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CGI-58/ABHD5-Derived Signaling Lipids Regulate Systemic Inflammation and Insulin Action

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Mutations of comparative gene identification 58 (CGI-58) in humans cause Chanarin-Dorfman syndrome, a rare autosomal recessive disease in which excess triacylglycerol (TAG) accumulates in multiple tissues. CGI-58 recently has been ascribed two distinct biochemical activities, including coactivation of adipose triglyceride lipase and acylation of lysophosphatidic acid (LPA). It is noteworthy that both the substrate (LPA) and the product (phosphatic acid) of the LPA acyltransferase reaction are well-known signaling lipids. Therefore, we hypothesized that CGI-58 is involved in generating lipid mediators that regulate TAG metabolism and insulin sensitivity. Here, we show that CGI-58 is required for the generation of signaling lipids in response to inflammatory stimuli and that lipid second messengers generated by CGI-58 play a critical role in maintaining the balance between inflammation and insulin action. Furthermore, we show that CGI-58 is necessary for maximal TH1 cytokine signaling in the liver. This novel role for CGI-58 in cytokine signaling may explain why diminished CGI-58 expression causes severe hepatic lipid accumulation (1). However, recent studies in mice with diminished levels of CGI-58 clearly show that ATGL-lipase (ATGL) (1). However, recent studies in mice with diminished levels of CGI-58 clearly show that ATGL-independent functions for CGI-58 also must exist (2,6). In addition to activating ATGL, CGI-58 catalyzes the acylation of lysophosphatidic acid (LPA) to generate the critical lipid second messenger phosphatidic acid (PA). Both the substrate (LPA) and the product (PA) of the LPA acyltransferase (LPAAT) reaction are well-known signaling lipids with critical roles in angiogenesis, cardiac development, carcinogenesis, and immunity (7–9). Furthermore, fibroblasts from CDS patients have dramatically altered rates of synthesis and turnover of other major lipids with signaling potential, including phosphatidylcholine (PC), phosphatidylinositol, and phosphatidylserine (10,11). Given the central importance of lipid mediators in growth factor and cytokine-mediated signal transduction (7–9), we reasoned that CGI-58 may be a novel source of signaling lipids. Unfortunately, conventional gene targeting of CGI-58 in mice results in premature lethality (6). To circumvent this, we used targeted antisense oligonucleotides (ASOs) to test whether CGI-58 plays a quantitatively important role in the generation of signaling lipids in vivo. Our findings show that CGI-58 is a novel source of signaling lipids that links inflammation to TAG and glucose metabolism.

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CGI-58/ABHD5-Derived Signaling Lipids Regulate Systemic Inflammation and Insulin Action

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C omparative gene identification 58 (CGI-58), also known as α/β hydrolase domain-containing protein 5 (ABHD5), recently has gained attention as the master regulator of triacylglycerol (TAG) hydrolysis and phospholipid metabolism (1–4). However, molecular mechanisms by which CGI-58 regulates these metabolic processes still are incompletely understood. Because the discovery that mutations in CGI-58 cause Chanarin-Dorfman syndrome (CDS) (5), several groups have studied CGI-58’s biochemical properties in vitro (1–4). An important advancement on this front came when it was demonstrated that CGI-58 indirectly promotes TAG hydrolysis by coactivating adipose triglyceride lipase (ATGL) (1). However, recent studies in mice with diminished levels of CGI-58 clearly show that ATGL-independent functions for CGI-58 also must exist (2,6). In addition to activating ATGL, CGI-58 catalyzes the acylation of lysophosphatidic acid (LPA) to generate the critical lipid second messenger phosphatidic acid (PA). Both the substrate (LPA) and the product (PA) of the LPA acyltransferase (LPAAT) reaction are well-known signaling lipids with critical roles in angiogenesis, cardiac development, carcinogenesis, and immunity (7–9). Furthermore, fibroblasts from CDS patients have dramatically altered rates of synthesis and turnover of other major lipids with signaling potential, including phosphatidylcholine (PC), phosphatidylinositol, and phosphatidylserine (10,11). Given the central importance of lipid mediators in growth factor and cytokine-mediated signal transduction (7–9), we reasoned that CGI-58 may be a novel source of signaling lipids. Unfortunately, conventional gene targeting of CGI-58 in mice results in premature lethality (6). To circumvent this, we used targeted antisense oligonucleotides (ASOs) to test whether CGI-58 plays a quantitatively important role in the generation of signaling lipids in vivo. Our findings show that CGI-58 is a novel source of signaling lipids that links inflammation to TAG and glucose metabolism.

RESEARCH DESIGN AND METHODS

Male C57BL/6N mice (Harlan) were maintained on standard rodent chow or a high-fat diet (HFD) for a period of 4–10 weeks and simultaneously injected with ASOs targeting knockdown (KD) of CGI-58, as previously described (2). The diets and ASOs used here have been described elsewhere (2). The HFD was prepared by our institutional diet core and contains ~45% of energy as fat (16.0 = 23.3, 18.0 = 15.9, 18.1 = 34.8, and 18.2 = 18.7%). The 20-mer phosphorothioate ASOs were designed to contain 2’-O-methoxymethyl groups at positions 1–5 and 15–20 and were synthesized, screened, and purified, as described previously (12), by ISIS Pharmaceuticals (Carlsbad, CA). The CGI-58 ASOs used in the current studies were described as CGI-58 ASO 9 in our previous work (2). All mice were maintained in an American Association for Accreditation of Laboratory Animal Care–approved specific pathogen-free environment on a 12:12-h light:dark cycle and allowed free access to regular chow and water. All experiments were performed with the approval of the institutional animal care and use committee.

Lipopolysaccharide-induced acute-phase response. Mice were injected with control or CGI-58 ASOs and maintained on standard chow or an HFD for a period of 4 weeks, as previously described (2). After 4 weeks of ASO treatment, mice were injected intraperitoneally with either saline or 5 μg lipopolysaccharide (LPS) (Escherichia coli 0111:B4). Following injection, plasma was collected at 1 h by submandibular puncture (for tumor necrosis factor [TNF] α measurements), and exactly 6 h after injection mice were killed with ketamine/xylazine (100–160 mg/kg ketamine and 20–32 mg/kg xylazine). Thereafter, a midline laparotomy was performed, and blood was collected by heart puncture. After blood collection, a whole-body perfusion was conducted by puncturing the inferior vena cava and slowly delivering 10 mL sterile 0.9% saline into the left ventricle of the heart to remove residual blood. Multiple tissues were collected and snap-frozen for subsequent analysis.
In vivo insulin-signaling analyses. Mice were injected with control or CGI-58 ASOs and maintained on standard chow or an HFD for a period of 4 weeks, as previously described (2). After an overnight fast (11:00 p.m. to 9:00 a.m.), mice were anesthetized with isoflurane (4% for induction and 2% for maintenance) and were placed in a 37°C heating pad to control body temperature. A minimal midline laparotomy was performed, and the portal vein was visualized. Sterile saline or recombinant human insulin (0.5 units/kg body wt; Novo Nordisk) was administered directly into the portal vein. Exactly 5 min later, tissues were excised without saline perfusion and immediately snap frozen in liquid nitrogen. Protein extracts from tissues were analyzed by Western blotting, as previously described (13–15), and lipid extracts were analyzed using mass spectrometry methods (16), as described in detail below.

Cytokine signaling in primary hepatocytes. After 4 weeks of ASO treatment, mouse primary hepatocytes were isolated by collagenase perfusion from chow-fed mice, as previously described (17). Hepatocytes were cultured for 3–6 h to dampen serum-driven signaling and then stimuolated with recombinant mouse TNFα (100 ng/mL, no. 410-MT; R&D Systems), interleukin (IL)-10 (10 ng/mL, no. 401-MI; R&D Systems), or IL-6 (10 ng/mL, no. 406-ML/CF; R&D Systems) over an acute time course. Protein extracts from tissues were analyzed by Western blotting for phospho- S6 ribosomal protein (Ser240/244), phospho- Akt (Thr183/Tyr185), Akt, p-actin, or hypoxanthine phosphoribosyltransferase 1 was used as invariant controls (no. TA09; R&D Systems) and were maintained on standard chow or an HFD for a period of 4 weeks, as previously described (2). After an overnight fast (11:00 p.m. to 9:00 a.m.), mice were anesthetized with isoflurane (4% for induction and 2% for maintenance) and were maintained on a 37°C heating pad to control body temperature. A minimal midline laparotomy was performed, and the portal vein was visualized. Saline or mouse recombinant TNFα (10 ng/mouse, no. 410-MT; R&D Systems) was administered directly into the portal vein. Exactly 5 min later, the liver was excised without saline perfusion and immediately snap frozen in liquid nitrogen. Protein extracts from tissues were analyzed by Western blotting, as previously described (13–15), and lipid extracts were analyzed using mass spectrometry methods (16), as described in detail below.

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RESULTS

CGI-58 KD paradoxically improves hepatic insulin signaling. Our original interest in CGI-58’s role in intracellular signaling was sparked by the unexpected “metabolic paradox” apparent in mice with diminished CGI-58 function (Fig. 1). We have discovered that CGI-58 KD results in striking hepatic steatosis (Fig. 1A) (2) yet paradoxically improves systemic glucose and insulin tolerance (2). It is well accepted that hepatic lipotoxicity, and more specifically the hepatic accumulation of signaling lipids such as DAG and ceramides, is linked to insulin resistance (22). However, hepatic lipid insult is not sufficient to cause insulin resistance in CGI-58 ASO-treated mice (Fig. 1) (2). Instead, CGI-58 KD actually improves systemic insulin action despite these metabolic abnormalities (2). To confirm that the hepatic steatosis seen in CGI-58 ASO-treated mice were indeed dissociated from primary defects in insulin signaling, we analyzed acute Akt and FoxO1 phosphorylation in response to portally administered insulin (Fig. 1B–G). In agreement with previous measures of systemic glucose and insulin tolerance (2), CGI-58 KD significantly improved hepatic insulin signaling (Fig. 1B and E). CGI-58 KD had no significant impact on insulin-stimulated Akt phosphorylation in skeletal muscle (Fig. 1C and F) and adipose tissue (Fig. 1D and G). Collectively, these data have uncovered an unexpected role for CGI-58 in dissociating hepatic steatosis from insulin resistance. This prompted us to examine the molecular basis for this dissociation.

CGI-58 KD prevents HFD-induced hepatic stress kinase activation. CGI-58 ASO-treated mice have elevated hepatic levels of multiple lipid species, TAG (Supplementary Fig. 1), DAG (Supplementary Fig. 2), MAG (Supplementary Fig. 3), and ceramides (2), yet accumulation of these lipid intermediates is insufficient to cause local insulin resistance (Fig. 1B and E). Thus, other factors must overcome this lipid insult to improve insulin signaling in the liver. In addition to the lipid hypothesis of insulin resistance (22), chronic elevation of proinflammatory TNFα cytokine action in metabolic tissues also promotes insulin resistance (23–25). It is noteworthy that both lipid- and cytokine-induced insulin resistance involve the chronic activation of stress kinase signaling pathways, such as IκB kinase β (IKKB) (24,25), S6 kinase 1 (S6K1) (26), the
mammalian target of rapamycin (mTOR) (26, 27), and JNK (28), which dampen insulin signaling by phosphorylating serine residues of insulin receptor substrate (IRS) proteins (IRS-1 and IRS-2). Hence, we examined circulating levels of proinflammatory cytokines and the activation state of cytokine-induced stress kinases (IKKβ, S6K1, mTOR, and JNK) in metabolic tissues of CGI-58 ASO-treated mice (Fig. 2). CGI-58 KD caused modest elevations in the plasma levels of several proinflammatory cytokines (IL12p40; monocyte chemoattractant protein-1 (MCP-1); macrophage inflammatory protein-2 (MIP-2); CXCL1) regulated upon activation, normal T-cell expressed, and secreted (RANTES).
CGI-58 GENERATES SIGNALING LIPOIDS

A

![Graph showing cytokine levels](image)

**FIG. 2.** CGI-58 KD alters HFD-induced inflammation: Evidence of hepatic cytokine resistance. C57Bl/6N mice were fed either a standard chow or HFD in conjunction with biweekly injections of either a non-targeting control ASO (●) or ASO targeting KD of CGI-58 (CGI-58 ASO: ■) for 10 weeks. A: Plasma levels of proinflammatory cytokines, including IL-6 and IL-12p40; monocyte chemoattractant protein-1 (MCP-1); macrophage inflammatory protein-2 (MIP-2); CXCL1 (KC); and regulated upon activation, normal T-cell expressed, and RANTES. Data represent the mean ± SEM from five mice per group, and values not sharing a common superscript letter differ significantly (P < 0.05). ND, levels below limit of detection. B: CGI-58 KD prevents maximal TNFα stimulation. In control ASO-treated mice, TNFα treatment elicited a small (18%) but significant increase in hepatic total PA levels, compared with saline treatment (Fig. 3A). It is noteworthy that CGI-58 KD prevented TNFα-induced PA generation (Fig. 3A). Neither TNFα nor CGI-58 KD significantly altered total hepatic LPAAT activity (Fig. 3A) or epididymal (p-IKKα/β, p-mTOR, and p-S6) phosphorylation in response to TNFα (32). In acute in vivo (2,6), TNFα signaling was interrogated based on its well-known ability to dampen insulin signaling (23) and promote TAG hydrolysis (34), two pathways that are regulated by CGI-58 in vivo (2,6). To examine acute TNFα signaling, we portally administered physiological levels of recombinant TNFα and analyzed signaling lipid generation at 5 min after stimulation. In control ASO-treated mice, TNFα treatment elicited a small (18%) but significant increase in hepatic total PA levels, compared with saline treatment (Fig. 3A). It is noteworthy that CGI-58 KD prevented TNFα-induced PA generation (Fig. 3A). Neither TNFα nor CGI-58 KD significantly altered total hepatic LPAAT activity (Fig. 3B), which was not surprising because multiple LPAAT enzymes are expressed in mouse liver (35). Of interest, CGI-58 KD specifically prevented TNFα-driven increases in 34:2 PA and 38:4 PA species (Fig. 3B), whereas other PA species were not altered (Supplementary Fig. 4). In addition, CGI-58 KD reduced basal levels of multiple hepatic glycerophospholipid species with signaling potential (36:4 PA, 34:1 PE, 34:2 PE, 36:1 PE, 36:2 PE, 36:4 PE, 36:2 PEp, 34:1 PC, 34:2 PC, and 40:6 PC) regardless of TNFα treatment (Supplementary Figs. 4–9). As previously reported (2), CGI-58 KD also caused large increases in hepatic phosphatidylglycerol levels, independent of TNFα treatment (Fig. 3A and Supplementary Fig. 10). Because TNFα signaling requires lipid second messengers (29), we examined whether CGI-58 KD blunted downstream signal transduction in the liver (Fig. 3C). Five minutes after TNFα administration in vivo, hepatic IκBα (a nuclear factor κB inhibitory protein) was hyperphosphorylated at serine 32 in control mice, whereas CGI-58 KD significantly attenuated hepatic IκBα phosphorylation (Fig. 3C). To determine whether the role of CGI-58 in hepatic cytokine action was cell autonomous, we isolated primary hepatocytes from CGI-58 ASO-treated mice. CGI-58 KD prevented JNK hyperphosphorylation and S6K1 activation in response to a time-course stimulation with TNFα (Fig. 3D), IL-1β (Fig. 3E), and IL-6 (Fig. 3F). Collectively, these data suggest that CGI-58-generated signaling lipids may participate in multiple cytokine signaling cascades, which deserves further exploration.

CGI-58 KD ALTERS THE SYSTEMIC RESPONSE TO ENDOTOXIN

Although TNFα cytokines (TNFα, IL-1β, and IL-6) clearly have been implicated in promoting chronic inflammatory conditions that accompany obesity (23–28), TNFα cytokine action has been best characterized in models of acute inflammation driven by microbial infection or tissue injury (36,37). In acute inflammation, TNFα cytokines are produced transiently by macrophages and mast cells to promote...
tissue reprogramming typified by the hepatic acute-phase response (34,36,37). Of interest, the acute-phase response in the liver also is associated with transient overproduction of VLDL-TAG (34) and insulin resistance (24,25), two pathologies closely correlated with inflammation, which includes serum amyloid A (SAA) and serum amyloid P-component (SAP) as components of the acute-phase response (34,36,37). Of interest, the acute-phase response also is associated with transient overproduction of peripheral TAG levels to the same levels seen in chow-fed CGI-58 ASO-treated mice (2) (Supplementary Fig. 1). To rule out this possibility, we fed mice an HFD for 4 weeks, which increased hepatic TAG accumulation of TAGs in the liver of CGI-58 ASO-treated mice (Supplementary Fig. 1). However, we were concerned that this effect may be simply a result of the abnormally high accumulation of TAGs in the liver of CGI-58 ASO-treated mice (2) (Supplementary Fig. 1). To rule out this possibility, we fed mice an HFD for 4 weeks, which increased hepatic TAG levels to the same levels seen in chow-fed CGI-58 ASO-treated mice by collagenase perfusion. Freshly isolated hepatocytes were stimulated for 15 min (15'), or 1 h with 100 ng/mL TNFα (D), 10 ng/mL IL-1β (E), or 10 ng/mL IL-6 (F). Downstream signaling was analyzed by immunoblotting for p-JNK (Thr183/Tyr185), phospho-S6 ribosomal protein (p-S6; Ser235/236), and β-actin. Data in D-F represent responses of hepatocytes isolated from three individual mice per condition.

FIG. 3. CGI-58–generated signaling lipids are necessary for maximal TNFα signaling in the liver. A–C: Mice were maintained on a chow diet for 4 weeks in conjunction with biweekly injections (25 mg/kg) of either a nontargeting control ASO (□) or ASO targeting knockdown of CGI-58 (CGI-58 ASO; ■). Mice were fasted for 10 h before injection of saline or TNFα (10 ng) into the portal vein. Exactly 5 min later, the liver was excised and immediately snap-frozen in liquid nitrogen for signaling analyses. A: Hepatic levels of PA and phosphatidyglycerol (PG) were analyzed by mass spectrometry. B: Total hepatic LPAAT activity. Data in A and B represent the mean ± SEM from four mice per group, and values not sharing a common superscript letter differ significantly (P < 0.05). C: Protein extracts from the liver were analyzed for p-IkBα (p-IκBα; Ser32), and phospho-IκBα (p-IκBα; Ser32); data from four representative animals are shown for each group. D–F: Acute stress kinase activation in primary hepatocytes. Following 4 weeks of ASO treatment, hepatocytes were isolated from control and CGI-58 ASO-treated mice by collagenase perfusion. Freshly isolated hepatocytes were stimulated for 15 min (15') or 1 h with 100 ng/mL TNFα (D), 10 ng/mL IL-1β (E), or 10 ng/mL IL-6 (F). Downstream signaling was analyzed by immunoblotting for p-JNK (Thr183/Tyr185), phospho-S6 ribosomal protein (p-S6; Ser235/236), and β-actin. Data in D-F represent responses of hepatocytes isolated from three individual mice per condition.
We then treated these HFD-fed mice with LPS to determine whether HFD-induced fatty liver could alter the acute-phase response in a similar fashion to CGI-58 ASO treatment. Of importance, HFD feeding did not mimic the effects of CGI-58 KD on LPS-driven plasma cytokine levels (Supplementary Fig. 12 C and D) or the hepatic acute-phase response (Supplementary Fig. 12 E). Moreover, CGI-58 ASO treatment increased plasma TH1 cytokines and blunted the acute-phase response of mice on both chow and HFDs (Supplementary Fig. 12 C–E), further supporting the idea that CGI-58 ASO-driven alteration in inflammatory signaling is an on-target effect of the ASO and not a result of hepatic TAG accumulation.

Given CGI-58’s documented role in promoting adipose lipolysis (1) and hepatic VLDL-TAG packaging (2), we examined these parameters in LPS-injected, CGI-58 ASO-treated mice. LPS treatment increased plasma nonesterified fatty acid levels by 18% in chow-fed control ASO-treated mice and 25% in chow-fed CGI-58 ASO-treated mice, indicating that LPS-driven adipose lipolysis was similar between groups (data not shown). However, in these same mice, the hepatic metabolic response to LPS was altered (Fig. 4 D and E). LPS treatment of chow-fed CGI-58 ASO-treated mice resulted in a significant (29%) increase in hepatic TAG levels (Fig. 4 D) but no comparable change in control ASO-treated mice. Of interest, LPS treatment caused a 73% increase in plasma TAG levels in chow-fed control ASO-treated mice yet caused no hypertriglyceridemia in CGI-58 ASO-treated mice (Fig. 4 E). These data suggest that hepatic CGI-58 plays a critical role in the overproduction of TAG-rich lipoproteins during infection. Collectively, these data suggest that CGI-58 function is critical to both the inflammatory and metabolic response to acute infection.
now alternatively propose that CGI-58’s ability to acylate LPA (3,4) also plays a critical role in CGI-58’s ability to modulate TAG metabolism and insulin signaling. The major findings of the current study are that CGI-58 KD in mice (1) improves insulin signaling in liver and skeletal muscle; 2) prevents HFD-induced stress kinase activation; 3) prevents the generation of PA and other glycerophospholipid species in response to TNFα, thereby attenuating downstream signaling; and 4) alters the integrated inflammatory response to endotoxin. In our current working model (Fig. 5), we propose that downstream of hepatic cytokine receptor activation, in response to inflammatory stimuli such as an HFD or LPS treatment, CGI-58 generates signaling lipids either directly through direct acylation of LPA or indirectly by coactivating ATGL-mediated TAG hydrolysis. CGI-58-generated PA, and likely other signaling lipids, can subsequently act as lipid second messengers to activate stress kinases such as IKK-β, S6K1, and mTOR. These stress kinases can then facilitate serine phosphorylation of critical residues on IRS-1, thereby dampening hepatic insulin signaling (Fig. 5). This role in cytokine signaling may partially explain why CGI-58 KD causes severe hepatic lipid insult and yet improves hepatic insulin signaling.

It has now been a decade since the causal link between CGI-58 mutations and CDS was established (5), yet molecular mechanism(s) by which CGI-58 prevents CDS has remained elusive. Early studies using skin fibroblasts isolated from patients with neutral lipid storage disease or CDS showed that these cultured cells had striking accumulation of intracellular TAGs under normal growth conditions (10,11,38–41). However, the TAG accumulation could not be explained by alteration in mitochondrial fatty acid uptake, β-oxidation, in vitro lipase activity, or TAG synthesizing enzyme activity (10,11,38–41). Instead, it was found that neutral lipid storage disease fibroblasts had impaired turnover of long-chain fatty acids from stored TAGs (38–41). We have likewise demonstrated that targeted knockdown of CGI-58 in hepatocytes impairs intracellular TAG hydrolysis in vitro and in vivo (2,42). Of interest, CGI-58 is a lipid-droplet–associated protein in adipocytes, achieving this subcellular localization by directly interacting with perilipin A (43,44). However, it is important to note that CGI-58 is not always associated with lipid droplets in nonadipocyte cell models (42–44), and the intracellular trafficking itinerary of CGI-58 under hormonal or cytokine stimulation deserves further study.

The product of the LPAAT reaction, PA, is a well-studied signaling lipid (7–9,45–47). In fact, PA participates in many cellular signal transduction pathways and regulates membrane trafficking (7–9,45–47). It is generally accepted that PA regulates cell signaling by physically interacting with target proteins through defined PA-binding motifs, thereby altering either membrane localization or activation state. Bona fide PA-binding proteins include protein kinases, phosphatases, phosphodiesterases, scaffolding proteins, and small guanine nucleotide exchange factors (7–9,45–47). Although the majority of acute cytokine-stimulated PA generation has been attributed to the enzymatic hydrolysis of PC through the action of phospholipase D (45–47) or the phosphorylation of DAGs by DAG kinases (48), there is growing evidence that LPAAT enzymes make substantial contributions to endotoxin- and cytokine-stimulated PA generation (29–33). In fact, pharmacologic inhibition of LPAAT activity protects mice against endotoxic shock, lung injury, and pancreatic islet dysfunction in response to endotoxin and IL-1 (32,33,49,50), implicating LPAAT-derived PA in promoting inflammatory disease. Undoubtedly, PA is a central lipid signaling molecule that can be synthesized or broken down by a number of enzymatic pathways (45–50). We propose that CGI-58–driven synthesis of PA represents a novel lipid-signaling pathway that may have important implications in human diseases, such as the metabolic syndrome and CDS. CGI-58 KD in mice prevents diet-induced obesity and decreases fat-pad mass (2), suggesting a defect in lipid storage by adipose tissue. The possibility that CGI-58–generated signaling lipids may regulate adipocyte function in vivo deserves further investigation. The signaling function of CGI-58 may also have implications for neurologic defects in CDS, including ataxia, mental retardation, and hearing loss. Given that global deficiency of CGI-58 results in postnatal lethality, tissue-specific CGI-58 knockout mice will be required to further dissect the role of CGI-58–generated signaling lipids in these other biological processes. In conclusion, these studies demonstrate that CGI-58 is a novel source of signaling lipids that integrate inflammation and nutrient metabolism.

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C.C.L., J.L.B., G.T., and M.A.D. conducted the experiments, analyzed the data, and aided in the manuscript preparation. P.T.I., S.B.M., D.S.M., and H.A.B. performed all lipidomic analyses and provided critical insights for these studies. S.C., M.L., and J.S.P. performed primary hepatocyte isolations. R.G.L., R.M.C., and M.J.G. provided antisense oligonucleotides and valuable discussion. D.L.B. aided in the measurements of hepatic LPAAT activity. M.B.F. and J.M. performed plasma cytokine analyses and discussed the data. J.M.B. planned the project, designed the experiments, designed the data, and wrote the manuscript, and 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. All authors were involved in the editing of the final manuscript.

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REFERENCES


47. Exton JH. Phospholipase D: enzymology, mechanisms of regulation, and function. Physiol Rev 1997;77:305–320

