Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels

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Abstract **The availability of mice containing an adipocyte lipid-binding protein (ALBP/aP2) gene disruption allowed for a direct examination of the presumed role of lipid-binding proteins in the mobilization and trafficking of intracellular fatty acids. Total body and epididymal fat pad weights, as well as adipose cell morphology, were unaltered in male ALBP/aP2 disrupted mice when compared to their wild-type littermates. Analysis of adipocytes isolated from wild-type and ALBP/aP2 null mice revealed that a selective 40- and 13-fold increase in the level of the keratinocyte lipid-binding protein (KLBP) mRNA and protein, respectively, accompanied the ALBP/aP2 gene disruption. Although KLBP protein was significantly up-regulated, the total lipid-binding protein level decreased 8-fold as a consequence of the disruption. There was no appreciable difference in the rate of fatty acid influx or esterification in adipocytes of wild-type and ALBP/aP2 null animals. To the contrary, basal lipolysis decreased approximately 40% in ALBP/aP2 nulls as compared to wild-type littermates. The glycerol release from isproterenol-stimulated ALBP/aP2 null fat cells was similarly reduced by ~35%. Consistent with a decrease in basal efflux, the non-esterified fatty acid (NEFA) level was nearly 3-fold greater in adipocytes from ALBP/aP2 nulls as compared to wild-type animals. The significant decrease in both basal and isoproterenol-stimulated lipolysis in adipose tissue of ALBP/aP2 null mice supports the model whereby intracellular lipid-binding proteins function as lipid chaperones, facilitating the movement of fatty acids out of the fat cell.**—Ribarik Coe, N., M. A. Simpson, and D. A. Bernlohr. **Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels.** ***J. Lipid Res.*** **1999.** 40: **967–972.**

Supplementary key words adipocyte • lipid binding proteins • lipolysis • fatty acid

The lipid-binding proteins are a family of intracellular, 15 kDa proteins capable of high-affinity, selective binding of hydrophobic ligands such as fatty acids, bile salts, and retinoids (1–3). Several tissues, including adipose, liver, intestine, heart, and brain express high levels of lipid-binding proteins. To date, there are over 15 members of the family, each expressed from a single gene although pseudogenes are present in at least two family members (4). It has been postulated that the diversity of lipid-binding proteins is necessary for the proper solubilization and trafficking of fatty acids within the varying intracellular milieu (3). Although these cytosolic proteins are highly expressed in tissues with active lipid metabolism, a specific physiological role has yet to be firmly established for any member of this family.

Adipocytes synthesize and store metabolic energy in the form of triacylglycerol in response to nutrient abundance and hydrolyze cellular lipid reserves in response to nutrient depletion (5). As such, fat cells carry out hormonally controlled, bi-directional lipid trafficking. Insulin promotes fatty acid internalization, esterification, and triacylglycerol storage while various catecholamines and glucagon promote triacylglycerol hydrolysis and fatty acid efflux from the adipocyte. Intracellular lipid-binding proteins are postulated to facilitate the intracellular trafficking of such lipids, although the exact mechanism of lipid exchange is largely uncharacterized. To date, the adipocyte lipid-binding protein (ALBP, also known as aP2) and the keratinocyte lipid binding protein (KLBP, epidermal-FABP) are the only LBPs found in adipose tissue. ALBP/aP2 forms a 1:1 complex with a variety of long-chain saturated and unsaturated fatty acids with affinities that range from 0.1 to 5.0 μm (6–8). KLBP is a minor lipid-binding protein found in fat cells (7). The amino acid sequence of KLBP is 70% similar and 50% identical to that of ALBP/aP2.

Hotamisligil and colleagues (9) have recently reported the construction of transgenic animals harboring a targeted disruption of the ALBP/aP2 allele. Animals homozygous for the disruption are phenotypically normal when maintained on low-fat (4% fat) diets. In contrast, when such animals are maintained on a high-fat diet (40% of calories from fat), the null animals remained in-

Abbreviations: ALBP/aP2, adipocyte lipid-binding protein; KLBP, keratinocyte lipid-binding protein; NEFA, non-esterified fatty acids; FABP, fatty acid binding protein; PCR, polymerase chain reaction.

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sulinormic and euglycemic while wild-type and heterozygotic littermates developed hyperglycemia and hyperinsulinemia (9), two hallmarks of obesity-induced non-insulin-dependent diabetes mellitus.

The availability of a murine ALBP/aP2 null line has allowed for the examination of the role of lipid-binding proteins in adipose cellular fatty acid metabolism. To avoid hormonal and metabolic complications due to high-fat diets (hyperglycemia and hyperinsulinemia), we have focused our attention on animals maintained on a low-fat (4% fat) diet. We report here metabolic evidence for the participation of ALBP/aP2 in intracellular fatty acid trafficking, and identify a molecular defect associated with basal and isoproterenol-stimulated lipolysis in ALBP/aP2 null mice.

EXPERIMENTAL PROCEDURES

C57Bl/6J wild-type and transgenic mice

A breeding pair of C57Bl/6J transgenic mice was a generous gift from Bruce Spiegelman and Gökhan Hotamisligil, Dana-Farber Cancer Institute, Boston, MA, and a transgenic colony was developed. All C57Bl/6J wild-type, heterozygous, or aP2 null adult male mice used for these were maintained on a 12 h light/12 h dark cycle at 70°F and fed a standard laboratory chow diet (Tech Lab, 4% fat) ad libitum. Wild-type and ALBP null mice were appropriately age-matched for all studies. Genetic integrity was maintained by appropriate matings of heterozygous siblings to ensure common ancestry within three subsequent generations. All animals were subjected to PCR analysis of tail DNA preparations as described (10) to provide genetic identity. All procedures involving mice were approved by the University of Minnesota Committee on Animal Care and Use.

Preparation of isolated adipocytes

Murine adipocytes were isolated based on the protocol established for Sprague-Dawley rat adipocytes with minor modifications (11). For metabolic studies, murine epididymal fat pads were removed, pooled, and digested at 37°C for 60 min with shaking. The digestion buffer contained 1–3 mg/mL collagenase (CLS 1, Worthington Biochemicals) and 20 mg/mL bovine serum albumin (BSA type V, Sigma) in Krebs-Ringer solution (118 mm NaCl, 4.75 mm KCl, 1.2 mm KH_2PO_4, 2.44 mm $MgSO_4$, 25 mm $NaHCO_3$, 2.52 mm $CaCl_2$). Fat cells were recovered from the digested fat pads by slow-speed centrifugation at room temperature and washed with Krebs-Ringer solution by repeated centrifugation. Prior to all metabolic studies, cell viability was evaluated by trypan blue exclusion and cells were counted using an improved Neubauer hemacytometer (American Scientific Products). As assessed by light microscopy, the size distribution of fat cells was not different between wild-type and ALBP/aP2 null mice.

[^{3}H]oleate uptake studies

To determine the kinetics of fatty acid uptake, adipocytes were isolated (representing 6 mice for each genotype) in Krebs-Ringer solution and washed in incubation buffer (125 mm NaCl, 2.6 mm KCl, 5.7 mm Na_2HPO_4, 1.2 mm H_2PO_4, 10 mm Hepes, 5.5 mm glucose, pH 8.5) (12) three times with centrifugation (2000 *g*, 5 min, 25°C). [^{3}H]oleic acid (0.05 μCi, 10 Ci/mmol) and nonradioactive oleic acid (1.2 nmol) were dried under N_2 in a polystyrene tube prior to the addition of 36 μL bovine serum albumin (1.2 nmol in incubation buffer). The total concentration of oleic acid in the assay was 14 μm yielding an unbound concentration of oleic acid of approximately 100 nm. BSA and oleic acid were preincubated at room temperature for 30 min prior to the start of influx analysis which was initiated by the addition of adipocytes. The reaction was quenched by the addition of 5 mL of stop solution (ice-cold incubation buffer containing 400 μm phloretin and 0.1% BSA) and poured directly onto Whatman filters under vacuum. The filters were washed with an additional 5 mL of stop solution to reduce non-cell-associated fatty acids and the radioactivity remaining on the filters was determined by scintillation counting. Radioactivity due to the non-specific binding of [^{3}H]oleic acid to the filter was determined for all timepoints.

To determine the concentration dependence of fatty acid uptake, isolated adipocytes (representing at least 4 mice for each genotype) were washed in Krebs-Ringer HEPES buffer (118 mm NaCl, 4.75 mm KCl, 1.2 mm KH_2PO_4, 2.44 mM $MgSO_4$, 25 mm $NaHCO_3$, 10 mm Hepes, 2.52 mm $CaCl_2$, pH 7.4) (12) as described above and aliquoted into glass tubes. Uptake was initiated by the addition of 500 μL of [^{3}H]oleic acid to 50 μL of cell suspension (0.7 to 2.7 × 10^4 cells) and quenched after 20 sec in 5 mL ice-cold stop solution (0.1% BSA, 400 μm phloretin, phosphate buffered saline, pH 7.4). The quenched cell mixture was immediately poured onto Whatman filters under vacuum and extensively washed. The non-specific radioactivity was subtracted from all timepoints. To determine the distribution of lipids during this uptake experiment, filters were incubated with acidified chloroform and the released lipids were analyzed by silica gel G thin-layer chromatography (Fisher Scientific) using development in petroleum ether–diethyl ether–acetic acid 80:20:1 (v/v/v). The lipid regions (as determined by comparison to authentic standards of triglycerides and fatty acids) were scraped from the plates and counted to determine the radioactivity in each fraction.

Efflux studies

Fat cells were isolated from epididymal fat pads (n = 8) of wild-type or ALBP/aP2 null mice by collagenase digestion as described. Packed adipocytes were diluted in assay buffer (118 mm NaCl, 4.75 mm KCl, 1.2 mm KH_2PO_4, 2.44 mm $MgSO_4$, 25 mm $NaHCO_3$, 2.52 mm $CaCl_2$, 20 mm HEPES) to a final concentration of 10^5 cells/mL. One hundred μL of diluted adipocytes was used in each assay. Lipolysis was stimulated under standard conditions for 30 min with 100 μm isoproterenol (13, 14). Treatment of primary cultured adipocytes with 100 μm isoproterenol results in optimal lipolytic stimulation in primary cultured adipocytes and has no adverse effects on cell viability (14, 15). Released glycerol was quantitated using the radiometric assay of Bradley and Kaslow (16).

Extraction and separation of lipids from fat cells

Fat pads (n = 6 for each genotype) were combined, weighed, and subjected to collagenase digestion as described above. Total lipids were extracted from a slurry of packed cells of adipocytes by the method of Dole and Meinertz (17) as previously described in detail (7). The NEFA pool level for each genotype was determined utilizing the colorimetric NEFA kit according to the manufacturer's instructions (Wako, Richmond, VA). The data represent the average of pools of three mice (n = 6) analyzed in duplicate for each genotype. The results are presented as nmoles total NEFA per gram of fat tissue.

Western analysis

Detection of KLBP and ALBP in murine epididymal fat pads was accomplished using a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence (Amersham). The mass of ALBP/aP2 and KLBP in fat samples was determined from standard curves using purified lipid-binding proteins expressed in *Escherichia coli* (7, 8).

RESULTS

Hotamisligil and colleagues (9) have reported that in animals maintained on a high-fat diet, the level of KLBP mRNA increases as a consequence of disrupting the ALBP/aP2 allele. To date, ALBP and KLBP are the only members of the fatty acid-binding protein family that have been detected in white adipose tissue. We have previously demonstrated in preliminary studies with experimental animals maintained on low-fat diets, that KLBP mRNA is also uniquely up-regulated in ALBP/aP2 null adipocytes (7). To quantitatively determine the effects of the ALBP/aP2 disruption on KLBP and ALBP protein levels, Western immunoblots were performed. The analysis outlined in **Table 1** demonstrates the expected decline in ALBP/aP2 levels among the wild-type, heterozygous, and ALBP/aP2 null animals, and the subsequent increase in KLBP levels. KLBP is expressed at a low, but detectable, level in fat cells from wild-type C57Bl/6J mice, approximately 1% that of ALBP/aP2. The level of KLBP protein increased 10-fold above basal levels in the heterozygous mice and 13-fold in the ALBP/aP2 null animals. Although KLBP is massively up-regulated in adipose tissue of ALBP/aP2 null animals, it is still expressed at a level much lower than that of ALBP in normal adipose tissue. While we have not exhaustively characterized the expression of all family members in null adipocytes, we have no evidence to suggest that lipid-binding proteins other than ALBP/aP2 and KLBP are expressed in adipocytes (7).

As the intracellular lipid-binding proteins are hypothesized to facilitate fatty acid trafficking, we examined influx and efflux in adipocytes from wild-type and ALBP/aP2 null animals. We reasoned that if ALBP/aP2 played a role in influx and/or efflux, the 8-fold decrease in total lipid-binding protein level coupled with the isoform switch may have measurable effects on metabolism that would lead to inferences about the role(s) of ALBP/aP2 in normal cellular lipid metabolism. Experiments measuring [^{3}H]oleic acid uptake by primary adipocytes isolated from the epididymal fat pads of wild-type or ALBP/aP2 null mice revealed no statistically significant ($P > 0.1$) differences in the kinetic profile of fatty acid internalization (**Fig. 1A**). The concentration of fatty acids yielding half-maximal rates of influx, often referred to as the K_m for transport, was approximately 150 nm for adipocytes from both wild-type and ALBP/aP2 null mice (Fig. 1B), which is comparable to that previously reported in 3T3 cells (18). As with the kinetics of uptake, no statistically significant differences were noted in the apparent K_m for uptake in adipocytes between wild-type and ALBP/aP2 null mice. Moreover, the distribution of internalized ^{3}H-labeled fatty acids among lipid fractions within the adipocytes is essentially identical for wild-type and ALBP/aP2 null mice (**Fig. 2**), suggesting that influx was unaffected and the intracellular metabolism of such fatty acids was unaltered by the lack of ALBP/aP2 or the heightened presence of KLBP. These results do not eliminate a role for lipid-binding proteins in fatty acid influx and esterification, but under the conditions analyzed, we did not observe significant differences between wild-type and ALBP/aP2 null adipocytes in initial uptake.

To evaluate the potential role of ALBP/aP2 in lipolysis, we isolated primary adipocytes from wild-type and ALBP/aP2 null mice and measured glycerol release under basal and isoproterenol-stimulated conditions. In contrast to the influx studies, which indicated no statistically significant differences in uptake between wild-type and ALBP/aP2 nulls, efflux studies revealed a statistically significant difference in both basal and stimulated levels of glycerol release for the two genotypes (**Fig. 3**). In the absence of lipolytic stimulation, the basal level of fatty acid efflux is ~40% lower in ALBP/aP2 null fat cells (4.2 ± 0.8 nmol glycerol released/30 min/10^4 adipocytes) than in adipocytes from wild-type animals (7.4 ± 0.7 nmol glycerol released/30 min/10^4 adipocytes). Under conditions of lipolytic stimulation (100 μm isoproterenol), the amount of glycerol released from the ALBP/aP2 null fat cells was significantly compromised (35% lower) (12.5 ± 2.1 nmol glycerol released/10^4 adipocytes/30 min) compared to their wild-type counterparts (19.0 ± 2.9 nmol glycerol released/10^4 adipocytes/30 min) although the fold-stimulation was essentially unaltered. As shown in Fig. 3, if the maximum level of lipolysis observed after isoproterenol treatment is corrected for the contribution of the basal component, the stimulated component of lipolysis was similarly reduced in ALBP/aP2 null mice compared to their normal littermates. These results indicate that both the basal and isoproterenol-stimulated components of the lipolytic process are similarly diminished in the ALBP/aP2 null animals.

Previous analysis has shown that the composition of the triglyceride and fatty acid pools in ALBP/aP2 wild-type and null animals was not different (7). To determine whether the level of the fatty acid pool was altered as a consequence of the ALBP/aP2 disruption, the fatty acid level in adipose cells from wild-type and ALBP/aP2 null animals was determined. Importantly, we found that the total non-esterified fatty acid (NEFA) level was significantly increased in the adipose tissue of ALBP/aP2 null

TABLE 1. Comparison of total weight, fat pad weight, lipid-binding protein levels, and nonesterified fatty acid levels in adipose tissue of wild-type and ALBP/aP2 null mice

Genotype	Total Weight	Fat Pad Weight	ALBP	KLBP	Fatty Acids
	g	*g*	*mg/g*	*mg/g*	*nmol/g*
Wild-type	25.4 ± 0.3	0.27 ± 0.07	60	0.6	525 ± 152
ALBP/aP2 null	26.2 ± 0.3	0.27 ± 0.04	0	8	1474 ± 535[a]

The mass of lipid-binding protein from the adipose tissue of wild-type and ALBP/aP2 null mice (mg per g of total cellular protein) was immunochemically quantitated by Western analysis. Nonesterified fatty acid levels were measured from primary cultured adipocytes as detailed in Experimental Procedures. Mass of the nonesterified fatty acids is expressed as nmol NEFA per g fat tissue for each genotype. Values are mean ± SD of two separate pools of adipocytes. Each pool represents three mice (n = 6) assayed in duplicate.

[a] Indicates that the levels of NEFA in the adipose of ALBP/aP2 null mice is significantly greater than that in wild-type mice based on Student's *t*-test analysis ($P < 0.05$).

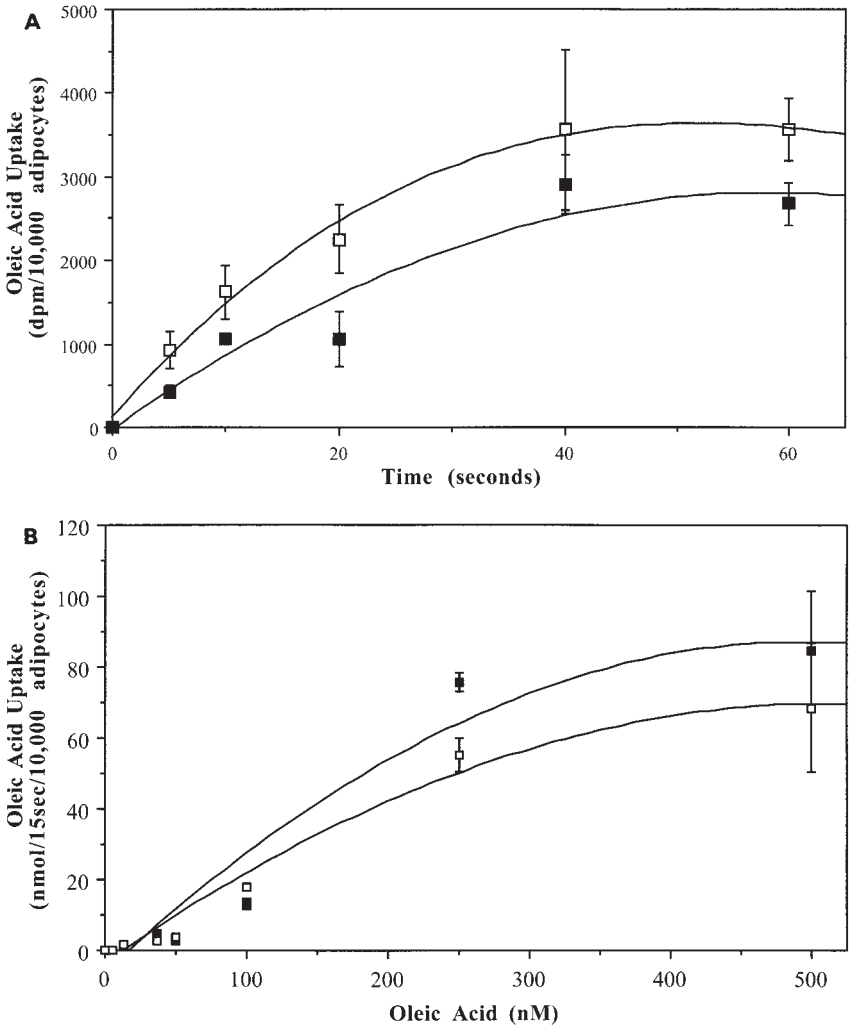

Fig. 1. Fatty acid influx in primary cultured adipocytes isolated from wild-type and ALBP/aP2 disrupted mice. Panel A: Uptake of [^{3}H]oleic acid (100 nm) by primary culture adipocytes isolated from wild-type and ALBP/aP2 null mice as a function of time. Adipocytes from mice (n = 6) of each genotype were obtained by collagenase digestion as described in Experimental Procedures. Time points were assayed in quadruplicate and bars represent the standard error of the mean. Panel B: Concentration dependence of [^{3}H]oleic acid uptake by primary culture adipocytes isolated from wild-type and ALBP/aP2 null mice. Uptake at the indicated fatty acid concentration was quenched in stop buffer after 15 sec as described in Experimental Procedures and assayed five times. Each point was assayed five times and bars represent the standard error of the mean. Closed and open squares represent data from wild-type and ALBP/aP2 null mice, respectively.

mice as compared to that found in adipose tissue of wild-type mice (Table 1). With the methods we used, we could not distinguish between NEFA that are found in the cytoplasm versus those that are membrane-associated. However, NEFAs are in rapid equilibrium with the aqueous environment of the cell and lipid-binding proteins have the capacity to affect the distribution of such lipids (19).

DISCUSSION

The present study demonstrates the up-regulation of the keratinoycte lipid-binding protein in adipocytes of ALBP/aP2 null mice. KLBP is highly up-regulated 40-fold at the mRNA level (7, 9) and greater than 10-fold at the protein level. Immunochemical analysis of other various tissues (brain, lung, intestine, spleen, testes, liver, kidney) isolated from wild-type and aP2 disrupted mice indicated that the up-regulation of KLBP is fat cell-specific (results not shown). The loss of the major lipid binding protein (ALBP/aP2) does not result in altered composition of the adipose fatty acid or triacylglycerol pools (7), changes in fatty acid uptake and reesterification (Fig. 1A, 1B) or differences in the mass (Table 1) or morphology of the adipose tissue. While it is tempting to consider the up-regulation of KLBP as an example of molecular compensation, careful analysis revealed that differences in fat cell metabolism exist between wild-type and null adipocytes.

Examination of the fat cells from such ALBP/aP2 disrupted animals indicated a decrease in both the basal and

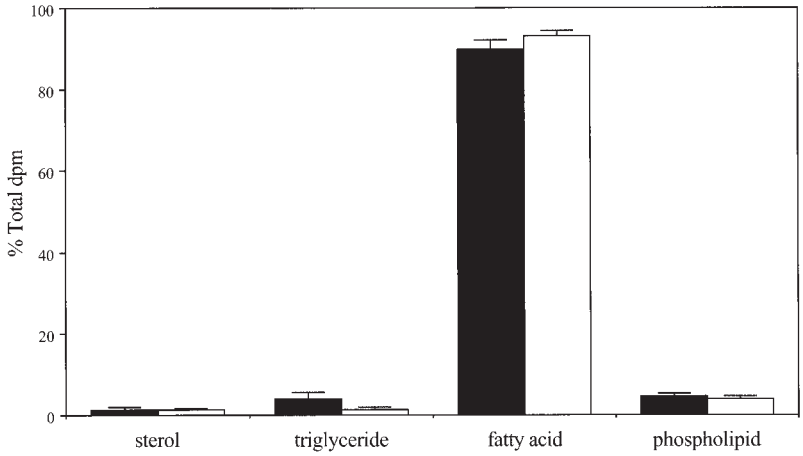

Fig. 2. Distribution of lipid species during influx in adipocytes from wild-type and ALBP/aP2 null mice. Adipocytes from each genotype were incubated with varying concentrations of [^{3}H]oleic acid (10–500 nm) for 15 sec and recovered by filtration. The recovered cells were extracted in acidified chloroform and the lipid species were separated by thin-layer chromatography. Various lipid class were identified by comparison to authentic standards. Results are presented as percentage of total radioactivity. Error bars represent the standard error of the mean of at least two samples assayed in duplicate. Closed and open bars represent data from wild-type and ALBP/aP2 null mice, respectively.

stimulated components of lipolysis and a concomitant 3-fold increase in the total NEFA pool. The inverse relationship between NEFA and the lipid-binding protein level is consistent with ALBP/aP2 as a chaperone for fatty acids. If ALBP/aP2 functioned as metabolic buffer for fatty acids, the measured fatty acid level would likely be directly proportional to the protein level. If, however, ALBP/aP2 functioned as a chaperone to carry a fatty acid between intracellular locales, the loss of the protein would likely result in the accumulation of the ligand. We measured an increase in fatty acid level occurring concomitantly with the decrease in ALBP/aP2 level, consistent with the chaperone model for lipid trafficking.

The decrease in the basal and stimulated components of lipolysis in ALBP/aP2 null mice can be interpreted in the context of model whereby cytosolic lipid-binding proteins facilitate the efflux process. During episodes of fasting or physical exercise, fat cell triacylglycerol stores are hydrolyzed by hormone-sensitive lipase resulting in the cleavage of fatty acids from the glycerol backbone of triacylglycerol. The release of one glycerol molecule is accompanied by efflux of approximately two fatty acids and the reesterification of one fatty acid (5). Although absolute differences in glycerol release (basal and isoproterenol-stimulated lipolysis) are significantly different when comparing adipocytes of wild-type and ALBP/aP2 null mice, the relative fold increases in glycerol release due to isoproterenol stimulation are quite similar: a 2.5-fold stimulation for wild-type and a 2.9-fold stimulation for the ALBP/aP2 null mice. This similar level of stimulation is a direct outcome of a similar 30–40% decrease in both the basal and isoproterenol-stimulated components of lipolysis. Lack of observed change in the fold stimulation of lipolysis (Fig. 3) implies maintenance of hormone-sensitive lipase responsiveness between the two phenotypes. Moreover, the increase in cellular NEFA levels would not likely occur if the effect of ALBP/aP2 on metabolism were on the signaling system resulting in phosphorylation, translocation, and activation of hormone-sensitive lipase. The accumulation of fatty acids intracellularly (Table 1) and compromised lipolysis suggests either a decrease in the efficacy of fatty acid release or a block in the reesterification of liberated fatty acids in adipose tissue of ALBP/aP2 null mice. As there was no difference in the esterification of exogenously provided fatty acids in adipose cells between wild-type and ALBP/aP2 null animals, it is likely that the molecular defect associated with accumulation of NEFA is linked to an inability to traffic fatty acids out of the cell rather than reesterification. Consistent with this, Hotamisligil et al. (9) found a statistically significant decrease in circulating triglycerides in ALBP/aP2 disrupted mice compared to their wild-type littermates.

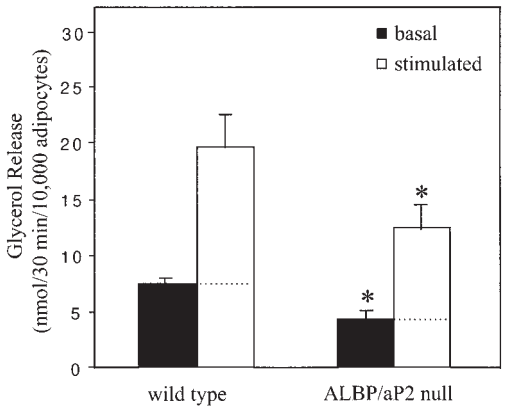

Fig. 3. Fatty acid efflux in primary cultured adipocytes isolated from wild-type and ALBP/aP2 disrupted mice. Glycerol release was determined from adipocytes either unstimulated (basal) or after a 30-min incubation with 100 μm isoproterenol (stimulated) as described in Experimental Procedures. Each bar graph point represents the average from a pool of adipocytes (n = 4 mice, assayed six times) ± standard deviation. The asterisk indicates that the levels of glycerol released are significantly lower in the adipose tissue of ALBP/aP2 null mice than of wild-type mice based on a Student's *t*-test analysis ($P < 0.01$) under both basal and isoproterenol-stimulated conditions. The dashed line indicates the level of the basal component of lipolysis under conditions of maximal stimulation.

Previous work has shown that ALBP/aP2 transfers its fatty acids to acceptor membranes via a collisional mechanism (20). If ALBP/aP2 also accepts fatty acids via a collisional mechanism, one potential site of interaction could be with hormone-sensitive lipase at the surface of the triacylglycerol fat droplet. To this end, it has been noted that a physical complex forms between ALBP/aP2 and hor-

mone-sensitive lipase (21). This interaction was identified using the yeast two-hybrid assay and verified biochemically using glutathione S-transferase fusion protein precipitation assays. Moreover, ALBP/aP2 and hormone-sensitive lipase co-immunoprecipitate in extracts from rat adipocytes. Overall, these results strongly implicate an ALBP/aP2–hormone-sensitive lipase complex as the initial step in mediating efflux of fatty acids from the adipocyte. In the absence of the lipid acceptor (ALBP/aP2) fatty acids accumulate intracellularly, possibly to levels high enough to reduce the intrinsic activity of the lipase (22). Therefore, the 30–40% decrease in both basal and stimulated lipolysis observed in ALBP/aP2 null mice may be due to a combination of lack of an appropriate fatty acid acceptor and/or product inhibition of the lipase from the accumulated fatty acids (22). It is not known whether KLBP forms a complex with the hormone-sensitive lipase. However, the electrostatic surface potential of KLBP is quite dissimilar from that of ALBP/aP2, suggesting that they interact with different molecular partners (23).

In summary, our results demonstrate an increase in NEFA levels and a decrease in cellular lipolysis in adipose tissue of ALBP/aP2 null mice. This report provides the first evidence from transgenic animals indicating a role for lipid-binding proteins in intracellular fatty acid trafficking and suggests that ALBP/aP2 serves the cell as a molecular chaperone for fatty acids destined for efflux.

The authors would like to thank members of the Bernlohr laboratory for helpful advice during the preparation of this manuscript. We would also like to thank Soren Nielsen for preparation of the keratinocyte lipid binding protein antibody. The helpful comments of Constantine Londos concerning lipolysis are gratefully acknowledged. In addition, we would like to thank Bruce Spiegelman and Gökhan Hotamisligil for providing a breeding pair of the ALBP/aP2 null mice, and Anne Johnston Smith and Jun Liu for maintaining the mouse colony. This work was supported, in part, by NIH DK 53189, DK 49807, and NSF 9506088 and 9816575 to DAB. N.R.C. is a recipient of a University of Minnesota Graduate School Doctoral Dissertation Fellowship.

Manuscript received 6 May 1998, in revised form 10 July 1998, and in re-revised form 27 January 1999.

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