Genes & Diseases (2023) 10, 72–75

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RAPID COMMUNICATION

Identification of Epsin1 as a regulator for hepatic lipid and glucose metabolism

Obesity related non-alcoholic fatty liver disease (NAFLD), which can progress to steatohepatitis and insulin resistance, has become a major chronic liver disease worldwide.[1] As an adaptor of clathrin-mediated endocytosis, Epsin1 plays a role in many diseases, including tumors, atherosclerosis and diabetic wound healing.[2] Epsins contain a conserved NH_2-terminal homology domain (ENTH), which binds to lipids on plasma membrane.[3] Here, we investigated the role of Epsin1 in the regulation of hepatic lipid and glucose metabolism. Results showed that hepatic *Epsin1* overexpression improved insulin tolerance test, and alleviated hepatic steatosis, hyperglycemia and hyperlipidemia in diet-induced obese (DIO) mice. Besides, *Epsin1* overexpression reduced the expression of gluconeogenic genes, lipogenic genes, and increased lipolytic genes in the liver. Proteomic data indicated that *Epsin1* overexpression reduced the levels of the proteins involved in lipid synthesis and gluconeogenesis.

The mRNA level of *Epsin1* was upregulated in the liver of obese mice compared with lean mice under both fed and fasted conditions, while the expression of *Epsin2* or *Epsin3* was not changed, except that *Epsin2* was slightly increased under fasted condition (Fig. 1A; Fig. S1A). Besides, immunohistochemistry data showed that Epsin1 protein mainly located on the membrane of hepatocytes, and the abundance of Epsin1 was much higher in the liver of obese mice than that of lean mice (Fig. 1B). Moreover, the expression of *EPSIN1*, but not *EPSIN2* or *EPSIN3*, was increased in the liver of obese pigs compared with lean pigs (Fig. S1B). These data indicated that Epsin1, but not Epsin2 or Epsin3, might be involved in the regulation of hepatic glucose and lipid metabolism.

We then explored the role of Epsin1 in the regulation of hepatic glucose and lipid metabolism with DIO mice, using the method of adenovirus mediated *Epsin1* overexpression. The mRNA and protein levels of Epsin1 were increased in mouse liver by AdEpsin1 injection compared with AdGFP injection (Fig. 1C; Fig. S2A, B). Though the body weight was not changed (Fig. S2C), blood glucose level and serum insulin concentration were significantly reduced by hepatic *Epsin1* overexpression compared with the control (Fig. 1D, E). Besides, the mRNA levels of gluconeogenic genes phosphoenolpyruvate carboxykinase (*Pepck1*) and glucose 6-phosphatase (*G6pc*) and their regulator peroxisome proliferator-activated receptor gamma coactivator 1 alpha ($Pgc1\alpha$) were lower in the liver of AdEpsin1 infected mice than those in the control mice (Fig. S2D). Moreover, insulin tolerance test showed that AdEpsin1 infected mice were more sensitivity to exogenous insulin than the control mice (Fig. 1F). These results indicated that Epsin1 could suppress hepatic gluconeogenesis.

The role of Epsin1 in the regulation of hepatic lipid metabolism was then investigated. Liver weight and hepatic triglyceride (TAG) content were significantly decreased by hepatic *Epsin1* overexpression compared with the control (Fig. 1G, H). Meanwhile, hematoxylin and eosin (H&E) staining and Oil-red O staining indicated that *Epsin1* overexpression reduced hepatic lipid droplet accumulation compared with control (Fig. 1I). Besides, serum content of TAG was also reduced by hepatic *Epsin1* overexpression compared with control (Fig. 1J). Upon Epsin1 overexpression, as the precursors of TAG, hepatic non-esterified fatty acids (NEFA) faced an increase while hepatic acetyl-CoA a decrease in their content, in contrast with the control (Fig. 1K, L); at the same time, serum NEFA level was not changed (Fig. S2E). Furthermore, *Epsin1* overexpression suppressed the expression of lipogenic genes including fatty acids synthetase (*Fasn*), stearoyl-CoA desaturase 1 (*Scd1*), acetyl-CoA carboxylase 2 (*Acc2*) and sterol regulatory element binding transcription factor 1 (*Srebf1*), and triglyceride synthetic genes, including diacylglycerol acyltransferase 1 (*Dgat1*) and *Dagt2* in the liver compared with the control treatment (Fig. S2F). In addition, the mRNA levels of lipid catabolic genes *Ppara*, *Cpt1a*, and *Acot3* were decreased, while expression of *Atgl* was increased, by *Epsin1* overexpression compared with control (Fig. S2G). Of the lipoproteins, only the mRNA level of *Apoa2* was decreased by *Epsin1* overexpression compared with control (Fig. S2H).

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.05.016

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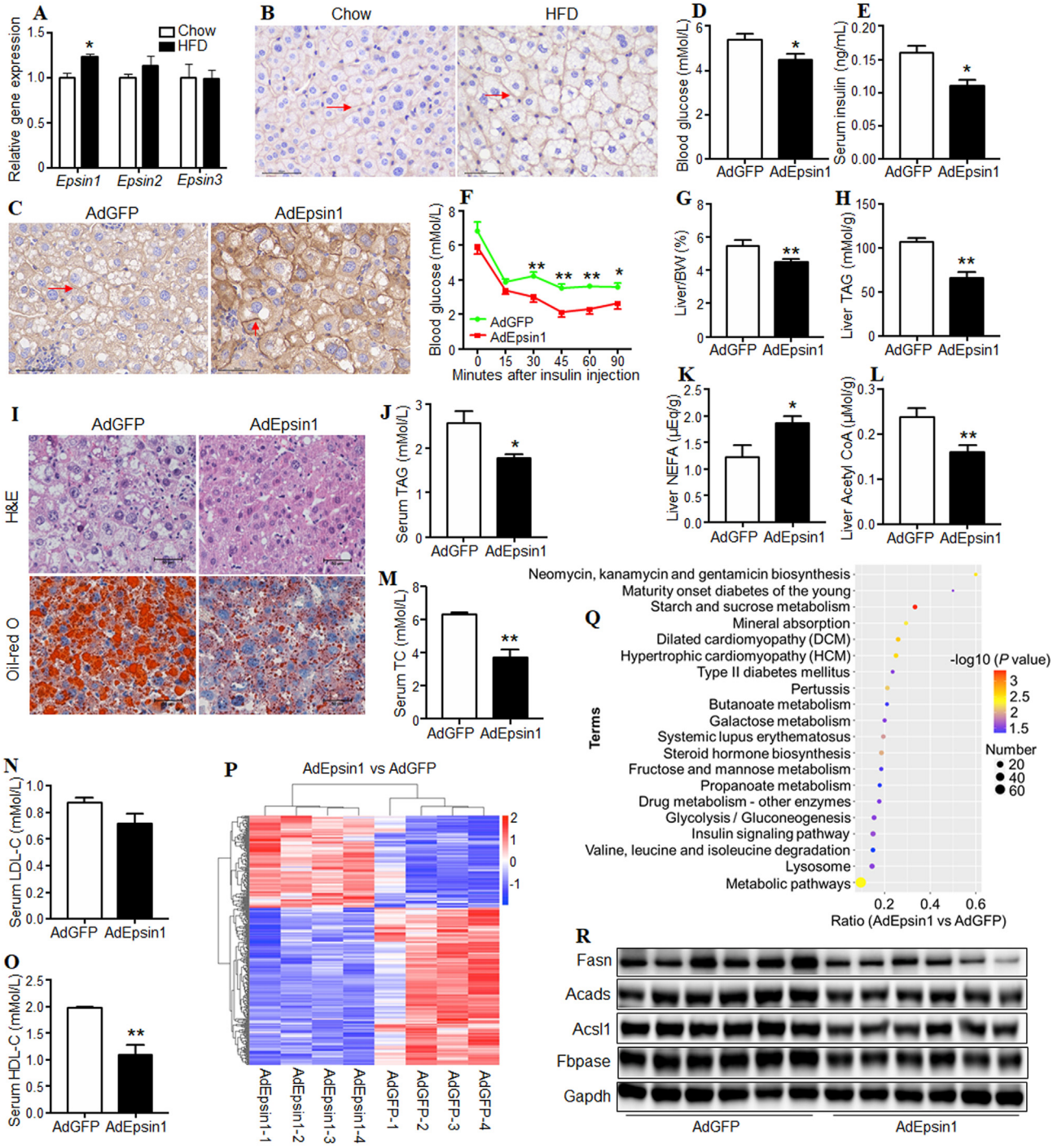

Figure 1 Epsin1 improved glucose and lipid metabolism in diet-induced obese (DIO) mice. **(A, B)** The livers of DIO mice and their littermate lean mice were harvested under fed condition ($N = 4$ for each group). (A) Gene expression levels of *Epsin1*, *Epsin2* and *Epsin3*. (B) Immunohistochemistry for Epsin1 in the livers of DIO mice and their littermate lean mice. Arrows indicate Epsin1 protein. Scale bars = 50 μm. **(C—R)** DIO mice were injected with AdGFP or AdEpsin1 through tail-vein, and were accessed to insulin tolerance study or harvested on day 7 of treatment ($N = 6$ per group). (C) Immunohistochemistry staining of liver samples with Epsin1 antibody. Blood glucose level (D) and serum insulin level (E) at harvest. (F) Insulin tolerance test study ($N = 6$ for each group). (G) Liver weight index. (H) Liver triglyceride (TAG) content. (I) Hematoxylin and eosin (H&E) staining and Oil red O staining with the liver sections. Scale bars = 50 μm. (J) TAG content in the serum. (K) Non-estesterified fatty acid (NEFA) content in the liver. (L) Acetyl-CoA content in the liver. (M) Total cholesterol (TC) content in the serum. (N) Low-density lipoprotein cholesterol (LDL-C) content in the serum. (O) High-density lipoprotein cholesterol (HDL-C) content in the serum. (P) Heatmap clustering was analyzed with differentially expressed proteins in the liver between AdGFP and AdEpsin1 groups. (Q) KEGG enrichment bubble chart. The color of the point indicates the *P*-value of the hypergeometric test, and the size represents their corresponding number of proteins in their pathway. (R) Western blot analysis of key proteins involved in lipid and glucose metabolism. Data were expressed as Mean ± SE. $^*P < 0.05$, $^{**}P < 0.01$.

These results indicated that Epsin1 could suppress lipogenesis and stimulate lipolysis in liver.

As another member of serum lipids, cholesterol is also an important indicator for metabolic diseases.[4] As compared with control group, hepatic *Epsin1* overexpression significantly decreased serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) contents, but did not change serum low-density lipoprotein cholesterol (LDL-C) content, while slightly increased hepatic TC content (Fig. 1M—O; Fig. S2I). Cholesterol is synthetized in liver and can be used to synthetize primary bile acid (BA) and secreted into blood circulation in the form of lipoprotein. The mRNA levels of low-density lipoprotein receptor (*Ldlr*) and ATP citrate lyase (*Acly*), key genes related to cholesterol uptake and biosynthesis, were decreased by *Epsin1* overexpression compared with control (Fig. S2J). Besides, cholesterol 7α-hydroxylase (Cyp7a1), sterol 12α-hydroxylase (Cyp8b1) and oxysterol 7α-hydroxylase (Cyp7b1) are key enzymes for BA synthesis,[5] while ATP binding cassette subfamily G member 5 and 8 (Abcg5 and Abcg8) are the main cholesterol transporters, which were all suppressed by *Epsin1* overexpression compared to control treatment (Fig. S2J). These data suggested that hepatic Epsin1 could improve hypercholesterolemia in DIO mice.

The role of Epsin1 in the regulation of lipid metabolism was also confirmed in hepatic *Epsin1* knocked down mice (Fig. S3A, B). Results showed that hepatic *Epsin1* knockdown increased liver TAG content (Fig. S3C, D), but did not change liver contents of TC, NEFA or acetyl-CoA, or serum contents of TAG, NEFA, TC, HDL-C or LDL-C (Fig. S3E—L).

To investigate the molecular mechanisms through which Epsin1 regulated glucose and lipid metabolism, iTRAQ-based proteomic analysis was performed on the liver of AdEpsin1 or AdGFP infected DIO mice. A total of 45,310 unique peptides were identified, and 4,789 proteins were detected. Sequence coverage of most proteins were less than 20% (Fig. S4A), and mass distribution of most proteins ranged from 20 to 60 kDa (Fig. S4B). The proteins with 20% CV between the two groups represented $>$80% of identified protein species (Fig. S4C). Heatmap cluster analysis demonstrated the distribution of differentially accumulated proteins (DAPs) (Fig. 1P). Besides, volcano plot analysis with the criteria of the 4,789 identified proteins with fold changes $>$1.20 or $<$0.83 and associated *P*-values $<$ 0.05 showed that 322 proteins were differentially expressed between the two groups (Fig. S4D and Table S1). Of them, 245 DAPs were categorized into biological process (BP), cellular component (CC) and molecular function (MF) based on Gene Ontology (GO) enrichment analysis (Fig. S4E). And, the most enriched functions were single-organism process (35.9%), regulation of biological quality (5.3%) and positive regulation of biological process (3.26%) in BP, cytoskeleton (5.30%) in CC, and oxidoreductase activity (9.38%) and cytoskeletal protein binding (6.12%) in MF (Fig. S4E). KEGG pathway enrichment analysis showed that the major enriched pathways of the DAPs were metabolic pathway, insulin signaling pathway, glycolysis/gluconeogenesis and Type 2 diabetes mellitus (Fig. 1Q). Other significant enriched KEGG pathways which related to lipid and glucose metabolism were listed in Table S2.

Of the 322 DAPs, some were linked to lipid and glucose metabolism (Table S3). And, some of them were consist with their gene expression (Fig. S2). To further validate the differently expressed proteins, we selected some of them for Western blot analysis. Consistently with the results of proteomics analysis, the protein levels of long-chain acyl-CoA synthetase 1 (Acsl1), acyl-CoA dehydrogenase short chain (Acads), Fasn and fructose-1,6-Bisphosphatase (Fbpase) were downregulated by *Epsin1* overexpression compared with the control (Fig. 1R).

In summary, our study demonstrated that Epsin1 might be an important regulator for hepatic lipid and glucose metabolism by suppressing lipogenesis and gluconeogenesis. Thus, Epsin1 might be a candidate target for the therapy of metabolic syndrome.

Author contributions

B. Feng, D. Wu and X. Huang conceived and designed the experiments; X. Huang, L. Jin, Z. Fang, L. Che, Y. Lin, S. Xu and Y. Zhuo performed the experiments; B. Feng, X. Huang and M. Li analyzed the data; X. Huang wrote the paper; B. Feng and D. Wu revised the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interests.

Funding

This study was supported by the National Natural Science Foundation of China (No. 31900834) and the China's Overseas Expertise Introduction Project for Discipline Innovation ("111 Project") (No. D17015).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.05.016.

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2 February 2022
Available online 28 May 2022