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EDITORIALS

Lipotoxicity: Why do saturated fatty acids cause and monounsaturates protect against it?

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Saturated fatty acids (SFA) (e.g. palmitate [16 : 0]) are almost universally toxic to cells in culture, whereas the monounsaturated fatty acids (MUFA) (e.g. oleate [18 : 1]) are either non-toxic or cytoprotective. The opposing effects of SFA and MUFA have been observed in multiple cell types including islet β-cells,[1] endothelial cells,[2] cardiomyocytes,[3] breast cancer cell lines,[4,5] and in hepatocyte cell lines as shown by Ricchi *et al.* in this issue of the Journal.[6] Importantly, the addition of MUFA to cell cultures dose-dependently inhibits SFA-induced cell death. Elevated glucose clearly increases the toxicity of palmitate in β-cells, a process called glucolipotoxicity.[1] The role of elevated glucose on lipotoxicity in other cell types has been under-investigated. An understanding of the mechanisms by which SFA are cytotoxic and MUFA are cytoprotective may give us clues to novel therapeutic approaches for relevant conditions, whether by diet or pharmacotherapeutic means. In most circumstances (e.g. steatohepatitis complicating non-alcoholic fatty liver disease [NAFLD]) the aim will be to inhibit cytotoxicity and/or promote cytoprotection. In some situations, however, inhibition of MUFA-induced cytoprotective mechanisms may have a role (e.g. in cancer therapy). So, why do the differing fatty acid types behave so differently with respect to cell survival?

Fatty acids and their metabolites have numerous biological functions.[7] Not only are lipids the major form by which energy is stored, they are also involved in cell structure, and participate in intracellular, extracellular and whole animal (endocrine) signaling processes. It should be no surprise, therefore, that the metabolism and behavior of the various types of fatty acids differs greatly. Considering this, it is probable that the mechanisms and/or pathways involved in SFA-induced cytotoxicity will be multiple and differ from those of MUFA-mediated cytoprotection.

Fatty acids may exert their effects directly, for example as ligands to cell surface receptors (e.g. G-protein coupled receptors [GPCR]) or to intracellular transcription factors (e.g. peroxisome proliferator-activated receptors [PPAR]). Alternatively, fatty acids may need to be metabolized intracellularly to have their effects. There is evidence for both these direct and indirect effects influencing cell viability. The mechanisms, however, remain to be clearly defined.

Direct effects of oleate cytoprotection via GPCR?

A few previously orphaned GPCR have been discovered to be cell surface receptors for fatty acids (GPR40, 41, 43 and 120).[8] The most studied is GPR40, which is highly expressed in pancreatic islet β-cells and brain.[9] This receptor is known to have a role in fatty acid augmentation of glucose-stimulated insulin secretion.[9,10] Interestingly, GPR40 may have a role in oleate-induced protection against palmitate-induced apoptosis, as shown in the mouse β-cell line NIT1,[11] and in various breast cancer cell lines.[12] The effects are possibly via the phospholipase C, mitogen-extracellular signal-regulated kinase 1/2, Src, and phosphatidyl 3-kinase/protein kinase B signaling pathways.[12] However, medium to long chain SFA and MUFA seem to be similarly active ligands for GPR40,[9] such that it is difficult to explain why oleate induces cytoprotection via this pathway, yet SFA do not. While GPR40 is believed to be minimally expressed in liver, in certain situations its expression might be induced as has recently been demonstrated in primary chicken hepatocytes cultured in the presence of linoleic acid.[13] There may be other cell surface receptors that respond more specifically to MUFA or their metabolites. For example, GPR119 is another GPCR for which *N*-oleoylethanolamine (OEA), an endogenous cannabinoid-like compound, is a ligand.[14] Further studies are required to clarify whether GPCR have any roles in mediating lipotoxicity.

Does PPAR activation underlie the cytoprotection mechanisms of MUFA?

Peroxisome proliferator-activated receptors are a family of nuclear receptors (PPARα, β/δ and γ) that are activated by fatty acid ligands. They play key roles as lipid/nutritional state sensors and transcriptional regulators of lipid metabolism.[15] As MUFA are more potent PPAR ligands than SFA, it is plausible that PPAR activation may explain the different effects of MUFA on cell viability.[15] Interestingly, the endogenous cannabinoid-like compounds, including OEA, have also been found to be PPARα agonists and to be neuroprotective following stroke in mice.[16] PPAR activation results in altered intracellular fatty acid metabolism, such that the intracellular partitioning of all fatty acids will be altered. Thus, direct PPAR activation by oleate may promote oxidation or sequestration of palmitate, thereby preventing subsequent cytotoxicity.

Activated PPARα not only induces the transcription of genes of peroxisomal and mitochondrial β-oxidation,[15,17] but also inhibits the pro-inflammatory nuclear factor (NF)-κB. In pancreatic β-cells and in breast cancer cells, fatty acid oxidation activation by pharmacological means reduced lipotoxicity, whereas its inhibition promoted cell apoptosis.[1,5] Hence, promotion of fatty acid oxidation, via MUFA activation of PPARα is likely to be a mechanism of cytoprotection. Consistent with this is the finding that cardioprotective fatty acids, such as oleate, increased the expression of genes promoting fatty acid oxidation.[18]

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Peroxisome proliferator-activated receptor-γ is predominantly expressed in adipose tissue, but is present at lower levels in many other tissues, including liver. PPARγ activation is a potent stimulator of adipocyte differentiation and lipid storage.[15] Therefore, activation of PPARγ could be cytoprotective by promoting partitioning of fatty acids into safe storage pools (see below).

In summary, activation of the PPAR family by MUFA may detoxify fatty acids, including SFA, as well as dampening effects on the activation of pro-inflammatory pathways, such as NF-κB, which have been implicated in lipotoxicity. Other fatty acid receptor/signal transduction pathways may also be involved, particularly in the processes of cytoprotection mediated by MUFA.

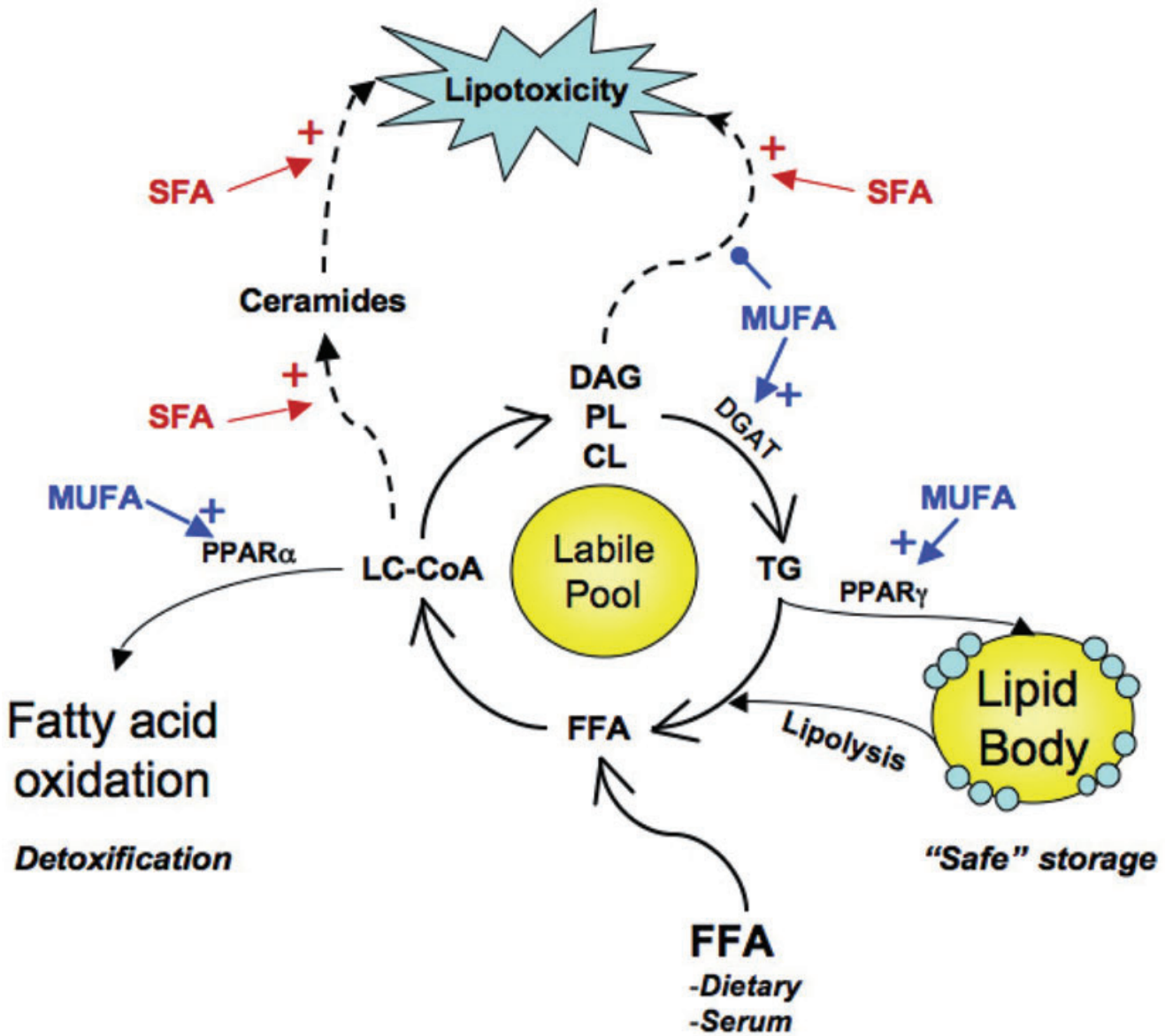

Figure 1 Model depicting effects of saturated fatty acids (SFA) compared to monounsaturated fatty acids (MUFA) on lipid partitioning and lipotoxicity. SFA are less well incorporated into triglycerides (TG) than are MUFA, as the enzyme diacylglycerol acyltransferase (DGAT) preferentially incorporates monounsaturated acyl-chains. SFA are also required for ceramide synthesis. SFA lead to greater accumulation of diacylgycerides (DAG) and a pattern of phospholipids (PL) with reduced cardiolipin (CL) production. This SFA pattern of lipid partitioning is associated with greater lipotoxicity. MUFA are well incorporated into TG and into lipid droplets that form a safe means of lipid storage. In this way fatty acids are removed from the functionally active labile pool of lipids. MUFA rather than SFA also activate the nuclear transcription factors PPARα and PPARγ which respectively promote lipid detoxification via fatty acid oxidation and safe fatty acid storage. Importantly, MUFA promote the safer partitioning of SFA into TG and fatty acid oxidation pathways.

Do MUFA favor cytoprotective intracellular fatty acid partitioning?

Saturated fatty acid cytotoxicity occurs after these fatty acids have been taken up by cells, and converted to long chain acyl-CoA (LC-CoA), a process of fatty acid activation.[1,5] The subsequent metabolism of the LC-CoA from SFA differs from MUFA LC-CoA in that their esterification into triglycerides (TG) is not as efficient.[5,6] Instead, intracellular diacylglycerides (DAG) and ceramides accumulate,[5] and the pattern of phospholipids synthesized differs.[5] Of particular note, incubation of breast cancer cell lines in SFA reduces production of the specialized phospholipid, cardiolipin.[5] One factor that undoubtedly plays a role in this differential metabolism of SFA and MUFA, is that oleoyl-CoA and palmitoleoyl-CoA (both MUFA-derived LC-CoA) are the preferred substrates for the TG synthesis enzyme, diacylglycerol acyltransferase. Thus, SFA derived LC-CoA need to be converted to monounsaturated-CoA by stearoyl-CoA desaturase 1 (SCD-1).[7] Dependent on cell type and possibly the underlying genetic susceptibility,[19] cell apoptosis induced by SFA may occur from altered mitochondrial function with release of cytochrome C due to reduction in cardiolipin,[5] endoplasmic reticulum (ER) stress[20] and/or oxidative stress.[3] This lipotoxicity is likely enhanced when SFA are not being converted to TG as rapidly as their monounsaturated counterparts. In INS1 β-cells, however, palmitate toxicity was shown to be associated with the accumulation of tripalmitin within dilated ER, perhaps as the result of misdirection of TG synthesis.[21] Recent bioinformatic profiling in cardiomyocytes has confirmed that SFA increased markers of oxidative and ER stress.[18]

So does oleate stop these effects of SFA via altering SFA metabolism? It appears that oleate can promote detoxification of SFA by altering their partitioning within the cell. The first is via promotion of fatty acid esterification into TG such that potentially toxic lipid can safely be stored within lipid droplets. The second is via promotion of their clearance via the induction of fatty acid oxidation.

Interestingly, co-incubation of SFA with oleate tends to promote SFA esterification processes, with improved TG synthesis and recovery of cellular cardiolipin levels.[5] Consistent with this, enhanced capacity for TG accumulation was observed in co-incubation studies by Ricchi *et al.* in this issue.[6] Furthermore, it has previously been shown that islets with greater capacity for TG storage are relatively protected from lipotoxic damage,[22] as are β-cell lines with higher expression levels of SCD-1.[23] Again consistent is the finding that induction of SCD-1 in human arterial endothelial cells protects them against lipotoxicity.[24] A fascinating finding in the MDA-MB-231 breast cancer cell line was that preincubation for 24 h in the presence of oleate markedly enhanced serum-free cell survival to at least 10 days, a phenomenon associated with the presence of large numbers of lipid droplets and enhanced glycerolipid/fatty acid cycling.[4]

Of relevance to NAFLD and NASH, we recently showed that high-fat feeding of lean *wildtype* mice develop simple steatosis without evidence of hepatocellular damage.[25] This occurs in association with upregulation of mRNA levels of SCD-1 and fatty acid oxidation processes. Obese *Alms1* mutant mice fed high fat, however, develop steatohepatitis (NASH) in the absence of the induction of SCD-1, PPARα or fatty acid oxidation gene expression.[25]

In summary, we propose a model (Fig. 1) in which MUFA promote processes of detoxification of SFA through: (i) enhancing their esterification and incorporation into stable lipid droplets; and (ii) via enhancement of their clearance by fatty acid oxidation. Thus, the SFA are denied the chance of being directed via alternative cytotoxic pathways. MUFA activation of PPAR is likely to be involved in their effects on re-partitioning SFA. Furthermore, MUFA may have additional beneficial effects via direct signaling through various fatty acid receptors, such as GPR40. As a note of caution, the findings of the *in vitro* cell culture experiments described here still need to be largely substantiated *in vivo*. Improved knowledge of the mechanisms underlying cytotoxicity and cytoprotection of differing fatty acids and fatty acid derivatives such as OEA should lead to the development of novel therapies for conditions such as steatohepatitis, for which lipotoxicity is now conceptualized as a major pathogenic pathway.

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Bile duct injuries associated with cholecystectomy

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The era of laparoscopic cholecystectomy has been associated with a minor increase in the frequency of significant bile duct injuries. Contemporary studies indicate a frequency of approximately 0.5% for laparoscopic procedures when compared to a frequency of 0.1–0.3% for open procedures.[1–3] These complication rates decrease with increasing experience of the surgeon in some[4] but not all[1] studies. The mechanisms of biliary injury are varied but include unrecognized stones and strictures in the lower bile duct and mistakes in the identification of the cystic duct and common hepatic duct in the triangle of Calot. Other factors include the excessive use of cautery, excessive traction, inadvertent duct laceration and the inappropriate application of clips or sutures.[5] In addition, rare patients have congenital anomalies of the biliary system, particularly an anomalous branch of the right hepatic duct that may enter the bile duct close to the cystic duct.[5] There is now persuasive population-based data indicating that the routine use of intraoperative cholangiography reduces the risk of bile duct injuries by approximately 30%.[4,6]

Bile duct injuries have been classified in a variety of ways but none are entirely satisfactory. One classification has four categories: type A, bile leaks from the cystic duct stump or peripheral hepatic duct; type B, bile leaks from a major hepatic duct; type C, strictures of the common hepatic duct without leakage; and type D, complete transection of the common hepatic duct. Approximately two-thirds of patients can be categorized as type A while types B, C and D have frequencies of approximately 15%, 10% and 10%, respectively.[7] In this issue of the Journal, Weber *et al.*[8] provide additional information on the frequency and management of these complications in Germany. Observations from this and other studies can be summarized as follows.

Bile leaks from the cystic duct stump

The function of the sphincter of Oddi generates a pressure gradient between the bile duct and the duodenum. This gradient is approximately 6 mmHg in humans[9] and similar gradients have been identified in laboratory animals. In dogs, cholecystectomy without ligation of the cystic duct stump results in a bile leak that persists for an average of 7 days.[10] Whether this also applies to humans remains unclear although in occasional patients, closure of the cystic duct is difficult or impossible because of extensive inflammation.

The most frequent cause of leaks through the cystic duct stump is elevation of the intrabiliary pressure because of distal biliary obstruction associated with stones, benign strictures or malignant strictures. After cholecystectomy, bile leaks through the cystic duct stump account for 70–80% of bile duct leaks[11–13] and 50–60% of all bile duct injuries.[7] Some of these leaks are identified at the time of surgery, others because of bile drainage through a drain-tube and the remainder because of the development of abdominal pain, fever and abdominal tenderness at 2–14 days following surgery. Investigations will be influenced by the clinical setting but may include ultrasound studies, computed tomography scans and magnetic resonance cholangiopancreatography (MRCP). Patients with symptomatic biliary collections (bilomas) are normally treated by percutaneous drainage. Thereafter, endoscopic retrograde cholangiography (ERC) is used to confirm the site of the fistula and to provide appropriate therapy. This may include endoscopic sphincterotomy and extraction of stones, endoscopic sphincterotomy for

