The acylation stimulating protein (ASP) is a 76-amino acid peptide that has been proposed as a potent mediator of triglyceride synthesis and, when functionally impaired, as a major cause of hyperapobetalipoproteinemia (HyperapoB). Purification and sequence analysis of ASP from human sera have revealed that ASP is identical to the complement C3-derived activation peptide C3ades-Arg. Because C3 is the precursor for C3ades-Arg and therefore ASP, a deficiency in C3 would be predicted to result in a phenotype characteristic of HyperapoB. To test this hypothesis in vivo, the current study was undertaken in which ASP(C3ades-Arg)-deficient mice were used as a model system. No significant differences were found in the triglyceride, cholesterol, or free fatty acid concentrations in the plasma of fasted normal and ASP(C3ades-Arg)-deficient animals. In addition, plasma lipoprotein analyses indicated that the very low density lipoprotein, low density lipoprotein, and high density lipoprotein cholesterol and triglyceride concentrations as well as the apolipoprotein B-48 and B-100 levels were not significantly different in the plasma of ASP(C3ades-Arg)-deficient and wild-type mice. Furthermore, when challenged with an oral fat load, the ASP(C3ades-Arg)-deficient mice showed no impaired ability to clear triglycerides and free fatty acids from their circulation when compared with their wild-type littermates. Collectively, these results indicate that ASP(C3ades-Arg) deficiency does not cause HyperapoB in mice and that the physiological importance of impaired ASP(C3ades-Arg) function as a cause of hyperapobetalipoproteinemia needs to be reevaluated.

The acylation stimulating protein (ASP) is a 76-amino acid serum protein that was initially discovered in the course of studies to determine the metabolic defect in patients with hyperapobetalipoproteinemia (HyperapoB). The HyperapoB syndrome is a major presentation of familial combined hyperlipidemia, an autosomal dominant disorder that constitutes the most common form of genetic hyperlipidemia. The molecular basis of HyperapoB has not been fully elucidated. Adipocytes and skin fibroblasts from a subset of patients with HyperapoB are impaired in their capacity to synthesize triglycerides (7). Based on this observation, it has been hypothesized that reduced triglyceride synthesis results in a metabolic disruption of the adipocyte-hepatocyte free fatty acid axis (8–10). This disruption will then produce a reduced rate of free fatty acid uptake by adipocytes with an enhanced delivery of free fatty acids and partially triglyceride-depleted chylomicron remnants to the liver. This would in turn cause an increased production of B-100-containing lipoprotein particles, yielding the HyperapoB phenotype.

A protein was subsequently partially purified that stimulated triglyceride synthesis in both fibroblasts and adipocytes in vitro and was called ASP (11). Additional studies suggested that the impairment in triglyceride synthesis in cells from HyperapoB patients was because of a difference in response to ASP (12), which was later suggested to be caused by a decrease in the ASP cell-surface receptor concentration (13). On further purification and characterization, ASP was found to be identical to C3ades-Arg (14), the inactive product of the complement anaphylatoxin peptide, C3a (15). This finding, together with the knowledge that adipocytes secrete proteins of the alternative pathway of complement (C3, Factor B and Factor D/adipsin) (16), led to the proposal of an adipocyte-ASP pathway as a means to generate C3ades-Arg by adipocytes (10, 17–20).

Collectively, these studies have led to a widely accepted hypothesis that ASP is a potent modulator of triglyceride synthesis that, when functionally impaired, would result in the HyperapoB syndrome (10). The recent generation of complement C3 “knock-out” mice (21) has provided an excellent opportunity to test the validity of this hypothesis in vivo. Because the complement protein C3 is the precursor of C3ades-Arg, normal arginine has been removed, also called the acylation stimulating protein; HyperapoB, hyperapobetalipoproteinemia; LDL, low density lipoprotein; FPLC, fast protein liquid chromatography.
which is identical to ASP, these mice will be totally deficient in ASP and would be predicted to exhibit phenotypic characteristics of hyperapobetalipoproteinemia. We present here data examining these mice as a possible murine model of this disease.

**EXPERIMENTAL PROCEDURES**

*Mice*—Generation of the ASP-deficient mice has been described elsewhere (21). Animal care was done in accordance with institution approval and NIH guidelines. Mice were weaned at 3 weeks of age and fed a normal rodent chow diet, containing 22.0% protein, 5% fat, 5% fiber, and 6% ash (PROLAB ISOPRO RMH 3000, SP75) (Purina, Brentwood, MO). The mice were maintained and bred in microisolator cages under a 12 h light-dark cycle in a specific pathogen-free animal facility. Mice were genotyped using tail genomic DNA, polymerase chain reaction, and Southern blotting procedures described previously (21). All experiments were carried out using 6–12-week-old littermates.

**Determination of Total Cholesterol, Triglycerides, and Free Fatty Acids**—After a 4–6 h fast, 300 μl of blood was collected, and plasma was isolated. Total plasma cholesterol was measured in a microtiter assay using a colorimetric enzymatic kit according to the manufacturer’s directions (Sigma). Total plasma triglyceride was measured in a similar microtiter assay (Sigma). Plasma free fatty acids were measured using the Wako Nefna C kit (Wako Chemicals, Richmond, VA) according to the manufacturer’s instructions.

**Lipoprotein Measurements**—Plasma lipoproteins were fractionated from plasma of individual mice by FPLC chromatography using an Amersham Pharmacia Biotech FPLC with two Superose-6 columns (Amersham Pharmacia Biotech, Uppsala, Sweden) connected in series (22). Fractions were collected, and very low density lipoprotein, LDL, and high density lipoproteins peaks were identified and assayed for triglycerides and cholesterol using the enzymatic kits described above.

**Quantitative Analysis of Apolipoproteins** B-48 and B-100—Western immunoblot analysis was performed as described previously (23). Briefly, after denaturation in the presence of 5% 2-mercaptoethanol, plasma samples were separated by 6% SDS-polyacrylamide gel electrophoresis (1 μl of plasma/lane) and transferred onto a nitrocellulose membrane. Detection of apolipoproteins was carried out by incubation with sheep anti-rat apoB followed by peroxidase-conjugated rabbit anti-sheep IgG (Roche Molecular Biochemicals) and detection by ECL (Amersham Pharmacia Biotech). The relative densities of bands on the immunoblot were quantified using an AlphaImager 2000 (Alpha Innotech Corporation).

**Oral Fat Load**—Following an overnight fast, an oral fat load of 400 μl of olive oil was administered by gavage. Blood samples (40 μl) were collected into tubes containing 1 μl of 200 mM EDTA at 0, 1, 2, 3, 4, and 6 h after administration of the fat load, and plasma was isolated. Triglyceride and free fatty acid measurements were done as described above.

|                  | Male (Mean ± S.D.) | Female (Mean ± S.D.) |
|------------------|--------------------|----------------------|
| **Triglycerides (mg/dl)** | 61.3 ± 23.2 (31) | 58.9 ± 24.6 (27) |
| **Cholesterol (mg/dl)**   | 76.3 ± 24.3 (27)  | 75.6 ± 20.3 (18)   |
| **Free fatty acids (mM)** | 0.76 ± 0.2 (11)  | 0.79 ± 0.2 (14)    |

**Results**

Table I summarizes the plasma lipid and apolipoprotein concentrations in wild-type and ASP-deficient male and female mice. The average triglyceride, cholesterol, and free fatty acid concentrations in the plasma of ASP-deficient male mice were 61.3 ± 23.2 mg/dl, 76.3 ± 24.3 mg/dl, and 0.76 ± 0.2 mM, respectively. These concentrations were not significantly different from the plasma concentrations determined in the wild-type male mice (triglycerides, 58.9 ± 24.6 mg/dl; cholesterol, 75.6 ± 20.3 mg/dl; free fatty acids, 0.79 ± 0.2 mM). Similarly, female mice, whether wild-type or ASP-deficient, had similar fasting plasma triglyceride, cholesterol, and free fatty acid concentrations (Table I).

To determine if the absence of ASP resulted in any change in the plasma lipoproteins, the plasma samples from fasted wild-type and ASP-deficient mice were fractionated by FPLC using two serially connected Superose-6 columns. Fractions of 0.5-ml were collected, and protein, cholesterol, and triglyceride concentrations were determined for each fraction. Shown in Fig. 1 are the FPLC profiles obtained from plasma samples of 9 wild-type and 9 ASP-deficient male mice. The FPLC analysis indicated that the very low density lipoprotein, LDL, and high density lipoprotein cholesterol and triglyceride concentrations were not significantly different in ASP-deficient and wild-type male mice (Fig. 1). The study was repeated using female mice. No significant difference was observed in the very low density lipoprotein, LDL, and high density lipoprotein cholesterol and triglyceride concentrations of the ASP-deficient and wild-type female mice (data not shown). Collectively, the data in Table I and the FPLC profiles (Fig. 1) demonstrate that the plasma lipid and lipoprotein concentrations in fasting mice are not affected by the absence of ASP.

Because no differences in fasting levels of plasma lipids and lipoproteins were detected in the ASP-deficient and wild-type animals, experiments were performed to determine if ASP deficiency alters plasma lipid concentrations following an oral fat load, as persistent postfat load hypertriglyceridemia has been documented in HyperapoB patients (5, 6). This experiment was performed by orally administering 0.4 ml of olive oil to fasted wild-type (10 males, 9 females) and ASP-deficient mice (11 males, 10 females). Blood samples (0.04 ml) were then collected at 1-h intervals, and free fatty acid and triglyceride plasma concentrations were determined at each time point. The results from this study are shown in Fig. 2. There was no significant difference in the plasma concentrations of free fatty acids or triglycerides in the 6 h following the olive oil gavage in ASP-deficient and wild-type mice. Triglyceride concentrations in all animals increased 2–3-fold up to 2 h following the oral fat load. Within 6 h after the olive oil gavage, the plasma triglyceride concentrations returned to fasting levels in both wild-type and ASP-deficient mice. There was a trend toward faster triglyceride clearance in the ASP-deficient male mice, although the difference was not significant (Fig. 2C). These data indicate that, in addition to the similar fasting lipid concentrations, ASP-deficient mice do not have significantly altered levels of plasma triglycerides and free fatty acids following an oral fat load.

As discussed, it has been postulated that a defective ASP...
response causes an enhanced production of hepatic apoB-100-containing lipoprotein particles, the metabolic hallmark of HyperapoB. Mice produce two forms of hepatic apoB, apoB-48, and apoB-100 (25–27). If defective ASP function does cause increased production of hepatic apoB lipoproteins, then the ASP(C3ades-Arg)-deficient mice would be expected to have elevated levels of plasma apolipoproteins B-48 and B-100. To test this possibility, quantitative Western blot analysis was performed on total plasma samples from fasted ASP(C3ades-Arg)-deficient and wild-type mice using a previously characterized sheep anti-rat apoB antibody (23). The results of the Western blot experiment from male and female wild-type and ASP(C3ades-Arg)-deficient littermates are shown at the top of Fig. 3. Densitometric measurements, normalized to an albumin standard, were determined and illustrated as bar graphs at the bottom of Fig. 3. It is clear by Western blot analysis there was no significant difference in the levels of apolipoproteins B-48 and B-100 in the ASP(C3ades-Arg)-deficient and wild-type littermates.

In summary, based on numerous observations in vitro it has

![Fig. 1. FPLC column chromatography of lipoproteins from ASP(C3ades-Arg)-deficient and wild-type littermates. Plasma samples from fasted individual male wild-type (closed circles, n = 9) and ASP(C3ades-Arg)-deficient mice (open circles, n = 9) were fractionated by FPLC using a Superose 6 column as described under “Experimental Procedures.” Fractions of 0.5 ml were collected. Absorbance at 280 nm (top panel), cholesterol (middle panel), and triglyceride concentrations (lower panel) were determined for each fraction. Results are expressed as mean ± S.D.](image-url)
Fig. 2. Kinetic measurements of free fatty acids and triglycerides in ASP(C3ades-Arg)-deficient and wild-type littermates following an oral fat load. Concentrations of free fatty acids and triglycerides were measured in male (A and C) and female (B and D) wild-type and ASP(C3ades-Arg)-deficient mice subjected to an oral gavage of olive oil (400 μl). Closed circles depict the values determined from wild-type mice (10 males, 9 females), and open circles correspond to the concentrations determined from the ASP(C3ades-Arg)-deficient mice (11 males, 10 females). The results are presented as mean ± S.D.

Fig. 3. Western blot analysis of apolipoproteins B-48 and B-100 from plasma of ASP(C3ades-Arg)-deficient and wild-type littermates. Quantitative analysis of apolipoproteins B-48 and B-100 was performed by Western immunoblotting of plasma samples separated by SDS-polyacrylamide gel electrophoresis as described under "Experimental Procedures." A blot showing the results from male (n = 4) and female (n = 6) wild-type and ASP(C3ades-Arg)-deficient mice is shown at the top of the figure. The relative densities of the bands were determined and depicted as column diagrams (mean ± S.D.) at the bottom of the figure.

been proposed that a defect in ASP function is the underlying defect in HyperapoB and that ASP deficiency would lead to the development of this disease. However, the in vivo data obtained in the current investigation, using a mouse model deficient in ASP, indicate that the possible role of impaired ASP function as a cause of HyperapoB needs to be reexamined. If impaired ASP function is a primary cause of hyperapobetalipoproteinemia, then it is difficult to explain why no significant phenotypic differences characteristic of HyperapoB are observed in the plasma lipid and lipoprotein profiles of the ASP(C3ades-Arg)-deficient mice compared with normal littermates. If indeed ASP dysfunction does cause increased production of hepatic apoB lipoproteins, it is surprising that at least some elevation of apolipoproteins B-48 and B-100 was not observed in the ASP(C3ades-Arg)-deficient animals. In addition, plasma lipid clearance was not impaired in ASP(C3ades-Arg)-deficient mice as observed in HyperapoB patients (5, 6). Although ASP function has been found to be similar in mouse and human cells (20), it is possible that mice may not be an ideal model system for these in vivo studies. However, our observations are consistent with other observations in humans. For example, several unrelated human families have been discovered with complete complement protein C3 deficiency (28); these C3-deficient individuals do not appear to display an increased predisposition to hyperlipidemia or to coronary artery disease. Moreover, a recent study indicates that elevated serum C3 levels are associated with an increased prevalence of myocardial infarction (29). This is the opposite of the expected effect of C3, the obligate ASP precursor, if functionally impaired ASP causes HyperapoB, which predisposes individuals to myocardial infarction. In conclusion, our observations indicate that ASP deficiency does not cause HyperapoB in mice and that the role of ASP in HyperapoB in humans needs to be reevaluated.

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