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Lipolysis of Visceral Adipocyte Triglyceride by Pancreatic Lipases Converts Mild Acute Pancreatitis to Severe Pancreatitis Independent of Necrosis and Inflammation

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Visceral fat necrosis has been associated with severe acute pancreatitis (SAP) for over 100 years; however, its pathogenesis and role in SAP outcomes are poorly understood. Based on recent work suggesting that pancreatic fat lipolysis plays an important role in SAP, we evaluated the role of pancreatic lipases in SAP-associated visceral fat necrosis, the inflammatory response, local injury, and outcomes of acute pancreatitis (AP). For this, cerulein pancreatitis was induced in lean and obese mice, alone or with the lipase inhibitor orlistat and parameters of AP induction (serum amylase and lipase), fat necrosis, pancreatic necrosis, and multisystem organ failure, and inflammatory response were assessed. Pancreatic lipases were measured in fat necrosis and were overexpressed in 3T3-L1 cells. We noted obesity to convert mild cerulein AP to SAP with greater cytokines, unsaturated fatty acids (UFAs), and multisystem organ failure, and 100% mortality without affecting AP induction or pancreatic necrosis. Increased pancreatic lipase amounts and activity were noted in the extensive visceral fat necrosis of dying obese mice. Lipase inhibition reduced fat necrosis, UFAs, organ failure, and mortality but not the parameters of AP induction. Pancreatic lipase expression increased lipolysis in 3T3-L1 cells. We conclude that UFAs generated via lipolysis of visceral fat by pancreatic lipases convert mild AP to SAP independent of pancreatic necrosis and the inflammatory response. *(Am J Pathol 2015, 185: 808—819; http://dx.doi.org/10.1016/j.ajpath.2014.11.019)*

Visceral fat necrosis has been noted to occur with pancreatitis for over 100 years.[1,2] This fat is typically located in or around the pancreas[3,4] and is a major component of necrotizing pancreatitis and peripancreatic necrosis.[5,6] Despite being a part of the criteria for staging the severity of acute pancreatitis (AP) in humans[7–9] and being included as a separate entity in the recently revised Atlanta criteria,[6] the pathogenesis and role of fat necrosis, that is, whether it is a marker or mediator of severe AP (SAP), remains unclear.

Clinical correlates of the relevance of fat necrosis include the several epidemiological studies that show individuals with increased intra-abdominal fat[10–13] or obese patients being at an increased risk for SAP.[11,14–20] Peripancreatic fat necrosis may occur independent of pancreatic necrosis[5] and is associated with an increased risk for severe attacks.[21,22] Although there is basolateral leakage of pancreatic enzymes during pancreatitis,[23–26] and lipases have been noted in fat necrosis in human disease,[27,28] it is unclear whether these lipases cause fat necrosis or are a remnant of pancreatic damage.

Triglycerides compose 80% to 90% of the volume of adipocytes[29–31] and can be hydrolyzed by lipases released

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basolaterally during pancreatitis.[23–26] Although *in vitro* studies done more than 2 decades ago showed lipase inhibition to reduce pancreatic acinar injury on co-incubation with pancreatic homogenates and triglycerides,[32] *in vivo* lipase inhibition in SAP models showed no benefit[33] until recently, when this was studied in the context of increased visceral fat.[34,35]

Previous studies have shown that long-chain unsaturated fatty acids (UFAs) form the majority of the nonesterified fatty acids in necrotic collections[36,37] and are more toxic than are saturated fatty acids.[32,34,35,38] Since obese (*ob/ob*) mice and obese humans may have a similar visceral fat composition,[3,35] we chose to study whether a classically mild model of pancreatitis, that is, cerulein pancreatitis, would result in different outcomes in these mice versus lean mice and explore the role of fat necrosis in these outcomes. Our previous work has shown a benefit of pharmacological inhibition of pancreatic lipases in biliary, IL-12–induced, and IL-18–induced pancreatitis[34,35]; we therefore used this as a tool to influence outcomes. Since obesity is also known to be associated with an exaggerated baseline inflammatory state, we went on to study whether the inflammatory response was different between the groups that had different outcomes. Additionally, since SAP may occur along with severe pancreatic necrosis or with multisystem organ failure (MSOF) with insignificant necrosis,[39–41] we also compared pancreatic necrosis between groups with different outcomes.

Reasons for preferring a pharmacological approach[34,35] over a genetic one include: i) previous validation in mechanistically distinct models,[34,35] ii) the redundant roles of pancreatic lipases[42] (which are supported by this study), iii) the deletion of the two lipases of interest being embryonically lethal,[43] and iv) the inability to make mice with a genetic deletion of lipases obese on a high-fat diet (unpublished data). Our results, in concert with those from previous studies,[34,35] show that pancreatic lipase–dependent visceral fat lipolysis worsens outcomes of AP, unrelated to the initiator of the disease, and suggest alternate ways to interpret the parameters of severity in AP, along with suggesting a potential approach to improving SAP outcomes.

Materials and Methods

Animal Studies

Male *ob/ob* (B6.V-lepob/J) mice or C57bl6 (lean) mice (8 to 10 weeks) were purchased from Jackson Laboratories (Bar Harbor, ME) and were acclimatized for at least 2 days before use. All animals were housed with a 12-hour light/dark cycle at temperatures from 21°C to 25°C, fed standard laboratory chow, and allowed to drink *ad libitum*. AP was induced in lean (C57bl6) and *ob/ob* mice by hourly i.p. injections of cerulein (50 μg/kg) × 12 doses on 2 consecutive days (CR group). Obese mice were administered orlistat [50 mg/kg b.i.d. in the CRO2 group (sacrificed at the end of 2 days) and in the CRO5 group (sacrificed at the end of 5 days)] as described previously[35] or its vehicle [10% triolein in phosphate-buffered saline, 100 μL per 30 g body weight in the CR + vehicle (CRV) group] i.p. for 2 days. The first injection was administered 2 hours after the induction of pancreatitis. The mice were followed up over a course of 5 days or until they were moribund or died overnight. Lean mice were also electively sacrificed at the end of the first 24 hours (CR1) or 48 hours after the first injection (CR2). Similarly, mice given orlistat (CRO2 group) were also electively sacrificed at 48 hours for comparison to the CR and CRV groups since 50% to 100% of the mortality occurred in these groups at 48 hours. All of the experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh (Pittsburgh, PA) and at the Mayo Clinic (Scottsdale, AZ).

Cytokine Assays

Cytokine assays were done as previously described,[35,38] with the fluorescence-based capture sandwich immunoassay based on the Luminex FlowMetrix system (Life Technologies, Carlsbad, CA). The Milliplex mouse adipokine panel (7-plex) 1 kit (Millipore, Billerica, MA) was used for serum analysis. A Bio-Plex suspension array system, including a fluorescent reader and Bio-Plex Manager analytical software version 200 (Bio-Rad Laboratories, Hercules, CA), was used for analyzing samples at the University of Pittsburgh Cancer Institute.

Evaluation of Pancreatic Necrosis and Special Stains

Whole pancreas section slides stained by hematoxylin and eosin were examined by a trained morphologist (K.P.) blinded to the sample as described previously.[35,44] Briefly, all contiguous areas were imaged using a 4× objective and photographed. Total fat area, necrotic fat area, perifat acinar necrosis, and total acinar necrotic area were measured in pixels as a percentage of total acinar area calculated for each pancreas. Von Kossa staining was done as described previously.[35,38] Staining with terminal deoxynucleotidyl transferase dUTP nick end labeling was done on paraffin sections of the lungs and kidneys as described previously.[35] Serial sections of gonadal fat pads were stained with hematoxylin and eosin and von Kossa staining. Von Kossa staining was done to detect calcium salts following the manufacturer's instructions (von Kossa stain kit, American MasterTech, Lodi, CA).

Nonesterified Fatty Acid Analysis

Long-chain fatty acid was analyzed using gas chromatography as described previously.[35,38] UFA amounts were calculated by adding individual C16:1, C18:1, C18:2, and C20:4 fatty acids. Saturated fatty acid amounts were calculated by adding individual C12:0, C14:0, C16:0, and C18:0 fatty acids.