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 E\(_2\) (PGE\(_2\)), which influences adipocyte signaling. We found that a selective inhibitor of human pla2g2a (5-(4-benzyloxyphenyl)-(4-cyto signaling. We found that a selective inhibitor of human pla2g2a (5-(4-benzyloxyphenyl)-(4-cyto signaling. We found that a selective inhibitor of human pla2g2a (5-(4-benzyloxyphenyl)-(4-cyto signaling. We found that a selective inhibitor of human pla2g2a (5-(4-benzyloxyphenyl)-(4-cyto signaling. 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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-benzyloxyphenyl)-(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-CAMP 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 corticosteroid (CS) and high-carbohydrate high-fat (HCHF) diets have been previously described (21). KH064 (5 mg/kg/day suspended in olive oil) was administered directly 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, mesenteric and epididymal fat pads 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 as a major component of abdominal fat. Retroperitoneal rat whole adipose tissue was digested using collagenase B and was differentially centrifuged to derive floating adipocyte and stromal vascular cell (SVC) fractions. To assess lipolysis, retroperitoneal whole adipose tissue explants were incubated in Kreb's Ringer bicarbonate HEPES for 2 h, with or without PGE2. Glycerol levels were measured using Free Glycerol Reagent (Sigma-Aldrich).

Gene expression. RNA was extracted using Qiazol reagent according to the RNeasy mini kit (Qiagen). RT-PCR was performed as previously described (22). Primer sequences are listed in Supplementary Table 1.

Immunoblot. Protein levels were detected using antibodies against pla2g2a (Abcam), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Sigma-Aldrich), phospho-hormone-sensitive lipase (HSL) (Ser563), and HSL (Cell Signaling). Bands were analyzed using ImageJ 1.40e software.

Cell culture. Human breast cancer cell line (MCF-7) cells were cultured in Iscove's modified Dulbecco's medium (IMDM). Jurkat E6.1 and human acute monocytic leukemia cell line (THP-1) cells were cultured in RPMI. Human peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (HMDMs) were cultured in IMDM. 3T3-L1 fibroblasts were differentiated 2 days after confluence, and fully differentiated adipocytes were used for experiments such as the cAMP-Glo assay (Promega).

PBMC and HMDM isolation. PBMCs and HMDMs were harvested from buffy coat of anonymous human donors (Australian Red Cross Blood Service, Brisbane). For HMDMs, cluster of differentiation (CD) 14+ monocytes were isolated using magnetic sorting (Miltenyi Biotech), then differentiated using macrophage-colony stimulating factor.

Enzyme-linked immunosorbent assay. Cells were pretreated with KH064 (10 μmol/L) for 15 min before experiments. Culture supernatant, serum, and adipose tissue homogenate PGF and insulin concentrations were determined using a PGF2 metabolite and a rat insulin kit, respectively.

Physiologic parameters. Oral glucose tolerance and clearance tests were previously described. For glucose tolerance, a glucose load of 2 g/kg body weight was used for glucose tolerance and an insulin load of 0.3 IU/kg was used for insulin tolerance (21). Plasma lipids and enzyme concentrations were measured at one of Queensland Veterinary Pathology Service (21). Systolic blood pressure was measured as described (23).

Echocardiographic studies, heart preparation, and histopathologic analysis. Echocardiographic examination was performed after 16 weeks as described (21). Histopathologic analysis of the heart, liver, and adipose was performed as described (21,23).

Total lipid content. Extraction of tissue and fecal lipids was undertaken by manual solvent extraction as described (24).

Drug. The inhibitor KH064 of pla2g2a was synthesized, purified, and enzymatically characterized as described (compound 2b in (25)). It binds in the active site of human pla2g2a (25) and is a potent inhibitor of enzymatic activity (half-maximal inhibitory concentration [IC50] 0.029 μmol/L). This enzyme is similar to human macrophage pla2g5 enzyme against which KH064 is 100 times less potent (IC50 2 μmol/L) (26). The rat homolog of human pla2g2a was not commercially available. KH064 does not bind or inhibit cPLA2, or iPLA2, enzymes (unpublished data from D.P.F.). We have reported pharmacokinetics for KH064 given to rats intravenously (27) and orally (28). At 5 mg/kg p.o. (as used herein), KH064 was detectable in plasma within 15 min at relatively constant concentrations for 6 h with Cmax −0.2 μg/mL, Tmax = 2 h, and ~4% oral bioavailability (area under the curve orally/area under the curve intravenously). KH064 was −0.05 μg/mL in plasma 18-24 h after oral dosing, suggesting partitioning to tissues (28). KH064 has been administered to mice at 300 mg/kg, in a single dose with no substantive toxic effects, and it has no significant off-target effects in a screen against 25 enzymes and 30 human G protein-coupled receptors (unpublished data from D.P.F.). KH064 is progressing to clinical development.

Statistical analysis. Data were plotted and analyzed using GraphPad Prism v5.00 software (GraphPad Software, San Diego, CA). Statistical differences in pairwise comparisons between treatments were assessed using the Student t test. Two-way comparisons in both weight and body weight were assessed by two-way ANOVA (+P < 0.05, **P < 0.01, and ***P < 0.001). All values of independent parameters are mean ± SEM (n ≥ 3 independent experiments) unless stated otherwise.

RESULTS

Pla2g2a but not pla2g16 is upregulated in rat adipose 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-Gαs-CAMP 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 isoforms recognized for their roles in inflammation (pla2g2a, pla2g4a, pla2g5, pla2g7, and pla2g10) or lipid metabolism (pla2g6 and pla2g16). Expression of pla2g2a, pla2g6, pla2g9a, pla2g7, 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 obese mice. 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. 1B).

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)

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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 (Fig. 3A and B). Adipose tissue immunohistochemistry demonstrated that treatment with KH064 also prevented the significant increase in crown formation and macrophage infiltration measured by ED1 staining (Supplementary Fig. 1C and D). Further, investigating the polarization of infiltrated and resident macrophages in adipose tissue using inflammatory/surface marker gene expression to profile M1- versus M2-macrophages (29) suggested that HCHF-fed rats had increased expression of M1-specific genes in adipose tissue in contrast to CS-fed 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 Gαi-proteins in this process (Supplementary Fig. 2A and B). In line with our in vitro results, exogenous addition of PGE2 to rat whole
adipose tissue explants decreased glycerol release consistent with reduced lipolysis (Supplementary Fig. 2). 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. 3). 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 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 triacylglyceride 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 attenuated this increase in liver enzymes (Fig. 5E). Further, the increased lipid content in liver was not attenuated with KH064 treatment from weeks 8 to 16 (Fig. 5D). 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 compared with CS-fed and HCHF-fed rats, respectively (Fig. 6A). Plasma nonesterified fatty acids were increased in HCHF rats but increased or normalized in HCHF rats treated with KH064 (Fig. 5G). In skeletal muscle, carnitine palmitoyltransferase 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. 5G, 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 (mgP DH) (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–Gαi-cAMP signaling during diet-induced obesity. PGE2 regulates adipocyte dysfunction and contributes to antilipolytic pathways via EP 3 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 (14,22–44). However, both pla2g16<sup>−/−</sup> and pla2g6b<sup>−/−</sup> phenotypes exhibited severe defects in insulin secretion. In pla2g16<sup>−/−</sup> 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>−/−</sup> mice were refractory to glucose-stimulated insulin release (43). Similarly, contradictory evidence on deposition of lipids in the liver was reported; pla2g16<sup>−/−</sup> mice showing increased fat deposition in the liver, whereas pla2g4a<sup>−/−</sup> and pla2g6b<sup>−/−</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-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 PGE<sub>2</sub> 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.

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