Protection against diet-induced obesity and obesity-related insulin resistance in Group 1B PLA2-deficient mice

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Received 11 March 2002; accepted in final form 23 July 2002


The TH Phospholipase A2 (PLA2) lipolytic enzyme is an abundant protein secreted by the pancreas in response to food intake. It belongs to the Group 1 class B type of secretory PLA2 (7) and is capable of hydrolyzing the fatty acyl bond at the sn-2 position of phospholipids to generate free fatty acids and lysophospholipids in the intestinal lumen. In addition to its prominent expression in the pancreas, the Group 1B PLA2 is also expressed in other tissues (14, 30, 31, 33). Phospholipase A2 has been one of the most extensively studied enzymes in terms of structure and mechanism of action, due to its abundant availability, stability, and ease of isolation. Despite the wealth of information known about the biochemical and structural characteristics of this enzyme (7, 8), the exact physiological function of PLA2 has not been completely delineated.

Phospholipids entering the digestive tract from the diet and bile comprise the second most abundant dietary lipid class found in the intestinal lumen (5). Therefore, it has been suggested that PLA2 functions in hydrolyzing these phospholipids to forms that can be absorbed by the enterocytes. Various in vitro and in vivo studies have also implied that PLA2 hydrolysis of phospholipids in the intestinal lumen is required for the efficient absorption of cholesterol from the diet. Studies in rats and humans have shown that intraduodenal infusion of phosphatidylcholine results in decreased cholesterol absorption compared with subjects infused with lower levels of phosphatidylcholine (2, 17). Mackay et al. (24) identified PLA2 as the major protein in pancreatic extract that mediates cholesterol transport in Caco-2 cells. The addition of PLA2 relieved the phosphatidylcholine inhibition of cholesterol transport from bile salt micelles to Caco-2 cells (18). In addition, our laboratory (39) has shown that PLA2 hydrolysis of phospholipids on the surface of lipid emulsions was required before pancreatic lipase digestion of triglycerides in the core of lipid emulsions, therefore suggesting a role for PLA2 in fat absorption.

To investigate the role of PLA2 in intestinal lipid digestion and transport, we recently generated mice lacking PLA2. Using both lymph fistula and single-dose, dual-isotope fecal recovery methods, we demonstrated that mice deficient in PLA2 had no differences in the absorption of dietary lipids compared with wild-type mice (30). We concluded that, although phospholipid digestion in the intestinal lumen is a prerequisite for efficient absorption of dietary lipids, additional enzyme(s) in the digestive tract can compensate for the
lack of PLA2 in catalyzing phospholipid digestion and facilitating lipid absorption in the PLA2 knockout mice (29). However, these studies were performed under basal chow-fed dietary conditions; therefore, dietary lipid absorption in the PLA2 knockout mice consuming a high-fat diet is not known.

The goal of the present study was to assess the lack of PLA2 in mice fed a western-type, high-fat/high-cholesterol diet. We hypothesize that the compensatory mechanisms present to compensate for the absence of PLA2 under basal chow-fed conditions may be overcome by feeding mice a high-fat diet. We report that PLA2-deficient mice are resistant to high-fat diet-induced obesity and that the mechanism for this effect is most likely due to decreased dietary fat absorption.

**EXPERIMENTAL PROCEDURES**

Generation and maintenance of PLA2-deficient mice. The strategy used to disrupt the PLA2 gene to generate PLA2-deficient mice was described previously (29). All animals used in these studies were back-crossed seven times into the C57BL/6 background and were genotyped by PCR as previously described (29). The mouse colony was maintained in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle and fed a rodent chow (LM485; Harlan-Teklad, Madison, WI) with free access to water. All animal protocols used in this study were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Diet-induced obesity study. Wild-type (PLA2+/+) and PLA2-deficient (PLA2−/−) mice were fed a standard mouse chow (LM485) or a western-type, high-fat/high-cholesterol diet containing 21% fat and 0.15% cholesterol by weight (TD88137, Harlan Teklad) for 16 wk. For the insulin tolerance test studies, PLA2+/+ and PLA2−/− mice were fed a high-fat/high carbohydrate diet (no. F3282; Bioserve Industries, Frenchtown, NJ), which contained 35.5% (wt/wt) fat (primarily lard) and 36.6% carbohydrate (primarily sucrose), for 14 wk. Mice had free access to water during the study period. At the beginning of the diet study, mice were 8–10 wk of age. Body weights were recorded throughout the experimental feeding period.

Food consumption studies. At week 14 of the experimental diet period, individually caged mice were given preweighed food, and the amount of food consumed was determined over a 24-h period for 5 days. The results are expressed as grams of food consumed per day.

Glucose tolerance tests. After an overnight fast, PLA2+/+ and PLA2−/− male mice consuming either the basal low-fat or the western-type diet for 15 wk were injected intraperitoneally with a bolus load of glucose (2 g/kg body wt). Blood was obtained from the tail vein before and 15, 30, 60, and 120 min after glucose administration. Blood glucose was measured using an automated glucose analyzer (Elite XL; Bayer, Elkhart, IN).

Insulin tolerance tests. After a 4-h fast, PLA2+/+ and PLA2−/− female mice consuming either the basal low-fat or high-fat/high-carbohydrate diet for 14 wk were injected intraperitoneally with bovine insulin (1 U/kg body wt; Sigma Chemical, St. Louis, MO). Blood was obtained for glucose determination from the tail vein before and 15, 30, and 60 min after insulin administration.

Euthanasia. After the 16-wk experimental feeding period, the mice were fasted for 4 h and then anesthetized by intraperitoneal injection with a solution composed of ketamine (80 mg/kg body wt; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (16 mg/kg; Butler, Columbus, OH) diluted in 0.9% saline. Body temperature was determined using a rectal thermometer. Blood was removed by cardiac puncture into tubes containing 1 mM EDTA. Adipose (epididymal and uterine fat pads) and brown adipose (intrascapular) tissue, and liver, heart, spleen, lungs, and kidneys were removed, and wet weight was recorded.

**RESULTS**

Mice lacking PLA2 have normal growth, lipid metabolism, and reproductive functions while maintained on a basal low-fat diet (Ref. 29 and unpublished observations). To assess the lack of PLA2 on mice fed a western-type high-fat/high-cholesterol diet for an extended period of time, wild-type (PLA2+/+) and PLA2 knockout (PLA2−/−) male and female mice were fed either a standard mouse chow or a high-fat/high-cholesterol (21% fat, 0.15% cholesterol) diet for 16 wk. There was no difference in body weight between PLA2+/+ and PLA2−/− mice when fed the basal low-fat diet (males: 25.3 ± 0.34 vs. 23.8 ± 0.40 g; females: 23.9 ± 0.40 vs. 21.6 ± 0.60 g, respectively). In contrast, the PLA2+/+ male and female mice gained significantly more weight than the PLA2−/− mice upon being fed the western diet (Fig. 1). This resulted in ∼57 and 40% more weight
Fig. 1. Body weights of phospholipase A₂ \( PLA₂^{+/+} \) and \( PLA₂^{-/-} \) mice after feeding on a western-type diet for 16 wk. Male (A) and female (B) mice 8–10 wk of age were placed on diet containing 21% fat and 0.15% cholesterol (wt/wt) for 16 wk. Body weight of wild-type (●) and PLA₂-null (○) mice was determined every 2 wk. The inset in each panel shows the weight gained from \( PLA₂^{+/+} \) (filled bars) and \( PLA₂^{-/-} \) (hatched bars) mice after 16 wk. Data points represent means ± SD from 4 animals in each group. *Significant difference between the groups, \( P < 0.05 \).

Fig. 2. Tissue weights. White adipose, brown adipose, and liver wet weights from chow-fed male (A), chow-fed female (B), 16-wk western-fed male (C) and 16-wk western-fed female (D) \( PLA₂^{+/+} \) (filled bars) and \( PLA₂^{-/-} \) (open bars) mice are shown. Data are expressed as means ± SD from 4 animals in each group. Statistically significant differences were determined by Student’s t-test.

gained by the male (Fig. 1A, inset) and female (Fig. 1B, inset) \( PLA₂^{+/+} \) mice compared with the \( PLA₂^{-/-} \) mice after feeding on the high-fat diet for 16 wk.

To account for the differences in weight gain between the \( PLA₂^{+/+} \) and \( PLA₂^{-/-} \) mice, various tissues were removed and weighed. Under low-fat dietary conditions, there were no differences in epididymal and uterine fat pad, brown fat, and liver weights between male and female \( PLA₂^{+/+} \) and \( PLA₂^{-/-} \) mice (Fig. 2, A and B). In contrast, the \( PLA₂^{-/-} \) mice had ~50% lower epididymal and uterine fat pad weight compared with \( PLA₂^{+/+} \) mice (Fig. 2, C and D). The increased adiposity was specific for white fat, as there was no difference in brown adipose mass between the two genotypes after high-fat dietary treatment (Fig. 2, C and D). Male \( PLA₂^{-/-} \) mice also had ~36% lower liver weight compared with the \( PLA₂^{+/+} \) mice (Fig. 2C). However, this difference in liver weight was not apparent in the female mice (Fig. 2D). There were also no significant differences in heart, spleen, lung, and kidney weights between the \( PLA₂^{+/+} \) and \( PLA₂^{-/-} \) mice fed the western diet (data not shown). These data suggest that the observed weight difference among the mice fed the western diet was due to increased adiposity in the wild-type mice.

In addition to differences in body weight gain and adiposity upon feeding on a high-fat/high-cholesterol diet, the \( PLA₂^{-/-} \) mice had decreased fasting plasma leptin and insulin concentrations compared with the \( PLA₂^{+/+} \) mice (Table 1). The difference in leptin levels is most likely due to changes in adipose tissue observed between the two groups of mice. In contrast, no signif-
significant difference was observed in fasting plasma glucose and free fatty acid concentrations (Table 1). However, there was a consistent trend toward decreased (20%) fasting glucose levels in the PL'A_{2}^{-/-} mice. Plasma cholesterol and triglyceride levels were similar between the PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice after high-fat dietary treatment (Table 1).

It appears that the high-fat-fed PL'A_{2}^{+/+} mice are more insulin resistant than the high-fat-fed PL'A_{2}^{-/-} mice because the increased fasting plasma insulin concentrations are necessary to maintain normal plasma glucose concentrations. To test glucose metabolism directly in these animals, glucose tolerance tests were performed on PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice under basal low-fat dietary conditions when the animals were similar in weight and adiposity and after feeding on a high-fat diet. There was no difference in glucose metabolism between the PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice consuming the chow diet (Fig. 3A). In contrast, after high-fat feeding, the PL'A_{2}^{-/-} mice displayed lower blood glucose concentrations 15 and 30 min after intraperitoneal glucose administration compared with those observed in the PL'A_{2}^{+/+} mice (Fig. 3B). In addition, insulin levels 30 min after glucose injection were 45% lower (1.15 ± 0.28 vs. 0.63 ± 0.08 ng/ml; P < 0.05) in the PL'A_{2}^{-/-} mice. These data demonstrate that high-fat-fed PL'A_{2}^{+/+} mice have impaired glucose tolerance due to the development of obesity-related insulin resistance, whereas high-fat-fed PL'A_{2}^{-/-} mice maintained normal glucose tolerance.

To test directly the development of obesity-related insulin resistance in these animals, insulin tolerance tests were performed. Both the PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice were fed a low-fat or a high-fat/high-carbohydrate diet for 14 wk. The latter diet has been shown previously to induce obesity and insulin resistance in C57BL/6 mice (36, 37). Interestingly, female PL'A_{2}^{-/-} mice exhibited increased glucose disposal 30 min after insulin injection compared with PL'A_{2}^{+/+} mice under low-fat feeding conditions (Fig. 4A). After feeding on the high-fat/high-carbohydrate diet for 14 wk, the PL'A_{2}^{-/-} mice had lower blood glucose levels 15, 30, and 60 min after insulin injection compared with PL'A_{2}^{+/+} mice (Fig. 4B). These results confirmed that PL'A_{2}^{-/-} mice were protected against the development of high-fat-induced insulin resistance. The data also suggest that PL'A_{2}^{-/-} mice have improved insulin sensitivity compared with PL'A_{2}^{+/+} mice, even under low-fat feeding conditions.

To examine potential mechanisms for the resistance to diet-induced obesity in the PL'A_{2}^{-/-} mice, we performed studies to measure food consumption, body temperature, and postprandial fat absorption. There was no difference in the amount of food consumed per day or resting body temperature between the PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice (Table 1). These data suggest that caloric intake and energy expenditure are not contributing to the resistance to diet-induced obesity in the PL'A_{2}^{-/-} mice. To examine postprandial fat absorption, we injected the mice fed the basal low-fat diet or the high-fat diet for 4 wk with Triton WR-1339 to inhibit lipolysis and suppress lipoprotein clearance from circulation. A bolus load of olive oil containing [3H]triolein was then fed to each mouse by gastric gavage. Lipid absorption efficiency was determined on the basis of the appearance of [3H]triglyceride in the plasma. Results, as shown in Fig. 5A, indicated no difference in the appearance of [3H]triglyceride in the plasma of PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice fed the chow diet. Interestingly, PL'A_{2}^{-/-} mice previously maintained on a high-fat diet had decreased appearance of [3H]triglyceride in the plasma 4 and 6 h after oil administration (Fig. 5B). In addition, there were no significant differences in [3H]triglyceride present in the intestinal wall between PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice (data not shown). These data suggest that the

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**Table 1. Food intake and serum chemistries in PL'A_{2}^{+/+} and PL'A_{2}^{-/-} mice fed western diet**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PL'A_{2}^{+/+}</th>
<th>PL'A_{2}^{-/-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature, °C</td>
<td>36.8 ± 0.5</td>
<td>36.5 ± 0.8</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>2.5 ± 0.4</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>58.5 ± 7.4</td>
<td>61.6 ± 8.2</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>1397 ± 12.0</td>
<td>1368 ± 11.8</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>22.5 ± 6.8</td>
<td>6.3 ± 1.8*</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.92 ± 0.34</td>
<td>0.38 ± 0.10*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>179 ± 32</td>
<td>140 ± 24</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.24 ± 0.02</td>
<td>0.36 ± 0.12</td>
</tr>
</tbody>
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Values are means ± SD. *P < 0.05.
decreased postprandial fat absorption observed in the PLA2<sup>−/−</sup> mice was not due to increased intestinal retention of radiolabel, thus suggesting suboptimal lipid digestion and/or uptake in the intestinal lumen of PLA2<sup>−/−</sup> mice.

The possible difference in fat absorption efficiency between PLA2<sup>+/+</sup> and PLA2<sup>−/−</sup> mice was addressed directly by measuring their fecal lipid output after they were fed the western diet for 4 wk. Fecal lipids were extracted and analyzed by TLC analysis. There was increased lipid in the form of triglycerides, fatty acids, and a lipid band migrating with cholesteryl ester in the PLA2<sup>−/−</sup> mice compared with that observed in PLA2<sup>+/+</sup> mice (Fig. 6). Because the diet contains relatively small amounts of cholesteryl ester, the identity of the fastest migrating band may be retinyl ester, which is known to co-migrate with cholesteryl ester. These data provided additional supporting evidence to document that mice lacking PLA2 have increased fecal lipid output when maintained on a high-fat diet.

**DISCUSSION**

Obesity has developed into a significant health problem in westernized societies over the past 20 years due to its association with various chronic diseases such as non-insulin-dependent diabetes (type 2 diabetes), cardiovascular disease, and cancer (10, 19). The increased prevalence of obesity has been attributed to the increased availability and consumption of fat-rich foods and reduced physical activity (35). This has led many researchers to develop animal models that will allow for the study of the mechanisms by which diet-induced obesity contributes to various disease states.

The C57BL/6 mouse has been shown previously to be a good model for studying diet-induced obesity and diabetes. It develops obesity, insulin resistance, and hyperlipidemia resembling human type 2 diabetes after feeding on a western-type, high-fat diet (22, 36–38). Results obtained from our wild-type mice are consistent with these previous reports. Interestingly, C57BL/6 mice with a PLA2-null mutation were resistant to diet-induced obesity. This resulted in these animals being hypoinsulinemic, hypoleptinemic, and more insulin sensitive compared with their obese wild-type counterparts. The phenotype of these PLA2<sup>−/−</sup> mice is similar to that observed in mice lacking the acyl-CoA:diacylglycerol transferase (Dgat) gene (34) and to the phenotype of animals lacking the protein tyrosine phosphatase-1B (PTP-1B) gene (9). In the Dgat<sup>−/−</sup> mice, obesity resistance was attributed to increased energy expenditure and increased activity (34). Obesity resistance and increased insulin sensitivity in the PTP-1B<sup>−/−</sup> mice was due to alteration in fat metabolism and increased energy expenditure as a conse-

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Fig. 4. Insulin tolerance tests. Insulin sensitivity is shown in female PLA2<sup>+/+</sup> (●) and PLA2<sup>−/−</sup> (○) mice fed a chow (A) or high-fat/high-carbohydrate diet (B) for 14 wk. Animals were injected ip with bovine insulin (1 U/kg body wt) after a 4-h fast. Blood was obtained from tail vein for glucose analysis. Data are expressed as %fasting glucose levels (means ± SD) from 8–10 animals in each group fed the chow diet and 5 animals in each group fed the high-fat/high-carbohydrate diet. ∗Significant difference from wild-type animals, P < 0.05.

Fig. 5. Postprandial fat absorption. Fat absorption was determined by measuring the appearance of [3H]triglyceride after an intragastric gavage of [3H]triolein in 50 μL of olive oil. After an overnight fast, male PLA2<sup>+/+</sup> (●) and PLA2<sup>−/−</sup> (○) mice fed a chow (A) or western diet (B) for 4 wk were injected via the retroorbital plexus with 12.5 mg of Triton WR-1339. Ten minutes after injection, the mice received an intragastric load of 2 μCi of [3H]triolein in 50 μL of olive oil. Blood samples were taken 1, 2, 4, and 6 h after gavage by tail bleeding. Radioactivity appearing in plasma was determined by liquid scintillation counting. Data are expressed as means ± SD from 7 animals in each group fed the chow diet and 4 animals in each group fed the western diet. ∗Significant difference from wild-type animals, P < 0.05.
PLA\textsubscript{2} in the intestine can partially compensate for the lack of previous observations that additional phospholipase(s) are not sufficient on a high-fat diet suggested that these compensatory phospholipases are not contributing to the resistance to diet-induced obesity in these animals after chronic feeding. One possible mechanism by which this may occur is its putative role in the release of digestive enzymes and/or hormones from the pancreas. It is well established that high-fat feeding results in an increase in pancreatic lipase synthesis and secretion from pancreatic acinar cells (4). This presumably occurs because more lipase is needed to digest the increased amounts of fat entering the small intestine. PLA\textsubscript{2} may be necessary for the upregulation of lipase from pancreatic acinar cells under high-fat conditions. This may occur either through hydrolysis of membrane phospholipids, thereby modifying the properties of the membranes favoring the secretory process, or, alternatively, PLA\textsubscript{2}-catalyzed hydrolysis of phospholipids may generate lipid-signaling molecules, such as lysophospholipids, that are mediators of the secretory process.

Another possible route by which PLA\textsubscript{2} may influence dietary lipid absorption is through an indirect mechanism mediated by its regulation of secretin release. This gastrointestinal hormone is known to be important in the pancreatic adaptation to dietary fat (4). It has also been shown to stimulate insulin secretion and enhance the insulin response to glucose (13). Recent studies have identified the Group 1B PLA\textsubscript{2} as the secretin-releasing factor in the intestinal lumen (6, 21). Because secretin release is stimulated by fat feeding, it is possible that PLA\textsubscript{2}\textsuperscript{−/−} mice have a reduced secretin level due to their lack of this secretin-releasing factor. This, in turn, may influence the fat-stimulated release of lipase leading to reduced fat absorption in these animals. Additional studies will need to be conducted to test this hypothesis.

Although the decreased level of fat absorption is the most likely cause of the protection against diet-induced obesity and obesity-related insulin resistance observed in the PLA\textsubscript{2}\textsuperscript{−/−} mice, other mechanisms may also contribute to this observed phenotype. The increased glucose disposal in response to insulin challenge observed in the PLA\textsubscript{2}\textsuperscript{−/−} mice under low-fat-feeding conditions suggests that PLA\textsubscript{2} gene inactivation may also alter insulin-signaling pathways in a manner similar to that observed in the PTP-1B\textsuperscript{−/−} mice (9, 20). Previous studies have shown that the Group 1B PLA\textsubscript{2} is also expressed in other tissues in addition to its prominent expression in the pancreas (14, 30, 31, 33). The peripheral PLA\textsubscript{2} interacts with specific receptors and modulates cell functions (15). Thus it is possible that defects in PLA\textsubscript{2} interaction with PLA\textsubscript{2} receptors, such as those observed in PLA\textsubscript{2}-deficient mice, may influence insulin sensitivity in a favorable manner. In support of this hypothesis is the report that PLA\textsubscript{2} receptor-defective mice are more resistant to endotoxic shock due to the reduced plasma level of tumor necrosis factor-\textalpha\ (TNF-\textalpha) (16). This cytokine confers insulin resistance in cells by regulating glucose transporter synthesis and by interfering with insulin signaling (26). Accordingly,
Group 1B PLA2 deficiency may lower TNF-α production and increase insulin sensitivity in this manner. Also, cell culture data have suggested that PLA2 participates directly in insulin secretion from pancreatic islets (11, 12, 27). Challenging the PLA2−/− mice with a high-carbohydrate diet without fat may help to elucidate a role for PLA2 in insulin signaling.

In summary, we report that mice lacking Group 1B PLA2 are resistant to diet-induced obesity. This most likely occurs through suppression of dietary fat absorption under high-fat-feeding conditions. Breeding the PLA2−/− mice with a high-carbohydrate diet without fat may help to elucidate a role for PLA2 in insulin signaling.

We thank Drs. Patrick Tso, Laura Woollett, and Phillip Howles for valuable discussions, and Dr. David D’Alessio for assistance with the insulin assay. Nick Schildmeyer and Tara Riddle provided excellent technical assistance to this study. This research was supported by a Program Project Grant (DK-54504) from the National Institutes of Health (NIH). K. W. Huggins was the recipient of a National Research Service Award from the NIH (F32 DK-10065), and A. C. Boileau received a Post-Doctoral Fellowship in Interdisciplinary Nutrition Science from the Dannon Institute.

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