

## Commentary

# Anarchy in the UPR: a Ca<sup>2+</sup>-insensitive PKC inhibits SERCA activity to promote ER stress

Carsten Schmitz-Peiffer<sup>1,2</sup><sup>1</sup>Diabetes and Metabolism Division, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia; <sup>2</sup>St Vincent's Clinical School, University of New South Wales, Sydney, NSW 2010, Australia**Correspondence:** Carsten Schmitz-Peiffer (c.schmitz-peiffer@garvan.org.au)

Nonalcoholic fatty liver disease (NAFLD) is highly prevalent in Western countries, and is linked to the development of liver cancer and Type 2 diabetes (T2D). It is strongly associated with obesity, but the dysregulation of liver lipid storage is not fully understood. Fatty acid oversupply to hepatocytes can establish a vicious cycle involving diminished protein folding, endoplasmic reticulum (ER) stress, insulin resistance and further lipogenesis. This commentary discusses the recent findings of Lai et al. published in *Bioscience Reports*, that implicate protein kinase C delta (PKC $\delta$ ) activation by fatty acids in the inhibition of the SERCA Ca<sup>2+</sup> pump, resulting in reduced ER Ca<sup>2+</sup> loading and protein misfolding. PKC $\delta$  therefore represents a target for the treatment of both steatosis and insulin resistance, key to the prevention of NAFLD and T2D.

Nonalcoholic fatty liver disease (NAFLD), characterized by the accumulation of hepatic triglycerides and cholesterol, is now the most common chronic liver disease in Western countries, affecting up to 25% of the population [1]. The disease is associated with obesity and increases the risk of nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma. In addition, NAFLD is linked to liver insulin resistance, a key feature of Type 2 diabetes (T2D) which is reaching epidemic proportions [2]. In combination with pancreatic  $\beta$ -cell dysfunction, insulin resistance leads to the failure of glucose homeostasis and results in long-term complications such as cardiovascular disease, kidney failure, and neuropathy. Therefore, the development of therapeutic strategies to prevent NAFLD are of key importance. However, liver lipid storage is normally tightly controlled in healthy subjects, and the mechanisms leading to its dysregulation are not well understood. In addition, the hierarchical relationship between excessive hepatic lipid deposition (steatosis) and insulin resistance is not clear, especially because there is evidence that each condition can play a causal role in the development of the other [3].

One mechanism that appears to link obesity and increased lipid availability to both steatosis and insulin resistance is the development of endoplasmic reticulum (ER) stress in hepatocytes [4]. If fatty acid delivery exceeds the ability of the hepatocyte to metabolize acyl chains by  $\beta$ -oxidation, the intracellular levels of triglyceride (TG) and the lipid intermediates in its synthesis will rise. Lipids have been proposed to negatively affect protein folding and trafficking in the ER in several ways [5-7], leading to the accumulation of unfolded proteins which in turn initiate the adaptive unfolded protein response, which increases protein folding capacity while reducing overall protein synthesis [8]. If this is insufficient to resolve the situation, chronic ER stress can lead to the induction of inflammatory pathways and insulin resistance, in part through Jnk activation and inhibition of insulin signal transduction [9].

Fatty acid oversupply can lead to perturbations in intracellular Ca<sup>2+</sup> homeostasis which in turn diminish protein folding capacity by affecting chaperone activity [10]. Lipid accumulation in hepatocytes has been reported to affect Ca<sup>2+</sup> entry into cells through store-operated Ca<sup>2+</sup> channels [11] and also to decrease both the activity [6] or expression [12] of the sarco/endoplasmic reticulum calcium ATPase (SERCA), a

Received: 05 November 2017  
Revised: 05 February 2018  
Accepted: 07 February 2018Accepted Manuscript Online:  
08 February 2018  
Version of Record published:  
16 March 2018

key component of  $\text{Ca}^{2+}$  homeostasis that transports  $\text{Ca}^{2+}$  ions into the ER. However, the mechanism by which lipids affect SERCA has not been elucidated.

Recently, in *Bioscience Reports*, Lai et al. show [13] that protein kinase C delta (PKC $\delta$ ), a member of the lipid-activated PKC family, regulates SERCA function in LO2 hepatocytes treated with the fatty acid palmitate, an *in vitro* model of hepatic steatosis. The PKC family of kinases consists of 10 isoforms grouped by their sensitivity to activators. The conventional PKCs (PKC $\alpha$ , PKC $\beta$ I, PKC $\beta$ II and PKC $\gamma$ ) are  $\text{Ca}^{2+}$  dependent, whereas the novel PKCs (PKC $\delta$ , PKC $\epsilon$ , PKC $\theta$ , and PKC $\eta$ ) and atypical PKCs (PKC $\zeta$  and PKC $\iota/\lambda$ ) are not. Both conventional and novel PKCs are, however, sensitive to diacylglycerol (DAG). This lipid is a second messenger released predominantly at the plasma membrane by acute phospholipase C (PLC) activation in response to extracellular signals, but is also an intermediate in fatty acid metabolism that accumulates at the ER in cells and tissues exposed to lipid excess [14]. Through the phosphorylation of their protein substrates, PKC isoforms regulate a wide variety of cellular processes, including gene expression, cell growth and division, and cytoskeletal reorganization [15]. The conventional and especially the novel isoforms are also important candidates for mediating the effects of lipid oversupply, because of their activation by DAG [14].

When cells sequester fatty acids as TG in lipid droplets, the flux through the DAG intermediate occurs as a result of the actions of acyl transferases, that esterify fatty acids onto a glycerol phosphate backbone, and lipins (phosphatidic acid phosphatases) that subsequently remove the phosphate group to generate DAG [16]. Finally, diacylglycerol acyl-transferases esterify further fatty acid molecules with DAG to form TG which enters lipid droplets forming at the ER. DAG generated in this way is therefore well-situated to activate PKC $\delta$  at a location where SERCAs are found, in contrast with second messenger DAG molecules released from plasma membrane phospholipids by PLC. In each case, the DAG stereoisomer generated (sn-1,2 DAG) is capable of activating PKC [17], whereas the DAG stereoisomers released from lipid droplets during lipolysis (primarily sn-1,3 and sn-2,3 DAG) are unlikely to participate [18].

Lai et al. demonstrate that palmitate-treated LO2 cells accumulate TG in lipid droplets, and this is associated with not only an increase in the expression of ER stress markers and dysregulation of intracellular  $\text{Ca}^{2+}$ , but also the activation of PKC $\delta$  [13]. Furthermore, knockdown of PKC $\delta$  improves intracellular  $\text{Ca}^{2+}$  regulation and reduces the induction of ER stress markers by the lipid, as well as reducing TG accumulation itself. A key finding is the restoration of SERCA activity, which is greatly reduced by palmitate treatment, through PKC $\delta$  depletion. This occurs in the absence of an effect on SERCA2 protein levels, suggesting a modulation of ATPase function rather than expression.

This is a novel observation which further implicates PKC $\delta$  as a mediator of the deleterious effects of lipid excess, and as a potential therapeutic target. PKC activation has previously been implicated in the reduction of ER  $\text{Ca}^{2+}$  content [19], and the effects of PKC $\delta$  on SERCA function may be mediated directly through phosphorylation of the protein. This would be similar to the PKC-dependent phosphorylation and inhibition of store-operated  $\text{Ca}^{2+}$  channels [11,20], and such a mechanism would be fully consistent with the alleviation of ER stress by PKC $\delta$  knockdown.

On the other hand, the data reported by Lai et al. lend support to the idea that PKC $\delta$  represents a key node in a more complex network of interactions between lipid oversupply, ER stress and insulin resistance. While it has long been proposed that aberrant PKC activity directly interferes with proximal insulin signaling and hence with the suppression of hepatic glucose output [21], investigations of PKC isoform deletion in mice have also implicated other roles, including the induction of ER stress [22,23] and the dysregulation of fatty acid metabolism itself [24]. Indeed, it appears that PKC isoforms not only respond to intracellular lipid signals, but also in turn regulate *de novo* lipid synthesis, fatty acid esterification, lipolysis, and  $\beta$ -oxidation [14]. Thus PKC $\delta$  deletion reduces lipogenesis in the liver of mice fed a high fat diet [25], which may be mediated by increased signaling to the lipogenic transcription factor Srebp1c through an mTORC1/p70S6K-dependent manner [26]. This would represent a positive feed-back loop, since further fatty acid synthesis promoted by PKC $\delta$  is likely to lead to additional elevations in intracellular DAG. Deletion of PKC $\delta$  in mice also promotes hepatic insulin sensitivity and improved glucose homeostasis [26], which could be due direct effects at the level of proximal insulin signaling, or indirectly through alleviation of lipid accumulation or ER stress. Supporting the latter, Greene et al. showed that PKC $\delta$  levels and activation were increased in *in vivo* and cellular models of NASH, induced by methionine and choline deficiency (MCD), together with markers of ER stress [27]. PKC $\delta$  knockdown alleviated ER stress, although the link between PKC $\delta$  and ER stress also appeared to be bidirectional, in that ER stress also contributed to PKC $\delta$  activation [27], as also reported for TNF $\alpha$ -induced ER stress [28].

Consistent with the *in vivo* studies of high fat diet-fed PKC-deficient mice [23,25,26], but differing from the findings of MCD [27], Lai et al. also observed that knockdown of PKC $\delta$  reduced the accumulation of TG and lipid droplets in palmitate-treated LO2 hepatocytes [13]. This supports the idea of a more complex interaction between PKC $\delta$ , fatty acid excess, ER stress, and steatosis. If a simple, linear hierarchy existed from fatty acids, through increased DAG flux



not all affect insulin sensitivity [34]. An alteration in lipid partitioning towards esterification rather than  $\beta$ -oxidation may also protect hepatocytes during acute fatty acid excess, avoiding the accumulation of reactive oxygen species. In any case, it is clear that PKC $\epsilon$  and PKC $\delta$ , while both potential targets for the treatment of insulin resistance and glucose intolerance, play non-redundant roles in lipid metabolism.

A PKC $\delta$ -dependent stimulation of lipogenesis through ER stress may also account in part for the selective insulin resistance of glucose metabolism but not of lipogenesis that can occur in hepatocytes [36]. While unresolved ER stress can lead to Jnk activation and interference with proximal insulin signaling, so that the down-regulation of gluconeogenic genes through the Akt2-FoxO1 pathway is diminished, the concomitant activation of Srebp1c through ER stress may augment the effects of the hormone on lipid synthesis [30]. DAG-activated signaling through PKC $\delta$  would be expected to promote this paradoxical state.

A question that is raised by the work of Lai et al. is the nature of the effect of PKC $\delta$  on SERCA activity. The Ca<sup>2+</sup>-sensitive conventional isoform PKC $\alpha$  inhibits SERCA2 activity in cardiomyocytes by directly phosphorylating protein phosphatase inhibitor-1. This in turn promotes the dephosphorylation of the SERCA2 inhibitor phospholamban and its interaction with SERCA2, diminishing contractility by reducing sarcoplasmic reticulum Ca<sup>2+</sup> loading and release [37]. Whether PKC $\delta$  plays a role in a similar mechanism, or even directly phosphorylates SERCA, is not known. Interestingly, a key difference is the Ca<sup>2+</sup>-insensitivity of this novel PKC. While PKC $\alpha$  can mediate Ca<sup>2+</sup>-dependent feedback inhibition, PKC $\delta$  provides a nexus between lipid signaling and Ca<sup>2+</sup> homeostasis.

The report by Lai et al. thus provides a new insight into the role of PKC in the development of steatosis and insulin resistance. An effect of PKC $\delta$  on SERCA activity and thus Ca<sup>2+</sup> homeostasis and ER stress may help to explain some of the links between these pathologies, and further highlights the potential therapeutic value in targeting this family of kinases for the treatment of obesity-related disease.

## Competing interests

The author declares that there are no competing interests associated with the manuscript.

## Abbreviations

DAG, diacylglycerol; ER, endoplasmic reticulum; MCD, methionine and choline deficiency; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PKC $\delta$ , protein kinase C delta; PLC, phospholipase C; TG, triglyceride; T2D, Type 2 diabetes.

## References

- 1 Younossi, Z. and Henry, L. (2016) Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. *Gastroenterology* **150**, 1778–1785, <https://doi.org/10.1053/j.gastro.2016.03.005>
- 2 Chatterjee, S., Khunti, K. and Davies, M.J. (2017) Type 2 diabetes. *Lancet*, [https://doi.org/10.1016/S0140-6736\(17\)30058-2](https://doi.org/10.1016/S0140-6736(17)30058-2)
- 3 Farese, Jr, R.V., Zechner, R., Newgard, C.B. and Walther, T.C. (2012) The problem of establishing relationships between hepatic steatosis and hepatic insulin resistance. *Cell Metab.* **15**, 570–573, <https://doi.org/10.1016/j.cmet.2012.03.004>
- 4 Hotamisligil, G.S. (2010) Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* **140**, 900–917, <https://doi.org/10.1016/j.cell.2010.02.034>
- 5 Pineau, L., Colas, J., Dupont, S., Beney, L., Fleurat-Lessard, P., Berjeaud, J.M. et al. (2009) Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids. *Traffic* **10**, 673–690, <https://doi.org/10.1111/j.1600-0854.2009.00903.x>
- 6 Fu, S., Yang, L., Li, P., Hofmann, O., Dicker, L., Hide, W. et al. (2011) Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* **473**, 528–531, <https://doi.org/10.1038/nature09968>
- 7 Boslem, E., Weir, J.M., MacIntosh, G., Sue, N., Cantley, J., Meikle, P.J. et al. (2013) Alteration of endoplasmic reticulum lipid rafts contributes to lipotoxicity in pancreatic beta-cells. *J. Biol. Chem.* **288**, 26569–26582, <https://doi.org/10.1074/jbc.M113.489310>
- 8 Han, J. and Kaufman, R.J. (2016) The role of ER stress in lipid metabolism and lipotoxicity. *J. Lipid Res.* **57**, 1329–1338, <https://doi.org/10.1194/jlr.R067595>
- 9 Fu, S., Watkins, S.M. and Hotamisligil, G.S. (2012) The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. *Cell Metab.* **15**, 623–634, <https://doi.org/10.1016/j.cmet.2012.03.007>
- 10 Coe, H. and Michalak, M. (2009) Calcium binding chaperones of the endoplasmic reticulum. *Gen. Physiol. Biophys.* **28**, F96–F103
- 11 Wilson, C.H., Ali, E.S., Scrimgeour, N., Martin, A.M., Hua, J., Tallis, G.A. et al. (2015) Steatosis inhibits liver cell store-operated Ca<sup>2+</sup> entry and reduces ER Ca<sup>2+</sup> through a protein kinase C-dependent mechanism. *Biochem. J.* **466**, 379–390, <https://doi.org/10.1042/BJ20140881>
- 12 Park, S.W., Zhou, Y., Lee, J., Lee, J. and Ozcan, U. (2010) Sarco(endoplasmic reticulum Ca<sup>2+</sup>-ATPase 2b is a major regulator of endoplasmic reticulum stress and glucose homeostasis in obesity. *Proc. Natl. Acad. Sci.* **107**, 19320–19325, <https://doi.org/10.1073/pnas.1012044107>
- 13 Lai, S., Li, Y., Kuang, Y., Cui, H., Yang, Y., Sun, W. et al. (2017) PKCdelta silencing alleviates saturated fatty acid-induced ER stress by enhancing SERCA activity. *Biosci. Rep.* **37**, <https://doi.org/10.1042/BSR20170869>
- 14 Schmitz-Peiffer, C. (2013) The tail wagging the dog – regulation of lipid metabolism by protein kinase C. *FEBS J.* **280**, 5371–5383, <https://doi.org/10.1111/febs.12285>

- 15 Parker, P.J. and Murray-Rust, J. (2004) PKC at a glance. *J. Cell Sci.* **117**, 131–132, <https://doi.org/10.1242/jcs.00982>
- 16 Brasaemle, D.L. and Wolins, N.E. (2012) Packaging of fat: an evolving model of lipid droplet assembly and expansion. *J. Biol. Chem.* **287**, 2273–2279, <https://doi.org/10.1074/jbc.R111.309088>
- 17 Boni, L.T. and Rando, R.R. (1985) The nature of protein kinase C activation by physically defined phospholipid vesicles and diacylglycerols. *J. Biol. Chem.* **260**, 10819–10825
- 18 Eichmann, T.O., Kumari, M., Haas, J.T., Farese, Jr, R.V., Zimmermann, R., Lass, A. et al. (2012) Studies on the substrate and stereo/regioselectivity of adipose triglyceride lipase, hormone-sensitive lipase, and diacylglycerol-O-acyltransferases. *J. Biol. Chem.* **287**, 41446–41457, <https://doi.org/10.1074/jbc.M112.400416>
- 19 Ribeiro, C. M.P., McKay, R.R., Hosoki, E., Bird, G. S.J. and Putney, J.W. (2000) Effects of elevated cytoplasmic calcium and protein kinase C on endoplasmic reticulum structure and function in HEK293 cells. *Cell Calcium* **27**, 175–185, <https://doi.org/10.1054/ceca.2000.0108>
- 20 Kawasaki, T., Ueyama, T., Lange, I., Feske, S. and Saito, N. (2010) Protein kinase C-induced phosphorylation of Orai1 regulates the intracellular Ca<sup>2+</sup> level via the store-operated Ca<sup>2+</sup> channel. *J. Biol. Chem.* **285**, 25720–25730, <https://doi.org/10.1074/jbc.M109.022996>
- 21 Petersen, M.C., Vatner, D.F. and Shulman, G.I. (2017) Regulation of hepatic glucose metabolism in health and disease. *Nat. Rev. Endocrinol.* **13**, 572–587, <https://doi.org/10.1038/nrendo.2017.80>
- 22 Liao, B.M., McManus, S.A., Hughes, W.E. and Schmitz-Peiffer, C. (2016) Flavin-containing monooxygenase 3 reduces endoplasmic reticulum stress in lipid-treated hepatocytes. *Mol. Endocrinol.* **30**, 417–428, <https://doi.org/10.1210/me.2015-1217>
- 23 Liao, B.M., Raddatz, K., Zhong, L., Parker, B.L., Raftery, M.J. and Schmitz-Peiffer, C. (2014) Proteomic analysis of livers from fat-fed mice deficient in either PKCdelta or PKCepsilon identifies Httip2 as a regulator of lipid metabolism. *Proteomics* **14**, 2578–2587, <https://doi.org/10.1002/pmic.201400202>
- 24 Raddatz, K., Turner, N., Frangioudakis, G., Liao, B.M., Pedersen, D.J., Cantley, J. et al. (2011) Time-dependent effects of Prkce deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. *Diabetologia* **54**, 1447–1456, <https://doi.org/10.1007/s00125-011-2073-0>
- 25 Frangioudakis, G., Burchfield, J.G., Narasimhan, S., Cooney, G.J., Leitges, M., Biden, T.J. et al. (2009) Diverse roles for protein kinase C delta and protein kinase C epsilon in the generation of high-fat-diet-induced glucose intolerance in mice: regulation of lipogenesis by protein kinase C delta. *Diabetologia* **52**, 2616–2620, <https://doi.org/10.1007/s00125-009-1543-0>
- 26 Bezy, O., Tran, T.T., Pihlajamaki, J., Suzuki, R., Emanuelli, B., Winnay, J. et al. (2011) PKCdelta regulates hepatic insulin sensitivity and hepatosteatosis in mice and humans. *J. Clin. Invest.* **121**, 2504–2517, <https://doi.org/10.1172/JCI46045>
- 27 Greene, M.W., Burrington, C.M., Ruhoff, M.S., Johnson, A.K., Chongkraitanakul, T. and Kangwanpornsiri, A. (2010) PKC{delta} is activated in a dietary model of steatohepatitis and regulates endoplasmic reticulum stress and cell death. *J. Biol. Chem.* **285**, 42115–42129, <https://doi.org/10.1074/jbc.M110.168575>
- 28 Greene, M.W., Ruhoff, M.S., Burrington, C.M., Garofalo, R.S. and Orena, S.J. (2010) TNFalpha activation of PKCdelta, mediated by NFkappaB and ER stress, cross-talks with the insulin signaling cascade. *Cell Signal.* **22**, 274–284, <https://doi.org/10.1016/j.cellsig.2009.09.029>
- 29 Baumbach, J., Hummel, P., Bickmeyer, I., Kowalczyk, K.M., Frank, M., Knorr, K. et al. (2014) A Drosophila in vivo screen identifies store-operated calcium entry as a key regulator of adiposity. *Cell Metab.* **19**, 331–343, <https://doi.org/10.1016/j.cmet.2013.12.004>
- 30 Baiceanu, A., Mesdom, P., Lagouge, M. and Foufelle, F. (2016) Endoplasmic reticulum proteostasis in hepatic steatosis. *Nat. Rev. Endocrinol.* **12**, 710–722, <https://doi.org/10.1038/nrendo.2016.124>
- 31 Arruda, A.P., Pers, B.M. and Parlakgul, G. (2014) Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat. Med.* **20**, 1427–1435, <https://doi.org/10.1038/nm.3735>
- 32 Kang, S., Dahl, R., Hsieh, W., Shin, A., Zsebo, K.M., Buettner, C. et al. (2016) Small molecular allosteric activator of the sarco/endoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA) attenuates diabetes and metabolic disorders. *J. Biol. Chem.* **291**, 5185–5198, <https://doi.org/10.1074/jbc.M115.705012>
- 33 Monetti, M., Levin, M.C., Watt, M.J., Sajan, M.P., Marmor, S., Hubbard, B.K. et al. (2007) Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metab.* **6**, 69–78, <https://doi.org/10.1016/j.cmet.2007.05.005>
- 34 Minehira, K., Young, S.G., Villanueva, C.J., Yetukuri, L., Oresic, M., Hellerstein, M.K. et al. (2008) Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. *J. Lipid Res.* **49**, 2038–2044, <https://doi.org/10.1194/jlr.M800248-JLR200>
- 35 Nagle, C.A., Klett, E.L. and Coleman, R.A. (2008) Hepatic triacylglycerol accumulation and insulin resistance. *J. Lipid Res.* **50**, S74–S79, <https://doi.org/10.1194/jlr.R800053-JLR200>
- 36 Brown, M.S. and Goldstein, J.L. (2008) Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab.* **7**, 95–96, <https://doi.org/10.1016/j.cmet.2007.12.009>
- 37 Braz, J.C., Gregory, K., Pathak, A., Zhao, W., Sahin, B., Klevitsky, R. et al. (2004) PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nat. Med.* **10**, 248–254, <https://doi.org/10.1038/nm1000>