited no activation using p-nitrophenyl butyrate. The activation by A-FABP was dependent upon its fatty acid binding properties because a non-fatty acid binding mutant, R126Q, failed to activate HSL. These results suggest that binding and activation of HSL by FABPs are separate and distinct functions and that HSL contains a site for fatty acid binding that allows for FABP association.

Lipolysis is a complex metabolic process carried out by adipocytes during times of nutrient deprivation and/or stress in which fatty acids and glycerol are liberated from the triacylglycerol storage droplet and released from the cell. Released free fatty acids are used for β-oxidation and ketogenesis, whereas the glycerol is largely channeled to the hepatocellular gluconeogenic pathway. Although described in detail in several excellent reviews (1–3), the salient features of the regulatory processes controlling lipolysis include G protein-coupled receptor activation of protein kinase A and subsequent phosphorylation of two key proteins: the droplet associated perilipin A and the cytoplasmic hormone-sensitive lipase (HSL).1 Phosphorylation of perilipin A allows for an ill defined dynamic restructuring of droplet surface architecture, redistribution of perilipin A to smaller microdroplets, and concomitant translocation of the lipase to the lipid surface where it gains access to its substrate (4). HSL is responsible for ~50% of the fatty acids generated by white adipose tissue triacylglycerol hydrolysis (5) and is the rate-limiting enzyme in lipolysis.

HSL is an 84-kDa cytoplasmic protein composed of two distinct structural domains. The ~48-kDa C-terminal domain contains the catalytic triad essential for triacylglycerol and cholesterol ester hydrolysis and has broad, albeit modest, amino acid similarity to several fungal and bacterial lipases (6). Inserted within the C-terminal catalytic domain is a regulatory module containing sites of phosphorylation (7) by the cAMP-dependent protein kinase A and the AMP-activated protein kinase. The catalytic domain is linked via a protease-sensitive hinge region to a ~36-kDa N-terminal domain that exhibits no sequence similarity to other known proteins. The N-terminal domain has recently been shown via a combination of yeast two-hybrid analysis, GST pull-downs, and co-immunoprecipitation studies to be the site of interaction with the major fatty acid-binding protein (FABP) of the adipocyte and has been referred to as a docking domain (8). This interaction has been mapped to a region on the lipase between amino acids 190 and 200 containing two key charged amino acids, His193 and Glu198, that have been shown by alanine scanning mutagenesis to be essential for FABP association (9). Incubation of extracts containing the A-FABP with HSL as well as co-transfection studies of HSL and A-FABP in Chinese hamster ovary cells have indicated that A-FABP stimulates the activity of the lipase (9).

The FABPs as a class are ~15-kDa soluble proteins that function by facilitating the intracellular diffusion of fatty acids between cellular compartments and/or enzymes (10). Structurally, the FABP fold has been described by a combination of x-ray and multidimensional NMR studies as 10 anti-parallel β-strands arranged into two five-strand β-sheets that fold together to form a barrel. Side chain packing closes one end of the barrel, whereas a helix-turn-helix motif caps the opposite barrel end and forms an interior water-filled ligand-binding cavity (11). Adipocytes express two FABPs, a major form A-FABP and

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To pursue the hypothesis that the molecular interaction of FABP with HSL forms a component of the lipolytic complex, in this report we characterize the properties and specificity of such an association. We describe herein that in addition to A-FABP, HSL also associates with E-FABP, but not with FABPs from liver (L-FABP) or intestine (I-FABP) and the origins of such metabolic effects remain to be determined (12–14). Importantly, A-FABP null mice exhibit decreased lipolysis both in situ and in vivo, whereas E-FABP overexpression in fat results in transgenic mice with adipocytes that exhibit increased lipolysis (15–18). The results from a combination of studies indicate that lipolysis is directly proportional to the total FABP abundance in the adipocyte and not a specific isoform.

EXPERIMENTAL PROCEDURES

Materials—Oleate and purified fatty acid standards used for gas chromatography analysis were purchased from Nu Chek Prep (Elysian, MN), and 1,8-anilinonaphthalene-8-sulfonic acid (1,8-ANS) was purchased from Molecular Probes (Eugene, OR). All other materials were reagent grade obtained from the best available source.

Protein Expression and Purification—Hi-Five baculovirus cells were infected with plasmids to express His6-HSL as previously described (19). The extract was subjected to centrifugation at 20,000 rpm for 15 min at 4 °C, and the supernatant was recovered. The detergent-solubilized His6-HSL was immediately applied to a Ni-NTA-agarose resin (Qiagen), washed in lysis buffer containing 20 mM imidazole, pH 8.0, and eluted with lysis buffer containing 250 mM imidazole, pH 8.0. Pooled fractions containing purified His6-HSL protein were dialyzed against buffer containing 50 mM NaPO4, pH 7.0, 20% glycerol, and 0.5% Nonidet P-40 and stored at 4 °C until use. Approximately 1 mg of homogenous His6-HSL could be isolated from a liter of culture and exhibited a specific activity of 70 milliunits/mg of protein. Alternatively, p-nitrophenyl butyrate was used as a water soluble substrate as described by Osterlund et al. (20) to determine the enzyme activity of HSL.

Lipid Extraction and Analysis—Fatty acids bound to bacterially derived His-FABPs were extracted using a mixture of chloroform-methanol-water (2:1:1.5 v/v/v) in a volume five times the sample volume after addition of C15:0 as an internal standard. The samples were vortexed briefly and subjected to low speed centrifugation at 2500 rpm for 10 min. The organic layer was removed, and the extraction was repeated twice. Free fatty acids were separated from the other lipid classes as described in Ref. 16. The free fatty acids were recovered, pooled, dried under nitrogen, and then esterified using 14% boron trifluoride in methanol. The fatty acid methyl esters were separated by gas chromatography using a HP 5890 gas chromatograph (Agilent Technologies) equipped with a flame ionization detector and integrator.

RESULTS

Previous studies have demonstrated that A-FABP forms a physical complex with hormone-sensitive lipase and that co-incubation results in an increase of HSL activity (2–2 fold) (9). Similarly, co-expression of HSL and A-FABP into Chinese hamster ovary cells resulted in a ~50% increase in HSL activity. However, in those studies the relationship of binding to catalysis was not addressed, nor was the specificity of the interaction with respect to other fatty acid-binding proteins.

Fatty Acid Binding Studies—The fatty acid binding properties of each protein were evaluated using the fluorescent probe 1,8-ANS as a surrogate ligand as described by Jenkins et al. (21).
Moreover, studies in Chinese hamster ovary cells have been criticized because they do not eliminate the possibility that a FABP regulates another protein that in turn activates HSL. To address these issues, His-tagged HSL was expressed in baculovirus and purified by two successive rounds of affinity chromatography using Ni-NTA-agarose resin. The resulting purified fusion protein had an apparent molecular mass of 84 kDa as determined by SDS-PAGE and was at least 95% homogeneous (Fig. 1A). Five different FABPs were subcloned into pRSET to produce histidine-tagged protein and expressed into bacteria where they were each found to be soluble and purified similarly. In addition, a non-fatty acid-binding mutant of A-FABP (R126Q) was expressed and purified similarly. Depending upon the specifics of the cloning procedures, each fusion protein exhibited a molecular mass between 17 and 19 kDa, and was essentially homogenous as evaluated by SDS-PAGE (Fig. 1B).

To verify that the histidine tag did not affect fatty acid binding by the FABPs, fluorescence binding and displacement assays using the surrogate ligand 1-anilinonaphthalene-8-sulfonic acid was performed. Each of the fusion proteins exhibited similar affinities for 1,8-ANS. Displacement of the bound fluorophore using oleic acid as a competing ligand revealed that each of the FABPs showed a similar $K_v$ value for oleate (Table I). The calculated $K_v$ values did not differ substantially from the nontagged FABP forms, suggesting that the presence of the N-terminal extension does not significantly affect the fatty acid binding properties of the protein (22). This is not unexpected because the N terminus of each FABP form is essentially 180° from the portal region (21), the site of ligand entry/exit into the binding cavity.

Gas chromatography was used to determine the fatty acid composition of the lipid pool endogenously bound to each of the FABP isoforms when purified from bacterial extracts. Gas chromatography analysis revealed that the different FABPs co-purify with varying amounts of bound fatty acids (Table II) with the most common being palmitate, oleate, and stearate. In contrast, when the FABPs were purified using Ni-NTA-agarose in the presence of 0.5% detergent, the fatty acids partition from the protein into the detergent micelle, and the proteins are isolated in their apo forms. To delipidate the FABPs without the use of detergent, chromatography through Lipidx resin (23) was employed and was found to be an effective method to remove endogenous fatty acids (Table II). As such, various FABP forms in either their holo or apo forms, in the presence or absence of detergent, could be prepared readily. Moreover, the addition of fatty acids to a solution containing FABPs in detergent results in partitioning of the fatty acids into the micelle.

This point is important and demonstrates that even when fatty acids have been added, FABPs in detergent solution are essentially apo protein, devoid of bound fatty acid.

To evaluate the interaction between each FABP and HSL in the absence of catalysis, isothermal titration calorimetry was employed in the presence of detergent to solubilize the HSL. Isothermal titration calorimetry has the additional benefit of providing thermodynamic parameters associated with the interaction, thereby allowing for a quantitative assessment of association (24). Controls for isothermal titration calorimetry included titrating buffer into buffer, buffer into HSL, and FABP into buffer, and the heat generated/consumed was subtracted from the experimental data to focus on specific heat of association. The corrected data were analyzed using Origin 2.9 software and experimentally fitted using several different model parameters. Analysis of the data revealed a best fit line using a simple single site binding model. Binding isotherms for four different FABPs are shown in Fig. 2 and are representative of three independent experiments. The thermodynamic data derived from such progress curves is presented in Table III. It should be noted that due to the presence of 0.5% Nonidet P-40 in the determinations, all of the thermodynamic parameters should be considered apparent values. Importantly, no physical association between any of the FABP forms with HSL could be detected in the absence of fatty acids. In contrast, when calorimetry was performed in the presence of oleate (10 μM), only A-FABP and E-FABP interacted with HSL; L-FABP and I-FABP exhibited no interaction. Because in these experiments the oleate partitions into the detergent micelle (0.5% Nonidet P-40), the concentration of free fatty acid in the aqueous phase is extremely low, and the results are simply reported relative to the total amount of fatty acid added. As shown by gas chromatography analysis, even though fatty acids have been added to the binding reaction, the FABPs are in their apo protein form. As such, in these experiments, the fatty acids are not bound to the FABPs but are found in the detergent micelle.
in potential association with the HSL. Under the conditions utilized, A-FABP and E-FABP each interacted with HSL with a stoichiometry (n) of 0.8–0.9 (Table III), indicative of the formation of a binary complex between the FABPs and HSL. The estimated dissociation constants derived from the thermodynamic parameters for A-FABP and E-FABP indicated a tight binding between the FABPs and HSL in the presence of fatty acids (Kd/HnM, 0.5 and 3 nM, respectively) (Table III).

To assess the effect of FABP association on the activity of HSL, the cholesteryl-oleate esterase activity of HSL was evaluated in the presence and absence of each FABP isoform. As shown in Fig. 3, when the activity was measured in the presence of each FABP isoform, HSL catalytic activity was enhanced about 2-fold. The stimulation with the different isoforms was dependent on the ratio of FABP to HSL in the assay. At maximal values each of the FABPs stimulated HSL activity.

Glutathione S-transferase, a non-fatty acid-binding protein, did not activate HSL to any extent over a wide concentration range. These results suggested that binding to HSL and activation of HSL activity were not equivalent because L-FABP

### Table II

| Fatty acid | L-FABP | H-FABP | E-FABP | I-FABP | A-FABP |
|-----------|--------|--------|--------|--------|--------|
|           | %      | %      | %      | %      | %      |
| C14:0     | trace  | 0      | 10     | 0      | 0      |
| C14:1     | trace  | 74     | 22     | trace  | trace  |
| C16:0     | 0      | 15     | 22     | trace  | trace  |
| C16:1     | 0      | 0      | 4      | 0      | 0      |
| C18:0     | 0      | 85     | 26     | 0      | 0      |
| C18:1*    | 0      | 0      | 43     | 0      | 0      |

*The sum of 18:1 plus 18:1-hydroxylated FA forms.

### Table III

| Protein | Kd | n    | ΔG° | ΔH° | ΔS° |
|---------|----|------|-----|-----|-----|
| A-FABP  | 0.5 ± 0.2 | 0.8 ± 0.3 | -13 ± 0.2 | -35 ± 2.4 | -22.5 ± 2.7 |
| E-FABP  | 3.1 ± 0.3 | 0.9 ± 0.5 | -8.5 ± 0.5 | -45 ± 2.3 | -37.3 ± 2.0 |

**Fig. 2. Isothermal titration calorimetry demonstrating the FABP-HSL interaction.** Isothermal titration calorimetry in the presence or absence of 10 μM oleate was used to evaluate the association between FABP and HSL. 5 μM FABP solutions were titrated into 0.5 μM HSL solutions, the heat was measured, and the data were analyzed using Origin 2.9 software. The open squares represent no fatty acid, and the closed squares represent the presence of fatty acid. The results shown are from one representative experiment of three separate experiments (including controls) carried out.
and I-FABP activated yet did not demonstrate any physical association.

To evaluate the relationship between binding and activation, a mutant of A-FABP (R126Q) that exhibits a 100-fold lower affinity for fatty acids as measured using the ADIFAB method (25) and fluorescence binding to 1,8 ANS (Fig. 4A) was used. The side chain of Arg<sup>126</sup> is oriented into the ligand-binding cavity of A-FABP and forms an essential component of a hydrogen-bonding network involving the bound fatty acid carboxylate (26). Although no crystal structure for R126Q A-FABP exists, all other mutant A-FABP structures reveal no significant structural changes accompany changes in cavity residues (26). In addition, the tryptophan fluorescence spectrum for R126Q is identical to that for native A-FABP (results not shown). Together these observations imply that the structural properties of R126Q are comparable with that of the native protein. No stimulation in the esterase activity of HSL was observed (Fig. 4B) when A-FABP (R126Q) was added to HSL (Fig. 4B) and in fact was modestly diminished at high protein concentrations. As such, activation of HSL by A-FABP required fatty acid binding and is consistent with the concept that FABPs provide relief of product inhibition and that binding to HSL by FABPs was unlinked to activation, at least in vitro.

To further evaluate whether the activation of HSL by the FABPs was a result of their ability to relieve product inhibition by fatty acid binding, HSL activity was examined using a water-soluble substrate, p-nitrophenyl butyrate. The product of hydrolysis in this case is the short chain fatty acid butyric acid that is not a ligand for FABPs. In this case, when each FABP isoform was incubated with HSL, no stimulation of lipase activity was observed (Fig. 5).

**DISCUSSION**

The process of lipolysis is tightly regulated to ensure that the flux of energy from the adipocyte is synchronized to the immediate metabolic needs of peripheral tissues and is governed by the overall nutritional status of the body. To that effect, hormone-sensitive lipase, the rate-limiting enzyme in this process, is subject to several levels of regulation including phosphorylation/dephosphorylation, translocation, and association with regulatory proteins. The protein-protein interactions detailed within this report represent a unique mechanism by which HSL, and the overall process of lipolysis, is regulated within the adipocyte. Herein, we examine the interaction of different FABPs with HSL using isothermal titration calorimetry in the presence or absence of fatty acids.

Using isothermal titration microcalorimetry, our results demonstrated that A-FABP and E-FABP physically associate with HSL in a fatty acid-dependent manner with high affinity and specificity (K<sub>d</sub> = 0.5 and 3 nM, respectively). The thermodynamic parameters suggest significant enthalpic and entropic components to the reaction, consistent with the mapping of the FABP-HSL binding site to His<sup>194</sup> and Glu<sup>199</sup> (9) and to interfacial binding. Two other family members, I-FABP and L-FABP, that exhibit the same overall fold yet have very different amino acid compositions did not interact with HSL under any conditions.

Second, in an attempt to distinguish binding from catalysis, the effects of the different FABPs on HSL hydrolytic activity were examined in vitro using cholesteryl oleate and pNPB as substrates. Unexpectedly, all of the FABPs (A-FABP, E-FABP, H-FABP, L-FABP, and I-FABP) stimulated HSL activity ~2-fold when using cholesteryl oleate as substrate. Moreover, a non-fatty acid binding mutant of A-FABP (R126Q) as well as glutathione S-transferase did not activate HSL. Importantly, the activation of HSL by the FABPs was dependent on their ability to bind fatty acids and not physical association, because L-FABP and I-FABP did not associate with HSL in the calorimetry experiments. None of the FABPs was able to stimulate HSL activity when pNPB, a water-soluble substrate producing a short chain fatty acid product, was used to assess HSL esterase activity. Although this is consistent with the interpretation that the activation of HSL by the FABPs is dependent on their ability to bind fatty acids, it should be noted that conformational changes accompanying protein kinase A-induced phosphorylation of HSL increase hydrolytic activity against cho-

**FIG. 3. Effect of FABPs on HSL activity in vitro.** HSL cholesterol esterase activity assays were performed in the presence of different amounts of FABPs. The results are the averages ± S.D. of triplicate samples and are representative of at least three independent experiments. The white bars represent HSL alone, and the light gray, dark gray, and black bars represent 0.1:1,1:1, and 10:1 ratios of FABPs to HSL, respectively. The asterisks indicate significance with p < 0.05 by comparing the activity of HSL in the presence of the indicated FABP with that with GST.
lesteryl ester and triacylglycerol substrates but have no effect on hydrolysis of pNPB.

The results presented in this report also describe E-FABP as also potentially involved in forming part of the lipolytic complex in adipose tissue. Results from FABP animal models paralleled these observations (15, 16). Overexpression of E-FABP in fat cells of transgenic mice resulted in a surprisingly significant increase in lipolysis, suggesting that E-FABP, like A-FABP is a likely partner with HSL in mediating lipolysis. Interestingly, the pattern of HSL expression in different tissues is very similar to that of FABPs. HSL is expressed in adipose tissue, ovary, testes, heart, skeletal muscle (27, 28), pancreatic β cells (29), and adrenal gland (30) and has recently been identified in the intestine (31). The present studies did not detect any interaction between HSL and liver or intestinal FABP. The liver and the kidney (27) do not express HSL, although other lipases are present in these tissues. As such, interaction of HSL with L-FABP would not likely occur physiologically. Although the expression of HSL in the intestine is localized to the same mucosal cells where I-FABP is expressed, an interaction between the two proteins is unlikely because of the divergent structural differences between I-FABP and the other FABPs.

Our results demonstrate that all FABP forms are capable of activating HSL and that activation is linked to the relief of product inhibition. This result is consistent with the long standing observation that albumin, a nonphysiological fatty acid-binding protein, is needed for optimal in vitro activity. This point is supported by two experimental findings. First, the non-fatty acid binding A-FABP mutant (R126Q) and GST fail...
to activate HSL. Second, none of the FABPs stimulated HSL activity when pNPB was used as a substrate. This conclusion is somewhat at odds with previous studies where a different non-fatty acid-binding protein mutant form (R126Q,Y128F) was shown to partially protect HSL against product inhibition (9). However, in those experiments, FABPs were provided as GST fusion proteins, and the assay conditions differed by the absence of detergent and a less stringent control of the fatty acids present.

Unexpectedly, the interaction between the FABPs and HSL was dependent on the presence of fatty acids. The fatty acids present in the calorimetry experiments partition into the detergent and are either associated with HSL directly or induce an altered micelle structure that in turn results in an HSL conformation that allows for FABP association. Previous work has shown that fatty acids at high concentration inhibit the lipase (9), presumably by binding to the product site of the reaction. Our results suggest that such fatty acids may induce a conformational change in the protein so as to allow access of FABPs to the N-terminal domain. Although speculative, this concept is not without precedent within the family of neutral lipases. Derewenda et al. (32) have suggested on the basis of x-ray analysis of fungal lipases that the tertiary flexibility linked to “interfacial activation” is complex and involves multiple conformational states dependent, in part, on the occupancy of the catalytic domain by monoacyl lipids. This view is schematically depicted in Fig. 6. In this model, fatty acids produced through catalysis induce a conformational change that allows for apo-FABP binding to the region surrounding amino acids 190–200 of HSL. Close physical proximity of FABP to the catalytic C-terminal domain facilitates efficient release of fatty acids from the active site and relieves product inhibition. Subsequent release of holo-FABP from the N-terminal docking domain allows for the cycle to be repeated. Metabolically, the requirement of fatty acids associated with HSL for the interaction with FABPs suggests a unique feed forward mechanism and implies that the lipolytic complex formed by A-FABP and HSL is likely to occur at the lipid droplet surface where relatively high concentrations of fatty acids are generated during lipolysis and not within the cytoplasm. This proposal is currently being evaluated.

In summary, the present work demonstrates that HSL interacts with A-FABP and E-FABP in a fatty acid-dependent manner and that such an interaction represents a novel feed forward mechanism for regulating HSL activity. It should be noted, however, that the work described was carried out utilizing nonphosphorylated HSL. Similar studies are currently underway using in vitro phosphorylated HSL to assess the role of protein phosphorylation in FABP association.

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![Fig. 6. Schematic model of HSL activation by A-FABP and fatty acids.](image-url)
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REFERENCES
1. Londos, C., Brasaemle, D. L., Schultz, C. J., Adler-Wailes, D. C., Levin, D. M., Kimmel, A. R., and Rondinone, C. M. (1999) *Ann. N. Y. Acad. Sci.* **892**, 155–168
2. Helm, C., Osterlind, T., Laurell, H., and Contreras, J. A. (2000) *Ann. Rev. Nutr.* **20**, 365–393
3. Kraemer, F. B., and Shen, W. J. (2002) *J. Lipid Res.* **43**, 1585–1594
4. Tornqvist, H., Belfrage, P., and DeGraff, P. (1972) *J. Lipid Res.* **13**, 424–426
5. Fredrikson, G., Tornqvist, H., and Belfrage, P. (1986) *Biochim. Biophys. Acta* **876**, 288–293
6. Contreras, J. A., Karlsson, M., Osterlind, T., Laurell, H., Svensson, A., and Helm, C. (1996) *J. Biol. Chem.* **271**, 31426–31430
7. Holm, C., Belfrage, P., and Fredrikson, G. (1987) *Biochem. Biophys. Res. Commun.* **148**, 99–105
8. Helm, C., Belfrage, P., and Fredrikson, G. (1987) *Biochem. Biophys. Res. Commun.* **148**, 99–105
9. Kraemer, F. B., Patel, S., Saedi, M. S., and Sztalryd, C. (1993) *J. Lipid Res.* **34**, 663–671
10. Winzell, M. S., Svensson, H., Arner, P., Ahren, B., and Holm, C. (2001) *Diabetes* **50**, 2225–2230
11. Cook, K. G., Lee, F. T., and Yeaman, S. J. (1981) *FEBS Lett.* **132**, 10–14
12. Grober, J., Lucas, S., Sorbeda-Winzell, M., Zagini, I., Mairal, A., Contreras, J. A., Besnard, P., Holm, C., and Langin, D. (2003) *J. Biol. Chem.* **278**, 6510–6515
13. Derewenda, U., Swenson, L., Wei, Y., Green, R., Kobos, P. M., Joergen, R., Haas, M. J., and Derewenda, Z. S. (1994) *J. Lipid Res.* **35**, 524–534