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学位論文題目	The effects of CREBH on ChREBP-mediated hepatic fructose metabolism (CREBHによるChREBPを介した肝臓フルクトース代謝の機能分析)		
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## Abstract of thesis

In this doctoral dissertation, Wang Y describes the pathophysiology of non-alcoholic steatohepatitis. The content is summarized as follows.

### Purpose

Non-alcoholic steatohepatitis (NASH) is the most common chronic liver disease in adults. Epidemiologic and animal studies implicate overconsumption of dietary sugar, particularly fructose, is suspected to be a critical contributor to the development of NASH. Carbohydrate response element binding protein (ChREBP) is a key transcriptional regulator of de novo lipogenesis (DNL). ChREBP is reported as an important factor in response to fructose-induced hepatic steatosis and hepatitis by regulating both gene expression and secretion of fibrosis growth factor 21 (FGF21). However, mechanisms underlying fructose modulation of ChREBP-FGF21 axis are still unclear. cAMP responsive element binding protein H(CREBH) is a membrane-bound transcription factor which regulates lipid metabolism. CREBH and peroxisome proliferator-activated receptor alpha (PPARα) co-activate hepatic Fgf21 expression and exert effects on energy metabolism through the modulation of plasma FGF21 levels. Previously, the group reported that deficiency of CREBH exacerbates non-alcoholic fatty liver disease (NAFLD) and NASH. Here, the author investigated to define if CREBH affects ChREBP-FGF21 axis in response to hepatic fructose metabolism.

### Material and method

The author fed 8-week-old male wild type (WT) mice and CREBH KO (CKO) mice with a high-fructose diet

(HFruD) or 60% fructose water (60% FW) for 8 weeks or 24 hours, respectively. Body weight, liver weight, plasma parameters, FGF21 levels, liver TC and TG content of mice were measured. The author performed RT-PCR, western blot, chromatin immunoprecipitation assay (ChIP) using liver samples. Liver sections were stained by haematoxylin and eosin (H&E) staining or Masson trichrome (M&T) staining. For in vitro experiments, 293 cells were transfected using lipofectamine 3000 and immunoprecipitated by specific plasmids to check protein-protein interactions. Mouse primary hepatocytes were isolated from the and challenged with fructose or glucose.

## Results

The author fed WT mice and CKO mice HFruD for 8 weeks and found that CKO mice showed severer fatty liver and liver injury. The loss of CREBH led a significant reduction of ChREBP protein level and *Chrebp $\beta$*  gene expression as well as *Fgf21* gene expression and its secretion in circulation. Furthermore, the author revealed that chromatin ChIP suggested the recruitment of ChREBP to ChoRE reporter on FGF21 promoter was significant lower in CKO mice compare with that of WT mice after 8 weeks HFruD. By WGA purification, the author found that one of ChREBP post-transcriptional modifications (PTMs), O-GlcNAcylation was reduced both in CKO mice and CREBH knockdown mouse primary hepatocytes. In addition, the author found that both exogenous and endogenous CREBH combined with O-Linked N -Acetylglucosamine Transferase (OGT) by using 293A cell line and mouse primary hepatocytes under high fructose condition. And exogenous CREBH increased the interaction between ChREBP and OGT. Taken together, the data suggested that CREBH plays a crucial role in fructose induced ChREBP-FGF21 axis hepatic fructose metabolism.

## Discussion

The author revealed a novel role of CREBH-mediated adaptive pathway that prevent the liver from fatty liver change and liver damage in response to fructose consumption. In vivo and in vitro data suggested that CREBH plays an essential role on O-GlcNAcylation induced ChREBP activation, which lead to the upregulation of FGF21 gene expression and secretion in response to fructose influx to prevent the liver from fructose overconsumption induced fatty liver change and liver injury.

## Abstract of assessment result

### (General Comments)

The author revealed a novel gene and nutrition interaction as a survival mechanism. Also, the author identified a new PTM of CREBH in response to fructose which allows CREBH to reduce fructose triggered ChREBP induced TG accumulation, mice with genetic defects in *Crebh* might progress to NASH or even cirrhosis likely due to the reduction of FGF21 secretion. In this article, the findings provide convincing evidences that CREBH plays a crucial role in hepatic lipid metabolism. The data also implicates that CREBH could be a potential therapeutic target for NAFLD, NASH or cirrhosis. The final examination committee conducted a meeting as a final examination on Jan. 8, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination. The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.