Published in final edited form as:

Diabetes Manag (Lond). 2014 ; 4(4): 323–326. doi:10.2217/dmt.14.29.

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

Anil K Agarwal^{*,1} and Shireesha Sankella¹

¹Division of Nutrition & Metabolic Diseases, Center for Human Nutrition, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA

Keywords

gluconeogenesis; hepatic steatosis; insulin resistance; lipodystrophy; lysophosphatidic acid; phosphatidic acid; rare disease

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

* Author for correspondence: Tel.: +1 214 648 7685; Fax: +1 214 648 0553; anil.agarwal@utsouthwestern.edu.

For reprint orders, please contact: reprints@futuremedicine.com

Financial & competing interests disclosure

The authors were supported by the NIH grant R01-DK54387, by the Southwestern Medical Foundation and Center for Human Nutrition at UT Southwestern Medical Center. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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 approximately 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 approximately 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 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, 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 approximately 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 approximately 400 million people are affected by T2D worldwide and projects this number to increase to approximately 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 PPAR γ 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, such as 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.

Acknowledgments

The authors would like to thank Katie Tunison for linguistic accuracy.

References

1. Agarwal AK, Garg A. Genetic disorders of adipose tissue development, differentiation, and death. *Annu Rev Genomics Hum Genet.* 2006; 7:175–199. [PubMed: 16722806]
2. Garg A. Clinical review: Lipodystrophies: genetic and acquired body fat disorders. *J Clin Endocrinol Metab.* 2011; 96(11):3313–3325. [PubMed: 21865368]
3. Boycott KM, Vanstone MR, Bulman DE, Mackenzie AE. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. *Nat Rev Genet.* 2013; 14(10):681–691. [PubMed: 23999272]

4. Agarwal AK, Arioglu E, De Almeida S, et al. AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat Genet.* 2002; 31(1):21–23. [PubMed: 11967537]
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.* 2009; 9(2):165–176. [PubMed: 19187773]
6. Agarwal AK, Garg A. Congenital generalized lipodystrophy: significance of triglyceride biosynthetic pathways. *Trends Endocrinol Metab.* 2003; 14(5):214–221. [PubMed: 12826327]
7. Agarwal AK. Lysophospholipid acyltransferases: 1-acylglycerol-3-phosphate O-acyltransferases. From discovery to disease. *Curr Opin Lipidol.* 2012; 23(4):290–302. [PubMed: 22777291]
8. Sankella S, Garg A, Horton JD, Agarwal AK. Hepatic gluconeogenesis is enhanced by phosphatidic acid which remains uninhibited by insulin in lipodystrophic *Acp2^{-/-}* mice. *J Biol Chem.* 2014; 289:4762–4777. [PubMed: 24425876]
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 *Acp2^{-/-}* gene lipodystrophic mice. *J Biol Chem.* 2011; 286(43):37676–37691. [PubMed: 21873652]
10. Ryu D, Oh KJ, Jo HY, et al. TORC2 regulates hepatic insulin signaling via a mammalian phosphatidic acid phosphatase, LIPIN1. *Cell Metab.* 2009; 9(3):240–251. [PubMed: 19254569]
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.* 2012; 109(5):1667–1672. [PubMed: 22307628]
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.* 2013; 103(2):150–160. [PubMed: 24300018]
13. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev.* 2013; 93(1):137–188. [PubMed: 23303908]
14. Grunberger G. Novel therapies for the management of Type 2 diabetes mellitus: part 1. Pramlintide and bromocriptine–QR. *J Diabetes.* 2013; 5(2):110–117. [PubMed: 23452312]
15. Mehanna A. Antidiabetic agents: past, present and future. *Future Med Chem.* 2013; 5(4):411–430. [PubMed: 23495689]

Figure 1. Overview of the structure of phosphatidic acid, generation of phosphatidic acid and proposed role of phosphatidic acid in hepatic gluconeogenesis

(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 *Plid*; 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 that 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: Cytidine diphosphate; DAG: Diacylglycerol; G3P: Glycerol-3-phosphate; LPA: Lysophosphatidic acid; PA: Phosphatidic acid; PC: Phosphatidylcholine; PI: Phosphatidylinositol; PIP2: PI-4,5-bisphosphate.

Data taken from [8].

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript