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Pancreatic acinar cell-specific over-expression of group 1B phospholipase A_2 exacerbates diet-induced obesity and insulin resistance in mice

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Abstract

Genome wide association studies have identified significant association between polymorphisms of the Group 1B phospholipase A_2 (*PLA2G1B*) gene with central obesity in humans. Previous studies have shown that Pla2g1b inactivation decreases postprandial lysophospholipid absorption and as a consequence increases hepatic fatty acid oxidation and protects against diet-induced obesity and glucose intolerance in mice. The current study showed that transgenic mice with pancreatic acinar cell-specific over-expression of the human *PLA2G1B* gene gained significantly more weight and displayed elevated insulin resistance characteristics, including impaired glucose tolerance, compared to wild type mice when challenged with a high fat/carbohydrate diet. Pre- and post-prandial plasma β-hydroxybutyrate levels were also lower, indicative of decreased hepatic fatty acid oxidation, in the hypercaloric diet-fed *PLA2G1B* transgenic mice. These, along with earlier observations of *Pla2g1b*-null mice, document that Pla2g1b expression level is an important determinant of susceptibility to diet-induced obesity and diabetes, suggesting that the relationship between *PLA2G1B* polymorphisms and obesity may be due to differences in *PLA2G1B* expression levels between these individuals. The ability of pancreas-specific over-expression of *PLA2G1B* to promote obesity and glucose intolerance suggests that target phospholipase activity in the digestive tract with nonabsorbable inhibitors should be considered as therapeutic option for metabolic disease therapy.

Keywords

Phospholipase A_2; diet-induced obesity; diabetes; fatty acid oxidation

Introduction

Genome wide association studies have identified significant association ($P<0.01$) between *PLA2G1B* gene polymorphisms and central adiposity in humans.[1] This gene encodes the Group 1B phospholipase A_2 protein (PLA2G1B) that hydrolyzes the fatty acyl bond at the sn-2 position of phospholipids to generate free fatty acids and lysophospholipids. More recent lipidomic analyses of obesity-discordant twins revealed lysophosphatidylcholine elevation in plasma is a major obesity risk factor in humans.[2] Thus, the obesity differences in humans with *PLA2G1B* polymorphisms may involve PLA2G1B-mediated lysophospholipid production.

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Conflict of interest - None.

Our earlier studies with *Pla2g1b*$^{-/-}$ mice showed that the absence of Pla2g1b reduces postprandial lysophospholipid levels due to decreased lysophospholipid absorption, but has no effect on absorption of other fat and fat-soluble nutrients.[3] Nevertheless, the *Pla2g1b*$^{-/-}$ mice are protected against obesity and insulin resistance induced by a high fat/carbohydrate diet.[4,5] The reduction in lysophospholipid levels in *Pla2g1b*$^{-/-}$ mice increases postprandial hepatic fatty acid oxidation, and as a consequence reduces fatty acid availability to extrahepatic tissues resulting in obesity protection.[3] The reduced fatty acid availability also increases glucose utilization by extrahepatic tissues, thereby providing protection against glucose intolerance and diabetes.[5] These results suggest that PLA2G1B inhibition may be a novel therapeutic strategy for treatment of obesity and diabetes.

The *PLA2G1B* gene is expressed primarily in the acinar cells of the pancreas with less amounts of mRNA present in lung type II alveolar epithelial cells.[6,7] The PLA2G1B protein is also present in insulin secretory granules of pancreatic islet beta cells and is secreted with insulin upon glucose stimulation.[8] The importance of pancreatic acinar cell-derived PLA2G1B versus enzymes synthesized in lung and islet β-cells toward diet-induced obesity and hyperglycemia has not been defined. This information is necessary to target PLA2G1B inhibitors to specific anatomic sites where they can be effective in suppressing diet-induced obesity and diabetes with minimal adverse effects. This study examined the impact of transgenic over-expression of the human *PLA2G1B* gene in mouse pancreatic acinar cells on diet-induced obesity and insulin resistance.

Methods

Animals

A 3.3-kB chimeric gene was constructed in PUC13 vector by ligating a 508-bp DNA fragment containing the −500 to +8 region of the rat elastase-1 gene[9] to the 5'-untranslated region of the 2.2-kB human growth hormone (*hGH*) gene, followed by insertion of a 560-bp human *PLA2G1B* cDNA into the unique *Bam*HI site at the 5'-untranslated region within exon 1 of the *hGH* gene (Fig. 1A). The resulting chimeric gene containing the rat elastase-1 promoter and transcription initiation site, the *PLA2G1B* cDNA, and the *hGH* gene with its accompanying introns, transcription termination, and polyadenylation signal was excised from the plasmid vector and then used to produce transgenic mice as described previously.[10] Founder mice were identified by PCR amplification of genomic DNA with *hGH*-specific primers, and mated with C57BL/6 mice for 10 generations to obtain transgenic mice in congenic C57BL/6 background. The animals were fed either a basal diet (D12328; Research Diets, New Brunswick, NJ, USA) or a hypercaloric diet (D12331; Research Diets) composed of 58.5% fat, 25% sucrose, and 16.5% protein. Body weight gain was monitored biweekly. All animal protocols were approved by Institutional Animal Care and Use Committee at the University of Cincinnati in accordance with National Institutes of Health guidelines.

Human PLA2G1B expression in transgenic mice

Offsprings of founder mice were screened for expression of the chimeric transgene by reverse polymerase-PCR amplification of mRNA isolated from the pancreas and other tissues and normalized to the expression levels of cyclophilin A. Pancreatic phospholipase A_2 activity was determined by monitoring the hydrolysis of 6.5 mM 1-palmitoyl-2-[^{14}C]oleoyl-phosphatidylcholine (0.05 μCi) with 50 μg of pancreatic extracts in 50 μl buffer containing 500 μM Tris-HCl, pH 8.0, 135 mM NaCl, 1 mM $CaCl_2$, and 7.95 mM cholic acid. Protein expression was detected by Western blot analysis with anti-phospholipase A_2 antibodies that react with both mouse and human Pla2g1b.[11] Pancreas from *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ C57BL/6 mice were used as positive and negative controls, respectively.

Blood chemistry and glucose tolerance tests

Blood was collected from the tail vein of mice after an overnight fast. Blood glucose level was determined with an Accu-Chek Glucometer (Roche Applied Science, Indianapolis, IN, USA). Insulin and β-hydroxybutyrate levels in plasma were measured by ELISA and colorimetric assay kits as described previously.[3] Glucose tolerance tests were conducted by measuring blood glucose levels before and at various time periods after feeding a bolus glucose-lipid mixed meal containing 50% glucose, 2.6 mM egg phosphatidylcholine, 13.33 mM triolein, and 2.6 mM cholesterol.[5] Insulin sensitivity and resistance were estimated by homeostasis model assessment index, calculated as (fasting insulin concentration in μU/mL × fasting glucose concentration in mmol/L)/22.5.[12]

Data analysis

All results are reported as means ± se with the sample size as indicated. Significance ($P<0.05$) was evaluated using unpaired 2-tailed Student's *t* test assuming equal variance. All statistical analyses, including the Pearson correlation, were conducted using SigmaPlot 10.0 (Systat Software, San Jose, CA, USA).

Results

Western blot analysis of various tissues in wild type and *PLA2G1B*-transgenic mice showed increased PLA2G1B in pancreas but not in the liver, lung, heart, and muscle tissues of the transgenic mice (Fig. 1B). Reverse transcriptase-PCR analysis of pancreatic mRNA isolated from the 4 independent transgenic mouse lines revealed different levels of human *PLA2G1B* expression, with the Tg-2 line showing the most robust expression level whereas expression of the transgene in the Tg-4 line was minimal (Fig. 1C). Phospholipase A_2 enzymatic activities in the pancreas of the transgenic mice were consistent with the mRNA expression data, with pancreas from Tg-2 mice displaying ~3.5-fold higher phospholipase A_2 activity compared to the non-transgenic wild type mice, whereas enzymatic activities in the pancreas of the other 3 transgenic lines showed variable phospholipase A_2 activities that were not different from that observed with non-transgenic mouse pancreas (Fig. 1D).

Initial characterization of the 4 transgenic mouse lines showed that the animals were healthy and breed normally with no obvious signs of distress. Plasma lipid levels and body weights were also similar between wild type and *PLA2G1B*-transgenic mice when they were maintained on regular chow diet. In contrast, the Tg-2 *PLA2G1B* transgenic mice were significantly larger and gained more weight compared to wild type and other lines of *PLA2G1B* transgenic mice when the animals were fed the hypercaloric high fat/high carbohydrate diet (Fig. 2A). Homeostasis model assessment of fasting plasma glucose and insulin levels revealed an accelerated rate of diet-induced insulin resistance in the Tg-2 *PLA2G1B* transgenic mice (Fig. 2B). Glucose tolerance test administered to these animals 19 weeks after feeding the hypercaloric diet also revealed delayed glucose excursion in the *PLAG1B* transgenic mice (Fig. 2C).

Previous studies have shown improved glucose tolerance and obesity resistance in *Pla2g1b*-null mice due to elevated hepatic fatty acid oxidation as a consequence of reduced lysophospholipid absorption.[3] In the current study, plasma β-hydroxybutyrate levels, a surrogate measurement of hepatic fatty acid oxidation,[3] were significantly lower in the Tg-2 *PLA2G1B* transgenic mice compared to wild type mice under both fasting conditions as well as throughout a 3 hr postprandial period after a fatty meal (Fig. 2D). Thus, reduced hepatic fatty acid oxidation may also account for the increase in diet-induced obesity and glucose intolerance in the transgenic mice.

Discussion

Polymorphisms in the *PLA2G1B* gene associated with central obesity in humans are either polymorphisms in non-coding introns or synonymous base substitutions in exons that do not result in sequence alterations of the PLA2G1B protein.[1] Therefore, the mechanism by which these *PLA2G1B* polymorphisms contribute to central obesity remains an enigma. The current study shows over-expression of *PLA2G1B* in the pancreas reduces hepatic fatty acid oxidation and promotes diet-induced body weight gain and glucose intolerance. These results, together with our previous studies showing decreased Pla2g1b activity is protective against diet-induced obesity and diabetes in mice,[3–5,13] indicate that varying levels of PLA2G1B expression and activity in the digestive tract is an important determinant of susceptibility to diet-induced obesity and glucose intolerance. Thus, the association between *PLA2G1B* polymorphisms with obesity may be related to PLA2G1B expression level in the pancreas.

The contributory role of PLA2G1B in promoting diet-induced obesity and glucose intolerance suggests that pharmacologic inhibition of its enzyme activity may be a viable strategy to prevent these metabolic disorders. In fact, we have shown in a proof of concept study that oral administration of a generic phospholipase A_2 inhibitor methyl indoxam effectively reduces plasma lysophospholipid levels and protects against diet-induced obesity and glucose intolerance in mice.[13] The bioavailability of orally fed methyl indoxam is ~13%[13] and is capable of inhibiting other phospholipases.[14] Thus, methyl indoxam may incur undesirable adverse effects in the systemic that limits its utility as a pharmacologic inhibitor of PLA2G1B for obesity and diabetes intervention. The current study showing that specific elevation of PLA2G1B expression in pancreatic acinar cells is sufficient to reduce hepatic fatty acid oxidation and promotes diet-induced obesity and diabetes. These results suggest that development of non-absorbable inhibitors that specifically target PLA2G1B in the digestive tract may be a safer and effective option for obesity and diabetes intervention.

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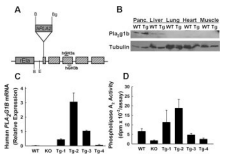

Figure 1.

Transgenic expression of human *PLA2G1B* in mice. *Panel A* shows the schematic diagram of transgene. The shaded boxes represent exons of *hGH* gene connected by their respective introns. The rEla box represents −500 to +8 region of the rat pancreatic elastase-1 gene, and the *hPLA2* box represents the 560-bp BamHI/BglII fragment of human *PLA2G1B* cDNA inserted into exon 1 of the *hGH* gene. The positions of the primers used for PCR amplification detection of the transgene are indicated as hGH3a and hGH3b, respectively. B denotes BamHI restriction site and E denotes EcoRI restriction site in the chimeric transgene. *Panel B* shows Western blot of extracts from wild type (WT) and transgenic (Tg) pancreas (Panc.), liver, lung, heart, and skeletal muscle with rabbit antibodies against PLA2G1B. Blots with anti-tubulin antibodies were used as loading control. *Panel C* shows human *PLA2G1B* mRNA detected in pancreas of wild type (WT), *Pla2g1b*-null (KO), and the 4 transgenic lines (Tg1–4) relative to expression levels of cyclophilin detected by RT-PCR. *Panel D* shows phospholipase A_2 eyzmatic activity in 50 μg pancreatic extracts from the respective mouse lines based on the hydrolysis of 1-palmitoyl-2-[^{14}C]oleoyl phosphatidylcholine. Each bar represents mean ± s.e. of 3–4 animals in each group.

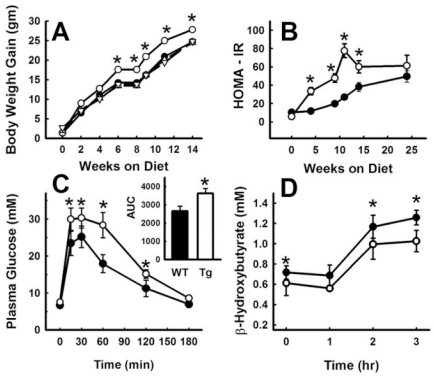

Figure 2.

Diet-induced obesity and diabetes in wild type and *PLA2G1B* transgenic mice. *Panel A* shows body weight gain of wild type (*filled circles*) and PLA2G1B transgenic mouse lines Tg-1 (*filled triangles*), Tg-2 (*open circles*), Tg-3 (*open triangles*), Tg-4 (*open inverted triangles*) in response to high fat/carbohydrate diet. Data represent mean ± s.e. from 8 mice in each group. Note that the lines for WT, Tg-1, Tg-3, and Tg-4 are superimposable. *Panel B* shows homeostasis assessment – insulin resistance HOMA-IR) index of high fat/carbohydrate-fed wild type mice (*filled circles*) and Tg-2 *PLA2G1B*-transgenic mice (open circles) (n=8 in each group). *Panels C and D* show plasma glucose and β-hydroxybutyrate levels in wild type (*filled circles*) and Tg-2 *PLA2G1B*-transgenic mice (open circles) after overnight fast (time = 0) and after feeding a bolus lipid meal (n=4 mice in each group). The inset to *panel C* shows areas under the curve determinations of the glucose tolerance tests. * denotes statistical significant differences from the wild type group at $P<0.05$.