Phosphatidic acid: a new therapeutic lead to suppress hepatic glucose production

Anil K Agarwal*,1 & Shireesha Sankella[1]

We view studying rare diseases with metabolic complications, like lipodystrophy, could also be a window into understanding the pathophysiology of more common diseases, such as Type 2 diabetes (T2D). The liver is the most important organ in mammals for glucose homeostasis. Upon meal ingestion, insulin is released from the pancreas (β-cells) and suppresses the liver glucose production while at the same time increasing the glucose uptake by the skeletal muscles and adipose tissue. This relationship is altered in T2D. T2D patients develop insulin resistance and thus the ability of insulin to suppress liver glucose production is reduced.

To understand the role of adipose tissue in mammalian physiology and metabolism, we have been studying rare cases of genetic lipodystrophies [1,2], a rare disease (defined as one affected individual per 200,000 in the USA or one affected individual per 20,000 in Europe) [3], in which subjects are born with undetectable adipose tissue referred to as congenital generalized lipodystrophy (CGL) or in those subjects who undergo loss of adipose tissue later in life known as partial lipodystrophies. It is counterintuitive to think that such a loss of adipose tissue will result in many of the same features of obesity including T2D. So how do humans develop diabetes that is similar to T2D in the total absence of adipose tissue? Using a positional cloning approach, we had previously identified mutation in the *AGPAT2* gene in patients with CGL [4]. AGPAT2 is key enzyme in the pathway that synthesizes glycerophospholipids, like phosphatidic acid (PA) and neutral lipids, namely diacylglycerol and triacylglycerol. When we created the murine model for this disease by homologous *Agpat2* gene deletion [5], these animals recapitulated all the features of lipodystrophy–insulin resistance, hepatic steatosis, hypertriglyceridemia and diabetes.

We then set out to search for the molecular mechanism for the development of diabetes in the *Agpat2*$^{-/-}$ mice. The enzyme AGPAT converts lysophosphatidic acid (LPA) to PA [6]. There are at least 11 known isoforms of this enzyme [7]. The AGPAT enzymatic activity in the livers of *Agpat2*$^{-/-}$ mice was ~10% of those of the wild-type mice [5]. With such a decrease in the liver AGPAT enzymatic activity, we would expect a significant decrease in the PA levels. However, when we measured the LPA and PA levels by a combination of techniques including organic extraction, HPLC separation, enzymatic digestion of PAs and mass spectrometry, we detected an increase in the PA levels [8]. The structure of one such C16:0/18:1 PA is shown in **Figure 1A**. Furthermore, this increase was due to only a few molecular species of PA in the livers of *Agpat2*$^{-/-}$ mice [8].

Since the *Agpat2*$^{-/-}$ mice are insulin resistant and diabetic, we then measured whether PA would affect the hepatic glucose production (HGP) in primary hepatic cells obtained from *Agpat2*$^{-/-}$ mice. We tested only those PAs in combinations or individually that were significantly elevated in the livers of *Agpat2*$^{-/-}$ mice: C16:0/18:1 PA, C18:1/20:4 PA and C16:0/18:2 PA in the molar ratio of 1:0.5:0.5 (as found in the livers of *Agpat2*$^{-/-}$ mice). This PA mixture increased HGP ~1.5-fold. When each individual PA species was tested in primary hepatocytes obtained from *Agpat2*$^{-/-}$ mice, we observed a 2–2.5-fold increase in glucose output in the

[1]Division of Nutrition & Metabolic Diseases, Center for Human Nutrition, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390, USA
*Author for correspondence: Tel.: +1 214 648 7685; Fax: +1 214 648 0553; anil.agarwal@utsouthwestern.edu
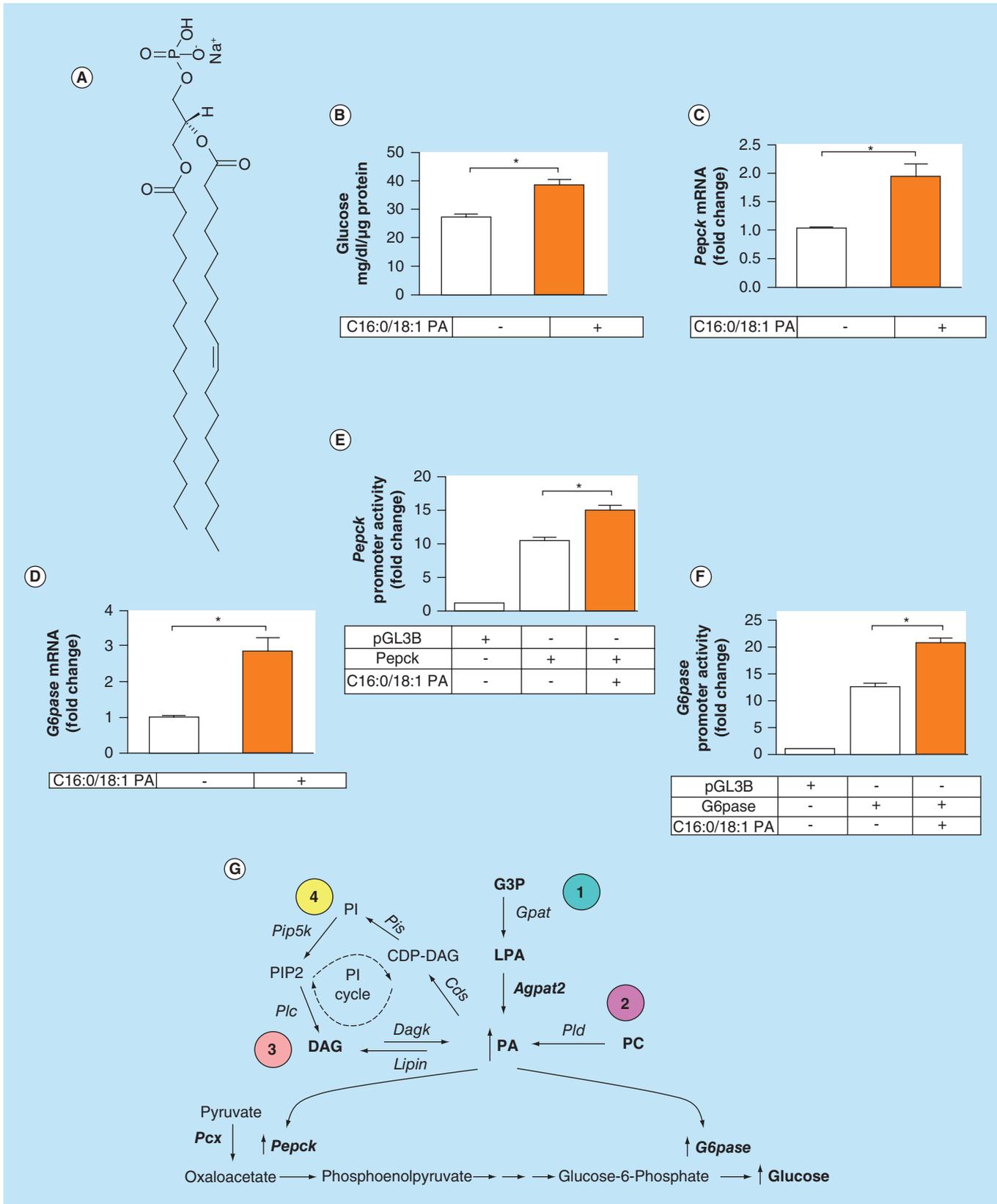
Future Medicine part of fsg

Figure 1. Overview of the structure of phosphatidic acid, generation of PA and proposed role of PA in hepatic gluconeogenesis (see facing page). (A) Chemical structure of C16:0/18:1 PA. This molecular species of PA was among several PAs that were found to be elevated in the livers of *Agpat2*$^{-/-}$ mice. This PA increased the glucose output when added to primary mouse hepatocytes obtained from *Agpat2*$^{-/-}$ mice. PA is a signaling molecule and the novel finding was that PA increased the glucose production **(B)** in primary hepatocytes isolated from *Agpat2*$^{-/-}$ mice. The increase in mRNA expression of key gluconeogenic genes, *Pepck* and *G6pase* **(C & D)** and increase in reporter activity of *Pepck* and *G6pase* **(E & F)** in the presence of C16:0/18:1 PA. **(G)** Various pathways leading to the synthesis of PA and its effect on gluconeogenesis. PA can be formed in four different ways: G3P is acylated by *Gpat* to form LPA with further acylation of LPA to PA by *Agpat2*; hydrolysis of PC to PA by *Pld*; phosphorylation of DAG to PA by *Dagk*; and by the PI cycle. In the PI cycle, PA is converted to CDP-DAG an intermediate for the synthesis of PI in the presence of *Cds*. CDP-DAG is converted to PI by *Pis*. The conversion of PI to PIP2 is carried out by *Pip5k*. DAG can be formed by hydrolysis of PIP2 by *Plc*. This is a speculative PA synthesis cycle which awaits experimental confirmation. Shown also is the gluconeogenesis pathway activated by key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (*Pepck* and *G6pase*).
*$p < 0.05$ considered statistically significant.
↑: Increased expression; CDP-DAG: Cytidine diphosphate-diacylglycerol; DAG: diacylglycerol; G3P: Glycerol-3-phosphate; LPA: Lysophosphatidic acid; PA: phosphatidic acid; PC: phosphatidylcholine; PI: Phosphatidylinositol; PIP2: PI-4,5-bisphosphate; Pepck: phosphoenolpyruvate carboxykinase; G6pase: glucose-6-phosphatase.
Data taken from [8].

presence of C16:0/18:1 PA **(Figure 1B)**. The increase in HGP in the presence of C16:0/18:2 PA was not statistically significant, indicating only a few molecular species of PA are responsible for the increase in HGP. We further confirmed that this increase in HGP is due to the increased expression of key gluconeogenic enzymes, glucose-6-phosphatase (*G6pase)* and phosphoenolpyruvate carboxykinase (*Pepck)* and that this increased expression is transcriptionally regulated by PA **(Figure 1C & 1D)**. For these experiments, we expressed the proximal promoter regions of *G6pase* and *Pepck* fused to a luciferase reporter gene in primary hepatocytes in the presence of PA. The relative luciferase units for *G6pase* and *Pepck* were also increased ~1.3 in the presence of C16:0/18:1 PA **(Figure 1E & 1F)** [8].

So what are likely source(s) of increased PA levels in the absence of AGPAT enzymatic activity? The increased PA levels might be generated from alternate pathways such as *Pld* and/or *Dagk* **(Figure 1G)** [8]. Although, from our studies, it is unclear which pathway is important for the generation of PA in *Agpat2*$^{-/-}$ mice livers, both the Pld and Dagk were upregulated at the mRNA and enzymatic levels. Our previous studies negate the idea that this could be due to AGPAT activity. When AGPAT2 was overexpressed in the livers of *Agpat2*$^{-/-}$ mice using recombinant adenovirus, it did not result in any improvement in diabetes [9], suggesting that the elevated PAs are not the products of the AGPAT2 enzyme. Further studies are required to determine which of these pathways is employed for the generation of PA. PA is a signaling molecule and is involved

in several signaling pathways at various subcellular levels [8]. This study now provides another layer of specificity for PA transduction as to HGP and several of these pathway(s) might require specific molecular species of PAs. Although our study now directly demonstrates the role of specific molecular species of PA in gluconeogenesis, the molecular mechanism as to how PA activates hepatic gluconeogenesis remains to be studied. Two previous studies also attempted to show the role of PA in glucose metabolism [10,11], although these studies did not directly demonstrate the role of PA in glucose production.

The International Diabetes Federation estimates ~400 million people are affected by T2D worldwide and projects this number to increase to ~600 million over the next 20 years [12]. T2D also affects microvascular disease, which includes blindness, kidney disease, diminished wound healing and amputation [13]. Thus, there is a great need to understand and seek new ways to tackle this ever growing disease. In fact, there are many physiological studies on the development of T2D and its associated molecular mechanism. However, there are very few studies on developing new leads/targets to treat T2D.

The main approaches for treating T2D have included sulfonylureas and nonsulfonylureas, which activate insulin release from pancreatic β-cells (e.g., glimepiride and metiglinides); biguanides (e.g., metformin), which suppress HGP; the thiazolidinedione class of PPARg agonists, which increases cellular sensitivity to insulin and adipocyte proliferation; α-glucosidase inhibitors, which inhibit hydrolysis of complex carbohydrates in intestines, thereby reducing

absorbable glucose; incretins, like glucagon-like peptide-1 (GLP-1) analogs and DPP-4 inhibitors, which prolong the action of the GLP-1; amylin and amylinomimetics, which control glucose levels by delaying gastric emptying, suppressing glucagon secretion and decreasing desire to eat; SGLT-2 inhibitors, which decrease glucose reabsorption by the kidney; the dopamine D2 receptor agonist bromocriptine-QR [14], enhances the lowered dopaminergic tone in patients with T2D; and, most important of all, insulin, which lowers HGP (reviewed in [15]).

With a large population affected worldwide by T2D and a limited collection of drugs, there is always a great need to seek newer targets and drugs to combat this disease. We have now demonstrated a new function of PA in enhancing HGP. Thus, maintaining appropriated PA levels in the liver by developing specific enzyme modulators that produce PA could lead to a novel method for treating T2D. In the coming years we hope this strategy might yield significant advancements in developing novel therapeutic agents and increasing our understanding of T2D.

References

1   Agarwal AK, Garg A. Genetic disorders of adipose tissue development, differentiation, and death. *Annu. Rev. Genomics. Hum. Genet.* 7, 175–199 (2006).

2   Garg A. Clinical review: Lipodystrophies: genetic and acquired body fat disorders. *J. Clin. Endocrinol. Metab.* 96(11), 3313–3325 (2011).

3   Boycott KM, Vanstone MR, Bulman DE, Mackenzie AE. Rare-disease genetics in the era of next–generation sequencing: discovery to translation. Nature reviews. *Genetics* 14(10), 681–691 (2013).

4   Agarwal AK, Arioglu E, De Almeida S *et al.* AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat. Genet.* 31(1), 21–23 (2002).

5   Cortes VA, Curtis DE, Sukumaran S *et al.* Molecular mechanisms of hepatic steatosis and insulin resistance in the AGPAT2– deficient mouse model of congenital generalized lipodystrophy. *Cell Metab.* 9(2), 165–176 (2009).

6   Agarwal AK, Garg A. Congenital generalized lipodystrophy: significance of triglyceride biosynthetic pathways. *Trends Endocrinol. Metab.* 14(5), 214–221 (2003).

7   Agarwal AK. Lysophospholipid acyltransferases: 1-acylglycerol-3-phosphate O-acyltransferases. From discovery to disease. *Curr. Opin. Lipidol.* 23(4), 290–302 (2012).

8   Sankella S, Garg A, Horton JD, Agarwal AK. Hepatic gluconeogenesis is enhanced by phosphatidic acid which remains uninhibited by insulin in lipodystrophic Agpat2–/– mice. *J. Biol. Chem.* 289, 4762–4777 (2014).

9   Agarwal AK, Sukumaran S, Cortes VA *et al.* Human 1-acylglycerol-3-phosphate O-acyltransferase isoforms 1 and 2: biochemical characterization and inability to rescue hepatic steatosis in Agpat2($^{-/-}$) gene lipodystrophic mice. *J. Biol. Chem.* 286(43), 37676–37691 (2011).

10   Ryu D, Oh KJ, Jo HY *et al.* TORC2 regulates hepatic insulin signaling via a mammalian phosphatidic acid phosphatase, LIPIN1. *Cell Metab.* 9(3), 240–251 (2009).

11   Zhang C, Wendel AA, Keogh MR, Harris TE, Chen J, Coleman RA. Glycerolipid signals alter mTOR complex 2 (mTORC2) to diminish insulin signalling. *Proc. Natl Acad. Sci. USA* 109(5), 1667–1672 (2012).

12   Beagley J, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults for 2013 for the IDF Diabetes Atlas. *Diabetes Res. Clin. Pract.* 103(2), 150–160 (2013).

13   Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol. Rev.* 93(1), 137–188 (2013).

14   Grunberger G. Novel therapies for the management of type 2 diabetes mellitus: part 1. pramlintide and bromocriptine–QR. *J. Diabetes* 5(2), 110–117 (2013).

15   Mehanna A. Antidiabetic agents: past, present and future. *Future Med. Chem.* 5(4), 411–430 (2013).