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Group 1B Phospholipase A_2–Mediated Lysophospholipid Absorption Directly Contributes to Postprandial Hyperglycemia

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Abstract

Postprandial hyperglycemia is an early indicator of abnormality in glucose metabolism leading to type 2 diabetes. However, mechanisms that contribute to postprandial hyperglycemia have not been identified. This study showed that mice with targeted inactivation of the group 1B phospholipase A_2 (Pla2g1b) gene displayed lower postprandial glycemia than that observed in wild-type mice after being fed a glucose-rich meal. The difference was caused by enhanced postprandial glucose uptake by the liver, heart, and muscle tissues as well as altered postprandial hepatic glucose metabolism in the *Pla2g1b*$^{-/-}$ mice. These differences were attributed to a fivefold decrease in the amount of dietary phospholipids absorbed as lysophospholipids in *Pla2g1b*$^{-/-}$ mice compared with that observed in *Pla2g1b*$^{+/+}$ mice. Elevating plasma lysophospholipid levels in *Pla2g1b*$^{-/-}$ mice via intraperitoneal injection resulted in glucose intolerance similar to that exhibited by *Pla2g1b*$^{+/+}$mice. Studies with cultured hepatoma cells revealed that lysophospholipids dose-dependently suppressed insulin-stimulated glycogen synthesis. These results demonstrated that reduction of lysophospholipid absorption enhances insulin-mediated glucose metabolism and is protective against postprandial hyperglycemia.

Postprandial hyperglycemia is an early indicator of abnormality in glucose metabolism leading to type 2 diabetes. However, mechanisms responsible for postprandial hyperglycemia have not been identified to date. Previously, we reported that the *Pla2g1b*$^{-/-}$ mice defective in expression of pancreatic group 1B phospholipase A_2 (Pla2g1b) were resistant to diet-induced obesity and diabetes (1). The marginal decrease in lipid absorption efficiency cannot account for the resistance to diet-induced obesity and the increased insulin sensitivity in *Pla2g1b*$^{-/-}$ mice because more fat was absorbed by fat-fed *Pla2g1b*$^{-/-}$ mice than chow-fed *Pla2g1b*$^{+/+}$ mice, yet their weights and plasma glucose levels were similar after 4 months of being fed their respective diets (1). Moreover, increased insulin sensitivity was observed not only in fat-fed *Pla2g1b*$^{-/-}$ mice compared with *Pla2g1b*$^{+/+}$ mice, but also in *Pla2g1b*$^{-/-}$mice fed in basal low-fat/low-cholesterol dietary conditions, when lipid absorption efficiency was identical to that of *Pla2g1b*$^{+/+}$ mice. These results suggested that Pla2g1b has a direct contributory role in diet-induced obesity and diabetes.

Pla2g1b is a lipolytic enzyme that is secreted by the pancreas in response to the ingestion of a meal. Because of its abundant availability, stability, and ease of isolation, Pla2g1b is one of the most extensively studied enzymes in terms of structure and mechanism of action. However, despite the wealth of information known about the biochemical and structural characteristics of this protein (2,3), the exact physiological function of Pla2g1b has not been completely

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delineated. Based on its enzymatic activity against phospholipids and its abundant presence in the digestive tract, Pla2g1b has generally been thought to function only in nutrient absorption, catalyzing the hydrolysis of phospholipids in the intestinal lumen to facilitate dietary lipid absorption (4). However, our recent results with *Pla2g1b*$^{-/-}$mice showed that although phospholipid digestion in the intestinal lumen is a prerequisite for dietary lipid absorption (1, 5), additional enzyme(s) in the digestive tract can compensate for the lack of Pla2g1b for phospholipid digestion in the intestinal lumen of *Pla2g1b*$^{-/-}$ mice, and Pla2g1b is not required for normal lipid absorption under basal dietary conditions (5). Nevertheless, the *Pla2g1b*$^{-/-}$mice were more insulin sensitive and resistant to diet-induced obesity and diabetes than their wild-type counterparts. The current study was undertaken to identify the mechanism by which Pla2g1b contributes to diet-induced insulin resistance. Because Pla2g1b is secreted to the intestinal lumen in response to meal feeding, where it catalyzes phospholipid hydrolysis, we explored the possibility that lysophospholipids, the product formed by Pla2g1b hydrolysis of phospholipids, may promote postprandial hyperglycemia and contribute to diet-induced insulin resistance.

RESEARCH DESIGN AND METHODS

Pla2g1b$^{-/-}$ mice were generated by homologous recombination in embryonic stem cells and backcrossed 10 times into the C57BL/6 background as described previously (5). Wild-type C57BL/6j mice were used as *Pla2g1b*$^{+/+}$ controls. All animals were maintained in a temperature- and humidity-controlled room with a 12-h light/dark cycle. The animals were 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.

Glucose tolerance tests

Pla2g1b$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice were fed with either basal low-fat/low-glucose diet (D12328; Research Diets, New Brunswick, NJ) or diabetogenic high-fat/high-carbohydrate diet (D12331; Research Diets) for 1 week and fasted overnight for 12 h before experiments. On the morning of the study, *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice were fed 4 ml/kg body wt of a bolus glucose-lipid mixed meal containing 50% glucose, 2.6 mmol/l egg phosphatidylcholine, 13.33 mmol/l triolein, and 2.6 mmol/l cholesterol in PBS for oral glucose tolerance tests. Glucose tolerance tests were also conducted by injecting a 50% glucose-PBS solution (2 g glucose/kg of body wt) into the intraperitoneal cavity. Blood was obtained by tail vein bleeding before and at different times after administration of the test meal. Blood glucose and insulin levels were measured by colorimetric assay and radioimmunoassay kits obtained from Wako Chemicals (Richmond, VA) and Linco Research (St. Charles, MO), respectively. For glucose tolerance tests involving testing the effect of lysophosphatidylcholine (LPC), saline with or without LPC (Sigma-Aldrich, St. Louis, MO) was injected (32 mg/kg of body wt i.p.) 5 min before glucose bolus administration.

Tissue glucose uptake assay

Pla2g1b$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice fed with the basal low-fat/low-glucose diet were injected with glucose (2 g/kg body wt i.p.) containing 5 μCi 2-deoxy-[^{3}H]glucose after an overnight fast. Animals were killed after 30 min, and tissues were collected and homogenized. The amount of radiolabeled 2-deoxyglucose taken up by each tissue was quantified by liquid scintillation.

Phospholipid absorption studies

Mice were fasted overnight and then fed by gavage 100 μl of the glucose-lipid mixed meal containing trace amounts of phosphatidyl-[^{3}H]choline. The mice were anesthetized with

ketamine/xylazine after 2 h. Blood was collected from the mesenteric portal vein, and the liver was removed. Lipid was extracted with $CHCl_3$:methanol (2:1, vol/vol), and radioactivity was measured to determine the amount of phosphatidyl-[^{3}H]choline absorbed as lysophosphatidyl-[^{3}H]choline and/or phosphatidyl-[^{3}H]choline.

LPC assay

The concentrations of LPC in plasma and liver homogenates were determined by a modification of the enzymatic assay procedure described by Kishimoto et al. (6). Briefly, 8 μl of sample was preincubated for 5 min in 240 μl of reagent A (0.1 mol/l Tris-HCl, pH 8.0, 0.01% Triton X100, 1 mmol/l $CaCl_2$, 3 mmol/l N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium dihydrate; 10 kU/l peroxidase, 0.1 kU/l glycerophosphorylcholine phosphodiesterase, 1 kU/l choline oxidase) in 96-well plates. The initial absorbance at 600 nm was recorded before initiating the reaction with the addition of 80 μl of reagent B (0.1 mol/l Tris-HCl, pH 8.0, 0.01% Triton X100, 5 mmol/l 4-aminoantipyrine, and 1 kU/l phospholipase B). The incubation was continued for 30 min at 37°C, and the final absorbance at 600 nm was recorded. Total LPC concentration in each sample was calculated by comparing the change in absorbance between the standard and samples after subtraction of the background absorbance recorded before initiation of the reaction. The accuracy of this method was confirmed by comparing concentrations determined by this enzymatic assay with high-performance liquid chromatography analysis.

Cell culture experiments

Human HepG2 and rat H4-2E hepatoma cell lines were obtained from the American *Type Culture* Collection (Manassas, VA) and maintained at 37°C under 5% CO_2 atmosphere in low-glucose Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Confluent cultures were serum-starved with Dulbecco's modified Eagle's medium, and subsequent additions of 5 mmol/l [^{14}C]glucose (0.25 mCi/l), BSA, and LPC were made for a 30-min preincubation. Insulin or empty vehicle was then added to the samples indicated, and incubation was continued for 2 h at 37°C. At the end of the incubation period, medium was removed, and cells were washed extensively with ice-cold PBS and then lysed with 30% KOH containing 5 mg/ml carrier glycogen. The lysate was incubated for 1 h at 60°C, and the glycogen was precipitated with four volumes of ice-cold 95% ethanol. The precipitated glycogen was dissolved in 0.2 N HCl, and the amount of [^{14}C]glucose incorporated into glycogen was determined by liquid scintillation counting. For RNA isolation and quantification, we used cells in duplicate wells treated identically except without radioactive glucose.

RNA quantification

Total RNA was isolated from liver, using a micro-to-midi spin column purification system from Invitrogen (Carlsbad, CA). Then, 5 μg of total RNA was reverse-transcribed with oligo (dT), using a Superscript first-strand synthesis system obtained from Invitrogen. Real-time PCR assays were performed in a BioRad iCycler, using 50 ng of the prepared cDNA with 0.2 μmol/l of each primer indicated in Table 1, 1 × PCR buffer (Invitrogen), 0.2 mmol/l dNTPs, 0.5 × SYBR Green (Molecular Probes), 10 nmol/l fluorescein, and 1 unit *Taq* polymerase (Invitrogen). Each reaction also contained Mg^{2+}at various concentrations, as indicated in Table 1. The samples were denatured at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s and then 30 s at the temperature indicated in Table 1. Amplification of specific transcripts was confirmed by product melt curve analysis. Results are reported as arbitrary units and are normalized to the expression level of cyclophilin mRNA in each sample. The expression levels of each mRNA in wild-type mice under fasting conditions were set to equal 1.0. Statistical analyses were performed, using Student's *t* test.

RESULTS

Reduced lysophospholipid absorption in *Pla2g1b*$^{-/-}$ mice

Although we previously showed no difference between *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice in phospholipid digestion and absorption of the associated fatty acyl chains through the gastrointestinal tract, it remains to be clarified whether the phospholipids were digested and absorbed as nonesterified fatty acids and glycerol 3-phosphorylcholine or as fatty acids and lysophospholipids. The difference in glycerol 3-phosphorylcholine versus lysophospholipid absorption and transport to the liver may have significant impact on regulation of hepatic glucose metabolism. Therefore, additional experiments were performed to test this possibility. A glucose-lipid mixed meal containing phosphatidyl-[^{3}H]choline was prepared and fed to *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice after a 12-h fast. Blood was collected from the portal vein after 2 h, and lipids were extracted for scintillation counting to determine the amount of the [^{3}H]choline head group absorbed intact with the lipid backbone, likely as lysophosphophatidyl-[^{3}H]choline. Results showed that a significant amount of the [^{3}H] choline was absorbed as lipid moiety in portal blood of both *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice (Fig. 1*A*). Importantly, the lipid-associated [^{3}H]choline in portal blood of *Pla2g1b*$^{-/-}$ mice was fivefold lower than that in the *Pla2g1b*$^{+/+}$ mice (Fig. 1*A*).

Hepatic and circulating lysophospholipid levels in *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice

The difference between *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice in the absorption of luminal phospholipids as lysophospholipids was confirmed by measuring lysophospholipid concentrations in plasma and in the liver of these animals under fasting conditions as well as after feeding a glucose-lipid mixed meal. No significant difference in either plasma or liver lysopholipid concentrations was observed between *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice under fasting conditions (data not shown). Feeding of a glucose-lipid mixed meal resulted in the elevation of lysophospholipid levels in the plasma and liver of *Pla2g1b*$^{+/+}$ mice fed control diet (Fig. 1*B* and *C*, respectively). In contrast, lysophospholipid levels in plasma and liver of *Pla2g1b*$^{-/-}$ mice fed control diet remained at fasting levels after feeding the glucose-lipid mixed meal. Furthermore, whereas both mouse lines fed diabetogenic diet had increased hepatic LPC levels, an exaggerated decrease in circulating LPC levels was displayed in mice lacking Pla2g1b. Thus, the meal-induced increase in lysophospholipid levels is most likely caused by Pla2g1b digestion of the dietary and biliary phospholipids in the intestinal lumen and the absorption of the digested product lysophospholipid through the portal blood. The lack of postprandial increase in plasma and liver lysophospholipid levels in the *Pla2g1b*$^{-/-}$ mice is consistent with this interpretation.

Glucose tolerance in *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice

Our previous studies showed that *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice were similar in oral glucose tolerance when maintained on a low-fat chow diet. However, the *Pla2g1b*$^{-/-}$ mice were more insulin sensitive and displayed a more rapid glucose clearance than *Pla2g1b*$^{+/+}$ mice after feeding a high-fat/high-cholesterol Western-type diet for 15 weeks (1). In these studies, glucose tolerance tests were administered after intraperitoneal injection of a bolus load of glucose in saline solution. Because postprandial hyperglycemia is associated with type 2 diabetes after ingestion of a high-carbohydrate/high-lipid mixed meal (7), we compared plasma glucose levels in chow-fed *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice after feeding them an oral glucose meal (2 g/kg body wt) containing 2.6 mmol/l phosphatidylcholine, 2.6 mmol/l cholesterol, and 13.33 mmol/l triglyceride. In contrast to previous results showing no difference in glucose tolerance between chow-fed *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice (1), the inclusion of fat in the oral glucose test meal resulted in the *Pla2g1b*$^{-/-}$ mice displaying significantly less postprandial hyperglycemia compared with that observed in the wild-type *Pla2g1b*$^{+/+}$ mice (Fig. 2*A*). Furthermore, the difference in glucose tolerance after mixed-meal feeding was still observed

after chronic feeding of the animals with a high-fat/ high-glucose diet (data not shown). The age of the mice in which diet-induced hyperglycemia has been achieved is also known to affect glucose tolerance. As such, younger adult 13-week-old mice were tested for sensitivity to glucose via intraperitoneal injection of a bolus load of glucose in saline solution. Postprandial hyperglycemia was found to be reduced in *Pla2g1b*$^{-/-}$ mice compared with *Pla2g1b*$^{+/+}$ mice, in contrast to previous studies utilizing much older mice, despite no accompanying oral administration of a lipid bolus (Fig. 2*B*). These results suggest that postprandial hyperglycemia resistance observed in *Pla2g1b*$^{-/-}$ mice is not limited to a state of extended high-fat feeding, as previously reported, but is also readily apparent in younger chow-fed animals.

Altered luminal phospholipid absorption and decreased postprandial hepatic lysophospholipids may be contributing to the increased postprandial hyperglycemia resistance phenotype observed in *Pla2g1b*$^{-/-}$ mice. To test this possibility, intraperitoneal injections of saline with and without LPC (32 mg/kg body wt) were administered to *Pla2g1b*$^{-/-}$ mice 5 min before testing glucose tolerance with either oral or intraperitoneal delivery of a glucose bolus (2 g/kg body wt). Blood glucose levels were elevated after glucose ingestion in *Pla2g1b*$^{-/-}$ mice injected with LPC in saline compared with mice injected with saline alone (Fig. 2*C* and *D*). Plasma glucose levels in *Pla2g1b*$^{-/-}$ mice injected with LPC were similar to those of *Pla2g1b*$^{+/+}$ mice (Fig. 2*A*). Therefore, regardless of whether glucose is delivered orally or via intraperitoneal injection, the elevation of LPC in the circulation of *Pla2g1b*$^{-/-}$ mice, including hepatic portal circulation, results in a glucose tolerance reminiscent of *Pla2g1b*$^{+/+}$ mice. The reintroduction of LPC into postprandial *Pla2g1b*$^{-/-}$ mice and the associated elimination of the hyperglycemia resistance phenotype suggest a link between circulating LPC levels and glucose intolerance.

Additional experiments were also performed to evaluate the tissues responsible for increased glucose uptake in *Pla2g1b*$^{-/-}$ mice. In these experiments, *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice were injected intraperitoneally with a glucose load (2 g/kg body wt) containing 2-deoxy-[^{3}H] glucose after an overnight fast. Various high-energy metabolism tissues were harvested from the animals after 30 min to determine tissue partitioning of the deoxy-[^{3}H]glucose. Results showed that the *Pla2g1b*$^{-/-}$ mice accumulated significantly more radiolabeled 2-deoxyglucose in liver, heart, and muscle tissues compared with that observed in *Pla2g1b*$^{+/+}$ mice (Fig. 3*A*). The difference is less obvious in white adipose tissues, but a trend toward higher deoxy-glucose uptake was also observed in the *Pla2g1b*$^{-/-}$ mice. The increased glucose uptake by various tissues in *Pla2g1b*$^{-/-}$ mice was not caused by increased insulin secretion by the pancreas because postprandial insulin concentrations in plasma of *Pla2g1b*$^{-/-}$ mice were consistently lower but not statistically different from those observed in *Pla2g1b*$^{+/+}$ mice (Fig. 3*B*). Thus, the *Pla2g1b*$^{-/-}$ mice appeared to be more insulin sensitive, achieving greater glucose tolerance with similar or lower levels of postprandial insulin release than the *Pla2g1b*$^{+/+}$ mice.

Lysophospholipid-dependent reduction of insulin-stimulated glycogen synthesis in hepatoma cell lines

The mechanistic relationship between differences in post-prandial lysophospholipid levels and hyperglycemia in *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice was explored by examining the influence of LPC on glucose metabolism in hepatoma cell lines. The inclusion of LPC with BSA in the incubation medium had no effect on the ability of HepG2 cells to utilize glucose for glycogen biosynthesis under basal conditions (Fig. 4*A*). However, insulin-stimulated glycogen biosynthesis increased approximately twofold in the absence of LPC, and the inclusion of LPC in the incubation medium reduced insulin-stimulated glycogen biosynthesis in a dose-dependent manner. Similar results were found in rat hepatoma H4-2E cells, except that higher concentrations of LPC lowered glycogen synthesis below basal levels (Fig. 4*B*). Analysis of RNA isolated from these cells revealed LPC suppression of glucose kinase gene expression

under both basal and insulin-stimulated conditions, but had no effect on glucose-6-phosphatase (G6Pase) gene expression under both stimulated and unstimulated conditions (Fig. 5*A* and *C*). These results suggest that LPC may play a role in suppressing insulin-induced glycogen synthesis.

Expression of hepatic glucose kinase and G6Pase in *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice

Increasing recent evidence has indicated that one mechanism contributing to abnormal postprandial glycemia is the failure to suppress hepatic glucose production (7,8). Although the in vitro cell culture data showed no difference in G6Pase mRNA levels in cells incubated with or without LPC, it remains possible that the higher postprandial LPC level in *Pla2g1b*$^{+/+}$ mice resulted in less suppression of hepatic glucose production compared with that observed in *Pla2g1b*$^{-/-}$ mice. We isolated RNA from the liver of both fasting and glucose/lipid-fed mice to test this possibility. Results showed no difference between *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice in hepatic expression of glucose kinase and G6Pase under fasting conditions (Fig. 5*B* and *D*). The ingestion of a bolus glucose-lipid mixed meal did not alter hepatic expression of the gluconeogenic enzyme G6Pase in *Pla2g1b*$^{+/+}$ mice (Fig. 5*B*). However, hepatic G6Pase expression was reduced twofold in *Pla2g1b*$^{-/-}$ mice after feeding them the glucose-lipid mixed meal. In contrast, expression of glucose kinase was induced in both *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice after feeding the glucose-lipid mixed meal (Fig. 5*D*). Interestingly, the induction of glucose kinase expression was significantly greater in the *Pla2g1b*$^{-/-}$ mice compared with that observed in *Pla2g1b*$^{+/+}$ mice. These results suggest increased glucose uptake and/or utilization for glycogen biosynthesis in the *Pla2g1b*$^{-/-}$ mice compared with *Pla2g1b*$^{+/+}$ mice. The expression levels of glucose kinase and G6Pase in the livers of *Pla2g1b*$^{-/-}$ mice in both fasting and postprandial states are consistent with the hyperglycemia resistance phenotype reported.

DISCUSSION

Phospholipid hydrolysis in the intestinal lumen is required before triglyceride digestion and absorption of dietary fat and cholesterol through brush border membranes (9–13). The major enzyme for phospholipid hydrolysis in the intestinal lumen is Pla2g1b secreted by the pancreas in response to meal feeding. However, other lipolytic enzyme(s) can serve a compensatory function in catalyzing phospholipid hydrolysis in the absence of Pla2g1b to sustain fat and cholesterol absorption (5). The difference in phospholipid hydrolysis between *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice, as shown in this study, is the products formed and absorbed by the animals. The major phospholipid digestive products absorbed by *Pla2g1b*$^{+/+}$ mice are nonesterified fatty acids and LPC. The absorption of dietary phospholipids as lysophospholipids is consistent with previous reports demonstrating >90% of dietary phosphatidyl-[^{3}H]choline is normally converted to lysophosphatidyl-[^{3}H]choline and transported directly to the liver via the portal circulation (14). However, in animals lacking a functional *Pla2g1b* gene, significantly less dietary phosphatidylcholine was absorbed as LPC (Fig. 1*A*), even though they absorbed similar levels of the fatty acyl moiety of dietary phospholipids as the *Pla2g1b*$^{+/+}$ mice. Thus, the compensatory enzyme for phospholipid digestion in the absence of Pla2g1b is likely to be phospholipase B, a distal intestinal enzyme that hydrolyzes phospholipids at both the *sn*-1 and *sn*-2 positions to produce nonesterified fatty acids and glycerophosphocholine (15,16).

The physiological significance of LPC generated during luminal phospholipid digestion and absorption has not been investigated previously. The availability of *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice, with different capabilities of LPC absorption and transport, provided the opportunity to evaluate the impact of LPC absorption in physiology and pathophysiology. The results of this study showed that LPC absorbed after Pla2g1b digestion of phospholipids in the intestinal lumen contributes to postprandial hyperglycemia by inhibiting glucose uptake by the

liver, heart, and muscle tissues. The reduced level of LPC transported to the liver of *Pla2g1b*$^{-/-}$ mice also resulted in increased postprandial glucose kinase expression (Fig. 5). The increased expression of the glucose utilization enzyme glucose kinase implies that postprandial hepatic glucose uptake and its utilization for glycogen synthesis are more efficient in *Pla2g1b*$^{-/-}$ mice than in *Pla2g1b*$^{+/+}$ mice. The data also showed decreased expression of G6Pase after meal feeding in the *Pla2g1b*$^{-/-}$ mice, suggesting that the *Pla2g1b*$^{-/-}$ mice were capable of suppressing hepatic glucose production in response to the glucose-lipid mixed meal. In contrast, postprandial suppression of G6Pase expression was not observed in *Pla2g1b*$^{+/+}$ mice. This failure to suppress G6Pase expression after a glucose-lipid mixed meal may account for postprandial hyperglycemia and susceptibility to diet-induced insulin resistance and diabetes in *Pla2g1b*$^{+/+}$ mice. Likewise, the ability to downregulate hepatic glucose production, as well as elevated glucose uptake by the liver, heart, and muscle tissues, after glucose feeding may account for the resistance of the *Pla2g1b*$^{-/-}$ mice to diet-induced insulin resistance and diabetes. The increased glucose uptake in tissues of *Pla2g1b*$^{-/-}$ mice also provided evidence that LPC may serve as a negative regulator of this insulin-stimulated process (Fig. 3*A*). Previous studies have shown that LPC can directly inhibit protein-mediated glucose uptake via interaction with glucose transport proteins on cell membranes (17,18). Additionally, LPC may inhibit insulin-stimulated glucose kinase expression in the liver, as demonstrated in cell culture of both HepG2 and H4-2E hepatoma cell lines (Fig. 4), in promoting postprandial hyperglycemia. The LPC-induced decrease in glucose kinase mRNA levels in HepG2 cells, along with elevated glucose kinase mRNA levels observed in lipid/glucose-fed *Pla2g1b*$^{-/-}$ mice compared with those in *Pla2g1b*$^{+/+}$ mice, would suggest a direct role of LPC in glucose kinase gene regulation. Interestingly, despite the lack of an LPC effect on G6Pase mRNA levels in cell culture experiments, G6Pase mRNA levels were found to be lower in lipid/glucose-fed *Pla2g1b*$^{-/-}$ mice compared with those in *Pla2g1b*$^{+/+}$ mice. Thus, the reduced G6Pase mRNA levels observed in vivo may not be a direct effect of LPC, but possibly an indirect effect via communication with other cell types or organs in the intact animal.

The phenotype of resistance to diet-induced obesity and diabetes observed in *Pla2g1b*$^{-/-}$ mice (1) is similar to that observed in *JNK*$^{-/-}$ mice with targeted inactivation of the c-Jun NH_2-terminal kinase (JNK) gene (19). Because LPC has been shown to activate JNK in fibroblasts and HeLa cells (20), and JNK inhibits insulin signaling cascade by inhibiting tyrosine phosphorylation of insulin receptor substrate-1 via serine phosphorylation (21), it is possible that LPC inhibition of glucose uptake by liver, heart, and muscle tissues as well as its suppression of hepatic glucose metabolism are also mediated through activation of JNK. Alternatively, LPC may also inhibit insulin-stimulated glucose metabolism by activation of protein kinase C-α (PKC-α). Previously, LPC activation of PKC-α resulting in the inhibition of insulin-induced protein kinase B/Akt phosphorylation was observed in smooth muscle (22) and endothelial cells (23). Insulin signaling through phosphatidylinositol 3-kinase and protein kinase B/Akt activation was also enhanced in cells with an inactivated PKC-α gene (24,25). Interestingly, the mechanism by which PKC-α inhibits insulin signaling is also related to its ability to induce serine/threonine phosphorylation of insulin receptor substrates and block their tyrosine phosphorylation (26). Thus, LPC may inhibit postprandial insulin signaling in tissues via inhibiting tyrosine phosphorylation of insulin receptor substrates by activating either JNK or PKC-α pathways. It must be noted, however, that these two pathways need not be mutually exclusive. Our future effort will focus on determining whether LPC inhibition of insulin signaling is mediated through either one or both of these pathways.

Regardless of the signaling mechanism(s) by which LPC inhibits postprandial hyperglycemia, results of the current study have major clinical implications. Previous studies have already demonstrated that failure to suppress endogenous glucose production after a glucose-lipid mixed meal is a major contributing factor toward hyperglycemia and type 2 diabetes (7). The results of this study further document that the Pla2g1b-digested product LPC is directly

responsible for suppression of hepatic glucose metabolism. In view of studies showing increased LPC levels in patients with type 2 diabetes (27,28), the current study suggests that inhibition of Pla2g1b may be a viable strategy to prevent the onset of type 2 diabetes in individuals consuming a high-glucose/high-fat diet.

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Glossary

G6Pase
: glucose-6-phosphatase

JNK
: c-Jun NH_2-terminal kinase

LPC
: lysophosphatidylcholine

PKC-α
: protein kinase C-α

Pla2g1b
: group 1B phospholipase A_2

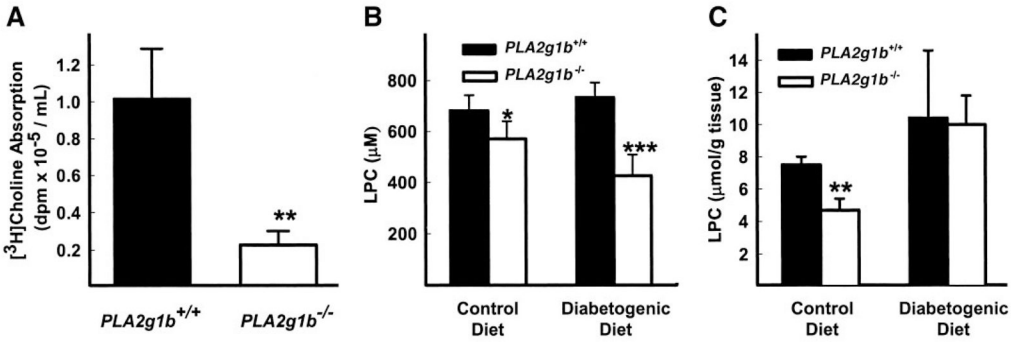

FIG 1.

Absorption of phosphatidylcholine (PC) and LPC levels in the livers and plasma of postprandial *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice. The *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice fed control diet or diabetogenic diet were fasted overnight and then fed a 0.1-ml glucose-lipid test meal containing phosphatidyl-[^{3}H]choline. After 2 h, hepatic portal blood was collected from the mesenteric portal vein to determine absorption of lipids associated [^{3}H]choline (*A*). Additionally, retroorbital blood (*B*), and liver tissue (*C*) were collected and assayed for LPC. Increased LPC levels were confirmed by comigration of a radioactive band with an LPC standard by thin-layer chromatography. Data are the means ± SE (n = 4–6) and are representative of two experiments. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

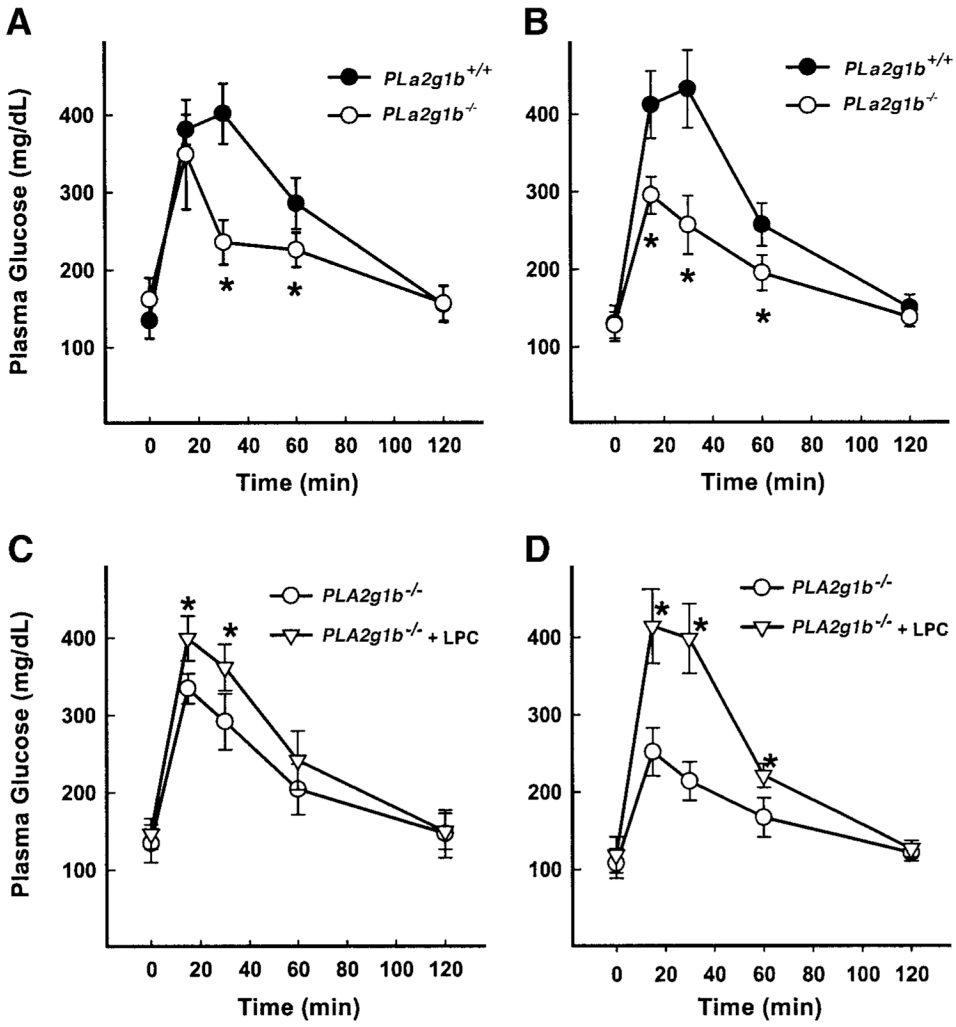

FIG 2.

Impact of *Pla2g1b* gene expression on oral glucose tolerance in mice. The *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice, age-matched at 15 weeks, were maintained on a basal low-fat diet and then subjected to an oral glucose tolerance test or an intraperitoneal glucose tolerance test. After an overnight fast of 12 h, each group was then fed by oral gavage (*A*) or injected intraperitoneally (*B*) with 50% glucose (~2 g/kg) in saline. Glucose tolerance tests were also performed, using *Pla2g1b*$^{-/-}$ mice injected with saline or saline plus LPC (32 mg/kg body wt) 5 min before oral (*C*) or intraperitoneal (*D*) administration of the glucose test meal. Blood was obtained via the tail vein before and 15, 30, 60, and 120 min after meal feeding for glucose analysis. Data are the means ± SE. Each panel is representative of at least two experiments of $n = 5$. *Statistical significant difference at $P < 0.05$.

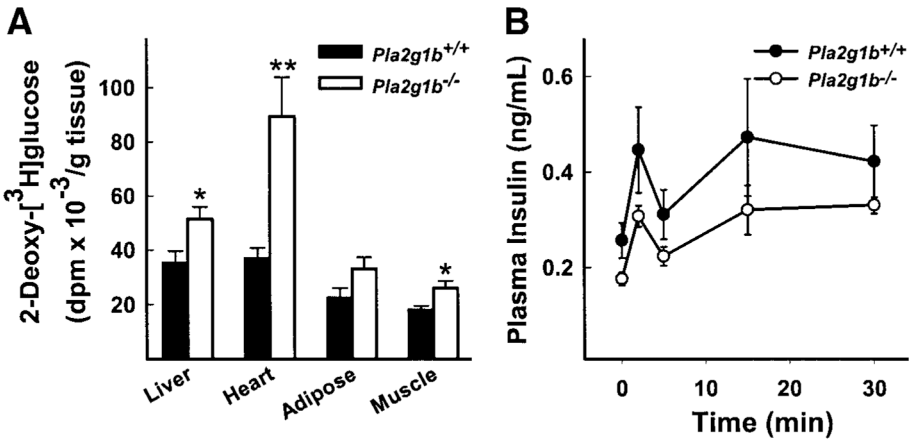

FIG 3.

Tissue glucose uptake and plasma insulin levels in *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice. We fed 4-month-old *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice control diet and injected them intraperitoneally with glucose (2 g/kg body wt) containing 2-deoxy-[^{3}H]glucose after a fast to measure glucose uptake (*A*). Liver, heart, white fat, and muscle tissues were collected after 30 min for determination of radioactivity. Plasma insulin levels in *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice after administration of a lipid-glucose test meal (*B*) were determined by radioimmunoassay. Data are the means ± SE (n = 4–7) and are representative of two experiments. *$P \leq 0.05$; **$P \leq 0.01$.

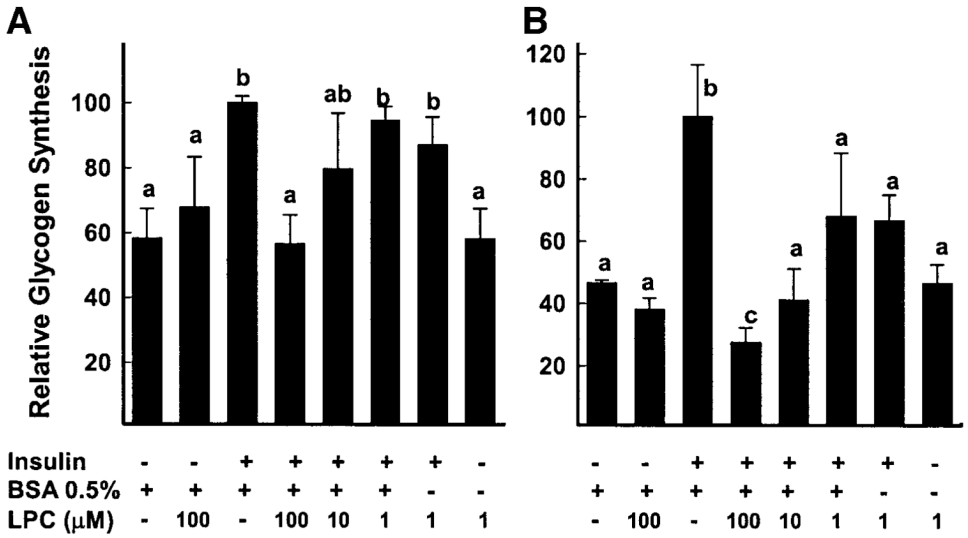

FIG 4.

Effects of LPC on insulin-induced glycogen synthesis. HepG2 (*A*) and H4- 2E→H4-2E (*B*) cells were serum starved for 12 h and then treated with [^{14}C]glucose (0.25 Ci), BSA, or LPC as indicated. All cells were subsequently incubated for 2 h after insulin or empty vehicle where indicated. Cells were washed and lysed, and after glycogen precipitation samples were assayed for radioactivity in a scintillation counter. Shown is a representative of at least three experiments. Data are expressed as the means ± SE (n = 4). Bars with different letters were significantly different at P <0.05.

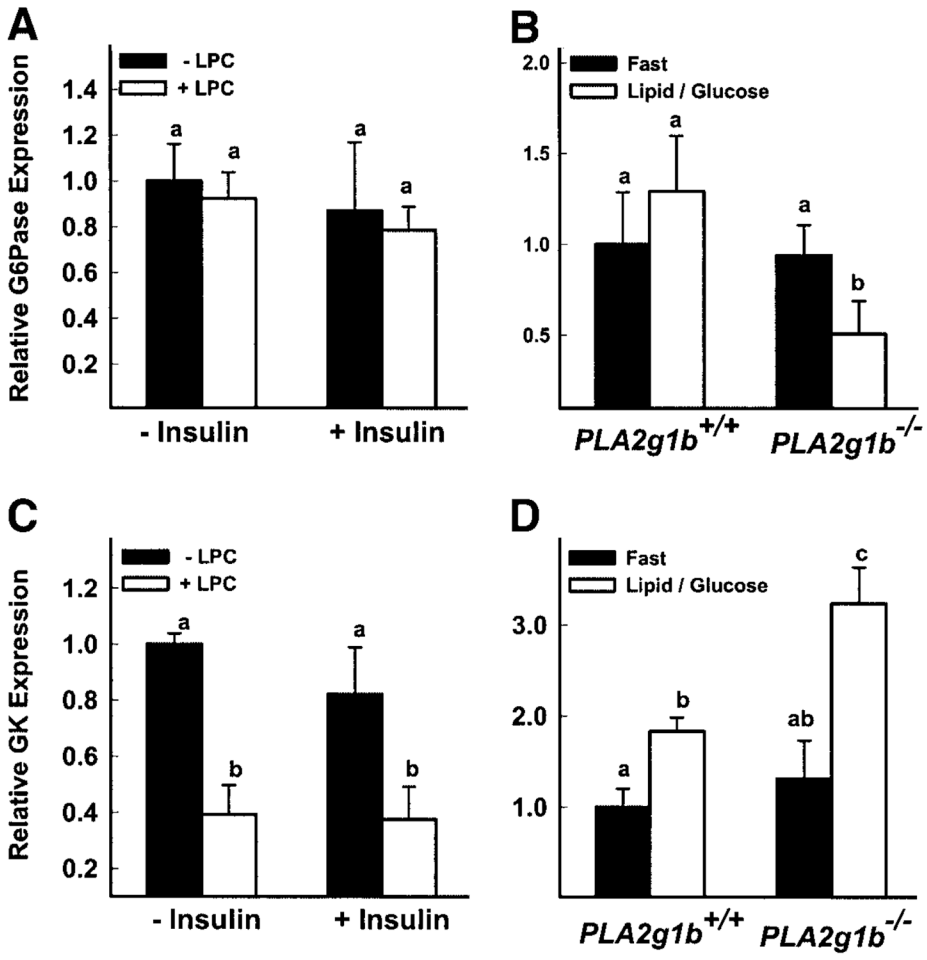

FIG 5.

Expression of G6Pase and glucose kinase in LPC-treated HepG2 cells (*A* and *C*) and in the livers (*B* and *D*) of *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice. Total RNA was isolated from liver or HepG2 cells after treatment and analyzed for expression of G6Pase (*A* and *B*) and glucose kinase (*C* and *D*) mRNA by real-time quantitative PCR. Livers were obtained from *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice after 18 h of fasting or after 2 h of feeding (after an 18-h fast) with a 0.1-ml glucose-lipid test meal containing 50% glucose (~2 g/kg body wt), 2.6 mmol/l egg phosphatidylcholine, 13.33 mmol/l triolein, and 2.6 mmol/l cholesterol. HepG2 cells were cultured to confluency in 12-well plates and serum-starved for 18 h before experiments. The cells were preincubated in the absence or presence of 100 nmol/l LPC, followed by an additional 2-h incubation with or without 20 mU/l insulin. Results were normalized to cyclophilin mRNA levels and are illustrated relative to wild-type fasting levels. Data are the means ± SE (n = 4). Bars with different letters were significantly different at P <0.05.

TABLE 1

Primers and reaction conditions used for real-time RT-PCR analyses of RNA

	Sense primers	Antisense primers	$[Mg^{2+}]$ used (mmol/l)	Reaction temperature (°C)
Mouse G6Pase	5′-TGCTGCTCACTTTCCCCACCAG	5′-TCTCCAAAGTCCACAGGAGGT	4	61
Mouse glucokinase	5′-CCCTGAGTGGCTTACAGTTC	5′-ACGGATGTGAGTGTTGAAGC	4	61
Mouse cyclophilin	5′-ATTCATGTGCCAGGGTGGTGACT	5′-TCAGTCTTGGCAGTGCAGAT	3	61
Human G6Pase	5′-ACATGTTCAGTGCCCAGG	5′-TTGGCTTTGTGGGAGGG	2	56
Human glucokinase	5′-GCTGGAATCAATTTCCCAGA	5′-CTCCCCACACAGGATGAGTT	2	52
Human cyclophilin	5′-AGGGTTCCTGCTTTCACAG	5′-ACTTGCCACCAGTGCC	2	56