Alcohol predisposes obese mice to acute pancreatitis via adipose triglyceride lipase-dependent visceral adipocyte lipolysis

We read with great interest articles by Wang *et al*[1] and Hegyi *et al*,[2] in which the authors reported that alcohol increased the risk of hereditary susceptibility to chronic pancreatitis. These results indicated an interaction effect between environmental and genetic risk factors on the development of pancreatitis. Epidemiological data suggested that alcohol abuse increased the risk of acute pancreatitis (AP) in people with type 2 diabetes mellitus (adjusted HR 86.3 (65.3–111.0)).[3] However, no study investigated the synergistic effect between obesity and alcohol excess on AP development. Here we report the combination of acute alcohol intake and obesity causes AP with multiorgan injury (MOI) in mice, mediated by visceral adipocyte tissue (VAT) lipolysis.

The schedule of high-fat feeding and ethanol administration is shown in figure 1A. Body weight was significantly higher in the high-fat (obese) than the chow (lean) group after 12 weeks (figure 1B). Acute ethanol administration in obese mice induced significant increases in pancreatic histopathology scores (oedema, inflammation and necrosis; figure 1C), elevated circulating pancreatic enzymes (figure 1D), pancreatic and lung myeloperoxidase, and serum interleukin-6 levels (figure 1E). Time-course changes in this obese alcoholic acute pancreatitis (OA-AP) model showed pancreatic injury parameters were significantly elevated from 3 to 6 hours after the first ethanol injection, with rises in MOI indices (online supplemental figure 1A–F); almost all parameters peaked at 12 hours. In contrast, acute ethanol administration in lean mice caused only mild pancreatic oedema without discernable pancreatic necrosis or elevations of MOI indices.

We speculated that lipolysis from excess abdominal fat is critical to OA-AP, releasing free fatty acids (FFAs) from ethanol-induced VAT lipolysis. Indeed, fat saponification was seen in the peritoneal cavity and around the pancreas of ethanol-treated obese mice (online supplemental figure 2A,B). Circulating baseline FFA levels were higher in obese mice than in lean mice, which were further increased after acute ethanol administration (figure 1F). FFA and glycerol release over 3 hours from freshly isolated epididymal VAT of ethanol-treated obese mice was higher than that of lean mice (figure 1G). While pancreatic amylase or pancreatic triglyceride lipase (PNLIP) were comparable (figure 1H), adipose triglyceride lipase (ATGL) of ethanol-treated epididymal VAT taken from obese mice was, however, significantly higher than from lean mice (figure 1I).

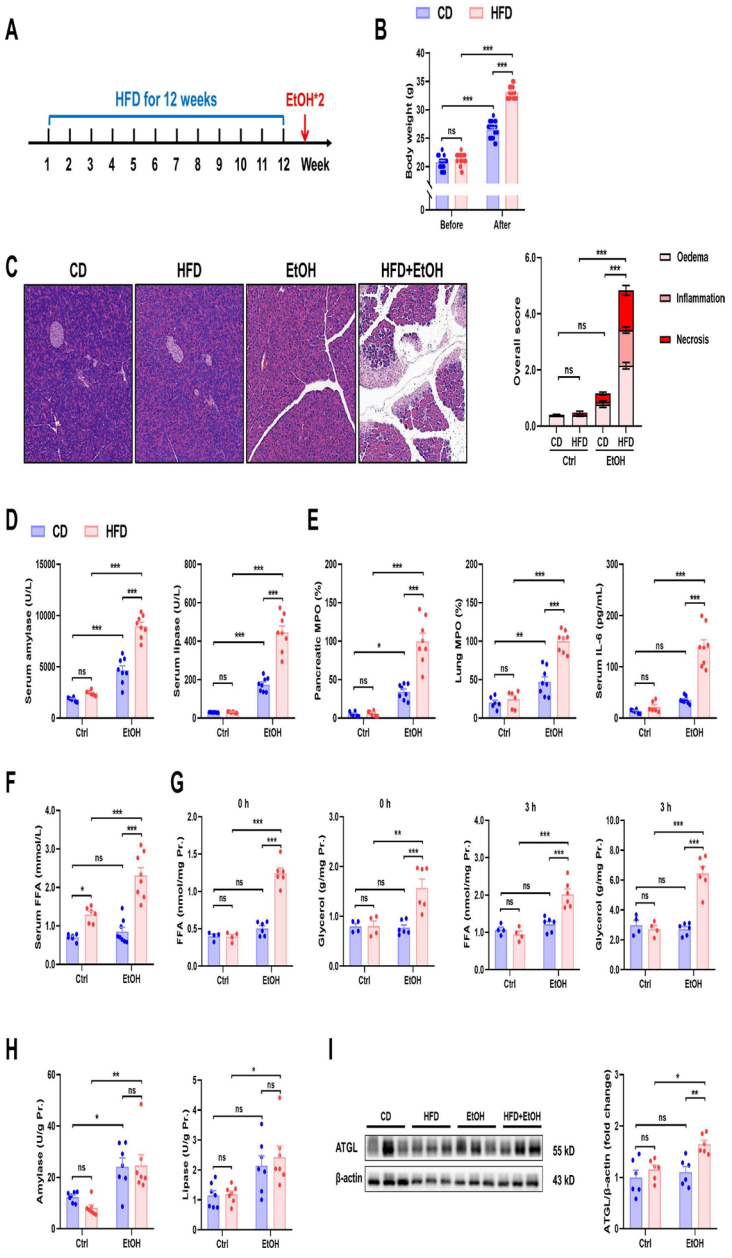

Figure 1 Alcohol predisposes obese mice to AP with systemic organ injury. (A) Experimental protocol of establishing OA-AP. C57BL/6 J mice were fed a CD (lean) or HFD (obese) for 12 weeks, then were injected intraperitoneally with 2 g/kg EtOH two times at 1 hour apart: (B) Body weight. (C) Representative images of pancreatic histopathology and histopathological scores (oedema, inflammation and necrosis; magnification ×200), (D) Serum amylase and lipase and (E) pancreatic MPO, lung MPO, and serum IL-6 levels of the OA-AP mice. (F) Serum FFA levels and (G) FFA and glycerol release in collected adipose tissues. (H) Amylase and lipase levels in epididymal adipose tissue. (I) Immunoblot analysis of ATGL proteins in epididymal adipose tissue. In all experiments, mice were sacrificed at 12 hours after the first injection of EtOH and assessed for disease severity and/or lipolytic parameters. *P<0.05, **P<0.01, ***P<0.001. AP, acute pancreatitis; ATGL, adipose triglyceride lipase; CD, control diet; Ctrl, control; EtOH, ethanol; FFA, free fatty acid; HFD, high-fat diet; IL, interleukin; MPO, myeloperoxidase; ns, not significant; OA-AP, obese alcoholic acute pancreatitis.

To confirm our hypothesis, we injected ethanol and adipocytes simultaneously

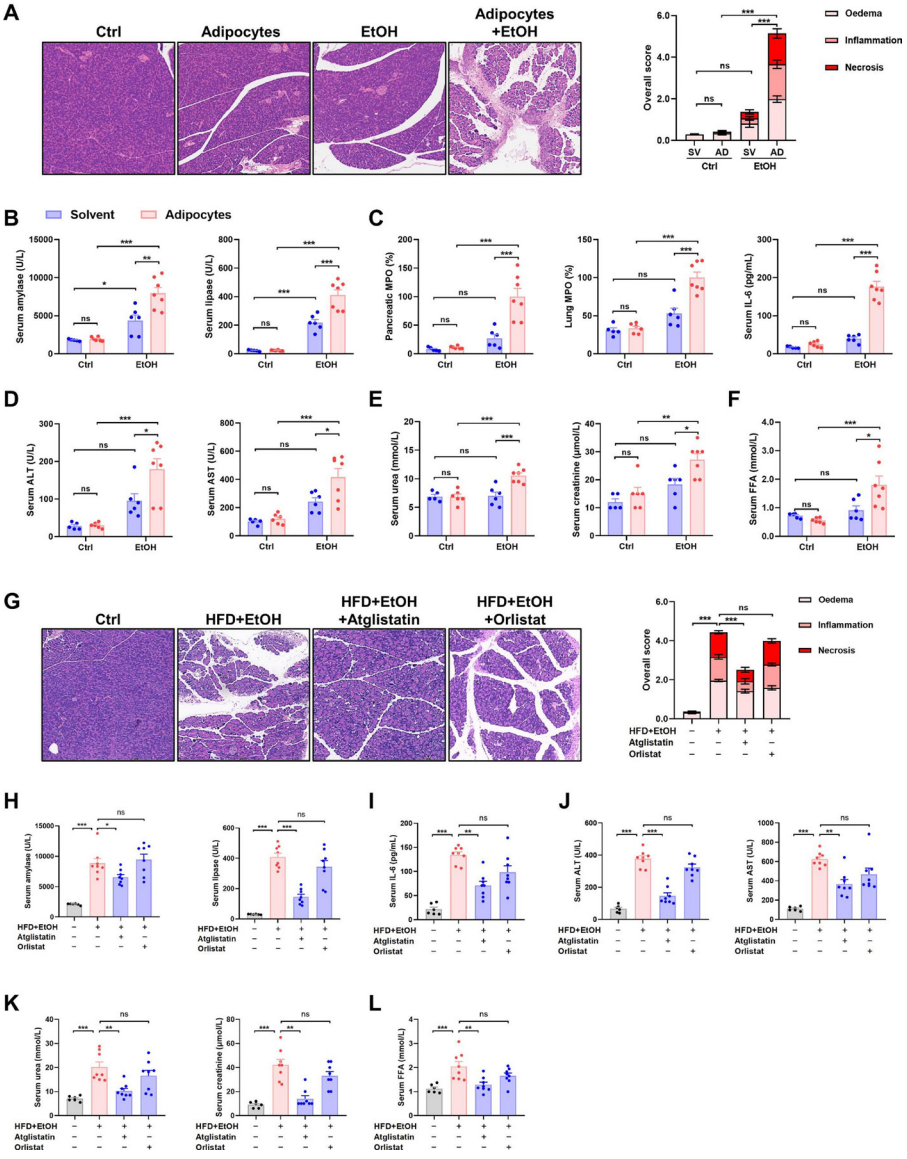

Figure 2 Obesity and acute alcohol intake cause AP through visceral adipocyte lipolysis mediated by ATGL. Lean mice received intraperitoneal injection adipocytes 1 hour prior to two injection of 2 g/kg EtOH at a 1-hour interval: (A) Representative images of pancreatic histopathology (magnification ×200) and histopathological scores. (B) Serum amylase and lipase, and (C) pancreatic MPO, lung MPO and serum IL-6 levels. (D) Serum ALT and AST levels, (E) serum urea and creatinine and urea levels, and (F) serum FFA levels. In another experiment, the effect of an ATGL inhibitor, atglistatin, and a pancreatic lipase inhibitor, orlistat, in the OA-AP mice was tested. (G) Representative images of pancreatic histopathology (magnification ×200) and histopathological scores. (H) Serum amylase and lipase activity, (I) serum IL-6 levels, (J) serum ALT and AST levels, (K) serum urea and creatinine levels, and (L) serum FFA levels. In all experiments, mice were sacrificed at 12 hours after the first injection of EtOH and were assessed for disease severity and/or lipolytic parameters. *P<0.05, **P<0.01, ***P<0.001. ALT, alanine aminotransferase; AP, acute pancreatitis; AST; aspartate aminotransferase; ATGL, adipose triglyceride lipase; Ctrl, control; EtOH, ethanol; FFA, free fatty acid; HFD, high-fat diet; IL, interleukin; MPO, myeloperoxidase; ns, not significant; OA-AP, obese alcoholic acute pancreatitis.

into the abdominal cavity of lean mice, which recapitulated all features of OA-AP (figure 2A–F). Inhibition of lipolysis using specific ATGL inhibitor atglistatin significantly reduced pancreas histopathology scores, serum pancreatic enzymes, serum MOI indices and serum FFA levels, while PNLIP inhibitor orlistat had a minimal effect (figure 2G–L). These findings indicate ethanol-induced VAT lipolysis via ATGL activation is central to the pathogenesis of OA-AP. This mechanism parallels the protective systemic effects of ATGL inhibition in burn injury,[4] which is distinct from systemic lipotoxicity consequent on leakage of PNLIP from the injured pancreas.[5] Interestingly, we found both atglistatin and orlistat were protective against caerulein-induced AP in obese mice (online supplemental figure 2C–G), mirroring patients with COVID-19 where PNLIP-mediated and ATGL-mediated lipotoxicity may both take place after disease onset.[6]

In summary, our study reports that obesity and alcohol act synergistically in the pathogenesis of onset and development of MOI in OA-AP through induction of ATGL-mediated VAT lipolysis. High amounts of ethanol alone may be insufficient to induce clinical AP and is not sufficient to induce murine experimental AP.[7] A genetic predisposition or a susceptible precondition may be required,[1 2 8 9] if not the presence of a cofactor, as in murine fatty acid ethyl ester AP induced by ethanol with palmitoleic or palmitic acid.[7 10] Obesity is an alternative, which our model suggests may be targeted by ATGL inhibition.

Xinmin Yang,[1,2] Linbo Yao,[1,3] Lei Dai,[4] Mei Yuan,[1] Wenhua He ,[5] Tingting Liu,[1] Xianghui Fu,[6] Jing Xue ,[7] Robert Sutton,[8] Qing Xia,[1] Wei Huang [1,2,3]

[1]Department of Integrated Traditional Chinese and Western Medicine, Sichuan Provincial Pancreatitis Centre and West China-Liverpool Biomedical Research Centre, West China Hospital, Sichuan University, Chengdu, People's Republic of China
[2]Institutes for Systems Genetics & Immunology and Inflammation, Frontiers Science Centre for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu, People's Republic of China
[3]West China Biobanks, Department of Clinical Research Management, West China Hospital, Sichuan University, Chengdu, People's Republic of China
[4]State Key Laboratory of Biotherapy and Cancer Centre, West China Hospital, Sichuan University and Collaborative Innovation Centre for Biotherapy, Chengdu, People's Republic of China
[5]Department of Gastroenterology, First Affiliated Hospital of Nanchang University, Nanchang, People's Republic of China
[6]Division of Endocrinology and Metabolism, State Key Laboratory of Biotherapy and Cancer Centre, West China Hospital, Sichuan University and Collaborative Innovation Centre for Biotherapy, Chengdu, People's Republic of China
[7]State Key Laboratory of Oncogenes and Related Genes, Stem Cell Research Centre, Ren Ji Hospital, School of Medicine, Shanghai Cancer Institute, Shanghai Jiao Tong University, Shanghai, People's Republic of China
[8]Liverpool Pancreatitis Research Group, Liverpool University Hospitals NHS Foundation Trust and Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK

Correspondence to Dr Wei Huang, Department of Integrated Traditional Chinese and Western Medicine, Sichuan Provincial Pancreatitis Centre and West China-Liverpool Biomedical Research Centre, West China Hospital, Sichuan University, 5B Floor 2nd Building, Tianfu Life Science Park of Hi-Tech Industrial Development Zone, No. 88 Keyuan South Road,

Chengdu, 610041, People's Republic of China; dr_wei_huang@scu.edu.cn and Dr Qing Xia, Department of Integrated Traditional Chinese and Western Medicine, Sichuan Provincial Pancreatitis Centre and West China-Liverpool Biomedical Research Centre, West China Hospital, Sichuan University, 10th Floor 3rd Inpatient Building, No. 37 Wannan Guoxue Alley, Chengdu, People's Republic of China; xiaqing@medmail.com.cn

Contributors XY and LY are joint first authors. QX and WHu are joint senior authors. WHu, QX and XY designed the study. QX and WHu obtained funding and supervised students. XY and WHu drafted the manuscript. XY, LY, MY, LD and TL planned and conducted the experiments and analysed the data. WHe, XF and RS had important intellectual input. JX and RS critically revised the manuscript. All authors read and approved the final version of the article.

Funding National Nature Science Foundation of China (number 81973632, to WHu; number 81860122, to WHe; number 81774120, to QX; number 81800575 to TL; numbers 92157205 and 81 970561 to XF); Programme of Science and Technology Department of Sichuan Province (number 22ZDYF1206, to XY); UK NIHR Senior Investigator Award (to RS).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2022-326958).

XY and LY are joint first authors.

QX and WH are joint senior authors.

To cite Yang X, Yao L, Dai L, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2022-326958

Received 12 January 2022
Accepted 12 March 2022

Gut 2022;**0**:1–3. doi:10.1136/gutjnl-2022-326958

ORCID iDs
Wenhua He http://orcid.org/0000-0001-5499-1346
Jing Xue http://orcid.org/0000-0002-6497-1792
Wei Huang http://orcid.org/0000-0001-8659-322X

REFERENCES

1 Wang Y-C, Mao X-T, Yu D, *et al*. Alcohol amplifies the association between common variants at *PRSS1-PRSS2* locus and chronic pancreatitis in a dose-dependent manner. *Gut* 2022. doi:10.1136/gutjnl-2021-326670. [Epub ahead of print: 07 Jan 2022].
2 Hegyi E, Tóth AZ, Vincze Áron, *et al*. Alcohol-dependent effect of *PRSS1-PRSS2* haplotype in chronic pancreatitis. *Gut* 2020;69:1713–5.
3 Lai S-W, Muo C-H, Liao K-F, *et al*. Risk of acute pancreatitis in type 2 diabetes and risk reduction on anti-diabetic drugs: a population-based cohort study in Taiwan. *Am J Gastroenterol* 2011;106:1697–704.
4 Kaur S, Auger C, Barayan D, *et al*. Adipose-specific ATGL ablation reduces burn injury-induced metabolic derangements in mice. *Clin Transl Med* 2021;11:e417.
5 de Oliveira C, Khatua B, Noel P, *et al*. Pancreatic triglyceride lipase mediates lipotoxic systemic inflammation. *J Clin Invest* 2020;130:1931–47.
6 Hegyi P, Szakács Z, Sahin-Tóth M. Lipotoxicity and cytokine storm in severe acute pancreatitis and COVID-19. *Gastroenterology* 2020;159:824–7.
7 Huang W, Booth DM, Cane MC, *et al*. Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca2+-dependent mitochondrial dysfunction and acute pancreatitis. *Gut* 2014;63:1313–24.
8 Whitcomb DC, LaRusch J, Krasinskas AM, *et al*. Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 2012;44:1349–54.
9 Farooq A, Richman CM, Swain SM, *et al*. The role of phosphate in alcohol-induced experimental pancreatitis. *Gastroenterology* 2021;161:982–95.
10 Maléth J, Balázs A, Pallagi P, *et al*. Alcohol disrupts levels and function of the cystic fibrosis transmembrane conductance regulator to promote development of pancreatitis. *Gastroenterology* 2015;148:427–39.