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Canagliflozin ameliorates hepatic fat deposition in obese diabetic mice: role of prostaglandin E_2

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ABSTRACT

Clinical and animal studies have suggested a possible beneficial effect of sodium-glucose cotransporter 2 (SGLT2) inhibitors on nonalcoholic fatty liver disease (NAFLD) including nonalcoholic steatohepatitis (NASH). Although SGLT2 inhibitors have been shown to reduce hepatic fat deposition in association with loss of body weight, the mechanism of this action has remained unknown. We here show that the SGLT2 inhibitor canagliflozin ameliorated fatty liver and hyperglycemia without affecting body weight or epididymal fat weight in obese diabetic KKAy mice. Lipidomics analysis based on liquid chromatography and tandem mass spectrometry revealed that canagliflozin treatment increased the amounts of prostaglandin E_2 (PGE_2) and resolvin E3 in the liver of these mice. We also found that PGE_2 attenuated fat deposition in mouse primary hepatocytes exposed to palmitic acid. Our results thus suggest that PGE_2 may play an important role in the amelioration of hepatic fat deposition by canagliflozin, with elucidation of its mechanism of action potentially providing a basis for the development of new therapeutics for NAFLD-NASH.

Keywords

SGLT2 inhibitor, Canagliflozin, Prostaglandin E_2 (PGE_2), Lipid mediator, Nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH)

Abbreviations

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SGLT2, sodium-glucose cotransporter 2; LTB_4, leukotriene B_4; LC-MS/MS, liquid chromatography–tandem mass spectrometry; FFA, free fatty acid; MRM multiple reaction monitoring; PG, prostaglandin; mPGES-1, microsomal prostaglandin E synthase–1.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent form of chronic liver disease in industrialized countries, affecting one-fourth of the global population [1–3]. NAFLD is characterized by excessive fat accumulation in the liver in association with obesity and insulin resistance. It ranges in severity from simple steatosis to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis with inflammation, ballooning degeneration, and fibrosis. NASH is estimated to account for 10% to 20% of NAFLD cases, and it can progress to cirrhosis and hepatocellular carcinoma [1–3]. There are currently no approved medications for NAFLD-NASH.

SGLT2 inhibitors are drugs that attenuate the reabsorption of glucose by suppressing the function of sodium-glucose cotransporter 2 (SGLT2) in the proximal tubules of the kidney and which thereby ameliorate hyperglycemia [4]. In addition to the well-established efficacy of these drugs for type 2 diabetes mellitus, they have been found to have a potential beneficial impact on NAFLD-NASH by attenuating hepatic fat deposition in clinical trials and animal experiments [5–7]. Although weight loss has been associated with the reduction in liver fat induced by SGLT2 inhibitors, some studies have found that the effect on liver fat is independent of the change in body weight [8,9], suggesting that mechanisms other than body weight loss might contribute to this action of SGLT2 inhibitors.

Recent studies have highlighted the importance of lipid mediators in the regulation of metabolism [10]. Leukotriene B_4 (LTB_4), a proinflammatory lipid mediator generated from arachidonic acid, plays an important role in the development of systemic insulin resistance in mice and humans [11–14], whereas lipoxin A_4, a proresolving lipid mediator also generated from arachidonic acid, has been found to ameliorate obesity-induced fatty liver and chronic kidney disease in mice [15]. However, whether lipid mediators might contribute to the beneficial effect of SGLT2 inhibitors on hepatic fat deposition has been unknown. We have now performed liquid chromatography and tandem mass spectrometry (LC-MS/MS)–based lipidomics analysis of liver tissue from obese diabetic KKAy mice treated with the SGLT2 inhibitor canagliflozin in order to obtain insight into the mechanism underlying the amelioration of hepatic fat deposition by this drug.

2. Materials and methods

2.1. Animal studies

Male KKAy mice at 7 weeks of age were obtained from CLEA Japan (Tokyo,

Japan), housed individually, fed a basal chow diet (CE-2, CLEA Japan), and acclimatized for 1 week. They were then divided into two groups and fed either a control diet or a diet supplemented with 0.01% canagliflozin (putative daily dose of ~17 mg/kg [16]) provided by Mitsubishi Tanabe Pharma Corp. (Osaka, Japan). Blood and plasma samples were collected after canagliflozin treatment for 3 weeks. The animals were anesthetized with isoflurane and killed by cervical dislocation for isolation of the liver after canagliflozin treatment for 4 weeks. This study was approved by the Institutional Animal Care and Use Committee of Kobe University (permission no. P171006-R1) and was performed according to Kobe University Animal Experimentation Regulations.

2.2. Blood parameters

Blood glucose was measured with a Glutest kit (Sanwa Kagaku Kenkyusho, Nagoya, Japan). Plasma triglyceride, free fatty acid (FFA), and cholesterol concentrations were determined with kits from Wako (triglyceride E-test, NEFA C-test, and cholesterol E-test). Plasma insulin was assayed with an ultrasensitive insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga, Yokohama, Japan).

2.3. LC-MS/MS analysis

Deuterated internal standards (d4-LTB_4, d8-5-hydroxyeicosatetraenoic acid, d4-prostaglandin E_2, and d5-resolvin D2) representing each chromatographic region of identified lipid mediators were added (500 pg each) to samples in order to facilitate quantification. Samples were subjected to solid-phase extraction on C18 columns as described previously [17] and were then subjected to LC-MS/MS with a Qtrap 6500 instrument (Sciex, Tokyo, Japan) equipped with an LC-30AD high-performance LC system (Shimadzu, Kyoto, Japan). The ZORBAX Eclipse Plus C18 column (100 by 4.6 mm, 3.5 μm; Agilent Technologies, Santa Clara, CA, USA) was subjected to elution with a gradient of methanol/water/acetic acid from 55:45:0.01 (v/v/v) to 98:2:0.01 at a flow rate of 0.4 ml/min. For monitoring and quantification of the targeted lipid mediators, a multiple reaction monitoring (MRM) method was developed with signature ion pairs (Q1 [parent ion]/Q3 [characteristic fragment ion]) for each molecule. Identification of each lipid mediator was based on published criteria for LC retention time, specific fragmentation patterns, and at least six diagnostic fragmentation ions. Quantification was performed on the basis of peak area on the MRM chromatograph, with linear calibration curves being obtained with authentic

standards for each compound.

2.4. Histology

The liver was fixed with 10% neutral buffered formalin and processed for staining of paraffin-embedded sections with hematoxylin-eosin or oil red O.

2.5. Cell studies

Mouse primary hepatocytes were prepared from male C57BL/6J mice (CLEA Japan) essentially as described previously [18]. Prostaglandin (PG) E_2 was obtained from Cayman (Ann Arbor, Michigan, USA). Lipids were extracted from mouse primary hepatocytes or mouse liver as described previously [18], and the concentration of triglyceride in the extracts was determined with a kit from Wako (triglyceride E-test). For staining with oil red O, mouse primary hepatocytes were washed twice with phosphate-buffered saline, fixed with 10% neutral buffered formalin for 1 h, and stained for 10 min.

2.6. Statistics

Data are presented as means ± SEM and were analyzed with the two-tailed Student's *t* test or Dunnett's multiple comparison test. A *P* value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of canagliflozin on blood parameters, body weight, and liver and fat weight in KKAy mice

Treatment with canagliflozin for 3 weeks induced a significant decrease in the blood glucose level of KKAy mice (Fig. 1A), whereas the plasma insulin concentration was not altered by such treatment (Fig. 1B). Although the plasma concentrations of cholesterol and FFAs were not affected, that of triglyceride was significantly reduced by canagliflozin treatment (Fig. 1C–E). Body weight was not altered during the observation period of up to 4 weeks after the onset of canagliflozin administration (Fig. 1F). Although the weight of epididymal adipose tissue was also not affected, canagliflozin treatment for 4 weeks induced a significant decrease in liver weight (Fig. 1G and H), suggesting that the effect of canagliflozin on liver weight was independent of body weight and fat weight.

3.2. Canagliflozin reduces hepatic fat in KKAy mice

Histological examination of the liver by hematoxylin-eosin and oil red O staining showed that the deposition of lipid droplets apparent in untreated KKAy mice was markedly ameliorated in canagliflozin-treated mice (Fig. 2A). This effect of the drug was confirmed by measurement of triglyceride content in the liver (Fig. 2B).

3.3. Effects of canagliflozin treatment on the lipid mediator profile of the liver of KKAy mice

To elucidate the mechanism underlying the amelioration of hepatic fat deposition by canagliflozin, we performed LC-MS/MS–based lipidomics analysis of liver tissue. The amounts of PGE_2 and resolvin E3 were significantly increased and that of PGD_2 tended to be increased in the liver of canagliflozin-treated KKAy mice compared with that of untreated mice (Fig. 3).

3.4. PGE_2 attenuates lipid droplet accumulation induced by palmitic acid in mouse primary hepatocytes

A beneficial effect of PGE_2 on the pathology of NASH was suggested by a recent study showing that diet-induced NASH is exacerbated in mice lacking microsomal PGE synthase 1 (mPGES-1), which mediates the synthesis of PGE_2 [19]. To investigate the possible role of PGE_2 in the attenuation of liver fat deposition by canagliflozin, we examined the effect of PGE_2 on lipid droplet accumulation induced by treatment of mouse primary hepatocytes with palmitic acid. Oil red O staining (Fig. 4A) as well as measurement of triglyceride content (Fig 4B) revealed that PGE_2 inhibited the accumulation of intracellular lipid droplets in hepatocytes in a concentration-dependent manner. These results suggested that PGE_2 may play an important role in the amelioration of liver fat deposition by canagliflozin.

4. Discussion

We found that administration of canagliflozin to KKAy mice markedly ameliorated hyperglycemia and hypertriglyceridemia. Consistent with the results of previous clinical and animal studies [20–22], canagliflozin also attenuated fat deposition in the liver. Although the loss of body weight or fat weight induced by SGLT2 inhibitors has been thought to be related to the accompanying reduction in hepatic fat content, the marked amelioration of hepatic fat deposition by canagliflozin treatment in the present study occurred in the absence of a reduction in body or fat weight, suggesting that the attenuation of hepatic fat accumulation by SGLT2 inhibitors may be independent of body and fat weight.

The mechanism underlying the improvement in liver fat content induced by SGLT2 inhibitors has been unclear. To obtain insight into this mechanism, we performed lipidomics analysis of liver tissue from KKAy mice, and we found that the amounts of lipid mediators including PGE_2 and resolvin E3 were altered by administration of canagliflozin. Furthermore, analysis of primary mouse hepatocytes in vitro revealed that PGE_2 inhibited the accumulation of lipid droplets in these cells, suggesting that PGE_2 might mediate the effect of canagliflozin on liver fat.

PGE_2 is generated from arachidonic acid in reactions catalyzed by cyclooxygenases and PGE synthases [23]. However, mRNA expression of these enzymes was not altered in liver of KKAy mice by canagliflozin treatment (data not shown). The increase in the hepatic abundance of PGE_2 induced by canagliflozin is therefore not likely attributable to up-regulation of the expression of enzymes related to its biosynthesis. Further studies will be required to investigate whether canagliflozin affects the activity of such enzymes or the expression or activity of enzymes responsible for PGE_2 degradation. It also remains unknown which cells in the liver serve as the source of the PGE_2 that accumulates in response to canagliflozin treatment. Although SGLT2 is thought to be exclusively expressed in the proximal tubules of the kidney and less expressed in the liver, it will be important to investigate whether the observed up-regulation of PGE_2 reflects a direct effect of canagliflozin on hepatocytes or other cells of the liver, or a secondary effect resulting from metabolic changes such as those affecting the circulating concentrations of glucose, insulin, and glucagon.

Four G protein–coupled receptors—EP1, EP2, EP3, and EP4—have been identified as receptors for PGE_2 and have been found to target different intracellular second messengers [24]. Activation of EP1 increases the cytosolic Ca^{2+} concentration; EP2 and EP4 are linked to G_s and increase the intracellular concentration of cAMP; and EP3 is linked to G_i and reduces the intracellular cAMP concentration. Studies with various agonists and antagonists should shed light on which receptors are responsible for the effect of PGE_2 on lipid deposition in hepatocytes.

NASH has a poor prognosis and can progress to cirrhosis and liver cancer. However, the pathophysiological mechanisms have remained unclear, and no specific therapeutic agent for NASH has been developed to date. Although evidence has suggested that SGLT2 inhibitors might have therapeutic efficacy for NAFLD-NASH [5–7], it has been unclear whether such efficacy is due only to the associated amelioration of hepatic fat deposition, or whether SGLT2 inhibitors

have other effects on inflammation and fibrosis. The recent finding that mice lacking the PGE synthase mPGES-1 show exacerbation of liver inflammation in a diet-induced NASH model [19] suggests that PGE_2 may have a protective effect on inflammation in NASH. Elucidation of the relevance of lipid mediators regulated by SGLT2 inhibitors to disease pathology and characterization of their mechanisms of action may provide a basis for the development of new therapeutics for NAFLD-NASH.

Declaration of competing interest

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References

[1] Z. Younossi, Q.M. Anstee, M. Marietti, T. Hardy, L. Henry, M. Eslam, J. George, E. Bugianesi, Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention, Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 11–20.
[2] M.E. Rinella, Nonalcoholic fatty liver disease: a systematic review, JAMA 313 (2015) 2263–2273.
[3] Z.M. Younossi, Non-alcoholic fatty liver disease—a global public health perspective, J. Hepatol. 70 (2019) 531–544.
[4] E.C. Chao, R.R. Henry, SGLT2 inhibition—a novel strategy for diabetes treatment, Nat. Rev. Drug Discov. 9 (2010) 551–559.
[5] A.J. Scheen, Beneficial effects of SGLT2 inhibitors on fatty liver in type 2 diabetes: a common comorbidity associated with severe complications, Diabetes Metab. 45 (2019) 213–223.
[6] L. Chrysavgis, A.M. Papatheodoridi, A. Chatzigeorgiou, E. Cholongitas, The impact of sodium glucose co-transporter 2 inhibitors on non-alcoholic fatty liver disease, J. Gastroenterol. Hepatol. (2020).
[7] Y. Sumida, M. Yoneda, Current and future pharmacological therapies for NAFLD/NASH, J. Gastroenterol. 53 (2018) 362–376.
[8] M.S. Kuchay, S. Krishan, S.K. Mishra, K.J. Farooqui, M.K. Singh, J.S. Wasir, B. Bansal, P. Kaur, G. Jevalikar, H.K. Gill, N.S. Choudhary, A. Mithal, Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: a randomized controlled trial (E-LIFT trial), Diabetes Care 41 (2018) 1801–1808.
[9] C. Komiya, K. Tsuchiya, K. Shiba, Y. Miyachi, S. Furuke, N. Shimazu, S. Yamaguchi, K. Kanno, Y. Ogawa, Ipragliflozin improves hepatic steatosis in obese mice and liver dysfunction in type 2 diabetic patients irrespective of body weight reduction, PLoS One 11 (2016) e0151511.
[10] M. Spite, J. Claria, C.N. Serhan, Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases, Cell Metab. 19 (2014) 21–36.
[11] I. Mothe-Satney, C. Filloux, H. Amghar, C. Pons, V. Bourlier, J. Galitzky, P.A. Grimaldi, C.C. Feral, A. Bouloumie, E. Van Obberghen, J.G. Neels, Adipocytes secrete leukotrienes: contribution to obesity-associated inflammation and insulin resistance in mice, Diabetes 61 (2012) 2311–2319.
[12] T. Hosooka, Y. Hosokawa, K. Matsugi, M. Shinohara, Y. Senga, Y. Tamori, C. Aoki, S. Matsui, T. Sasaki, T. Kitamura, M. Kuroda, H. Sakaue, K. Nomura, K. Yoshino, Y. Nabatame, Y. Itoh, K. Yamaguchi, Y. Hayashi, J. Nakae, D. Accili,

T. Yokomizo, S. Seino, M. Kasuga, W. Ogawa, The PDK1-FoxO1 signaling in adipocytes controls systemic insulin sensitivity through the 5-lipoxygenase-leukotriene B4 axis, Proc. Natl. Acad. Sci. USA 117 (2020) 11674–11684.
[13] M. Spite, J. Hellmann, Y. Tang, S.P. Mathis, M. Kosuri, A. Bhatnagar, V.R. Jala, B. Haribabu, Deficiency of the leukotriene B4 receptor, BLT-1, protects against systemic insulin resistance in diet-induced obesity, J. Immunol. 187 (2011) 1942–1949.
[14] P. Li, D.Y. Oh, G. Bandyopadhyay, W.S. Lagakos, S. Talukdar, O. Osborn, A. Johnson, H. Chung, M. Maris, J.M. Ofrecio, S. Taguchi, M. Lu, J.M. Olefsky, LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes, Nat. Med. 21 (2015) 239–247.
[15] E. Borgeson, A.M. Johnson, Y.S. Lee, A. Till, G.H. Syed, S.T. Ali-Shah, P.J. Guiry, J. Dalli, R.A. Colas, C.N. Serhan, K. Sharma, C. Godson, Lipoxin A4 attenuates obesity-induced adipose inflammation and associated liver and kidney disease, Cell Metab. 22 (2015) 125–137.
[16] Y. Watanabe, K. Nakayama, N. Taniuchi, Y. Horai, C. Kuriyama, K. Ueta, K. Arakawa, T. Senbonmatsu, M. Shiotani, Beneficial effects of canagliflozin in combination with pioglitazone on insulin sensitivity in rodent models of obese type 2 diabetes, PLoS One 10 (2015) e0116851.
[17] R.A. Colas, M. Shinohara, J. Dalli, N. Chiang, C.N. Serhan, Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue, Am. J. Physiol. Cell Physiol. 307 (2014) C39–C54.
[18] M. Matsumoto, W. Ogawa, K. Akimoto, H. Inoue, K. Miyake, K. Furukawa, Y. Hayashi, H. Iguchi, Y. Matsuki, R. Hiramatsu, H. Shimano, N. Yamada, S. Ohno, M. Kasuga, T. Noda, PKCλ in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity, J. Clin. Invest. 112 (2003) 935–944.
[19] J. Henkel, C.D. Coleman, A. Schraplau, K. Johrens, T.S. Weiss, W. Jonas, A. Schurmann, G.P. Puschel, Augmented liver inflammation in a microsomal prostaglandin E synthase 1 (mPGES-1)-deficient diet-induced mouse NASH model, Sci. Rep. 8 (2018) 16127.
[20] M. Inoue, A. Hayashi, T. Taguchi, R. Arai, S. Sasaki, K. Takano, Y. Inoue, M. Shichiri, Effects of canagliflozin on body composition and hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease, J. Diabetes Investig. 10 (2019) 1004–1011.
[21] K. Cusi, F. Bril, D. Barb, D. Polidori, S. Sha, A. Ghosh, K. Farrell, N.E. Sunny, S. Kalavalapalli, J. Pettus, T.P. Ciaraldi, S. Mudaliar, R.R. Henry, Effect

of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes, Diabetes Obes. Metab. 21 (2019) 812–821.
[22] K. Shiba, K. Tsuchiya, C. Komiya, Y. Miyachi, K. Mori, N. Shimazu, S. Yamaguchi, N. Ogasawara, M. Katoh, M. Itoh, T. Suganami, Y. Ogawa, Canagliflozin, an SGLT2 inhibitor, attenuates the development of hepatocellular carcinoma in a mouse model of human NASH, Sci. Rep. 8 (2018) 2362.
[23] B. Samuelsson, R. Morgenstern, P.J. Jakobsson, Membrane prostaglandin E synthase-1: a novel therapeutic target, Pharmacol. Rev. 59 (2007) 207–224.
[24] R.A. Coleman, W.L. Smith, S. Narumiya, International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes, Pharmacol. Rev. 46 (1994) 205–229.

FIGURE LEGENDS

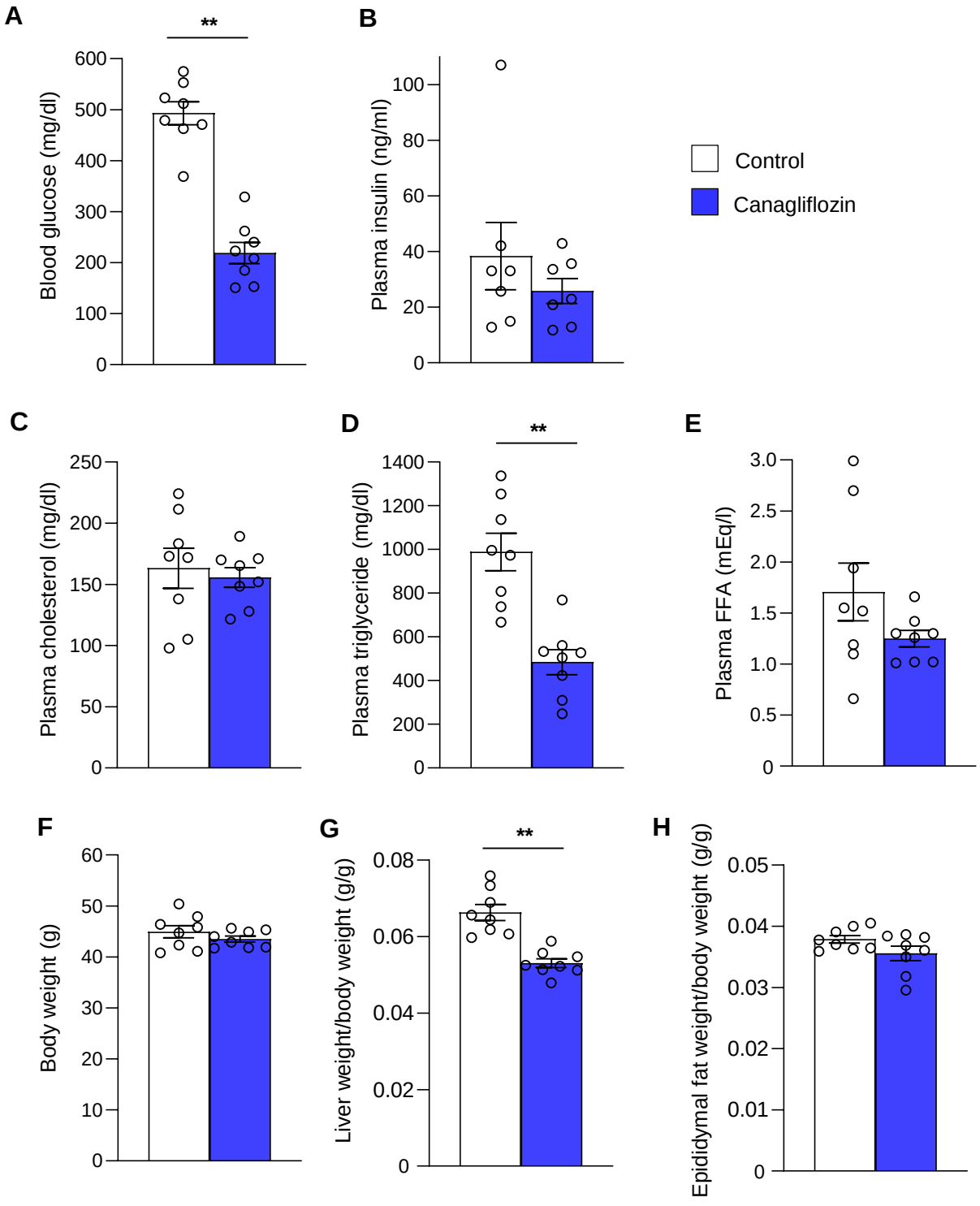
Fig. 1. Effects of canagliflozin treatment on blood glucose and plasma insulin and lipid concentrations as well as on body and tissue weight in KKAy mice. (A–E) Blood glucose (A) as well as plasma insulin (B), cholesterol (C), triglyceride (D), and FFA (E) concentrations were measured in control mice or mice treated with canagliflozin for 3 weeks. (F–H) Body weight (F) as well as the ratio of liver (G) or epididymal fat (H) weight to body weight were measured in control mice or mice treated with canagliflozin for 4 weeks. All data are means ± SEM (n = 8 mice per group). $^{**}P < 0.01$ (Student's t test).

Fig. 2. Canagliflozin ameliorates hepatic fat deposition in KKAy mice. (A) Hematoxylin-eosin (H-E) and oil red O staining of liver sections from control mice or mice treated with canagliflozin for 4 weeks. Scale bars, 200 μm. (B) Triglyceride content of the liver from control mice or mice treated with canagliflozin for 4 weeks. Data are means ± SEM (n = 8 mice per group). $^{**}P < 0.01$ (Student's t test).

Fig. 3. Effects of canagliflozin on the lipid mediator profile of the KKAy mouse liver. The concentrations of lipid mediators in liver extracts prepared from control mice or mice treated with canagliflozin for 4 weeks were determined by LC-MS/MS analysis. Data are means ± SEM (n = 8 mice per group). $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's t test). Abbreviations not defined in text: 12S-HHT, 12*S*-hydroxyheptadecatrienoic acid; HETE, hydroxyeicosatetraenoic acid; AA, arachidonic acid; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; DHA, docosahexaenoic acid; HYA, 10-hydroxy-*cis*-12-octadecenoic acid; HYC, 10-hydroxy-*trans*-11-octadecenoic acid; HYB, 10-hydroxy-octadecanoic acid; KetoA, 10-oxo-*cis*-12-octadecenoic acid; KetoB, 10-oxo-octadecanoic acid; KetoC, 10-oxo-*trans*-11-octadecenoic acid.

Fig. 4. PGE_2 attenuates fat deposition in mouse primary hepatocytes. Mouse primary hepatocytes were incubated with 0.5 mM palmitic acid for 24 h and then in the presence of the indicated concentrations of PGE_2 for 24 h, after which they were subjected to oil red O staining (A) or measurement of triglyceride content (B). Original magnification, ×100. Quantitative data are means ± SEM (n = 3 independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$ (Dunnett's multiple comparison test).

Fig. 1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500
400
300
200
100
0
B
Plasma insulin (ng/ml)
100
80
60
40
20
0
Control
Canagliflozin
C
Plasma cholesterol (mg/dl)
250
200
150
100
50
0
D
**
Plasma triglyceride (mg/dl)
1400
1200
1000
800
600
400
200
0
E
Plasma FFA (mEq/l)
3.0
2.5
2.0
1.5
1.0
0.5
0
F
Body weight (g)
60
50
40
30
20
10
0
G
**
Liver weight/body weight (g/g)
0.08
0.06
0.04
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0.03
0.02
0.01
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Fig. 2

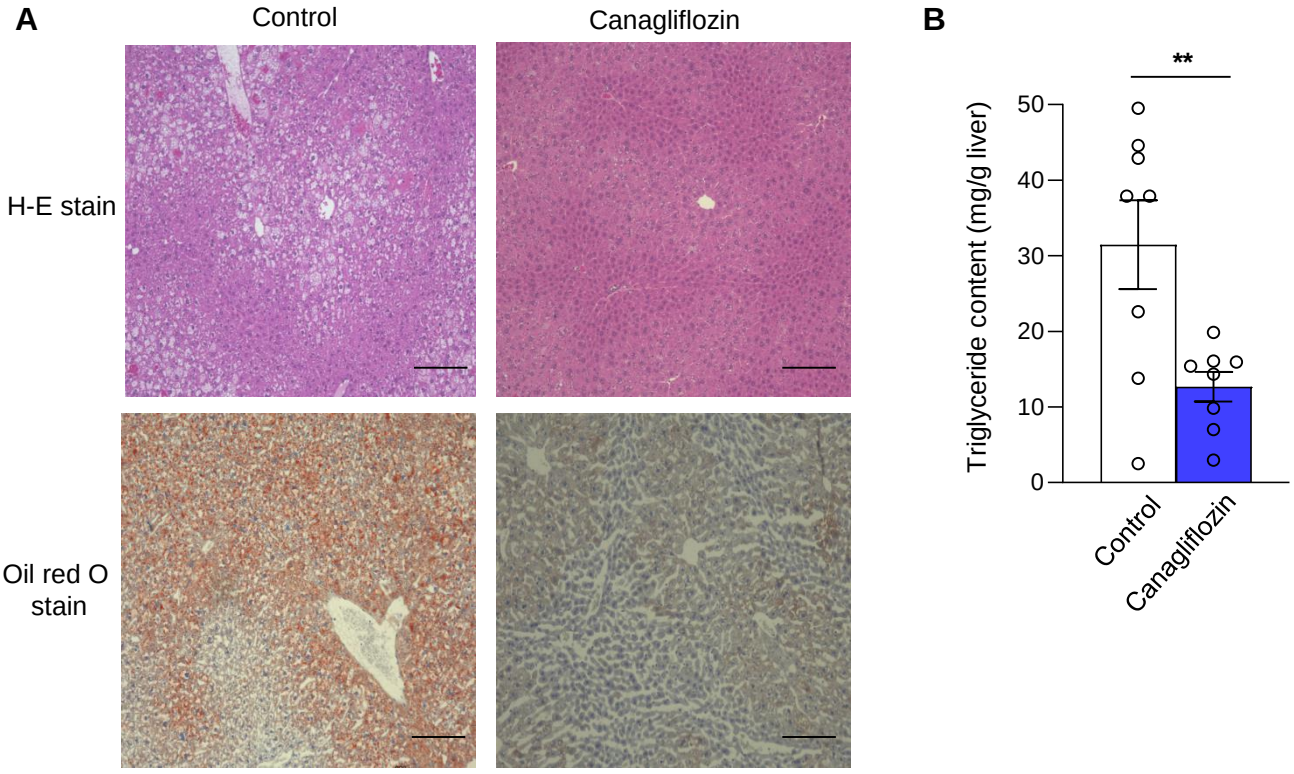
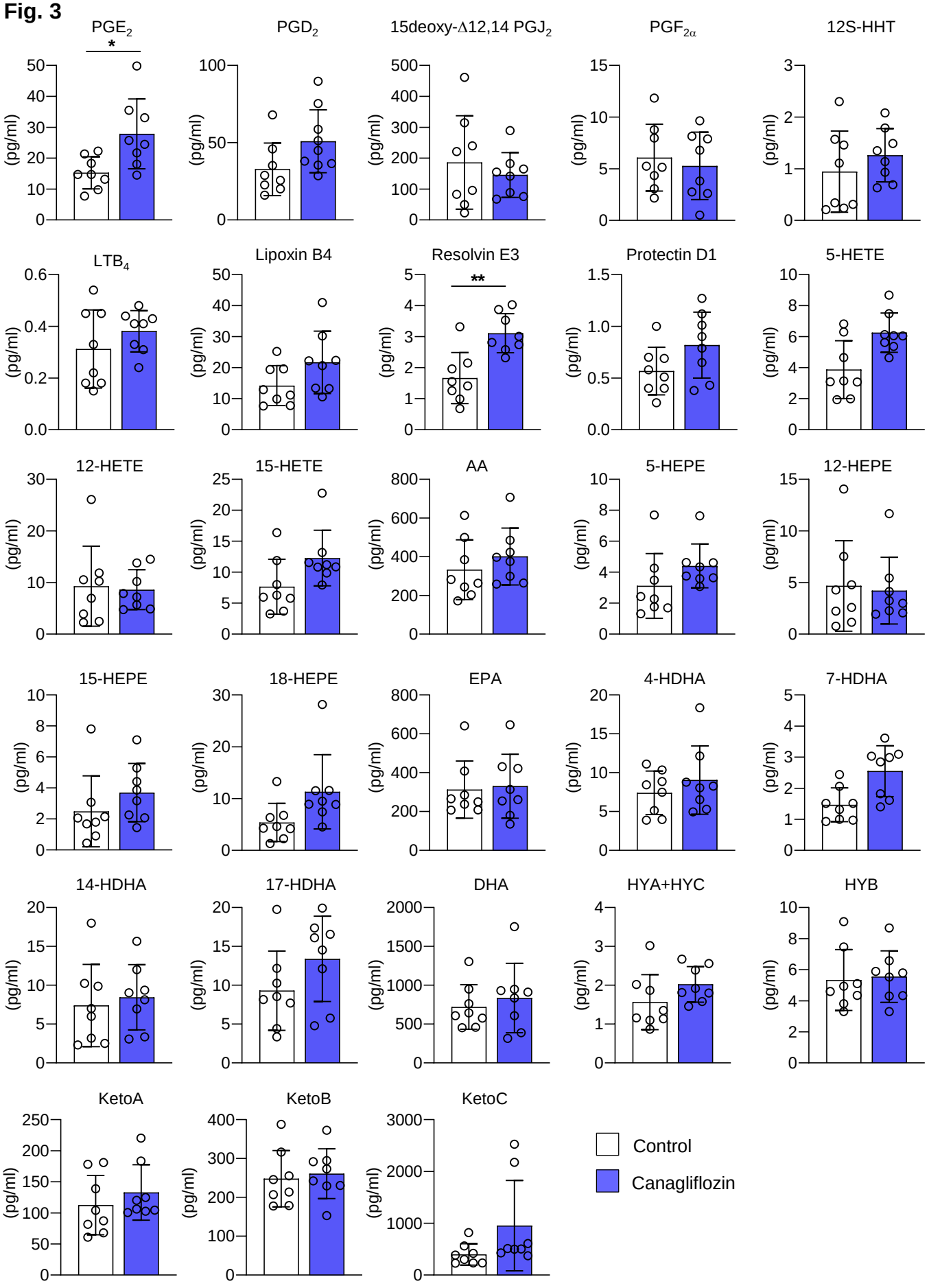

A
Control
Canagliflozin
H-E stain
Oil red O stain
B
**
Triglyceride content (mg/g liver)
50
40
30
20
10
0
Control
Canagliflozin

Fig. 3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PGF2α
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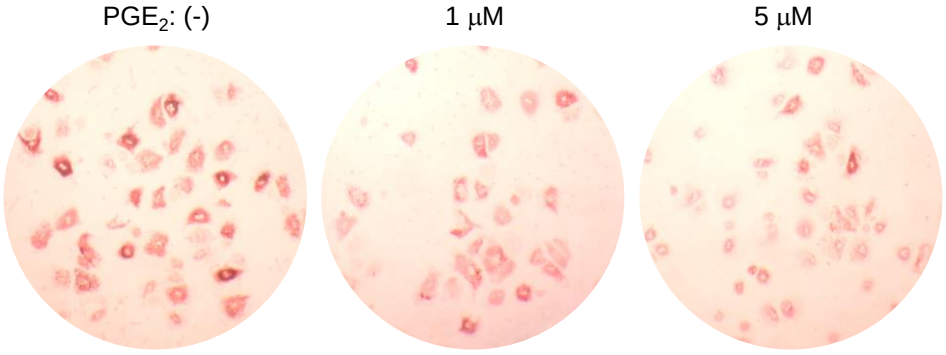
12-HETE
15-HETE
AA
5-HEPE
12-HEPE
15-HEPE
18-HEPE
EPA
4-HDHA
7-HDHA
14-HDHA
17-HDHA
DHA
HYA+HYC
HYB
KetoA
KetoB
KetoC
(pg/ml)
*
**
Control
Canagliflozin

Fig. 4

A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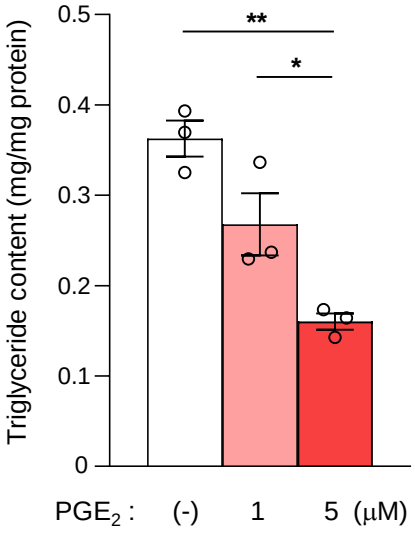
5 μM

B


Triglyceride content (mg/mg protein)
0.5
0.4
0.3
0.2
0.1
0
**
*
PGE2 : (-) 1 5 (μM)