

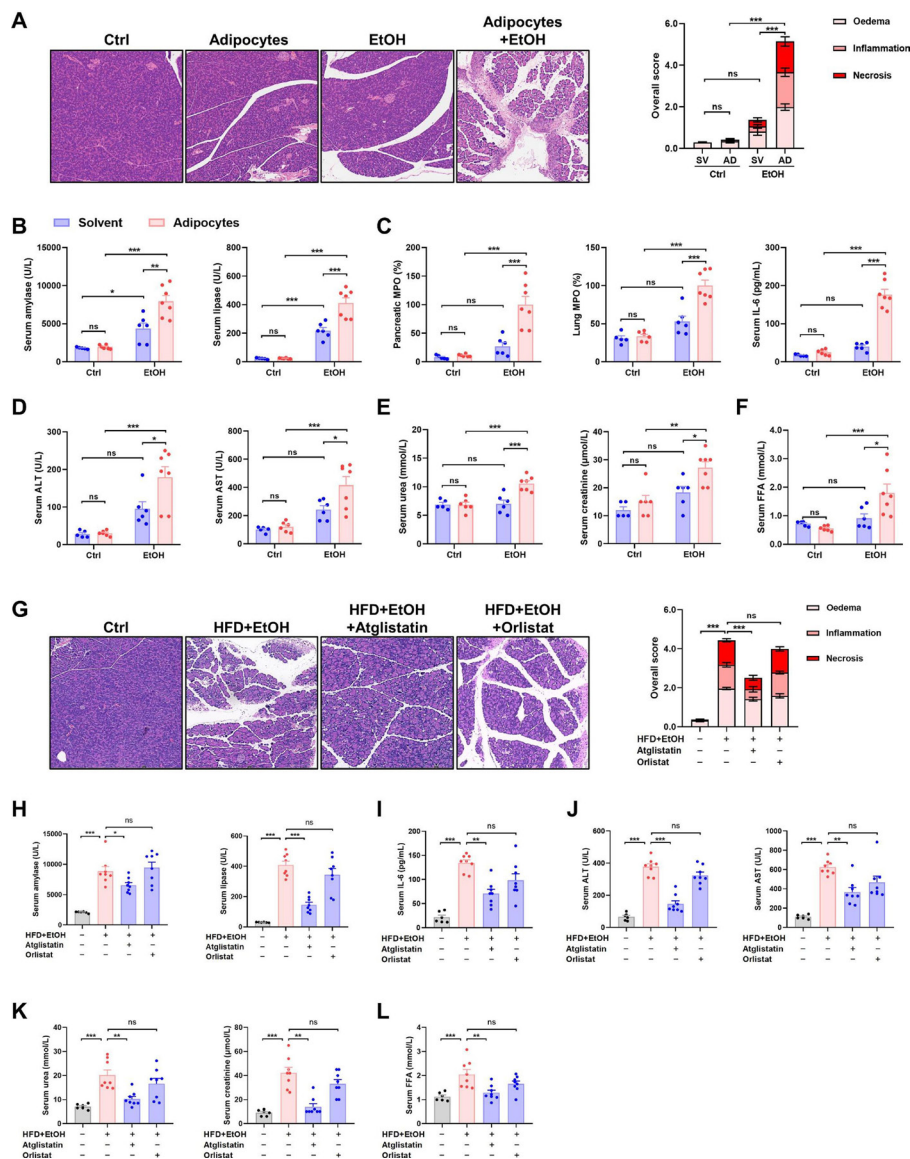
**Figure 1** Alcohol predisposes obese mice to AP with systemic organ injury. (A) Experimental protocol of establishing OA-AP. C57BL/6J mice were fed a CD (lean) or HFD (obese) for 12 weeks, then were injected intraperitoneally with 2 g/kg EtOH two times at 1 hour apart: (B) Body weight. (C) Representative images of pancreatic histopathology and histopathological scores (oedema, inflammation and necrosis; magnification  $\times 200$ ), (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.

## 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*<sup>1</sup> and Hegyi *et al*,<sup>2</sup> 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)).<sup>3</sup> 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,



**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  $\times 200$ ) 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, atglitastatin, and a pancreatic lipase inhibitor, orlistat, in the OA-AP mice was tested. (G) Representative images of pancreatic histopathology (magnification  $\times 200$ ) 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.

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).

To confirm our hypothesis, we injected ethanol and adipocytes simultaneously into the abdominal cavity of lean mice, which recapitulated all features of OA-AP (figure 2A–F). Inhibition of lipolysis using specific ATGL inhibitor atglitastatin 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,<sup>4</sup> which is distinct from systemic lipotoxicity consequent on leakage of PNLIP from the injured pancreas.<sup>5</sup> Interestingly, we found both atglitastatin 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.<sup>6</sup>

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 AI.<sup>7</sup> A genetic predisposition or a susceptible precondition may be required,<sup>1,2,8,9</sup> if not the presence of a cofactor, as in murine fatty acid ethyl ester AP induced by ethanol with palmitoleic or palmitic acid.<sup>7,10</sup> Obesity is an alternative, which our model suggests may be targeted by ATGL inhibition.

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WHu, 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.

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