Intestinal Lipolysis Mitigates Nonalcoholic Fatty Liver Disease: New Roles for Carboxylesterase 2c in the Intestine

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The health and financial impact of the obesity epidemic has become a worldwide challenge in the past decades. Despite widespread public health policies and efforts to combat the growing rate of obesity, over 2 billion people are obese or overweight.[1] Excessive weight is associated with an increased incidence of cardiovascular disease, hypertension, stroke, type 2 diabetes mellitus and malignancy, and obesity-associated insulin resistance is central to metabolic disturbances.[2] Nonalcoholic fatty liver disease (NAFLD), of which the spectrum ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), is a prominent obesity-associated comorbidity.

Although dyslipidemia is a hallmark of NAFLD, the mechanisms through which aberrant lipogenesis contributes to NAFLD are not fully understood.[3] Both genetic and environmental factors are associated with NAFLD development. Genetic factors include specific genotypes (PNPLA3, TM6SF2, and GCKR), which have been found to predict NASH and severe liver disease, but have no significant connection to type 2 diabetes.[4,5] Environmental factors include high caloric intake, which leads to hepatic lipid overflow.[6] Insulin resistance holds the critical mechanistic function of impairing insulin action, resulting in upregulation of hepatic *de novo* lipogenesis and triglyceride (TG) synthesis, and increased fatty acid delivery to the liver.[6] Regardless of phenotype, a critical histological and metabolic feature of NAFLD is the accumulation of TG in the liver, which when in excess can lead to hepatic steatosis, which may further promote cell death, inflammation, and hepatic stellate cell activation, developing NASH and fibrosis.[3,7] More recent literature has implicated carboxylesterases in NAFLD and obesity. Specific roles include regulation of both TG pools for very low density lipoprotein synthesis, the size and number of cytosolic lipid droplets, as well as the impact on blood fatty acid concentration and insulin sensitivity.[8] Two recent studies demonstrated reductions in hepatic carboxylesterase 2 (*CES2*) levels in the development of NAFLD in mice and in human obesity.[9,10] Notably, in diabetic and high-fat diet (HFD) fed mice, restoration of hepatic *CES2* expression reversed liver steatosis and insulin resistance.[10]

Although previous studies had provided evidence of the role of hepatic carboxylesterase 2c (*Ces2c*) in the development of NAFLD, *Ces2c* is highly expressed in the intestine. Therefore, in this issue of Hepatology Communications,[11] Maresch et al. have further demonstrated the impact of intestinal

Abbreviations: Akt, protein kinase B; CES2, carboxylesterase 2; Ces2c, carboxylesterase 2c; DG, diglyceride; HFD, high-fat diet; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TG, triglyceride; WT, wild type.

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Ces2c activity in NAFLD development and hepatic insulin sensitivity. The authors initially assessed the role of *Ces2c* and its proposed human orthologue *CES2* through transient overexpression in COS-7 cells. Overexpressed *Ces2c* or *CES2* increased neutral TG hydrolysis and diglyceride (DG) hydrolysis activities. Furthermore, *Ces2c* overexpression led to an increase fatty acid oxidation, suggesting that fatty acids are generated by lipid hydrolysis for further degradation.

To study the specific role of *Ces2c* in the intestine, the authors generated new intestine-specific *Ces2c* transgenic mice. These mice showed increased TG hydrolysis and fatty acid oxidation in the small intestine. In parallel, fatty acid oxidation–regulating genes, including *PPARα* and *CPT1a*, were up-regulated.

To address their central question of whether intestinal lipid metabolism regulated by *Ces2c* affects liver pathophysiology, the study further investigated the role of *Ces2c* activity in the development of HFD-induced weight gain, NAFLD, and insulin resistance. When mice were fed HFD, wild-type (WT) mice showed increased body weight, but the body weight of *Ces2c* transgenic mice was significantly low compared with WT controls. Interestingly, activity levels and daily/total food intake were comparable between both groups. Importantly, intestinal *Ces2c* transgenic mice displayed reduced hepatic TG content and histological steatosis, which is normally increased in WT mice when fed with HFD. In addition to reduced histological evidence of hepatic steatosis, inflammatory and fibrotic markers were also decreased in intestinal *Ces2c* transgenic mice.

In addition, the study evaluated systemic insulin resistance, which could be associated with the severity of NAFLD. In *Ces2c* transgenic mice, glucose clearance and insulin sensitivity were improved. Importantly, in intestinal *Ces2c* transgenic mice there was an increase in phosphorylation of protein kinase B (Akt), a downstream kinase of insulin signaling, suggesting improved hepatic insulin sensitivity. The authors commented on how intestinal *Ces2c* overexpression did not affect Akt phosphorylation in skeletal muscle, suggesting that the role that insulin signaling played in skeletal muscle is not strong. Therefore, the study suggested that intestinal *Ces2c* overexpression improved hepatic insulin signaling, rather than systemic insulin resistance. However, drawing this conclusion might require further investigation, as Akt is not only activated by insulin.

Finally, the question remains as to how increased intestinal *Ces2c* expression affects the observed hepatic phenotype and provides protection from HFD-induced insulin resistance in this study. Dietary fat absorption in intestinal Ces2cint mice was virtually identical to control mice, and no significant changes were observed in overall energy expenditure. Furthermore, the authors provide data demonstrating similar rates of lipid entry/secretion into the circulation in transgenic mice when compared with controls. This, together with the normal food intake previously mentioned in transgenic and control mice, suggest other mechanisms at play. Finally, the authors go on to assess postprandial lipidemia. Notably, enhanced chylomicron TG clearance and apolipoprotein B48 levels were observed after fat challenge orally in intestinal *Ces2c* transgenic mice. In parallel with previous data suggesting that increased chylomicron size enhances TG clearance, final experiments in this study demonstrated that isolated chylomicrons were larger in size and diameter. The authors go on to note increased postprandial TG clearance in intestinal *Ces2c* transgenic mice as the possible mechanism that lowers lipid flux and lipid accumulation in the liver, leading to protective effects from obesity-induced NAFLD. Given that messenger RNA expression of genes involved in fatty acid oxidation was increased in skeletal muscles, the authors speculate lipid mobilization into skeletal muscle as the site of lipid clearance. However, this requires further investigation as the evidence is not fully clear.

This study proposed at least two major mechanisms of the potential of intestinal *Ces2c* on lipid metabolism. One is that intestinal *Ces2c* overexpression increases the hydrolysis of TG and DG, which generates fatty acids for degradation through fatty acid oxidation. The other is that DG and monoglyceride generation from the hydrolysis of lipids, of which the re-esterification is enhanced, increases the apolipoprotein B48–containing particle size, further promoting enhanced chylomicron clearance from the circulation. Thus, enhanced intestinal *Ces2c* activity could mitigate NAFLD and insulin resistance by reducing hepatic, intestinal, and systemic lipid content through degrading and eliminating the lipids from the body. The most important argument of this study is that modulation of intestinal lipid

metabolism by enhancing intestinal *Ces2c* expression has the potential to affect hepatic and systemic lipid metabolism, regulating the development of NAFLD and systemic insulin resistance. This further suggests that medications to increase intestinal *CES2* activity can be a future effective therapy for NAFLD and diabetes.

Although this study has illustrated the important role of intestinal lipid metabolism, several questions remain to be answered. Given the difference between known carboxylase 2 family members in mice and humans, further studies are needed to investigate the biological similarities and cross activities against the proposed human carboxylase orthologue, *CES2*. As recent studies demonstrated a link between reduced hepatic expression of *Ces2c* and *CES2* in NAFLD development in obese mice and humans, respectively, it would prove useful to similarly characterize the expression in intestinal samples of obese humans and mice. Additionally, work must be done to further differentiate the advantages of targeting intestinal lipid carboxylase activity versus hepatic. Nonetheless, this study provides evidence highlighting the importance of intestinal lipid homeostasis in obesity and development of weight-associated comorbidities such as NAFLD.

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