An Inhibitor of Phospholipase A₂ Group IIA Modulates Adipocyte Signaling and Protects Against Diet-Induced Metabolic Syndrome in Rats

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Obesity, type 2 diabetes, and cardiovascular disease correlate with infiltration to adipose tissue of different immune cells, with uncertain influences on metabolism. Rats were fed a diet high in carbohydrates and saturated fats to develop diet-induced obesity over 16 weeks. This nutritional overload caused overexpression and secretion of phospholipase A₂ group IIA (pla2g2a) from immune cells in adipose tissue rather than adipocytes, whereas expression of adipose-specific phospholipase A₂ (pla2g16) was unchanged. These immune cells produce prostaglandin E₂ (PGE₂), which influences adipocyte signaling. We found that a selective inhibitor of human pla2g2a (5-(4-benzyloxyphenyl)-(4-cytosine signaling. We found that a selective inhibitor of human pla2g2a (5-(4-benzyloxyphenyl)-(4-

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This study has specifically investigated the role of pla2g2a, and the therapeutic potential of its inhibition, in adipose tissue during diet-induced obesity in a rat model relevant to human disease. The findings support a new hypothesis that inhibition of pla2g2a may reverse and protect against adiposity and metabolic dysfunction in diet-induced obese rats and suggest a mechanism of promoting lipolysis to enhance fat utilization and energy expenditure. The pharmacologic responses of a selective pla2g2a inhibitor (5-(4-benzoyloxyphenyl)-(4S)-(phenyl-heptanoylamino)-pentanoic acid [KH064]) were not related to direct effects on adipocytes but rather to decreasing PGE2 release from immune cells (e.g., macrophages, T cells, monocytes, and mast cells) also resident in adipose tissue. This inhibitor stimulated lipolysis in adipose tissue, suggesting a novel mechanism for immune cells regulating lipolysis through the PGE2-EP3-G-protein-coupled receptor pathway in diet-induced obesity.

RESEARCH DESIGN AND METHODS

Animals and diets. Male Wistar rats were bred at The University of Queensland Biological Resources facility. All experimental protocols were approved by the Animal Experimentation Ethics Committee of The University of Queensland. The corncob (CS) and high-carbohydrate high-fat (HCHF) diets have been previously described (21). KH064 (5 mg/kg/day suspended in olive oil) was administered daily by oral gavage to HCHF rats, starting at week 8 of the study protocol. C57BL/6 mice, used for gene expression studies, were fed with a high-fat diet for 16 weeks.

Adipose tissue analysis. Postmortem retroperitoneal, mesenteric, and epididymal fat depots were collected and weighed immediately. The retroperitoneal depot was defined as adipose tissue located behind the kidney, along the back of the abdomen. All molecular analyses have been done in retroperitoneal whole adipose tissue during diet-induced obesity in a rat model.

Gene expression. RNA was extracted using Qiagen reagent according to the RNeasy mini kit (Qiagen). RT-PCR was performed as previously described (22). Primer sequences are listed in Supplementary Table 1. Protein levels were detected using antibodies against pla2g2a (Abcam), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Sigma-Aldrich), and macrophage-colony stimulating factor (Abcam), and the therapeutic potential of its inhibition, in adipose tissue homogenate. Western blot analysis was performed as described (23). Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA) was performed as described (24). Total lipid content. Extraction of tissue and fecal lipids was undertaken by a modified Bligh and Dyer method (25).

RESULTS

Pla2g2a but not pla2g16 is upregulated in rat adipose tissue by HCHF feeding. Our first objective was to investigate which phospholipase could be a potential therapeutic target to prevent adiposity and metabolic dysfunction. Increased PLA2 expression in adipose tissue might dampen lipolysis through PGE2-EP3-G-protein-coupled receptor signaling, promoting adipocyte and metabolic dysfunction together with cardiovascular symptoms of metabolic syndrome (14). Rats were fed a HCHF diet to induce adiposity and symptoms of metabolic syndrome (21). Relative to rats fed a CS diet, those receiving the HCHF diet for 16 weeks became obese, gaining 54 ± 4% weight from weeks 0 to 16 and 112 ± 17% total visceral fat compared with CS rats (21). We measured mRNA expression in adipose tissue of PLA2 isozymes recognized for their roles in inflammation (pla2g2a, pla2g9a, pla2g9b, pla2g9c, pla2g9d, pla2g9e, and pla2g10) or lipid metabolism (pla2g9f and pla2g16). Expression of pla2g9a, pla2g9b, pla2g9c, pla2g9d, pla2g9e, pla2g9f, and pla2g10 genes was extremely low in CS-fed rats but significantly elevated in HCHF-fed obese rats. Among these, PLA2 enzymes were somewhat upregulated in response to HCHF feeding; pla2g2a mRNA expression was strikingly elevated by ~20-fold (Fig. 1A). Correspondingly, pla2g2a protein expression was also substantially increased in adipose tissue from HCHF-fed obese rats compared with CS-fed normal rats (Fig. 1B). Plasma and whole adipose tissue PGE2 concentrations were elevated by HCHF compared with CS feeding, correlating with increased adiposity (Fig. 1C and D). We compared pla2g16 mRNA expression in rat adipose tissue because this enzyme reportedly regulates adipocyte function and lipid metabolism in mice and is overexpressed in ob/ob mice adipose tissue. Pla2g16 was expressed in rat adipose tissue, but expression was unchanged by HCHF feeding (Fig. 1A and Supplementary Fig. 1A). In an isolated experiment, pla2g16 gene expression was unchanged in adipose tissue from lean mice and diet-induced obese mice after 16 weeks (Supplementary Fig. 1A).

KH064 attenuates adiposity in diet-induced obese rats. KH064 is an orally active, potent, and isoform-selective inhibitor of pla2g2a. We have reported a crystal structure for this inhibitor in complex with pla2g2a (25).
and anti-inflammatory activity for this compound (25–28). The increased adiposity exhibited by rats fed a HCHF diet for 16 weeks (Fig. 2) was attenuated by oral administration of KH064 (5 mg/kg/day) between weeks 8 and 16, with marked prevention of body weight gain (weeks 8–16 HCHF, 19 ± 1% +KH064, 9 ± 1%; Fig. 2A and B) and total visceral fat deposition (+KH064, Fig. 2C–E). Treatment with KH064 also attenuated retroperitoneal and omental fat rather than epididymal fat deposition (Fig. 2E). Further, administration of KH064 daily between weeks 8 and 16 to CS-fed rats, which expressed only low pla2g2a, did not exhibit any overt symptoms of toxic side effects or cause changes in body weight (data not shown). Besides improvements in adiposity, KH064 treatment from weeks 8 to 16 attenuated increases in plasma and whole adipose tissue PGE2 concentrations in HCHF rats (Supplementary Fig. 1E and F). Treatment with KH064 normalized this trend to a more M2-like population in the adipose tissue comparable to CS-fed rats (Supplementary Fig. 1E and F).

Pla2g2a overexpression and inhibitor action in immune cells, not adipocytes. Separation of whole adipose tissue into adipocyte and SVC fractions confirmed that pla2g2a was mainly expressed in the SVC fraction rather than adipocytes in adipose tissue. Furthermore, HCHF feeding induced overexpression of pla2g2a, but only in the SVC (Fig. 3C). In contrast, pla2g16 was predominantly expressed in the adipocyte fraction from whole adipose tissue (Supplementary Fig. 1B), and its expression levels were not affected by HCHF feeding (as shown in Fig. 1A).

The pla2g2a enzyme is an important generator of inflammatory lipid mediators during immune cell activation, including PGE2 produced by cyclooxygenase (15,19,20). We focused on PGE2 because it is implicated as a major paracrine antilipolytic factor in adipocytes (14,30). We found that PGE2 inhibited cAMP production in 3T3-L1 adipocytes and established the involvement of EP3 receptor and pertussis toxin-sensitive Goi-proteins in this process (Supplementary Fig. 2A and B). In line with our in vitro results, exogenous addition of PGE2 to rat whole

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**FIG. 1.** RT-PCR gene expression of different PLA2 enzymes and protein expression of pla2g2a in rat adipose tissue. **A:** Quantitative comparison of mRNA expression level of PLA2 enzymes in adipose tissue from rats fed CS vs. HCHF diets for 16 weeks. Values of PLA2 mRNA levels were normalized relative to the housekeeping gene 18S rRNA. **B:** Immunoblot of pla2g2a protein levels in adipose tissue with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as a loading control. Each lane represents adipose tissue homogenate from a single rat. Optical density of protein bands was determined using ImageJ software. Serum (C) and adipose (D) homogenate concentrations of PGE2 from CS vs. HCHF fed rats. Error bars represent means ± SEM (n = 3–5 animals). *P < 0.05, **P < 0.01, ***P < 0.001.
adipose tissue explants decreased glycerol release consistent with reduced lipolysis (Supplementary Fig. 2C).

To establish that pla2g2a was primarily expressed in the SVC and that this was where KH064 was acting, we pretreated SVC with KH064 before lipopolysaccharide (LPS) stimulation. LPS stimulated PGE2 production in the SVC, and this was inhibited by KH064 (Fig. 3D). Further, at a relatively high concentration (1 mmol/L), KH064 did not inhibit any PLA2 enzymatic activity in adipose tissue from CS-fed rats, confirming that KH064 did not inhibit pla2g16 or other PLA2 enzymes in adipose tissue (Supplementary Fig. 3). These results clearly trace the pharmacologic responses of KH064 on pla2g2a to the immune cell–rich SVC in whole adipose tissue and not the adipocyte fraction.

Regulation of lipolysis via pla2g2a and PGE2 in immune cells. Many types of resident and infiltrated immune cells, including macrophages, monocytes, T cells and mast cells, contribute to adipocyte dysfunction (5–7). However, their relative roles and importance in lipid homeostasis and adipocyte function in obesity are not yet clear. In this study, we investigated whether some or all of these cells regulate lipolysis via pla2g2a/PGE2 in adipose. Because we were not able to isolate infiltrated rat adipose tissue immune cells in sufficient quantity or purity for in vitro studies, we chose five primary or cultured human cell types that are closely associated with adiposity and metabolic dysfunction. The five immune cell types—HMDM, PBMC, HMC-1, Jurkat, and THP-1—all showed increased PGE2 production after stimulation with palmitic acid, the most common and abundant nutritional fatty acid in Western-style diets that plays a major role in inducing adipocyte and metabolic dysfunction in obese subjects (31). Treatment of each cell type with KH064 markedly reduced (two- to fivefold) this increased PGE2 production (Fig. 4). To support the notion that KH064 was inhibiting palmitic acid-induced PGE2 production via the pla2g2a enzyme in these cell types, LPS was separately used to elicit PGE2 production in these five immune cell types (Supplementary Fig. 4). HMDM, PBMC, and HMC-1 showed increased PGE2 production after LPS stimulation, and KH064 treatment markedly reduced the increased PGE2 production in these cells (Supplementary Fig. 4). However, there was no significant PGE2 increase in Jurkat and THP-1 cells after stimulation with LPS. This suggests that, in addition to macrophages (8), other immune cell types, such as monocytes, T cells, and mast cells, may also secrete anti-lipolytic factors, such as PGE2, to reduce lipolysis and free fatty acid concentrations, thereby promoting adiposity. 

Pla2g2a and PGE2 inhibition restores lipolysis in vivo. One approach to improve adipocyte function is to decrease fat stores in adipose tissue by stimulating lipolysis and oxidation of released fatty acids (13). Because the pla2g2a inhibitor KH064 normalized lipolysis and attenuated adiposity, we investigated whether inhibiting PGE2 in vivo stimulated enhanced release of fat from adipose toward better fat utilization and oxidation in the liver and skeletal muscle. Phosphorylation of HSL through cAMP-mediated activation of protein kinase A is a key mediator of increased lipolysis in adipose tissue (14). HCHF-fed rats decreased phosphorylation of HSL (Ser563) in adipose compared with CS-fed rats (Fig. 5A). Treatment with KH064 from weeks 8 to 16 normalized or increased lipolysis in adipose of HCHF rats by preventing this decrease in HSL phosphorylation, without altering total HSL protein expression (Fig. 5A). In addition, genes involved in lipid metabolism and the lipolytic cascade, such as peroxisome
proliferator-activator receptor-γ (Pparg), Ppargc1a, uncoupling protein 2 (Ucp2), adiponectin (Adipoq), adipose triglyceride lipase/desnutrin (Atgl), and Hsd1 in adipose tissue were all normalized or increased with KH064 treatment compared with CS-fed and HCHF-fed rats, respectively (Fig. 5B). Plasma nonesterified fatty acids were increased with KH064 treatment compared with untreated HCHF animals at week 16, consistent with increased lipolysis from adipose tissue stores (Fig. 5C).

During lipolysis, either through acute weight loss or metabolic disease, macrophages have been proposed as transporters of fatty acids from adipose and peripheral tissues to liver for metabolism and energy production, similar to involvement of macrophages in the reverse cholesterol transport pathway during atherosclerosis (8,9). KH064 treatment from weeks 8 to 16 (Fig. 5D) represented fat droplets (Supplementary Fig. 5 and Fig. 5E). Histology analysis showed that livers from KH064-treated rats had accumulated fat droplets (Supplementary Fig. 5 and Fig. 5E). Plasma concentrations of alkaline phosphatase increased in HCHF-fed rats but increased or normalized in HCHF rats treated with KH064 (Fig. 5G). In skeletal muscle, carnitine palmityltransferase 1 (Cpt1) and Ucp2 genes involved in energy expenditure were suppressed in HCHF rats but increased or normalized in HCHF rats treated with KH064 (Fig. 5H). Other genes involved in fatty acid oxidation and mitochondrial biogenesis, including Pparg, Ppargc1a, and pyruvate dehydrogenase kinase 4 (Pdk4) in adipose, liver, and skeletal muscle (32,33), were upregulated in KH064-treated compared with untreated HCHF-fed rats (Fig. 5B, G, and H). However, further investigation is needed on the exact mechanisms by which KH064 alters the expression of lipid-handling genes in liver and skeletal muscle and its direct responses on these metabolically relevant tissues.

**KH064 PREVENTS DIET-INDUCED ADIPOSY**

**FIG. 3.** KH064 treatment modulates PGE2 concentrations in Wistar rats and SVCs. PGE2 concentrations in serum (A) and whole adipose tissue (B) from rats fed a high carbohydrate high fat (HCHF) diet alone or with drug (KH064 5 mg/kg/day). C: Pla2g2a gene expression in whole adipose tissue, adipocyte, and SVC fractions. D: Ex vivo treatment with KH064 inhibits LPS-induced PGE2 production in SVCs. Error bars represent mean ± SEM of least three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

factor 1 (Srebf1), Ucp2, Pparg, and hepatic lipase (Lipc), were suppressed in HCHF rats but increased or normalized in HCHF rats treated with KH064 (Fig. 5G). In skeletal muscle, carnitine palmityltransferase 1 (Cpt1) and Ucp2 genes involved in energy expenditure were suppressed in HCHF rats but increased or normalized in HCHF rats treated with KH064 (Fig. 5H). Other genes involved in fatty acid oxidation and mitochondrial biogenesis, including Pparg, Ppargc1a, and pyruvate dehydrogenase kinase 4 (Pdk4) in adipose, liver, and skeletal muscle (32,33), were upregulated in KH064-treated compared with untreated HCHF-fed rats (Fig. 5B, G, and H). However, further investigation is needed on the exact mechanisms by which KH064 alters the expression of lipid-handling genes in liver and skeletal muscle and its direct responses on these metabolically relevant tissues.

**Pla2g2a inhibitor KH064 promotes lipolysis and protects against diet-induced metabolic syndrome.** Attenuation of adipocyte dysfunction or adiposity improves metabolic and cardiovascular symptoms of metabolic syndrome in animals and clinical studies (4,34–37). In this study, alterations in metabolic parameters in HCHF-fed compared with CS-fed rats included decreased expression of genes involved in glucose metabolism in the pancreas (Fig. 6A), increased plasma insulin concentrations (Fig. 6B), impaired glucose and insulin tolerance (Fig. 6C–E), increased systolic blood pressure (Supplementary Table 2 and Supplementary Fig. 6), and abnormalities in cardiac structure and function (Fig. 6F–H). Treatment with KH064 attenuated changes in glucose metabolism genes in the pancreas, such as glucose transporter 2 (glut2), hexokinase, and mitochondria glycerophosphate dehydrogenase (mGPDH) (38,39), while normalizing glucose and insulin tolerance (Fig. 6C–E). Furthermore, most
of the changes in cardiovascular structure and function in HCHF-fed rats were attenuated by KH064 (Fig. 6A–H, Supplementary Table 2, and Supplementary Fig. 6), including excessive collagen deposition in the left ventricle of the heart in HCHF rats.

**DISCUSSION**

This study reports important new evidence for involvement of secretory phospholipase A2 enzyme, pla2g2a, in diet-induced adiposity and metabolic and cardiovascular dysfunction. Phospholipases are important in pathogenesis of chronic inflammatory diseases and thought to be important in lipid metabolism. Here we show that the specific isozyme pla2g2a is an important mediator in the cross talk between immune and metabolic systems in adipose tissue, relevant to lipid and energy homeostasis, metabolic, and cardiovascular function. In HCHF-fed rats, pla2g2a was upregulated in the SVC but not in the adipocyte fraction of adipose tissue. The selective pla2g2a inhibitor (KH064) (25), administered orally to HCHF-fed rats, was found herein to inhibit the development of obesity, adiposity, insulin resistance, and glucose intolerance. The pla2g2a inhibitor also attenuated changes in other features of metabolic and cardiovascular dysfunction induced by nutritional overload.

These in vivo effects of KH064 were linked to preventing PGE2 release in adipose tissue from immune cells and not adipocytes. Immune cells infiltrate adipose tissue during obesity and metabolize fatty acids. Our hypothesis is that these immune cells overexpress pla2g2a, generating metabolites of phospholipids, such as PGE2, to act on secondary target cells (adipocytes) and inhibit lipolysis via a PGE2–EP3–cAMP pathway. An inhibitor of pla2g2a has been shown here to inhibit in vitro palmitate- or LPS-stimulated release of PGE2 from macrophages, T cells, monocytes, and mast cells, known to reside in adipose tissue of obese humans (5, 6, 40). The differential responses of immune cells to LPS- and palmitate-induced secretion of PGE2 may involve other, as yet unknown, signaling mechanisms. Palmitate-induced production of PGE2 may involve fatty acid receptors, such as FFA1 (GPR40), FFA2 (GPR43), FFA3 (GPR41), or GPR120 (41). Nonetheless, in vivo KH064 decreased plasma and adipose PGE2 concentrations, normalized and stimulated lipolysis in adipose tissue, and protected against adiposity in diet-induced obese rats. This suggests a novel role for immune cells in regulating lipolysis through EP3–Gor1–cAMP signaling during diet-induced obesity. PGE2 regulates adipocyte dysfunction and contributes to antilipolytic pathways via EP3 receptors, thereby decreasing cAMP concentrations (14). This is the first study to show that modulating PGE2 secretion, through inhibition of pla2g2a from key immune cells resident in and infiltrated to adipose, reverses diet-induced adiposity and metabolic syndrome. However, we cannot rule out improvements in metabolic parameters arising from synergistic responses from KH064 acting on other metabolically relevant tissues along with adipose, from decreased intestinal fat absorption and altered lipid metabolism, or through unknown off-target effects on nuclear receptors such as constitutive active/androstane receptor (CAR) or pregnane X receptor (PXR).

Some other PLA2 isozymes (pla2g16, pla2g6b, pla2g4a) may be important in metabolism because their genetic knockout prevented development of obesity (14, 42–44). However, both pla2g16−/− and pla2g6b−/− phenotypes exhibited severe defects in insulin secretion. In pla2g16−/− mice,
FIG. 5. In vivo responses of KH064 (5 mg/kg/day p.o., weeks 8–16) on the regulation of metabolic parameters that were elevated in rats fed HCHF vs. CS diets for 16 weeks. A: Immunoblot of phospho-HSL (Ser563) in adipose tissue with total HSL as the loading control. Optical density of protein bands was determined using ImageJ software. B: RT-PCR gene expression of genes in adipose tissue of rats fed CS and HCHF diets with and without KH064 treatment. C: Plasma concentrations for lipids nonesterified fatty acids (NEFA), triglycerides, and total cholesterol in rats of different groups (n = 5–10). D: Liver total lipid content in rats of different groups (n = 5). E: Plasma liver enzymes in rats of different groups (n = 5–10). F: Total soluble fecal fat in rats of different groups (n = 5). RT-PCR gene expression of different metabolic genes in liver (G) and skeletal muscle (H). Values of mRNA levels were normalized relative to 18S rRNA. Error bars represent means ± SEM (n = 3–5 animals). *P < 0.05, **P < 0.01, ***P < 0.001.
insulin resistance was observed in normal and high-fat diets (14), whereas pla2g6b<sup>2/2</sup> mice were refractory to glucose-stimulated insulin release (43). Similarly, contradictory evidence on deposition of lipids in the liver was reported; pla2g16<sup>2/2</sup> mice showing increased fat deposition in the liver, whereas pla2g4a<sup>2/2</sup> and pla2g6b<sup>2/2</sup> had decreased fat deposition in liver compared with wild types after high fat feeding (43–45). There is growing evidence suggesting that immune cells transport lipids from adipose to liver for metabolism, but those studies did not investigate whether deposition of lipids was transient or on-going or caused liver damage. Moreover, pla2g16 was upregulated in genetically modiﬁed mice (ob/ob and db/db) and in acute-fed fasted mice (14). In our much longer-duration study, pla2g16 gene expression was unchanged in adipose from rats and mice (Supplementary Fig.1A) after 16 weeks of HCHF feeding. Our ﬁndings differ from those reported for pla2g16 in fasted acute-fed mice (14). It is conceivable that pla2g2a might act early in a proinflammatory pathway required for immune cell inﬁltration. In the absence of pla2g16, any increase in adipose tissue inﬁammation and immune cell inﬁltration might have been blocked to prevent an increase in adipose tissue PLA2 activity. However, in our much longer chronic obesity experiments, it is clear that pla2g2a is unique among sPLA2 isoforms examined in being dramatically overexpressed in HCHF-fed rats compared with CS-fed rats, and importantly, the pla2g2a-selective inhibitor attenuated adiposity and most symptoms of metabolic syndrome in these rats.

Activating inﬂammatory lipid mediators associated with adipokines and cytokines to alter adipocyte and immune cell function has now become the prevailing hypothesis to explain mechanisms of metabolic and adipocyte dysfunction (4,46). Nutrient- and pathogen-sensing systems in humans may have evolved together, possibly for more efﬁcient management of energy homeostasis, metabolic function, and immunity by coordinating energy storage, transport, and metabolism (1,3). We speculate that recruiting immune cells into adipose during obesity, and associated metabolic dysfunction, is not only an inﬂammatory response to stress but also a mechanism to regulate energy metabolism. Weight loss by calorie restriction is associated with macrophage recruitment to white adipose tissue and regulation of lipid trafﬁcking and lipolysis (8,9). Our hypothesis is supported by recent studies in which there was increased local release of fatty acids during fasting, inducing recruitment of immune cells and secretion of antilipolytic factors such as PGE2 to reduce free fatty acid concentrations (8,9). Our results indicate that resident and inﬁltrating immune cells in adipose have
important signaling roles in regulating energy metabolism, such as lipolysis and oxidation of released fatty acids, and these signaling networks may be overloaded and dysfunctional in obesity. Pharmacologic modulation of immune cells can improve diet-induced adiposity and symptoms of metabolic syndrome. Contrary to current opinion, increasing or normalizing lipolysis as a pharmacologic strategy or elevating plasma free fatty acids in obese subjects may not necessarily translate into metabolic dysfunction or insulin resistance (47). More dynamic and refined studies are required to clarify relationships and roles of specific fatty acids in obesity and metabolic dysfunction, with greater emphasis on consequences of impaired or dysfunctional adipocyte fat storage, release, and function on adipokines/cytokines that affect metabolism (47).

In summary, pla2g2a has been shown to be by far the most upregulated Pla2 isozyme in adipose tissue of diet-induced obese rats. This protein is localized to immune cells in adipose tissue rather than to adipocytes. The study raises the possibility that systemic pla2g2a inhibition may decrease fat stores by preventing PGE2 biosynthesis from immune cells in adipose tissue to stimulate lipolysis and fatty acid oxidation. Importantly, KH064 is a potent and specific inhibitor of human pla2g2a and prevents diet-induced adiposity, insulin resistance, and metabolic and cardiac dysfunction in rats. These results indicate that modulation of adipose tissue homeostasis, by blocking an endocrine and paracrine stimulus from immune cells rather than adipocytes, may be a novel approach to treat diet-induced metabolic syndrome in humans.

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A.I. and J.L. performed experiments, collected data, and drafted the manuscript. H.P., J.Y.S., and J.W. assisted in data collection. R.C.R. synthesized the inhibitor. J.B.P. and J.P.W. provided advice on cellular studies. D.P.F. and L.B. directed the research, contributed intellectually, and developed the manuscript. D.P.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444: 860–867.
2. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011;29:415–445.
3. Hormet T, Hotamisligil GS. Linking the inflammasome to obesity-related disease. Nat Med 2011;17:164–165.
4. Iyer A, Fairlie DP, Prins JB, Hammock BD, Brown L. Inflammatory lipid mediators in adipocyte function and obesity. Nat Rev Endocrinol 2010;6:71–82.
5. Liu J, Divoux A, Sun J, et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med 2009;15:940–945.
6. Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009;15:930–939.
7. Nishimura S, Manabe I, Nagasaki M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009;15:914–920.
8. Kosteli A, Sugaru E, Haemmerle G, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest 2010;120:3456–3470.
9. Red Eagle A, Chawla A. In obesity and weight loss, all roads lead to the mighty macrophage. J Clin Invest 2010;120:3457–3440.
10. Spalding KL, Arner E, Westmark PO, et al. Dynamics of fat cell turnover in humans. Nature 2008;453:783–787.
11. Arner P, Bernard S, Salehpour M, et al. Dynamics of human adipose lipid turnover in health and metabolic disease. Nature 2011;478:110–113.
12. Koch L. Adipose lipid turnover—a new target in metabolic disease. Nat Rev Endocrinol 2011;7:694.
13. Langin D. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacol Res 2006;53:482–491.
14. Jaworski K, Ahmadian M, Duncan RE, et al. AdPLA inhibition increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. Nat Med 2009;15:159–168.
15. Scott DI, White SP, Browning JL, Rosa JJ, Gelb MH, Sigler PB. Structures of free and inhibited human secretory phospholipase A2 from inflammatory exudate. Science 1994;265:1000–1001.
16. Duncan RE, Sarkadi-Nagy E, Jaworski K, Ahmadian M, Sul HS. Identification and functional characterization of adipose-specific phospholipase A2 (AdPLA). J Biol Chem 2008;283:25428–25436.
17. Strong P, Coleman RA, Humphrey PP. Prostanoid-induced inhibition of lipolysis in rat isolated adipocytes: probable involvement of EP3 receptors. Prostaglandins 1992;43:559–566.
18. Kang S, Moua-Maouss S, Vastag S. Secretory, endocrine and autocrine/paracrine function of the adipocyte. J Nutr 2000;130:3110S–3115S.
19. Weir JP, Schevitz RW, Clawson DK, et al. Structure of recombinant human rheumatoid arthritic synovial fluid phospholipase A2 at 2.2 Â A resolution. Nature 1991;352:79–82.
20. Reddy ST, Herschman HR. Transcellular prostaglandin production following mast cell activation is mediated by proximal secretory phospholipase A2 and distal prostaglandin synthase 1. J Biol Chem 1996;271:169–181.
21. Panczel SK, Poutly H, Iyer A, et al. High-carbohydrate, high-fat diet-induced metabolic syndrome and cardiovascular remodeling in rats. J Cardiovasc Pharmacol 2011;57:611–624.
22. Suen JY, Gardiner B, Grimmond S, Fairlie DP. Profiling gene expression induced by protease-activated receptor 2 (PAR2) activation in human kidney cells. PLoS One 2010;5:e13809.
23. Iyer A, Fenning A, Lim J, et al. Antiadipogenic activity of an inhibitor of histone deacetylases in DOCA-salt hypertensive rats. Br J Pharmacol 2010;159:1408–1417.
24. Poutly H, Panczel SK, Waanders J, Ward L, Brown L. Lipid redistribution by o-linoleic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. J Nutr Biochem 2012;23:153–162.
25. Hansford KA, Reid RC, Clark CI, et al. D-Tyrosine as a chiral prursor to potent inhibitors of human nonpancreatic secretory phospholipase A2 (Ila) with antiinflammatory activity. ChemBioChem 2003;4:181–185.
26. Levick S, Loch D, Rolfe B, et al. Antiadipogenic activity of an inhibitor of group IIa secretory phospholipase A2 in young spontaneously hypertensive rats. J Immunol 2006;176:7000–7007.
27. Arumugam TV, Arnold N, Proctor LM, et al. Comparative protection against rat intestinal reperfusion injury by a new inhibitor of sPLA2, COX-1 and COX-2 selective inhibitors, and an LTC4 receptor antagonist. Br J Pharmacol 2003;140:71–80.
28. Woodruff TM, Arumugam TV, Shields IJ, et al. A potent and selective inhibitor of group IIa secretory phospholipase A2 protects rats from TNBS-induced colitis. Int Immunopharmacol 2005;5:883–892.
29. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007;117:175–185.
30. Mates MK, Thelen AP, Jump DB. Arachidonic acid and PGE2 regulation of hepatic lipogenic gene expression. J Lipid Res 1999;40:1045–1052.
31. Guilherme A, Virbasious JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:367–377.
32. Wende AR, Huss JM, Schaeffer PJ, Girgieri V, Kelly DP. PGC-lalpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha.
a mechanism for transcriptional control of muscle glucose metabolism. Mol Cell Biol 2005;25:10684–10694
33. Gerhart-Hines Z, Rodgers JT, Bare O, et al. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J 2007;26:1913–1923
34. Van Gaal LF, Mertens I, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875–880
35. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821–1830
36. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
37. Odegaard JJ, Ricardo-Gonzalez RR, Goforth MH, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 2007;447:1116–1120
38. Kjørholt C, Akerfeldt MC, Biden TJ, Laybutt DR. Chronic hyperglycemia, independent of plasma lipid levels, is sufficient for the loss of beta-cell differentiation and secretory function in the db/db mouse model of diabetes. Diabetes 2005;54:2755–2763
39. Thorens B, Weir GC, Leahy JL, Lodish HF, Bonner-Weir S. Reduced expression of the liver/beta-cell glucose transporter isoform in glucose-insensitive pancreatic beta cells of diabetic rats. Proc Natl Acad Sci USA 1990;87:6492–6496
40. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1706–1808
41. Stoddart LA, Smith NJ, Milligan G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFAR1, -2, and -3: pharmacology and physiological functions. Pharmacol Rev 2008;60:405–417
42. Song H, Wohltmann M, Bao S, Ladenson JH, Semenkovich CF, Turk J. Mice deficient in group VIB phospholipase A2 (iPLA2gamma) exhibit relative resistance to obesity and metabolic abnormalities induced by a Western diet. Am J Physiol Endocrinol Metab 2010;298:E1097–E1114
43. Hatakeyama S, Tsutsumi K, Sato T, Akiba S. Group IVA phospholipase A2 is associated with the storage of lipids in adipose tissue and liver. Prostaglandins Other Lipid Mediat 2008;6:12–17
44. Mancuso DJ, Sims HF, Yang K, et al. Genetic ablation of calcium-independent phospholipase A2gamma prevents obesity and insulin resistance during high fat feeding by mitochondrial uncoupling and increased adipocyte fatty acid oxidation. J Biol Chem 2010;285:36495–36510
45. Hatakeyama S, Tsutsumi K, Sato T, Akiba S. Group IVA phospholipase A2 is associated with the storage of lipids in adipose tissue and liver. Prostaglandins Other Lipid Mediat 2008;6:12–17
46. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes 2011;60:2441–2449
