Where does liver fat go? A possible molecular link between fatty liver and diabetes

Ectopic fat accumulation in insulin-target organs leads to the development of insulin resistance in each organ by altering oxidative stress and gene expression profiles. Specifically, the liver functions as a center to maintain whole-body energy homeostasis by sensing nutrient stimuli, and producing a variety of nutrients and bioactive substances. Liver fat is associated with not only enhanced hepatic glucose production, but also skeletal muscle insulin resistance, supporting a central role of fatty liver in systemic insulin resistance in patients with non-alcoholic fatty liver disease (NAFLD). Of the triacylglycerol accumulated in the liver of patients with NAFLD, 59.0% arises from non-esterified fatty acids (NEFAs), mainly derived from adipose tissue, whereas 14.9% arises from diet and 26.1% from de novo lipogenesis. Triglyceride (TG) itself is not a toxic lipid, but might rather protect against the toxic effects of free fatty acids. However, some free fatty acids are regarded as toxic lipids that cause hepatic insulin resistance through oxidative stress. In an in vitro fatty liver system using H4IIEC3 hepatocytes, a saturated fatty acid, palmitate, and not an unsaturated fatty acid, oleate, inhibits insulin-stimulated tyrosine phosphorylation of insulin receptor substrate 2 and serine phosphorylation of Akt through c-Jun NH2-terminal kinase activation. In that model, mitochondrial β oxidation-derived reactive oxygen species (ROS) play a causal role in the palmitate-induced c-Jun NH2-terminal kinase activation. Therefore, toxic lipid mitochondrial ROS might underlie the link between steatosis and insulin resistance in the liver. Indeed, in humans, genes involved in mitochondrial oxidative phosphorylation (OXPHOS)/electron transport chain are more coordinately upregulated and positively correlated with those involved in a ROS-related pathway in the livers of obese type 2 diabetic patients compared with those of non-obese type 2 diabetic patients. These findings suggest that mitochondria play a key role in governing energy homeostasis in the liver, and might be a potential therapeutic target for type 2 diabetes and NAFLD.

Recently, Burgess et al. proposed that an enhanced OXPHOS pathway in the liver causes elevated hepatic glucose production, oxidative stress and inflammation. They focused on substrate fluxes in the mitochondrial tricarboxylic acid (TCA) cycle and OXPHOS. Mitochondria play a central role in energy production in whole tissues, mostly in the form of adenosine triphosphate, through the TCA cycle, suggesting a relatively suppressed ketogenic pathway even under enhanced TCA cycle flux in human NAFLD.

Sunny et al. quantitated intrahepatic TG content (IHTG) using 1H magnetic resonance spectroscopy, and assayed anaplerotic substrate flux and cataplerotic substrate eflux in the mitochondrial TCA cycle by a tracer method using 3H and 13C in participants with NAFLD. Hepatic oxidative flux in the TCA cycle was doubly increased in participants with high IHTG compared with those with low IHTG; 50% higher rates of lipolysis increased mitochondrial anaplerosis, which increased cataplerotic eflux, leading to 30% higher rates of gluconeogenesis. There was a strong positive correlation between IHTG content and anaplerotic flux. Unexpectedly, ketone production assessed by tracer dilution of β-hydroxybutyrate was not different between participants with high and low IHTG, suggesting a relatively suppressed ketogenic pathway even under enhanced TCA cycle flux in human NAFLD.

Next, Satapati et al. examined the molecular mechanisms underlying increased hepatic anaplerotic flux in diet-induced mouse and rat models of obesity. In concert with the aforementioned human data, ketogenesis, oxidative flux in the TCA cycle, anaplerosis, cataplerosis and gluconeogenesis were all increased in the livers from fed mice perfused with high NEFA (0.8 mmol/L) compared with those perfused with low NEFA (0.2 mmol/L). Although insulin administration to the liver perfused with high NEFA suppressed glycojenolysis, it did not prevent the elevation in anaplerosis and gluconeogenesis. Therefore, NEFA
overload is considered sufficient to increase ex vivo hepatic oxidative metabolism, anaplerosis and gluconeogenesis. In agreement with the ex vivo liver perfusion, in the liver from rats in which circulating NEFA was increased by infusing intralipid for 6 h, ketone turnover, oxidative flux in the TCA cycle, and gluconeogenesis were increased together with increased circulating levels of TG and insulin. Thus, increased lipid delivery is sufficient to amplify anaplerosis and gluconeogenesis in vivo. In that in vivo model, messenger ribonucleic acid levels for mitochondrial superoxide dismutase 2, inflammatory cytokines, such as 

\[ \text{tnf}\alpha \text{ and } \text{Il}6, \text{ and lipid peroxidation were elevated in proportion to the rise in oxygen consumption that is strongly associated with anaplerosis/catablerosis flux and gluconeogenesis during intralipid infusion.} \]

In an in vitro study using H4IIE hepatocytes\(^1^0\), NEFA increased ROS production, which was attenuated with an inhibitor for phosphoenolpyruvate carboxykinase (PEPCK), which links catablerosis efflux from the TCA cycle and gluconeogenesis. Based on this in vitro finding, they investigated the therapeutic role of PEPCK in NAFLD in vivo. Systemic knockdown of Pck1 (PEPCK-C) retained the ability to suppress hepatic gluconeogenesis during a hyperinsulinemic–euglycemic clamp study. Also, insulin-induced phosphorylation of Akt was reduced in the liver of mice fed a high fat diet (HFD), which was rescued by Pck1 knockdown. Interestingly, Pck1 knockdown sufficiently prevented a fatty acid-induced rise in anaplerosis/catablerosis in mice fed a HFD, but not in mice fed a control diet. Why does Pck1 knockdown decrease anaplerosis/catablerosis? TCA cycle oxidation is regulated by redox states and product inhibition\(^1^1\). Pck1 knockdown reduced the hepatic mitochondrial and cytosolic nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide ratio estimated from the plasma acetacocetate/\(\beta\)-hydroxybutylate and liver lactate/pyruvate ratio, respectively. Because nicotinamide adenine dinucleotide serves as a coenzyme for dehydrogenases in the TCA cycle, a reduced nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide ratio suppresses TCA flux. Such a redox state is known to inhibit the forward reaction of the TCA cycle\(^1^1\). In addition, several TCA cycle metabolites are known to inhibit upstream enzymes, which is known as product inhibition\(^1^1\). Pck1 knockdown markedly increased oxaloacetate, one of the intermediate products produced during the TCA cycle, which inhibits upstream enzyme succinate dehydrogenase. As the TCA cycle is the major source of electrons for OXPHOS, a Pck1 knockdown-mediated decrease in anaplerosis/catablerosis might decrease ROS production through OXPHOS. Indeed, hepatic expression of the genes involved in oxidative stress, peroxidase and peroxiredoxins were upregulated by a HFD, which was canceled by Pck1 knockdown. Also, HFD-induced lipid peroxidation was canceled by Pck1 knockdown. Pck1 knockdown resulted in a more oxidized redox state of mitochondrial inner membranes. Furthermore, a reduced cytosolic redox state is favorable for the reduced form of glutathione and the clearance of peroxide by its anti-oxidant system. Pck1 knockdown accumulated some intermediates in the TCA cycle that activate anti-oxidant transcription factors, such as nuclear respiratory factor 1 and nuclear factor, erythroid-derived 2, like 2. Collectively, Pck1 knockdown reduces electron transport and activates anti-oxidant systems, and thus reduces ROS levels in the liver. In agreement with low oxidative stress, Pck1 knockdown also protected mice against HFD-induced inflammation.

Metformin suppresses hepatic glucose production, and is used as a first-line oral antidiabetic agent for type 2 diabetes. Metformin might prevent a rise in anaplerosis/catablerosis, as it suppresses Pck1 messenger ribonucleic acid levels in a concentration- and time-dependent manner\(^1^2\). Indeed, Satapati et al\(^1^0\) showed that metformin treatment reduces fasting hepatic gluconeogenesis by suppressing TCA flux and anaplerosis/catablerosis in mice fed a HFD. Also, metformin reduced the

**Figure 1 | Schematic of the hepatic tricarboxylic acid (TCA) cycle with the major anaplerotic and catablerotic pathways associated with gluconeogenesis, lipogenesis, and ketogenesis.**

A labeling of fatty acid delivery amplifies the TCA cycle flux with a rise in anaplerosis/cataplerosis, leading to an increase in fatty acid delivery amplifies the TCA cycle flux with an increase in anaplerosis/cataplerosis, leading to an increase in oxidative stress and inflammation in liver. The redox state and intermetabolites in the TCA cycle amplify or suppress the flux. Oxidative stress is causal for mitochondrial dysfunction. ATP, adenosine triphosphate; OXPHOS, oxidative phosphorylation; PC, pyruvate carboxylase; PCK1, phosphoenolpyruvate carboxykinase; ROS, reactive oxygen species.
hepatic expression of Tnfα and IL6 expressions in mice fed a HFD.

Finally, Satapati et al.\(^6\) examined the significance of oxidative metabolism in human NAFLD pathology. The histological NAFLD activity score of the human liver biopsy specimens correlated with oxygen consumption, indicating that activation of oxidative metabolism is associated with liver pathology in human NAFLD.

The Burgess et al.\(^3,10\) linked mitochondrial oxidative metabolism, including amplified TCA cycle flux and anaplerosis/cataplerosis, to hepatic oxidative stress, inflammation and/or insulin resistance in mice infused with NEFA. Notably, substrate flux into ketogenesis is differently regulated in humans\(^9\) and mice\(^10\). How substrate flux is regulated still remains unclear. Metformin protects mice fed a HFD from oxidative stress and inflammation by reducing mitochondrial oxidative metabolism\(^10\). However, in a clinical setting, metformin seems unsatisfactory in ameliorating human NAFLD pathology\(^13\). Instead, they have proposed another candidate therapeutic target for reducing mitochondrial oxidative metabolism. Unexpectedly, inhibition of PEPCK protects HFD-fed mice from oxidative stress and inflammation by reducing mitochondrial oxidative metabolism. As anaplerosis is elevated in people with NAFLD\(^9\), further studies are required to provide evidence of whether a PEPCK inhibitor is effective in ameliorating the pathology of NAFLD, including liver histology and insulin resistance, in humans.

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Doi: 10.1111/jdi.12573