Disruption of the murine intestinal alkaline phosphatase gene *Akp3* impairs lipid transcytosis and induces visceral fat accumulation and hepatic steatosis

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Departments of 1Biochemistry, 2Diabetes and Endocrinology, and 5Morphology, Faculty of Medicine, and 3School of Medical Technology and Health, Faculty of Health and Medical Care, Saitama Medical University, Saitama, Japan; 4Burnham Institute for Medical Research, La Jolla, California; 6Toxicology Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco, Kanagawa, Japan; and 7Department of Medicine, Washington University School of Medicine, St. Louis, Missouri

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Nakano T, Inoue I, Koyama I, Kanazawa K, Nakamura K, Narisawa S, Tanaka K, Akita M, Masuyama T, Seo M, Hokari S, Katayama S, Alpers DH, Millán JL, Komoda T. Disruption of the murine intestinal alkaline phosphatase gene *Akp3* impairs lipid transcytosis and induces visceral fat accumulation and hepatic steatosis. *Am J Physiol Gastrointest Liver Physiol* 292: G1439–G1449, 2007. First published March 1, 2007; doi:10.1152/ajpgi.00331.2006.—Intestinal alkaline phosphatase (IAP) is involved in the process of fat absorption, a conclusion confirmed by an altered lipid transport and a faster body weight gain from 10 to 30 wk in both male and female mice with a homozygous null mutation of the IAP coding gene (*Akp3*+/− mice). This study was aimed to delineate morphologically and quantitatively the accelerated lipid absorption in male *Akp3*+/− mice. Feeding a corn oil bolus produced an earlier peak of triacylglycerol in serum (2 vs. 4 h for *Akp3*+/− and wild-type mice, respectively) and an approximately twofold increase in serum triacylglycerol concentration in *Akp3*+/− mice injected with a lipolysis inhibitor, Triton WR-1339. A corn oil load induced the threefold enlargement of the Golgi vacuoles in male wild-type mice but not in *Akp3*+/− mice, indicating that absorbed lipids rarely reached the Golgi complex and that the transcytosis of lipid droplets does not follow the normal pathway in male *Akp3*+/− mice. Force feeding an exaggerated fat intake by a 30% fat chow for 10 wk induced obesity in both male *Akp3*+/− and wild-type mice, and therefore no phenotypic difference was observed between the two. On the other hand, the forced high-fat chow induced an 18% greater body weight gain, hepatic steatosis, and visceral fat accumulation in female *Akp3*+/− mice but not in female wild-type controls. These results provide further evidence that IAP is involved in the regulation of the lipid absorption process and that its absence leads to progressive metabolic abnormalities in certain fat-forced conditions.

fat absorption; metabolic abnormality; obesity; small intestine

**THE ISOZYMES OF ALKALINE PHOSPHATASE (AP; orthophosphoric monoester phosphohydrolase, EC 3.1.3.1)** are ectoenzymes anchored onto the cytoplasmic membrane via a phosphatidylinositol glycan moiety (23), and they are capable of catalyzing dephosphorylation and transphosphorylation reactions of a wide spectrum of substrates in vitro. The presence of AP isozymes in a range of species from bacteria to humans and their localization in proximity to the cell surface suggest that they play a role in facilitating the movement of substances across the cell membrane. The APs of mice are encoded by five genes: *Akp2, Akp3, Akp5, Akp6* (RIKEN sequence, new AP locus, chr1:87002298–87005230, accession no. AK008000), and *Akp-ps1* (28), which encode non-tissue-specific AP, intestinal AP (IAP), embryonic AP, AKP6 [tentative designation by Prof. J. L. Millán (28)], and a pseudogene, respectively. IAP expression is largely restricted to the gut, especially to the epithelial cells (enterocytes) of the small intestinal mucosa. No severe metabolic abnormalities have been observed in mice with disrupted *Akp3* or *Akp5* genes (30, 31), whereas mice with *Akp2*-null mutations exhibit a severe form of hypophosphatasia and abnormal vitamin B₃ metabolism (11, 32).

IAP has long been known to be associated with lipid absorption on the basis of the following evidence: 1) during fat absorption, parallel increases in IAP activity and triacylglycerol (TAG) concentration are observed in the thoracic duct lymph (15); 2) IAP is associated with chylomicron secretion (24, 25, 40) but not with chylomicron formation (33), and serum IAP levels are correlated with the levels of apolipoprotein B-48, a protein exclusive to intestinal chylomicrons in humans (29); 3) IAP is found in the membrane surrounding the neutral fat droplet in the villi of the intestinal mucosa during fat absorption (24, 25) and is thought to transport dietary lipids from the intestinal tract into the circulation as a component of unilamellar membranes called surfactant-like particles (SLPs) (40); 4) l-phenylalanine (Phe) (22) and somatostatin (2, 19), both of which are inhibitors of IAP activity, attenuate fat absorption in the small intestine. However, the exact biological functions and the mechanism for the involvement of IAP in lipid absorption are still unknown.

Narisawa et al. (31) developed mice with a homozygous null mutation in the IAP coding gene (*Akp3*+/− mice) and observed a faster body weight gain when these animals were fed a high-fat chow (11%). The morphological examination of the proximal intestine of *Akp3*+/− mice showed an increased lipid clearance when these mice were fed a single oral administration of corn oil, indicating that IAP participates in a rate-limiting mechanism that regulates fat absorption in association with SLPs (25). In the present study, we further define morphologically and quantitatively the effect(s) of a homozygous null mutation of *Akp3* in mice by examining histology and

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tissue lipid uptake and by identifying the differences in phenotypic expression between Akp3<sup>−/−</sup> and wild-type (WT) mice with a high-fat (30%) chow.

**MATERIALS AND METHODS**

**Reagents**

A hydrophobic surfactant, Pluronic L81 (BASEF, Washington, NJ), was a kind gift from Dr. Patrick Tso (Dept. of Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, OH). [H]<sub>3</sub>Trioctanoin (specific activity 17.0 Ci/mmole) was purchased from GE Healthcare (Tokyo, Japan). An L-homoarginine (10 mg/kg) was administered by gavage 1 h after a 10 ml/kg corn oil load (10 mg/kg). Mice in one group (n = 5) were fed 10 ml/kg of corn oil containing 50 mg/ml of Pluronic L81. Four hours after these treatments, the mice were killed under light ether anesthesia, and blood samples were collected for TAG measurement. The chylomicron fraction was floated onto saline (d = 1.006) by centrifuging at 16,000 rpm for 30 min. The resulting upper layer containing chylomicrons and VLDL (d < 1.006) was recovered and assayed for TAG.

**Biochemical Assay Methods**

The small intestine was removed from the mice after an overnight fast and cut into four segments of equal length. The segments were opened longitudinally, washed in cold 0.9% saline, and homogenized in 20 mmol/L Tris-HCl, pH 8.0, containing 1% Triton X-100 and 3 mmol/L MgCl<sub>2</sub>. The homogenates were centrifuged at 15,000 rpm for 15 min, and the supernatants were assayed for protein (Bradford method) and AP activity with phenolphosphate as a substrate (Phosphatase test K; Wako). A specimen of liver tissue (0.1 g) was homogenized in 1 ml of 0.1 mol/L phosphate buffer, pH 7.4, and the lipids in the tissue were extracted with isopropanol alcohol for a TAG assay (Triglyceride E test; Wako) or with chloroform-methanol [2:1 (vol/vol)] for a cholesterol assay (Cholesterol E test; Wako) according to the manufacturer’s instructions.

**Postprandial Increase in Serum TAG Level**

We measured the postprandial serum TAG increase in male Akp3<sup>−/−</sup> WT, 129/Sv, C57Bl/6J, and BALB/c mice. After an overnight fast, the mice received an intragastric load of 10 ml/kg corn oil by gavage between 7 AM and 9 AM and were allowed to absorb the oil for the indicated periods. Blood samples were obtained from the tail vein or postcaval vein before and at various times after the corn oil load.

**Effect of Triton WR-1339 on the Postprandial Increase in Serum TAG Level**

The postprandial increase in serum TAG was also evaluated after intravenous injection of Triton WR-1339 (Nacalai Tesque, Kyoto, Japan; 12.5 mg/100 μl phosphate-buffered saline per mouse) to prevent the effect of lipolysis on the postprandial TAG level. Male Akp3<sup>−/−</sup> and WT mice (n = 3 per group) were injected with Triton WR-1339 and were subsequently given or were not given a corn oil load (10 mg/kg). Blood samples were collected from the tail vein 2, 4, and 6 h after the Triton WR-1339 injection.

**Isotope Infusion Studies**

Fasting male mice were force-fed 50 μl of corn oil containing 1.65 × 10<sup>6</sup> cpm of [1<sup>4</sup>H]triolein. This dose was ~20% of the dose used in the postprandial TAG studies to maximize the specific activity of the radiotracer. The mice were returned to their individual cages to allow absorption of the lipid for various periods and were killed under diethyl ether anesthesia. Immediately, their abdomens were opened, and the mice were exsanguinated via the vena cavae. The small intestine, liver, and skeletal muscle (a mixture of the soleus, gastrocnemius, and vastus lateralis) were quickly isolated and frozen in liquid nitrogen. The small intestine was cut into two halves (proximal and distal) and opened longitudinally. The luminal contents were flushed out with a 0.9% saline solution containing 4 mmol/L of sodium taurocholate. The lipids in the tissues were removed, and the remaining small intestine was also extracted according to the method of Bligh and Dyer (3). After the lipid extractions, the chloroform phase was collected, dried in a glass vial, and resuspended in 10 ml of liquid scintillator (Ultima Gold; PerkinElmer, Wellesley, MA). The radioactivity in each vial was measured with a scintillation counter.

**Histology and Ultrastructure**

**Oil Red O staining.** Male Akp3<sup>−/−</sup> and WT mice (n = 2 per phenotype) were fasted overnight, and 10 ml/kg corn oil was administered by gavage. The mice were killed 7 h after the oil load, and the proximal jejunum was immediately removed and fixed overnight in 10% buffered formalin, immersed in phosphate-buffered saline containing from 10 to 20% sucrose, and embedded in optimum cutting temperature compound. Serial sections (6 μm thick) were stained with Oil Red O and were counterstained with hematoxylin and eosin. Liver sections obtained from animals fed a 30% fat chow for 10 wk were stained by the same procedure.

**Electron microscopy.** Male Akp3<sup>−/−</sup> and WT mice (n = 2 per experiment) were killed after fasting or at 7 h after a corn oil load as described above. Jejunum slices were trimmed into small blocks (1 mm<sup>3</sup>), fixed in 2.5% glutaraldehyde for 2 h, and postfixed in a 1% OsO<sub>4</sub> solution buffered with Sorenson’s phosphate at pH 7.4. Then, they were rapidly dehydrated in alcohol and embedded in Epon 812. Thin sections of representative areas were then examined by electron microscopy. Enterocytes visualized (n = 5 per experiment) at ×125,000 were scanned, and the area of the Golgi vacuoles was measured with Image J for Windows software (version 1.31; available at http://rsb.info.nih.gov/ij/). Liver tissue specimens obtained from both male and female Akp3<sup>−/−</sup> and WT mice (n = 2 per experiment) fed 30% fat chow for 10 wk were examined in the same manner.

**Statistical Analysis**

Data are shown as means ± SE. For parametric data, means were compared with the Student’s t-test. For nonparametric data, a Mann-Whitney U-test was used. The statistical analyses were performed with StatView version 5.0 for Windows software (SAS Institute).
RESULTS

AP Activity of the Small Intestine

IAP, AKP6, and embryonic AP are expressed in the small intestine of mice (Ref. 28 and S. Narisawa, M. Joylaerts, K. Doctor, D. H. Alpers, and J. L. Millán, unpublished observations). The two IAP genes are differently regulated in rats. The larger 3.0-kb mRNA encoding the isozyme rIAP-II in the rat intestine has been found to increase following fat feeding (36) or normal feeding of a high-fat chow to the suckling animal (7), whereas the 2.7-kb mRNA encoding the rIAP-I isozyme in the lung was increased with lipopolysaccharide (18), suggesting the different physiological substrates of these isozymes (17). The amino acid sequences of Akp3 and Akp6 reveal close homology to those of the intestinal AP isozymes of rats, rIAP-II (83%) and rIAP-I (91%), respectively (28). The Akp3 gene is the ortholog of the rat IAP II gene (26). These facts also imply the involvement of a murine IAP isozyme in lipid absorption.

The absence of IAP in Akp3<sup>-/-</sup> mice has been confirmed by molecular and immunological methods (31), but their intestinal tissues had never been examined for differences in AP activity. In the present study, we assayed AP activity in homogenates of the intestinal tissues and found that the total AP activity in the proximal one-fourth of the intestine of Akp3<sup>-/-</sup> mice was decreased approximately by half compared with that of WT mice (Fig. 1A), whereas no substantial differences were observed in the other fractions. When L-Phe, a specific inhibitor of intestinal AP isozymes, was added to the enzyme assay mixtures, there was little difference in the AP activity inhibition of the proximal fractions between Akp3<sup>-/-</sup> and WT mice (IC<sub>50</sub> 8.3 ± 0.25 mmol/l for WT and 7.6 ± 0.26 mmol/l for Akp3<sup>-/-</sup> mice; Fig. 1B). L-Homoarginine, an inhibitor of non-tissue-specific AP enzyme activity, had little inhibitory effect, consistent with the data that there are very small amounts of non-tissue-specific AP and embryonic AP in intestinal homogenates. The residual AP activity in the upper small intestine of Akp3<sup>-/-</sup> mice is probably attributable to another “intestinal” isozyme, most likely AKP6, because rIAP-I, which has close homology to AKP6, accounts for 50–90% of the AP activity in the duodenum and jejunum of rats (17).

Earlier Increased Postprandial TAG Peak in Akp3<sup>-/-</sup> Mice

Postabsorptive serum TAG measurement revealed a maximal TAG peak at 2 and 4 h in the male Akp3<sup>-/-</sup> and WT mice, respectively, with a 1.3-fold increase over baseline in the Akp3<sup>-/-</sup> mice and 1.6-fold increase over baseline in the WT mice at the respective peak periods (Fig. 2A). Moreover, in the Akp3<sup>-/-</sup> mice, the area under the curve of the TAG concentrations (over the mean serum TAG level at 0 h) was ~30% greater than in WT mice (Fig. 2B). These findings suggest that the transit of dietary lipids into the circulation is earlier in Akp3<sup>-/-</sup> mice than in WT and that the amount of lipids transferred is greater in Akp3<sup>-/-</sup> mice, supporting our hypothesis that lipids are more rapidly absorbed in Akp3<sup>-/-</sup> mice. The serum TAG responses of the WT and Akp3<sup>-/-</sup> mice to the corn oil load were clearly weaker than those of the C57Bl/6J and BALB/c mice, which exhibited approximately sixfold and threefold postprandial TAG increases, respectively, with peaks 2 to 3 h after the loading (Fig. 2A). This weak postprandial TAG increase in the transgenic and nontransgenic control mice is most likely because the Akp3<sup>-/-</sup> mice have a 75% 129/Sv genetic background; in that strain, no serum TAG increase was observed.

Postprandial Serum TAG Increase Under Inhibitory Conditions of Lipoprotein Lipase

Narisawa et al. (31) showed the rapid clearance of lipid in the small intestine of Akp3<sup>-/-</sup> mice morphologically and hypothesized that the absence of the Akp3 gene resulted in the accelerated fat absorption. Our findings reported above support this hypothesis, but the degree of acceleration is unclear because the postprandial TAG increase in both Akp3<sup>-/-</sup> and WT mice was weak, probably due to a faster lipid clearance from the circulation. We therefore used Triton WR-1339 to
block lipoprotein lipase-mediated lipolysis, and clear differences were observed. Corn oil load by gavage following Triton WR-1339 injection showed an approximately twofold greater TAG increase in $\text{Akp3}^{-/-} / \text{H11002} / \text{H11002}$ than in WT mice, whereas there was little difference between the two phenotypes in the increase of TAG without corn oil load (Fig. 2, C and D).

Increased Lipid Tissue Uptake in $\text{Akp3}^{-/-} / \text{H11002} / \text{H11002}$ Mice in a Lipid Tracer Experiment

Use of a radiolabeled tracer showed greater lipid uptake in the blood and tissue of $\text{Akp3}^{-/-} / \text{H11002} / \text{H11002}$ mice compared with WT mice except in the distal small intestine, although the two genotypes had similar lipid distribution (Fig. 3). The magnitude of the increase was consistent with the twofold increase in area under the curve of postprandial TAG concentrations after Triton WR-1339 injection (Fig. 2, C and D). Narisawa et al. (31) reported rapid clearance of lipid from duodenal enterocytes at 5 h (not at 3 h) after fat feeding in $\text{Akp3}^{-/-}$ mice. The duodenum of $\text{Akp3}^{-/-}$ and WT mice, however, contained similar radioactivity at 5 h after feeding (Fig. 3B). This label in the $\text{Akp3}^{-/-}$ mice could represent lipid that might still be in the form of TAG inside or outside the enterocytes or in the form of complex lipids in cellular membranes, and thus it is difficult to relate these numbers exactly to lipid absorption. Moreover, the lower dose of corn oil in these tracer experiments might obscure differences between the animals. IAP secretion into blood in rats, for example, is evident only with large doses (2 ml) of corn oil (10). The dose of corn oil was approximately one-fourth and one-tenth lower than the corn oil load test in the present experiments and in the studies by Narisawa et al. (31), respectively.

Morphology of Altered Lipid Transport in $\text{Akp3}^{-/-}$ Mouse Enterocytes After a Corn Oil Load

Narisawa et al. (31) observed rapid lipid clearance from the intestinal mucosa in male $\text{Akp3}^{-/-}$ mice after forced corn oil feeding. To better understand how a homozygous null mutation of $\text{Akp3}^{-/-}$ affects lipid metabolism in the enterocytes, we histologically examined sections of the proximal jejunum of WT and $\text{Akp3}^{-/-}$ male mice 7 h after they were fed a bolus of corn oil (10 ml/kg), in contrast to the chronic fat-feeding

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Fig. 2. A: comparison of postprandial serum triacylglycerol (TAG) increases after a corn oil load in different mouse strains and genotypes. Male mice were given 10 ml/kg of corn oil by gavage, and serum TAG levels were measured at the times indicated. The numbers of mice used at each point are shown in parentheses. *$P < 0.05$ and **$P < 0.01$ compared with serum TAG levels at 0 h. B: areas under the curves (AUCs) of the postprandial TAG increase over baseline (mean serum TAG level at 0 h). AUC was obtained from the mean serum TAG level during the period from 0 to 7 h after corn oil infusion by gavage. The areas below each baseline were not included in the calculation for AUC. C: postprandial TAG increase in male $\text{Akp3}^{-/-}$ and WT mice after the injection of Triton WR-1339, a lipoprotein lipase inhibitor. Triton WR-1339 was given intravenously just before the corn oil infusion (10 mg/kg) by gavage. The circulating TAG level was determined after 0, 2, 4, and 6 h after the injections. WT mice injected with (□) and without (○) a bonus of corn oil and $\text{Akp3}^{-/-}$ mice infused with (●) and without (▲) a bonus of corn oil. All mice were injected with 12.5 mg of Triton WR-1339. D: AUCs of increased circulating TAG level during the 6-h period. The area in the $\text{Akp3}^{-/-}$ mice infused with corn oil infusion was approximately twofold greater than that in the WT mice.
conditions used in the previous study (31), to more clearly show the lipid content in the mucosa. Our observations showed that fat droplets were much less evident in the mucosa of Akp3−/− mice than in that of WT mice (Fig. 4, A and B). These observations were consistent with our previous findings (31).

Absorbed fatty acids are resynthesized to TAG in the enterocytes and are formed into lipid droplets (nascent chylomicrons) that are transported to the Golgi complex (20). Enlarged Golgi vacuoles are observed after fat intake because the absorbed lipids accumulate in them (12). IAP is a component of the SLP (24, 25) and is involved in the transport of absorbed lipid; thus we examined the changes in size and morphology of the Golgi complex in the enterocytes of the proximal jejunum, the most active site of lipid absorption, before and after a corn oil load by using electron microscopy to investigate the effect of a null mutation of Akp3 on the lipid transport in the enterocytes. The Golgi vacuoles of the WT mice were massively enlarged with fat in the postprandial state, and their area was increased approximately threefold greater than in the fasting state (Fig. 4H). However, no significant differences were observed in the Akp3−/− mice (Fig. 4, C–F). Moreover, the Golgi complex of the Akp3−/− mice appeared to be more diffused than in the littermate controls (Fig. 4, E and F).

The basolateral cytoplasm of the enterocytes of Akp3−/− mice sometimes contained large lipid droplets close to the nucleus (Fig. 4G). Such large cytosolic lipid droplets have also been observed in conditions in which chylomicron formation was impaired, i.e., in swine chronically fed a chow containing low doses of Pluronic L81 (4) and in mice with a disrupted acyl-CoA:diacylglycerol acyltransferase gene chronically fed a high-fat chow (5). Moreover, exposure of animals to protein synthesis inhibitors results in the formation of similar large lipid droplets (13, 16), probably by preventing lipid transport out from the mucosal cells by impairing lipoprotein formation. In the absence of apolipoprotein B in the intestine, the endoplasmic reticulum lipid particles in enterocytes continue to accumulate lipid and increase in size (39). This interpretation does not fit with the evidence suggesting that lipid absorption is enhanced in the entire animal (Figs. 2 and 3) but does provide some support for a change in the Golgi trafficking of lipids in Akp3−/− mice.

**Acute Inhibition of IAP Activity in the Small Intestine Attenuates the Postprandial TAG Increase in Serum**

Findings obtained by Narisawa et al. (31) and in the present study indicate that a homozygous null mutation of Akp3 re-
Fig. 4. A and B: histological sections of microvilli (Oil Red O and counterstaining with hematoxylin and eosin) from Akp3−/− (A) and WT (B) male mice 7 h after a corn oil load. Bar = 50 \( \mu \)m. C–F: Golgi area of the enterocytes of male WT mice (C and D) and male Akp3−/− mice (E and F) before (C and E) and 2 h after a corn oil load (D and F). Bar = 1 \( \mu \)m; representative of \( n = 5 \) in each group. G: huge lipid droplets (arrowheads) were occasionally seen in the enterocytes of the Akp3−/− mice, but they were never seen in the enterocytes of the WT mice (data not shown). Bar = 1 \( \mu \)m. H: areas of the Golgi vacuoles in the enterocytes 2 h after a corn oil load. CO, corn oil; NS, not significant.
sulted in an accelerated fat absorption; however, other studies have shown that the inhibition of its enzymatic activity decreases intestinal TAG absorption in humans (2, 19, 22). We therefore investigated the effect of AP inhibitors on the postprandial serum TAG levels in C57Bl/6J mice, a strain with a clear TAG increase in serum with oil load by gavage (Fig. 2). Administration of L-Phe markedly inhibited the postprandial TAG increase in male C57Bl/6J mice, but L-homoarginine and D-Phe did not inhibit the increase at all (Fig. 5), indicating that the decreased postprandial triglyceridemia was associated with the inhibition of IAP activity. A similar effect was also seen when the mice injected with the chylomicron inhibitor Pluronic L81, which is known to inhibit lipid absorption (34) and to suppress the postprandial increase in serum TAG by 60–76% in rats (25).

**A Chronic High-Fat Chow Induces Hepatic Steatosis and Visceral Fat Accumulation in Akp3<sup>−/−</sup> Mice**

Narisawa et al. (31) previously observed faster body weight gain in the Akp3<sup>−/−</sup> mice fed an 11% fat chow, although there was no difference in the food intake between the genotypes [Akp3<sup>−/−</sup> vs. WT; P = 0.92 in females (n = 10; WT n = 6); P = 0.52 in males (n = 11; WT n = 6)]. This effect was evident in males rather than in females. Thus, the faster body weight gain in Akp3<sup>−/−</sup> mice was not a result of hyperphagia. To exaggerate the effect of the Akp3<sup>−/−</sup> mutation, mice were fed a 30% fat chow for 10 wk, beginning with the fifth week after birth. At the end of the 10 wk, an 18% greater mean body weight gain was observed in female Akp3<sup>−/−</sup> mice in these conditions, but there was no significant difference in the body weight of male mice (Fig. 6A). Both the male Akp3<sup>−/−</sup> and WT mice had equivalent lipid accumulation in the liver and abdomen (Fig. 6, B and D). It is possible that such a high fat intake overwhelmed the component of fat absorption that may be regulated by IAP.

In females, the mean amount of visceral fat was also approximately twofold greater in Akp3<sup>−/−</sup> mice than in controls (Fig. 6B). Visceral fat accumulation and hepatic steatosis are the major triggers of glucose intolerance in humans, but there were no significant differences in the fasting glucose levels between the genotypes in the male and female mice (Fig. 6C). After 10 wk of feeding on a 30% fat chow, hepatic steatosis was observed in the female Akp3<sup>−/−</sup> mice (Figs. 6 and 7), but no abnormal findings were observed in the livers of the female WT mice. The livers of female Akp3<sup>−/−</sup> mice showed pallor of the hepatic lobule and considerable lipid deposition (Fig. 7, B and F). Also, these livers had significantly higher mean TAG and cholesterol contents than those of the littermate controls (Fig. 6, E and F). Electron microscopic examination of the livers showed intracellular lipid inclusions and a number of lipid droplets, indicating greater lipid accumulation in the hepatocytes of female Akp3<sup>−/−</sup> mice (Fig. 7, G and H). The lipid droplets of Akp3<sup>−/−</sup> mice were brighter than those of WT mice, suggesting that the livers of Akp3<sup>−/−</sup> mice had a higher saturated fatty acid content of TAG (14). The liver weight of the Akp3<sup>−/−</sup> and WT mice did not differ significantly after correction for body weight (Fig. 6D). All male mice had hepatic steatosis and elevated liver TAG and cholesterol content.

**DISCUSSION**

Involvement of IAP in lipid absorption has long been proposed, with a number of experimental studies supporting this putative role. However, the extent to which this isozyme contributes to this process is still unclear. The results of the present study provide three further pieces of evidence relating the physiological functions of IAP to fat absorption: 1) faster dietary lipid transport in male Akp3<sup>−/−</sup> mice; 2) lack of enlargement in Akp3<sup>−/−</sup> mice of the enterocytic Golgi vacuoles with fat following corn oil gavage (6), indicating an
altered lipid transport by the null mutation; and 3) induction by 30% fat feeding of visceral fat accumulation and hepatic steatosis in the female Akp3−/− mice but not female WT mice. These findings further support the hypothesis that IAP is involved in a rate-limiting mechanism that regulates lipid absorption and that the Akp3−/− mutation leads to an accelerated lipid transport in the small intestine.

SLP, a product of the enterocytes, appears to be involved in fat absorption (25). Intracellular lipid droplets are surrounded by a membrane that very likely derives from SLP. IAP, a characteristic enzyme for SLP, is enriched in SLP (8, 9) and is present on the surface (surrounding membrane) of the lipid droplets. IAP expression enhances SLP production and secretion (37). In addition, IAP on the lipid droplets colocalizes with cubilin, a protein known to be involved in transcytosis and contained in SLP (24). These facts suggest that IAP plays a crucial role in the lipid transcytosis by SLP. Our hypothesis is that SLP regulates the transcytotic movement, possibly by protein-protein interactions, and thereby the absence of IAP disturbs the control and results in acceleration of fat transport. The degree of change noted in these studies suggests that IAP production may help to regulate the rate of fat absorption but is not required for the process to occur.

Consistent with this hypothesis, in the present study we have observed greater circulating TAG levels and increased tissue lipid uptake levels in male Akp3−/− mice after a corn oil load. Quantitative analysis showed approximately a twofold greater postabsorptive serum TAG increase in the male Akp3−/− mice. Furthermore, the absorbed fat did not appear to be properly transported to the Golgi complex by SLP in Akp3−/− mice. Normally, intracellular lipid droplets derived from dietary fat are transported to or retained by the Golgi complex and then are exocytosed basolaterally as chylomicrons. The enlargement of the Golgi vacuoles observed in the enterocytes of WT mice is consistent with this model, but such a phenomenon was not observed in the Akp3−/− mice. The finding is consistent with either a very rapid transit time through the Golgi complex, using the normal pathway for fat absorption, or an inability to utilize the Golgi for intracellular lipid trafficking. If the latter possibility is considered, one needs to ask how dietary lipids are delivered to the circulation and what pathway might be used to transport them in Akp3−/− mice. Although no data are

Fig. 6. Differences in the appearance of Akp3−/− and WT mice after 10 wk on 30% fat chow. A–C: body and visceral fat weights after 10 wk on 30% fat chow. Feeding a high-fat chow for 10 wk induced greater body weight and visceral fat of female Akp3−/− mice than in female WT mice, but no significant changes were found in the males. Fasting blood glucose levels were not significantly different between Akp3−/− and WT mice in both males and females. D–F: Liver weight (D), TAG (E), and cholesterol (F) contents in the liver of the male and female Akp3−/− and WT mice after 30% fat chow for 10 wk. The numbers of mice examined are shown in parentheses. KO, knockout, i.e., Akp3−/− mice.
available at present, several studies have reported that the small intestine appears to utilize other pathway(s) when the lipid absorption mechanism is disturbed, and such a compensatory mechanism might be operating in \textit{Akp3}^{-/-} mice. For example, Buhman et al. (5) observed no fat malabsorption in mice with disruption of the gene coding for acyl-CoA:diacylglycerol acyltransferase, the crucial enzyme for resynthesis of TAG in enterocytes. Moreover, the fat-absorption ability of the ileum was increased in rats whose jejunum had been removed (35) or in bile-fistula rats (21, 27), demonstrating the ability of the small intestine to compensate for decreased lipid absorption.

Oral administration of specific inhibitors for IAP isozyme, on the other hand, has been reported to attenuate fat absorption in the small intestine. We confirmed this effect with \textit{l}-Phe, a specific intestinal AP isozyme inhibitor, in C57Bl/6 mice (Fig. 5), which were used because they develop a robust postprandial rise in TAG concentration, unlike the 129/Sv strain, from which the \textit{Akp3}^{-/-} mice were derived (Fig. 2, A and B). The resulting greater increase in serum TAG level after a corn oil load in mice with a null mutation of \textit{Akp3} was inconsistent with this acute effect of inhibiting intestinal AP activity. This discrepancy may be explained by the compensatory ability of the small intestine to absorb lipids. Indeed, Brunelle et al. (4) reported that a single oral dose of Pluronics L81 inhibits fatty acid absorption, whereas the absorptive ability of rats chronically fed a chow containing the drug recovered to normal levels. The difference might also account for the fact that acute administration of \textit{l}-Phe only inhibits IAP on the luminal surface of the intestine (perhaps allowing more lipid endocytosis to occur), whereas the \textit{Akp3}^{-/-} mice produce a defect in IAP not only on the surface but also within the cells along the pathway used during fat absorption, as demonstrated by the fact that IAP is found on the membrane that surrounds intracellular fat droplets (25).

Narimasa et al. (31) observed that mice with a disrupted \textit{Akp3} gene showed faster body weight gain than control WT
mice. In that study, body weight in Akp3−/− mice did not begin to differ from WT mice until 10 wk of feeding, but at 30 wk the animals had gained considerably more weight, affecting males much more than females. In this study, we looked at animals after only 10 wk of feeding to diminish the effects of body weight on the studies of lipid metabolism and have been able to provide additional evidence for the role of IAP in lipid metabolism. In contrast to these previous findings (31), we found no significant differences between Akp3−/− and WT in male mice in the phenotypic appearances. The 30% fat chow might be too much to elicit the difference between them, because both male Akp3−/− and WT mice showed obesity and hepatic steatosis. In the females, however, significantly greater visceral fat accumulation and apparent hepatic steatosis were observed only in female Akp3−/− mice. Although we examined male mice only in the acute fat-feeding responses in the present study, the disruption of Akp3 gene would result in impaired lipid transport in male and female mice similarly. In fact, Narisawa et al. (31) previously found significant differences in the lipid transport of both male and female Akp3−/− mice, whereas the responses to a high-fat chow differed between the genders. It seems possible that IAP may play more of a role when the amount of fat to be absorbed is somewhat limited, either by a lower fat intake (11%) or by differences in the handling of fat in female vs. male mice. An interesting and relevant model is the knockout mouse for intestinal fatty acid binding protein (I-FABP), in which body weight gain was noted in male but not female mice (1). Male mice had changes in lipid morphology, but changes in glucose tolerance were observed only in female, not male, mice. Visceral fat accumulation induces multiple complications, including insulin resistance, hypertension, and cardiac enlargement, which is referred to as the “metabolic syndrome” (38); thus, Akp3−/− mice fed a high-fat chow may be a useful animal model to study the effects of altering lipid metabolism.

The results of the present study are consistent with the hypothesis by Narisawa et al. (31) that the null mutation of Akp3 reduces SLP production, thus disturbing the regulated transcytosis of lipid droplets by SLP. The increased circulating postprandial TAG levels after a corn oil load suggest that the absence of IAP induces faster lipid transport. However, the lipid transport pathway in the absence of IAP and the importance of IAP and SLP in the transcytosis of lipids are still to be clarified. The effect of the disruption of Akp3 is probably affected by the content of fat in the chow. In the present study, we did not find differences in body weight in males with a 30% fat chow, although Narisawa et al. (31) showed an evident difference in the body weight gain in males with an 11% fat chow. Dietary fat content and the period of observation may be important factors to elicit the differences in body weight gain and abnormalities in lipid metabolism. Further studies are needed to determine the most appropriate experimental conditions to elicit this response in male and female mice, respectively.

In conclusion, we quantitatively confirmed approximately up to twofold increase in the rate of lipid absorption in Akp3−/− male mice. In the absence of IAP, lipid droplets appeared not to be properly transported into the Golgi complex, suggesting that IAP is necessary for controlled transcytosis of lipid droplets in association with SLP. Since faster lipid transport occurred when the transcytosis system was impaired, a compensatory lipid transport pathway(s) may be utilized, if indeed lipid is excluded from transfer by the Golgi complex. Faster body weight gain and hepatic steatosis imply that a high-fat chow could induce abnormalities in lipid metabolism in Akp3−/− mice due to deregulated lipid transport in the small intestine.

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