Secretory phospholipase A$_2$ group IIA modulates insulin sensitivity and metabolism

Michael S. Kuefner, Kevin Pham, Jeanna R. Redd, Erin J. Stephenson, Innocence Harvey, Xiong Deng, Dave Bridges, Eric Boilard, Marshall B. Elam, and Edwards A. Park

Departments of Pharmacology, Physiology, and Pediatrics, College of Medicine, University of Tennessee Health Science Center, Memphis, TN; Department of Veterans Affairs Medical Center, Memphis, TN; Department of Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, MI; and Department of Infectious Diseases and Immunology, Faculté de Médecine de l’Université Laval, CHUQ Research Center and Division of Rheumatology, Quebec City, Canada

Abstract Secretory phospholipase A$_2$ group IIA (PLA2G2A) is a member of a family of secretory phospholipases that have been implicated in inflammation, atherogenesis, and antibacterial actions. Here, we evaluated the role of PLA2G2A in the metabolic response to a high fat diet. C57BL/6 (BL/6) mice do not express PLA2g2a due to a frameshift mutation. We fed BL/6 mice expressing the human PLA2G2A gene (IIA+ mice) a fat diet and assessed the physiologic response. After 10 weeks on the high fat diet, the BL/6 mice were obese, but the IIA+ mice did not gain weight or accumulate lipid. The lean mass in chow- and high-fat-fed IIA+ mice was constant and similar to the BL/6 mice on a chow diet. Surprisingly, the IIA+ mice were more insulin sensitive and glucose tolerant than the BL/6 mice, even when the IIA+ mice were provided the high fat diet. The IIA+ mice had increased expression of uncoupling protein 1 (UCP1), sirtuin 1 (SIRT1), and PPAR coactivator 1α (PGC-1α) in brown adipose tissue (BAT), suggesting that PLA2G2A activates mitochondrial uncoupling in BAT.

Our data indicate that PLA2G2A has a previously undiscovered impact on insulin sensitivity and metabolism.—Kuefner, M. S., K. Pham, J. R. Redd, E. J. Stephenson, I. Harvey, X. Deng, D. Bridges, E. Boilard, M. B. Elam, and E. A. Park. Secretory phospholipase A$_2$ group IIA modulates insulin sensitivity and metabolism. J. Lipid Res. 2017. 58: 1822–1833.

Supplementary key words high fat diet • insulin resistance • obesity • hepatic steatosis

Phospholipases A$_2$ (PLA$_2$s) are a group of esterase enzymes that hydrolyze the second carbon of membrane phospholipids to release nonesterified free fatty acids and lysophospholipids (1, 2). PLA$_2$ enzymes have been classified into four groups based on their Ca$^{2+}$ requirement and cellular localization. These include secretory phospholipase A$_2$ (sPLA$_2$), cytosolic PLA$_2$, calcium-independent PLA$_2$, and lipoprotein-associated PLA$_2$ (3, 4). The sPLA$_2$s are low molecular mass phospholipases (14–18 kDa) that are secreted into the extracellular environment in response to various stimuli. Currently, eleven sPLA$_2$s have been identified (3, 5). These phospholipases mediate multiple biologic actions by targeting various noncellular phospholipids, such as microbial membranes, dietary phospholipids, and lipoproteins (5).

sPLA$_2$ group IIA (PLA2G2A) was first purified from the platelets and synovial fluids of patients suffering from arthritis (6). It has high affinity for anionic phospholipids such as phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol (5). Many cell types, including hepatocytes, vascular smooth muscle cells, and endothelial cells, secrete PLA2G2A (7, 8). Expression of PLA2G2A is induced by various stimuli, including interleukin (IL)-1, IL-6, TNFα, lipopolysaccharides, and cyclic AMP (9–12). The finding that PLA2G2A is highly abundant in biological fluids of patients suffering from inflammatory diseases, like arthritis, sepsis, and myocardial infarctions, suggested that PLA2G2A promotes inflammation (5, 13, 14). In support of this concept, under inflammatory conditions PLA2g2a knockout BALB/c mice have attenuated joint inflammation compared with wild-type BALB/c mice (15).

Abbreviations: ACC1, acetyl-CoA carboxylase; BAT, brown adipose tissue; BL/6, C57BL/6; CLAMS, comprehensive laboratory animal monitoring system; CPT1a, carnitine palmitoyltransferase; eWAT, epididymal white adipose tissue; GGT, glucose tolerance test; HNE, 4-hydroxy-2-nonenal; IIA+, C57BL/6 mice expressing the human PLA2G2A gene; IL, interleukin; ITT, insulin tolerance test; LCFA, long chain fatty acid; PGC-1α, PPARγ coactivator 1α; PLA$_2$, phospholipase A$_2$; PLA2G2A, secretory phospholipase A$_2$ group IIA; RER, respiratory exchange ratio; SIRT1, sirtuin 1; sPLA$_2$, secretory phospholipase A$_2$; UCP1, uncoupling protein 1; WAT, white adipose tissue.

1M. S. Kuefner and K. Pham contributed equally to this work.

To whom correspondence should be addressed.
e-mail: epark@uthsc.edu

This article is available online at http://www.jlr.org
In addition to its inflammatory properties, PLA2G2A is proatherogenic with elevated levels of PLA2G2A being a biomarker for cardiovascular disease (16). Evidence of a role for PLA2G2A in atherosclerosis came from work utilizing PLA2G2A transgenic mice. C57BL/6 (BL/6) mice do not express PLA2g2a due to frameshift mutation in exon 3. The human PLA2G2A gene was introduced into BL/6 mice to create PLA2G2A-expressing (IIA+) mice (17, 18). When fed a high cholesterol diet, the IIA+ mice had increased atherosclerotic lesions, reduced plasma HDL, and slightly increased LDL (17). They also had elevated hepatic cholesterol levels, suggesting that PLA2G2A affects hepatic cholesterol uptake (19). Furthermore, bone marrow transplantation from IIA+ mice into LDL receptor-deficient mice increased atherosclerotic lesions, and expression of PLA2G2A in mouse macrophages accelerated the development of arterial wall lesions and the movement of cholesterol from LDL to foam cells (20, 21). Oral dosing of varespladib, an inhibitor of sPLA2g2a, for 16 weeks reduced aortic atherosclerosis in ApoE-deficient mice (22). However, the failure of varespladib to reduce myocardial infarction and cardiovascular death in clinical trials has called into question the view that PLA2G2A is pro-atherogenic (23, 24).

There is a relationship between PLA2G2A, inflammatory processes, and cardiovascular disease. However, the contributions of PLA2G2A to metabolic regulation have not been examined. In this work, we investigated the metabolic effects of PLA2G2A in relation to hypercaloric high fat feeding. Our findings demonstrated that IIA+ mice are resistant to body weight and fat mass gains in response to a high fat diet. Moreover, IIA+ mice have superior glucose tolerance and insulin sensitivity, and these changes are associated with elevations in total energy expenditure. This study has identified a novel role of PLA2G2A in the regulation of whole-body metabolism.

MATERIALS AND METHODS

IIA+ mice

The IIA+ mice, which express the human PLA2G2A gene under the regulation of the human promoter, were provided by Dr. Eric Boilard at the Université Laval (CHUL), Quebec, Canada (25). Male mice heterozygous for the gene were bred with BL/6 females. For these experiments, male BL/6 and IIA+ mice were used. Mice were placed on diets 8 weeks after birth. In each dietary group, we had 11 BL/6 chow-fed mice, 10 IIA+ chow-fed mice, 11 BL/6 high fat diet-fed mice, and 12 IIA+ high fat diet-fed mice. Mice were housed with a constant light and dark phase of 12 h at 20–23°C. All animal procedures were approved by the University of Tennessee Health Science Center Institutional Animal Care and Use Committee.

Diet and food intake

The chow diet was the Teklad LM-485 mouse/rat diet (7012) and the high fat diet was D12451 from Research Diets Inc. The high fat diet provided 45% of the calories from fat, while the chow diet contained 5% of the calories as fat. The mice were given free access to food throughout the study. The food was weighed at weekly intervals and the kilocalorie consumption was calculated based on grams of food eaten, the calorie density of the food, and the weight of the mice.

Body composition analysis

Mice were weighed and total fat and fat-free mass were determined weekly by EchoMRI-1100.

Glucose and insulin tolerance tests

Following 6 h fasts, baseline blood glucose concentrations were determined in blood collected from a small tail incision using a hand-held glucometer (AccuCheck). D-glucose in PBS [2 mg/kg fat-free mass; glucose tolerance test (GTT)] or insulin [1 U/kg fat-free mass; insulin tolerance test (ITT)] was injected intraperitoneally and blood glucose concentrations measured at 15, 30, 45, 60, 75, 90, 105, and 120 min postinjection (26). The data are presented as blood glucose levels versus time and the area under the glucose curve.

Comprehensive laboratory animal monitoring system

After 11 weeks of diet, mice were individually housed in the comprehensive laboratory animal monitoring system (CLAMS) chambers. They were maintained on either chow or high fat diet. Total energy expenditure was determined using indirect calorimetry (27). VO2 and heat production are expressed relative to fat-free mass. The respiratory exchange ratio (RER) was measured to determine energy substrate preference. Physical activity was determined by the number of infrared beam breaks.

RNA extraction and real-time PCR

Mice were allowed access to food prior to tissue collection for RNA and protein. The mice were anesthetized by isoflurane and then euthanized by cervical dislocation. Liver, quadriceps muscle, brown adipose tissue (BAT), and epididymal white adipose tissue (eWAT) were harvested and stored in liquid nitrogen. Total RNA was extracted from mouse liver by RNA-STAT 60 (Tel-Test). cDNA was synthesized via reverse transcription using Superscript III (Invitrogen). The parameters for RT-PCR were as follows: 95°C for 5 min, 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 10 s. Cyclophilin D was used as a reference gene. The quantification of the PCR products was carried out using the ∆∆Ct method (28).

| Gene          | Forward           | Reverse           |
|--------------|------------------|------------------|
| Cpt1a        | 5′-GGAGGACATCCAGAGGAGGATGAT-3′ | 5′-GGCAAGAGAACAGAGGATGAT-3′ |
| Gapdh        | 5′-GAGGTCATTGCCAAGAAGTCCAG-3′ | 5′-GGCTTCATTGCCAAGAAGTCCAG-3′ |
| Hprt1        | 5′-CAGAGACTAGAACACCTG-3′ | 5′-CCGATAGAACACCTG-3′ |
| Pparγ2       | 5′-GGGACATCCAGAGGAGGATGAT-3′ | 5′-GGCAAGAGAACAGAGGATGAT-3′ |
| Ppargc1a      | 5′-GGGACATCCAGAGGAGGATGAT-3′ | 5′-GGCAAGAGAACAGAGGATGAT-3′ |
| Sirt1        | 5′-GGTTCCTTGCGCCATGAGGCG-3′ | 5′-GGCTTCATTGCCAAGAAGGATGAT-3′ |
| Srebf1        | 5′-GGGACATCCAGAGGAGGATGAT-3′ | 5′-GGCAAGAGAACAGAGGATGAT-3′ |
| Usp1         | 5′-GGGACATCCAGAGGAGGATGAT-3′ | 5′-GGCAAGAGAACAGAGGATGAT-3′ |

Table 1. The forward and reverse primers used in the real-time PCR reactions are shown.
The forward and reverse primers used for real-time PCR are shown in Table 1. The PLAC2G2A primers were from Qiagen (catalog number PH05823B).

Western blots

Total protein from mouse livers, quadriceps, BAT, and eWAT was isolated in RIPA buffer containing protease inhibitors [50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM EDTA (pH 8.0), 1% Triton, 1 mM benzamidine, and 0.5 mM PMSF] (29). Protein lysates were prepared in loading buffer and equal amounts were loaded on 4–20% gradient Tris/glycine precast acrylamide gels. Proteins were separated using SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% nonfat milk in TBS-T prior to antibody incubation. Membranes were immunoblotted with the appropriate primary antibody in 5% BSA in TBS-T and secondary antibody in 5% nonfat milk in TBS-T. Immunoreactive proteins were detected using Super Signal West Femto chemiluminescent substrate (Thermo Scientific) (28). The following antibodies were used: acetyl-CoA carboxylase (ACC1), catalog number C83B10 from Cell Signaling Technology; p-p70S6K(Thr389), catalog number 108D2 from Cell Signaling Technology; p70S6K, catalog number 9202 from Cell Signaling Technology; Akt, catalog number C67E7 from Cell Signaling Technology; β-actin, catalog number 13E5 from Cell Signaling Technology; uncoupling protein 1 (UCP1), catalog number D9D6X from Cell Signaling Technology; UCP2, catalog number D105V from Cell Signaling Technology; GAPDH, catalog number 14C10 from Cell Signaling Technology; alpha-tubulin, catalog number 11H10 from Cell Signaling Technology; p-Akt (T308), catalog number 244F9 from Cell Signaling Technology; PLA2G2A, catalog number AB23705 from AbCam; sirtuin 1 (SIRT1), catalog number A21993 from Life Technologies; SREBP-1c, catalog number 5351581 from BD Pharmingen.

Lipids

Plasma cholesterol and lipoproteins in serum were measured at the University of Tennessee Endocrinology/Lipoprotein Laboratory. Blood was obtained by cardiac puncture from anesthetized mice and the serum was collected from the clotted blood. For hepatic triglyceride measurements, liver samples were first lysed in homogenization buffer [50 mM Tris (pH 8), 5 mM EDTA, 30 mM EDTA, 30 mM mannitol, and phosphatase inhibitors]. Lipids were then extracted with a chloroform/methanol mixture using the Folch extraction procedure (30). Once the chloroform phase was evaporated, hepatic triglycerides were

Fig. 1. Weight gain and body composition of HIA+ mice on high fat diets. BL/6 and HIA+ mice were placed on chow diet or HFD for 10 weeks, as described in the Materials and Methods. A: The weight gain (grams) of the mice in the four groups was assessed on a weekly basis. B, C: MRI analysis was performed weekly and used to determine the lean mass and percent body fat. D: The average kilocalories consumed per mouse per gram of body weight were calculated from the food intake and the calorie content of the various diets. In all groups, there were 10–12 mice. The differences in the various time points were determined by two-way ANOVA, using genotype and diet as two separate factors.
measured using the Sigma triglyceride assay kit as directed by the manufacturer.

Statistical analysis
Data analysis was performed using the JMP Statistical Discovery program. Data presented are the mean results of 3–4 replicate experiments ± the SE. Data were analyzed by Student’s one/two-tailed t-test or ANCOVA. P < 0.05 was considered to be statistically significant.

RESULTS
Effect of PLA2G2A on weight gain
Initially, we evaluated the effect of PLA2G2A on weight gain and lipid accumulation by monitoring the body composition in response to 10 weeks of high fat diet consumption in both BL/6 control and the IIA+ mice (15). In response to the high fat diet, BL/6 mice gained significantly more weight than the chow-fed BL/6 mice or the IIA+ mice on either the chow or high fat diet. The IIA+ mice were resistant to high fat diet-induced weight gain (Fig. 1A). The fat-free mass of BL/6 and IIA+ mice was similar on both the chow and high fat diets (Fig. 1B). BL/6 mice receiving the high fat diet had significantly elevated total body fat compared with their chow counterparts, whereas fat mass was slightly lower in the IIA+ groups regardless of the diet (Fig. 1C). We measured the caloric intake of the mice and found that over the 10 week feeding period the BL/6 and IIA+ mice consumed similar kilocalories of food per gram of body weight (Fig. 1D). Initially, the BL/6 mice on the high fat diet ate more kilocalories than the IIA+ mice, but after 8 weeks feeding was similar across all groups (data not shown). The unexpected observation was that the IIA+ mice on the high fat diet were protected from lipid accumulation, unlike the BL/6 mice on the high fat diet.

PLA2G2A and energy expenditure
Next, we assessed energy expenditure and physical activity using CLAMS. The BL/6 mice on the high fat diet had higher oxygen consumption than the chow-fed BL/6 mice (Fig. 2A). Surprisingly, the IIA+ mice on chow and high fat diets had higher oxygen consumption than the BL/6 controls, suggesting increased energy expenditure regardless of diet. In addition to the elevated VO2 consumption, the IIA+ mice had increased heat production (Fig. 2B). The BL/6 and IIA+ mice on the high fat diet had lower RER compared with their chow counterparts, indicating less carbohydrate utilization (Fig. 2C). The activity levels of the IIA+ mice were similar to the BL/6 mice on the same diets, indicating the increased energy expenditure was not due to increases in activity (Fig. 2D). However, the mice on the high fat diet were less active than those on the chow diet. Overall, the data in Figs. 1 and 2 suggest that the lean phenotype of the IIA+ mice was due to elevated basal metabolism.

PLA2G2A and insulin sensitivity
We examined the glucose and insulin tolerance of these mice after 10 weeks on the diets. Results from the GTT show that the IIA+ mice were able to clear glucose quite rapidly, even after 10 weeks on a high fat diet (Fig. 3A).
However, the BL/6 mice on the high fat diet had reduced glucose clearance and higher levels of blood glucose 2 h after glucose administration compared with the BL/6 chow-fed mice. There was no effect of the high fat diet on glucose uptake in the IIA+ mice. In Fig. 3B, the total blood glucose amount over the 2 h assay is shown. To assess insulin sensitivity, we conducted an ITT. The BL/6 mice on the high fat diet had reduced insulin sensitivity and little change in blood glucose in response to insulin administration. In contrast, the IIA+ mice remained insulin sensitive regardless of the diet (Fig. 3C) and blood glucose remained low 2 h after insulin administration. The total change in blood glucose level over the 2 h assay is shown in Fig. 3D. These data suggest that PLA2G2A enhances whole-body insulin sensitivity.

**Plasma lipid levels**

Because previous studies have shown that the IIA+ mice have a propensity to develop atherosclerosis, we evaluated the blood lipids of the mice (17, 20). The BL/6 mice on the high fat diet had significantly elevated serum cholesterol compared with all other groups. The IIA+ mice on the high fat diet had elevated cholesterol relative to the IIA+ chow-fed group, but the overall cholesterol was lower in the IIA+ mice when compared with the BL/6 mice (Fig. 4A). Plasma triglycerides were lowest in the IIA+ chow animals (Fig. 4B). Triglycerides were highest in the IIA+ mice on the high fat diet, but this elevation was only significant when comparing them to their chow diet counterpart. HDL was greatly elevated in the BL/6 mice on high fat diet, and HDL levels were lower in the IIA+ groups relative to their dietary BL/6 controls (Fig. 4C). LDL was higher in the IIA+ mice on a high fat diet, although there was great variability among the animals (Fig. 4D). The increased LDL may reflect the higher triglycerides in the high fat-fed IIA+ mice. Because energy expenditure was higher in the IIA+ mice on a high fat diet, although there was great variability among the animals (Fig. 4D). The increased LDL may reflect the higher triglycerides in the high fat-fed IIA+ mice. Because energy expenditure was higher in the IIA+ mice, we measured the T4 levels in the animals. There was no difference in the T4 between BL/6 and IIA+ mice, although the T4 was lower in both BL/6 and IIA+ animals on the high fat diet (Fig. 4E). Finally, we measured the triglyceride levels in

---

![Fig. 3. Glucose and insulin tolerance in IIA+ mice on the high fat diet. A: GTTs were conducted on all the mice in week 11 of the diet study, as described in the Materials and Methods. The blood glucose was measured at 15 min intervals. Each time point is the average of 11–15 mice. B: The total blood glucose from all time points is shown. C: ITTs were conducted on all the mice in week 12 of the diet study, as described in the Materials and Methods. The blood glucose was measured at 15 min intervals following insulin injection. Each time point is the average of seven to nine mice. D: Total blood glucose for all time points in the ITT is shown. Differences in the glucose levels were determined by the t-test. *P < 0.05, **P < 0.01, ***P < 0.001.](image-url)
PLA2G2A modulates metabolism 1827

the liver. The hepatic triglycerides were greatly elevated in the BL/6 mice on the high fat diet, but the lipid levels in the IIA+ mice were only slightly increased (Fig. 4G).

Tissue expression of PLA2G2A

To determine where PLA2G2A was present, we tested various tissues for the expression of PLA2G2A. We observed PLA2G2A mRNA in liver, muscle, eWAT, and BAT (Fig. 5A–D). The level of PLA2G2A mRNA in the liver was especially high. In the liver and muscle, PLA2G2A mRNA and protein abundance were increased by the high fat diet, indicating that the gene is stimulated by high fat feeding.

Metabolic gene expression in liver of IIA+ mice

To understand the molecular mechanisms underlying the metabolic effects of PLA2G2A, we measured the impact of PLA2G2A on the abundance of various mRNAs...
encoding metabolic genes in the liver (Fig. 6). First, we measured the mRNA abundance of genes involved in fatty acid synthesis and oxidation, including Sreb1, Acaca, and carnitine palmitoyltransferase (Cpt1a). Acaca mRNA levels trended higher in the chow-fed IIA+ mice compared with the IIA+ mice on high fat diet (P = 0.2871), but the levels were not significant. (Fig. 6A). Surprisingly, the Sreb1 mRNA was reduced in the IIA+ mice (Fig. 6B). Cpt1a was elevated in response to the high fat diet in BL/6 mice, but not in the IIA+ mice (Fig. 6C). Next, we measured the hepatic abundance of two genes important for mitochondrial biogenesis, Ppargc1a and Sirt1. The mRNA levels of Ppargc1a and Sirt1 were higher in the IIA+ mice on the chow diet, but not the high fat diet (Fig. 6D, E). To identify changes in protein levels, we measured the abundance of hepatic proteins involved in metabolic regulation by Western analyses. The abundance of ACC1 was elevated in both the BL/6 and IIA+ mice on high fat diet (Fig. 6D, E). To identify changes in protein levels, we measured the abundance of hepatic proteins involved in metabolic regulation by Western analyses. The abundance of ACC1 was elevated in both the BL/6 and IIA+ mice on high fat diet (Fig. 6D, E). To identify changes in protein levels, we measured the abundance of hepatic proteins involved in metabolic regulation by Western analyses. The abundance of ACC1 was elevated in both the BL/6 and IIA+ mice on high fat diet (Fig. 6D, E). To identify changes in protein levels, we measured the abundance of hepatic proteins involved in metabolic regulation by Western analyses. The abundance of ACC1 was elevated in both the BL/6 and IIA+ mice on high fat diet (Fig. 6D, E). To identify changes in protein levels, we measured the abundance of hepatic proteins involved in metabolic regulation by Western analyses. The abundance of ACC1 was elevated in both the BL/6 and IIA+ mice on high fat diet (Fig. 6D, E).
First, the IIA+ mice did not gain weight or accumulate fat mass on the high fat diet. Only the BL/6 mice on the high fat diet accumulated fat. The IIA+ mice were smaller than the BL/6 mice and consumed similar kilocalories per gram of body weight over the 10 week period. The increased energy expenditure was likely the major cause of the reduced weight gain by the IIA+ mice. Increased energy expenditure was not due to increased activity in the IIA+ mice, as they were not more active than the BL/6 mice (Fig. 2D). Elevated thyroid hormone concentrations were not responsible (Fig. 4E). In the liver, key regulators of proteins involved in fatty acid oxidation, such as PGC-1α and SIRT1, were not elevated. The transcriptional events that specify a brown adipogenic program have been well-characterized. PGC-1α and the transcription factor PR domain containing 16 (PDRM16) directly regulate BAT induction, and SIRT1 may promote adipose tissue browning as well (34–36). We observed increased expression of UCP1, PGC-1α, and SIRT1 in the BAT of the IIA+ mice, suggesting that PLA2G2A promotes mitochondrial uncoupling in BAT (Fig. 9).

It was previously discovered that lipoproteins modulated by sPLA2 were more susceptible to lipid peroxidation, resulting in the production of oxidized lipoproteins and the byproduct, 4-hydroxy-2-nonenal (4-HNE). The 4-HNE can induce mitochondrial uncoupling through covalent modification of UCPs and adenine nucleotide translocase (ANT), an ATP exporter (37). Given the increases we have observed in overall brown adipogenic programming through induction of PGC-1α and SIRT1, as well as induction of UCP1 itself, in the IIA+ mice, a possible explanation is that overexpression of PLA2G2A enhanced lipid peroxidation and, therefore, 4-HNE. The 4-HNE could activate
mitochondrial uncoupling through its stimulatory actions on proton conductance.

One of the many fatty acids released by sPLA₂ activity is arachidonic acid, a necessary precursor for biosynthesis of a diverse group of eicosanoids and other signaling molecules involved in the inflammatory response (38). However, it was recently discovered that arachidonic acid alone can also directly activate the mitochondrial proton leak caused by UCP1 in both beige and brown adipocytes through experiments using inner mitochondrial matrix mitoplasts (39). Moreover, a similar study found that UCP1 can be controlled by fatty acid products generated from phospholipid hydrolysis by cytosolic PLA₂ (40). The IIA+ mice on chow and high fat diets had elevated VO₂ (Fig. 2A) and heat dissipation (Fig. 2B). This may be due to enhanced release of arachidonic acid or other long chain fatty acid (LCFA) signaling molecules by sPLA₂. This LCFA then migrates to BAT and induces mitochondrial uncoupling through UCP1’s LCFA anion/H⁺ symporter activity.

Because PLA2G2A is frequently associated with inflammation and rheumatoid disease, we anticipated that the IIA+ mice would be insulin resistant, as inflammation has been frequently linked with insulin resistance (41, 42). However, another unexpected observation was that the IIA+ mice were much more sensitive to insulin, as demonstrated by both the GTT and the ITT (Fig. 3). Furthermore, the hepatic insulin signaling pathways were clearly activated, as shown by the increased abundance and phosphorylation of Akt (Fig. 8). One possibility is that as the IIA+ mice were refractory to high fat diet-induced obesity, the insulin sensitivity was secondary to the different obesity states. Previous work found that cholesterol accumulated in the liver of IIA+ mice on an atherogenic diet (19). The livers of the BL/6 mice on high fat diet contained much more triglyceride than those of all other groups (Fig. 4G). We observed a slight increase in hepatic triglycerides in the IIA+ mice on high fat diet compared with the chow groups. The attenuated accumulation of triglycerides in the IIA+ mice may provide an additional

Fig. 7. Changes in metabolic factors in livers of IIA+ mice. Proteins were collected from the livers at the end of the feeding period and the abundance of various proteins was analyzed by Western blot. Representative Western blots are shown and the data was quantified. ACC1 (A), precursor SREBP-1c (B), nuclear SREBP-1c (C), PGC-1α (D), SIRT1 (E), representative blots are shown (F). The data are expressed as relative protein abundance. Each point is the average of Western data blot from four animals. The data were analyzed by t-test. *P < 0.05, **P < 0.01, ***P < 0.001.
reason as to why the IIA+ mice on high fat diet are not insulin resistant.

Recent studies have found that several sPLA2 isoforms possess different and tissue-specific roles in obesity and metabolic dysfunction. Hui and colleagues reported that the knockout of \( \text{PLA2g1b} \) provided protection against diet-induced obesity and insulin resistance (43). \( \text{PLA2g1b} \) is secreted into the intestinal lumen from the pancreas and the protection arose from reduced lysophosphatidylcholine absorption. The \( \text{PLA2g1b} \) knockout mice also had elevated hepatic fatty acid oxidation, indicating that the livers relied more on fatty acids for energy (44, 45). Addition of lysophosphatidylcholine to isolated hepatocytes directly reduced mitochondrial oxidation of fatty acids (46). Work from Murakami and colleagues examined the interplay of \( \text{PLA2g5} \) and \( \text{PLA2g2e} \) in the adipose tissue with respect to metabolic actions (47). They found that \( \text{PLA2g5} \) knockout mice displayed insulin resistance and hyperlipidemia, and were increasingly prone to diet-induced obesity, suggesting that \( \text{PLA2g5} \) provides protection from obesity. In the same study, it was also discovered that knocking out \( \text{PLA2g2e} \) in mice decreased hyperlipidemia and diet-induced obesity, and improved overall liver health, indicating that \( \text{PLA2g2e} \) may promote obesity (47). A metabolic role for \( \text{PLA2g2a} \) was investigated in rats (48). With a high fat diet, the levels of \( \text{PLA2g2a} \) were elevated 20-fold in rat white adipose tissue (WAT) (48). In concurrence with these studies, we previously reported that BALB/c mice on a high fat diet had increased \( \text{PLA2g2a} \) mRNA in the liver and that the addition of oleic acid to rat hepatocytes increased \( \text{PLA2g2a} \) (25). In rats, chronic administration by gavage of the \( \text{PLA2g2a} \) inhibitor, KH064, protected against diet-induced weight gain and reduced fasting glucose concentrations (48). The authors proposed that \( \text{PLA2g2a} \) inhibition improved WAT function via stimulating lipolysis, thereby decreasing fat stores in WAT (48). These results differ from our observations that elevated PLA2G2A activity enhances energy expenditure in BAT and overall insulin sensitivity. It may be that the results in the rat study stem from an impairment of intestinal lipid absorption, thereby reducing the impact of the high fat diet, as Iyer et al. (48) observed increased fat in the feces of the KH064 treated rats. It is clear from multiple studies that the various secretory phospholipases can have isoform-specific and tissue-specific effects. For example, Boillard et al. (15) reported that \( \text{PLA2g5} \) has an anti-inflammatory role that counteracts the inflammation mediated by \( \text{PLA2g2a} \) in rheumatoid arthritis. Therefore, the role of \( \text{PLA2G2A} \) and other sPLA2 isoforms in metabolism requires further investigation.

In summary, we have found that \( \text{PLA2G2A} \) improves insulin sensitivity and provides resistance to high fat diet.

**Fig. 8.** Alterations in insulin signaling pathways in IIA+ mice. Proteins were collected from the livers at the end of the feeding period and the abundance of various kinases was analyzed by Western blot. Kinase abundance was measured and the ratio of phospho-kinase to kinase is shown. pAkt/Akt (A), pS6K/S6K (B), representative Western blots are shown (C). Each point is the average of proteins from four animals. The data were analyzed by \( t \)-test. *\( P < 0.05 \), **\( P < 0.01 \).
diet-induced weight gain in mice. Furthermore, PLA2G2A increases the energy expenditure of the mice independent of activity and diet, partly through UCP1 and induction of BAT. We have identified a previously undescribed role of this secretory phospholipase in the modulation of BAT and metabolism. Future studies will determine the mechanisms underlying this phenomenon.

REFERENCES

1. Lambeau, G., and M. H. Gelb. 2008. Biochemistry and physiology of mammalian secreted phospholipases A2. Annu. Rev. Biochem. 77: 495–520.
2. Murakami, M., H. Sato, Y. Miki, K. Yamamoto, and Y. Taketomi. 2015. A new era of secreted phospholipase A(2). J. Lipid Res. 56: 1248–1261.
3. Dennis, E. A., J. Cao, Y. H. Hsu, V. Magrioti, and G. Kokotos. 2011. Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem. Rev. 111: 6130–6185.
4. Kudo, I., and M. Murakami. 2002. Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat. 68–69: 3–58.
5. Murakami, M., Y. Taketomi, H. Sato, and K. Yamamoto. 2011. Secreted phospholipase A2 revisited. J. Biochem. 150: 233–255.
6. Puziński, W., and P. Vadas. 1988. Secretory synovial fluid phospholipase A2 and its role in the pathogenesis of inflammation in arthritis. J. Rheumatol. 15: 1601–1605.
7. Grass, D. S., R. H. Felkner, M. Y. Chiang, R. E. Wallace, T. J. Nevalainen, C. F. Bennett, and M. E. Swanson. 1996. Expression of human group II PLA2 in transgenic mice results in epidermal hyperplasia in the absence of inflammatory infiltrate. J. Clin. Invest. 97: 2233–2241.
8. Murakami, M., Y. Taketomi, C. Girard, K. Yamamoto, and G. Lambeau. 2010. Emerging roles of secreted phospholipase A2 enzymes: lessons from transgenic and knockout mice. Biochimie. 92: 561–582.
9. Massaad, C., M. Paradon, C. Jacques, C. Salvat, G. Bereziat, F. Berenbaum, and J. L. Olivier. 2000. Induction of secreted type IIA phospholipase A2 gene transcription by interleukin-1beta. Role of C/EBP factors. J. Biol. Chem. 275: 22686–22694.
10. Antonio, V., A. Brouillet, B. Janvier, C. Monne, G. Bereziat, M. Andreani, and M. Raymondjean. 2002. Transcriptional regulation of the rat type IIA phospholipase A2 gene by C/EBP, nuclear factor-kappaB and Ets transcription factors. Biochem. J. 368: 415–424.
11. Crowl, R. M., T. J. Stoller, R. R. Conroy, and C. R. Stoner. 1991. Induction of phospholipase A2 gene expression in human hepatoma cells by mediators of the acute phase response. J. Biol. Chem. 266: 2647–2651.
12. Couturier, C., V. Antonio, A. Brouillet, G. Bereziat, M. Raymondjean, and M. Andreani. 2000. Protein kinase A-dependent stimulation of rat type II secreted phospholipase A(2) gene transcription involves C/EBP-beta and -delta in vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 20: 2559–2565.
13. Mallat, Z., J. Benessiano, T. Simon, S. Ederhy, C. Schella-Arguelles, A. Cohen, V. Huart, N. J. Wareham, R. Luben, K. T. Khaw, et al. 2007. Circulating secretory phospholipase A2 activity and risk of incident coronary events in healthy men and women: the EPIC-Norfolk study. Arterioscler. Thromb. Vasc. Biol. 27: 1177–1183.
PLA2G2A modulates metabolism