Original Article

MOLECULAR METABOLISM

Intracellular lipids are an independent cause of liver injury and chronic kidney disease in non alcoholic fatty liver disease-like context

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ABSTRACT

Objective: Ectopic lipid accumulation in the liver and kidneys is a hallmark of metabolic diseases leading to non-alcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD). Moreover, recent data have highlighted a strong correlation between NAFLD and CKD incidences. In this study, we use two mouse models of hepatic steatosis or CKD, each initiated independently of the other upon the suppression of glucose production specifically in the liver or kidneys, to elucidate the mechanisms underlying the development of CKD in the context of NAFLD-like pathology.

Methods: Mice with a deletion of *G6pc*, encoding glucose-6 phosphatase catalytic subunit, specifically in the liver (L.G6pc$^{-/-}$ mice) or the kidneys (K.G6pc$^{-/-}$ mice), were fed with either a standard diet or a high fat/high sucrose (HF/HS) diet during 9 months. These mice represent two original models of a rare metabolic disease named Glycogen Storage Disease Type Ia (GSDIa) that is characterized by both NAFLD-like pathology and CKD. Two other groups of L.G6pc$^{-/-}$ and K.G6pc$^{-/-}$ mice were fed a standard diet for 6 months and then treated with fenofibrate for 3 months. Lipid and glucose metabolisms were characterized, and NAFLD-like and CKD damages were evaluated.

Results: Lipid depot exacerbation upon high-calorie diet strongly accelerated hepatic and renal pathologies induced by the *G6pc*-deficiency. In L.G6pc$^{-/-}$ mice, HF/HS diet increased liver injuries, characterized by higher levels of plasmatic transaminases and increased hepatic tumor incidence. In K.G6pc$^{-/-}$ mice, HF/HS diet increased urinary albumin and lipocalin 2 excretion and aggravated renal fibrosis. In both cases, the worsening of NAFLD-like injuries and CKD was independent of glycogen content. Furthermore, fenofibrate, *via* the activation of lipid oxidation significantly decreased the hepatic or renal lipid accumulations and prevented liver or kidney damages in L.G6pc$^{-/-}$ and K.G6pc$^{-/-}$ mice, respectively. Finally, we show that L.G6pc$^{-/-}$ mice and K.G6pc$^{-/-}$ mice developed NAFLD-like pathology and CKD independently.

Conclusions: This study highlights the crucial role that lipids play in the independent development of both NAFLD and CKD and demonstrates the importance of lipid-lowering treatments in various metabolic diseases featured by lipid load, from the "rare" GSDIa to the "epidemic" morbid obesity or type 2 diabetes.

Keywords Metabolic diseases; Lipids; Glycogen; Fenofibrate

1. INTRODUCTION

The rise in global epidemics of obesity and type 2 diabetes in westernized countries contributes to a worrying increase in the incidence of non-alcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD). Both pathologies are associated with high morbidity and mortality, and thus represent serious public health problems. Indeed, NAFLD and CKD have been associated with long-term complications such as hepatic tumorigenesis and renal failure, respectively [1,2]. A common metabolic feature of these complications is ectopic lipid

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Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; CKD, Chronic Kidney Disease; EMT, epithelial-mesenchymal transition; G6P, glucose-6 phosphate; G6Pase, glucose-6 phosphatase; GSDI, Glycogen Storage Disease type I; HCA, hepatocellular adenomas; HCC, hepatocellular carcinomas; HF/HS, high fat/high sucrose; PPARα, Peroxisome Proliferator Activated Receptor α; STD diet, standard diet; TG, triglyceride

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accumulation, which is suspected to induce organ damage and dysfunction [3]. Indeed, abnormal lipid content in non-adipose tissues is often considered as a major factor involved in cell injury, inflammation, necrosis, and activation of pathological pathways [4–9].

It is noteworthy that ectopic accumulation of lipids in both the liver and kidneys is an important hallmark of a rare disease named Glycogen Storage Disease type I (GSDI) [10,11]. GSDI is caused by glucose-6 phosphatase (G6Pase) deficiency, leading to severe hypoglycemia during short fasts [12–14]. Mutations in *G6PC*, encoding the catalytic subunit of G6Pase, are responsible for GSD type Ia (GSDIa), while mutations in *SLC37A4* encoding the transport subunit of the G6Pase, are responsible for GSD type Ib (GSDIb). G6Pase operates the hydrolysis of glucose-6 phosphate (G6P) in glucose thus allowing liver and kidneys, the main organs responsible for endogenous glucose production, to release glucose in the blood and regulate plasma glucose concentration [15]. The lack of G6Pase induces G6P accumulation in the liver and kidneys, leading to metabolic reprogramming in these organs [16]. The first consequence of G6P increase is abnormal accumulation of glycogen in both organs [11,14,17,18], which has given its name to the disease. However, hepatic and renal lipid metabolism are also markedly altered, characterized by a G6P-dependent increase in *de novo* lipogenesis and fatty acid chain elongation [10,11,17,19–21]. Thus, GSDI patients suffer from a combined hypertriglyceridemia and hypercholesterolemia associated with hepatic steatosis, characterized by a low-inflammatory state without fibrosis [22–24] and abnormal lipid deposition in the kidney cortex [17]. Interestingly, GSDI patients exhibit all NAFLD features, concomitantly with an elevated incidence of hepatocellular adenomas (HCA), which can later transform in hepatocellular carcinomas (HCC) [25,26]. Moreover, GSDI patients exhibit the first signs of CKD quite early during their youth, which can progress to renal failure with age [10,13,14].

For several years, we have used contrasting obesity/diabetes (characterized by over- or unrepressed production of glucose) and GSDI (characterized by a loss of glucose production) as a strategy to reciprocally unravel these two "mirror" pathologies, with special focus on liver and kidneys. This strategy has already allowed us to emphasize the striking similarities between hepatic and renal metabolism in GSDI and diabetes, an increased metabolism downstream of G6P being a common causal feature [20]. For that, we use mouse models of obesity/diabetes and two mouse models with targeted deletions of G6PC either in the liver (L.G6pc$^{-/-}$ mice) or in the kidney (K.G6pc$^{-/-}$ mice). These mice develop GSDIa hallmarks, including deregulated lipid metabolism in the targeted organ exclusively [11,17]. More precisely, the loss of G6Pase in the liver leads to severe hepatic steatosis, in the absence of fibrosis, and later to the development of HCA/HCC [11]. In kidneys, this leads to tubular damages and then to the development of interstitial fibrosis and glomerulosclerosis, associated with a thickening of the basal glomerular membrane, a loss of podocytes/pedicels and proliferation of mesangial cells, finally responsible for the loss of the renal filtration function [10,17].

It is noteworthy that, in the obesity/diabetes field, recent data have highlighted a strong correlation between NAFLD and CKD incidences. Moreover, arguments have suggested suggest that NAFLD may play a causal role in CKD development [27–29]. However, studies firmly unravelling a causal relationship or a mechanism to account for a link between these two pathologies are currently lacking.

In this study, to decipher the role of triglycerides (TG) in the mechanisms underlying both hepatic and renal complications leading to NAFLD or CKD, we first investigated whether and how a diet enriched in lipids could accelerate liver or kidney injuries in L.G6pc$^{-/-}$ and K.G6pc$^{-/-}$ mice. In parallel, using the same mouse models, we examined whether a drug activating intracellular lipid catabolism could prevent or delay NAFLD and CKD, respectively. Finally, a part of this study also allowed us to document whether NAFLD can influence the development of CKD or not.

2. METHODS

2.1. Experimental animal models

L.G6pc$^{-/-}$ mice and K.G6pc$^{-/-}$ mice were obtained by deletion of exon 3 of the *G6pc* specifically in the liver or kidneys (more precisely in the proximal tubules that specifically express G6Pase [30]), respectively, thanks to an inducible CRE-lox strategy, as previously described [11,17]. Briefly, B6.G6pc$^{ex3lox/ex3lox}$ mice were crossed with mice expressing the inducible CRE^{ERT2} recombinase under the control of the serum albumin promoter (B6.SA$^{CreERT2/w}$) or under the control of the kidney androgen-regulated protein promoter (B6.Kap$^{CreERT2/w}$) to generate L.G6pc$^{-/-}$ and K.G6pc$^{-/-}$ mice, respectively, after intraperitoneal injections of tamoxifen (five consecutive days in 6–8 weeks old male). Male C57Bl/6J mice (Charles Rivers Laboratories) were also treated with tamoxifen (here referred to as WT mice). Female mice were not used because *Kap* promoter is under androgenic control.

Mice were housed in the animal facility of Lyon 1 University (ALECS) under temperature controlled (22 °C) conditions and with a 12/12 h light/dark cycle, in enriched environment in groups of 4–6 mice. After tamoxifen treatment, mice were fed either a standard (STD) diet (3.1% fat, 60% carbohydrates, 16.1% proteins, Safe) or a HF/HS diet (36.1% fat, 35% carbohydrates composed by maltodextrine (50%, wt/wt) and sucrose (50%, wt/wt), 19.8% proteins, produced at the "Unité de Préparation des Aliments Expérimentaux"; UE0300 INRA, Jouy-en-Josas, France) for 9 months. Fenofibrate-treated mice were first fed a STD chow diet for 6 months and then a STD diet supplemented with 0.2% fenofibrate (Sigma, wt/wt) (Safe, Augy, France) for additional 3 months. All mice were killed 9 months after tamoxifen treatment by cervical dislocation at 6 h of fasting (with continuous access to water). Tissue was frozen by freeze-clamping in liquid nitrogen and then stored at −80 °C. All the procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals. The regional animal care committee (C2EA-55, Université Lyon 1, Lyon) approved all the experiments.

2.2. Histological analysis

A piece of fresh liver and a piece of kidney were fixed in formaldehyde and embedded in paraffin. The 4 μm-thick sections were stained with hematoxylin and eosin (H&E) or Masson's Trichrome staining. The slides were examined under a Coolscope microscope (Nikon). For transmission electron microscopy analyses, small pieces of the liver were immediately fixed in 2% glutaraldehyde at 4 °C. The sample was dehydrated in a grade series of ethanol and embedded in an epoxy resin. Tissue was surveyed with a series of 70 nm sections and observed with a Jeol 1400JEM transmission electron microscope equipped with a Orius 100 camera and digital micrograph.

2.3. Urine parameters

K.G6pc$^{-/-}$ mice were housed in individual metabolic cages (Ugo Basile) for urine collection during 24 h. Urea concentration was assessed using a BioAssay Systems colorimetric kit (Hayward, CA, USA). Uric acid concentration was measured with a colorimetric kit (DiaSys, Holzheim, Germany). Albuminuria and lipocalin 2 levels were assessed using a mouse albumin ELISA kit (Neobiotech, Clinisciences,

