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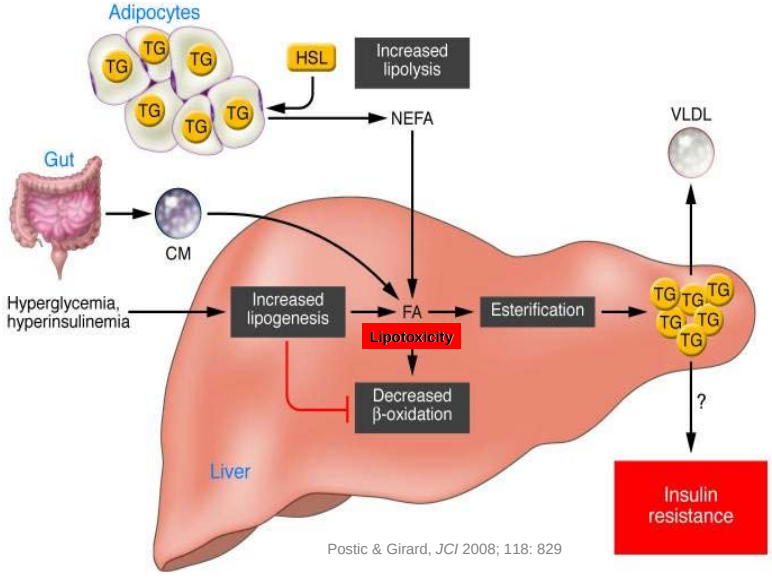
Role of hepatic triglyceride lipases in fatty liver disease

Background: The determinants responsible for progression of simple fatty liver (i.e. accumulation of triglycerides [TG]) to potentially progressive non-alcoholic steatohepatitis (NASH) remain largely unclear (Browning & Horton 2004). Liver specific inhibition of acyl-coenzyme A:diacylglycerol acyltransferase (DGAT2), the enzyme responsible for the last step in hepatic TG synthesis, improves hepatic steatosis in obese diabetic mice but, unexpectedly, exacerbates injury and fibrosis (Yamaguchi et al. 2007). These findings suggest that the ability to synthesize TG may, in fact, be protective against lipotoxicity of FA in liver (Unger 2002, Schaffer 2003). Conversely, the mobilization of stored intracellular TG is mediated by hormone-sensitive lipase (HSL) and a recently discovered adipose triglyceride lipase (ATGL) (Jaworski et al. 2007, Zechner et al. 2005), although the function of these two critical enzymes in liver is still poorly understood. Although the regulation of ATGL and its physiological function remain to be determined in liver, present data obtained in mice that lack or overexpress the enzyme permit the conclusion that ATGL is critically involved in the hepatocellular mobilization of FA and FA signaling (Jaworski et al. 2007, Zechner et al. 2005) ATGL-/- mice develop hepatic steatosis (Haemmerle et al. 2006) while transient adenoviral hepatic overexpression of HSL or ATGL promotes FA oxidation, stimulates direct release of FA, and ameliorates hepatic steatosis in both ob/ob mice and mice with high fat diet-induced obesity (Reid et al. 2008). Anti-angiogenic pigment epithelium-derived factor (PEDF) is highly expressed in hepatocytes and plays an important role in hepatic triglyceride homeostasis through binding to ATGL (Chung et al. 2008). Dysregulation of this pathway may also contribute to the pathogenesis of NAFLD and hepatocellular carcinoma (HCC). Recently, genetic variations of PNPLA3, another member of the patatin-like lipase family and close homologue to ATGL, have been identified as major determinant for susceptibility to NAFLD (Romeo et al. 2008), but the underlying molcular mechanisms and role for progression to NASH remain to be resolved. Recent evidence suggests that ATGL and HSL protein expression is decreased in white adipose tissue (WAT) from obese and insulin-resistant subjects (Jocken et al. 2007), but little is known about potential role of hepatocellular lipases in the progression of simple NAFL to NASH, cirrhosis and HCC.



While HSL is regulated through betadrenergic receptor-mediated increases in cAMP and PKA (anatgonized by insulin), little is known about the regulation of ATGL in adipose tissue and liver (Jaworski et al. 2007, Zechner et al. 2005). So far existing evidence suggest that ATGL may be regulated at the transcriptional level via PPARgamma and glucocorticoids as well as post-transcriptionally by protein-protein

interactions with CGI-58 and PEDF (Jaworski et al. 2007, Zechner et al. 2005). Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, emerging evidence suggests that bile acids (BA) may also play a key role as signalling molecules in the regulation of TG and energy homeostasis (Houten et al. 2006, Thomas et al. 2008). Although BA have been shown to regulate hepatic *de novo* lipogenesis, TG (VLDL) export and FA oxidation in brown adipose tissue (BAT) and skeletal muscle (Houten et al. 2006, Thomas et al. 2008), the role of BA in regulating hepatic and (by spillover possibly also WAT) lipase activity as first critical step in providing free FA has so far not been considered in current pathophysiologic and therapeutic concepts

We hypothesize that hepatic TG lipases (i.e. ATGL, its coregulators CGI-58 and PEDF, as well as HSL and PNPLA3) critically determine the progression from simple fatty liver to NASH with subsequent risk for progressive liver disease including liver cancer. Moreover, we hypothesize that potentially divergent regulation of ATGL and HSL in WAT and liver may determine the individual susceptibility to NAFLD and NASH in obese subjects. Finally, we aim to test the hypothesis, that the recently emerging metabolic hormonal action of bile acids may also target hepatic and WAT lipases. In line with this hypothesis, bile acids and their modified analogues may be useful to treat and/or prevent NASH. Collectively, the results of this WP should alter our understanding of the fundamental pathogenesis of NASH and result in novel treatment strategies for NAFLD and NASH targeting ATGL and other hepatic TG lipases.

Methods: These questions will be addressed in vivo in knockout and transgenic mouse models in collaboration with the lab of Rudolf Zechner within the SFB-LIPOTOX (http://lipotox.uni-graz.at/project.html). To extrapolate potential findings from mouse models to human diseases, we will crossvalidate the obtained key experimental findings in tissues from patients with fatty liver disease. These in vivo results will be complemented by transfection and siRNA studies in isolated mouse and human hepatocytes / cell lines. In order to test our key hypothesis that hepatic TG lipases critically determine the susceptibility to lipotoxicity and development of NASH, we will first challenge mice lacking or overexpressing key tissue lipases and their regulators (i.e. ATGL, HSL, CGI-58, PEDF) in liver with high fat, high sucrose and methionine choline-deficient diet. This *in vivo* approach in knockout and transgenic mice will be complemented by *in vitro* studies in isolated hepatocytes/hepatocellular cell lines lacking or overexpressing ATGL, CGI-58, PEDF and HSL (transient tranfection, siRNA strategies). Moreover, the potential role of bile acids as enterohepatic signal regulating hepatic TG lipase activity will be tested in bile acid challenged mice, cell lines and selcetd patients undergoing liver biopsy during bile acid (UDCA) therapy. Finally we will address potential differences in hepatic TG lipase activities in livers (and WAT) from patients with bland NAFL versus NASH to unravel whether individual differences in hepatic and WAT lipase patterns may determine the susceptibility to NAFLD and NASH.

Collaborations within the PhD program: Höfler, Zatloukal, Speicher, Kratky

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