BASIC AND TRANSLATIONAL—PANCREAS

Fibrosis Reduces Severity of Acute-on-Chronic Pancreatitis in Humans

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See Covering the Cover synopsis on page 265.

BACKGROUND & AIMS: Acute pancreatitis (AP) and chronic pancreatitis (CP) share etiologies, but AP can be more severe and is associated with a higher rate of mortality. We investigated features of CP that protect against severe disease. The amount of intrapancreatic fat (IPF) is increased in obese patients and fibrosis is increased in patients with CP, so we studied whether fibrosis or fat regulate severity of AP attacks in patients with CP. **METHODS:** We reviewed records from the University of Pittsburgh Medical Center/Presbyterian Hospital Autopsy Database (1998–2008) for patients with a diagnosis of AP (n = 23), CP (n = 35), or both (AP-on-CP; n = 15). Pancreatic histology samples from these patients and 50 randomly selected controls (no pancreatic disease) were analyzed, and IPF data were correlated with computed tomography data. An adipocyte and acinar cell Transwell coculture system, with or without collagen type I, was used to study the effects of fibrosis on acinar-adipocyte interactions. We studied the effects of nonesterified fatty acids (NEFAs) and adipokines on acinar cells in culture. **RESULTS:** Levels of IPF were significantly higher in nonobese patients with CP than in nonobese controls. In patients with CP or AP-on-CP, areas of IPF were surrounded by significantly more fibrosis than in controls or patients with AP. Fat necrosis–associated peri-fat acinar necrosis (PFAN, indicated by NEFA spillage) contributed to most of the necrosis observed in samples from patients with AP; however, findings of peri-fat acinar necrosis and total necrosis were significantly lower in samples from patients with CP or AP-on-CP. Fibrosis appeared to wall off the fat necrosis and limit peri-fat acinar necrosis, reducing acinar necrosis. In vitro, collagen I limited the lipolytic flux between acinar cells and adipocytes and prevented increases in adipokines in the acinar compartment. This was associated with reduced acinar cell necrosis. However, NEFAs, but not adipokines, caused acinar cell necrosis. **CONCLUSIONS: Based on analysis of pancreatic samples from patients with CP, AP, or AP-on-CP and in vitro studies, fibrosis reduces the severity of acute exacerbations of CP by reducing lipolytic flux between adipocytes and acinar cells.**

Keywords: Pancreas; Inflammation; Necrosis; Mechanism.

Several studies have shown that the risk of severe acute pancreatitis (SAP) and mortality increases in obese patients[1–5] (body mass index [BMI] $\geq$30 kg/m^2). Additionally, various studies[4,6,7] have shown that visceral fat depots (ie, intrapancreatic fat [IPF]) increase with body weight or BMI in children[8,9] and adults and that fat abutting acinar cells is involved in parenchymal damage in patients with acute pancreatitis (AP).[4,10,11] Fatty pancreas replacement may also occur in patients with chronic pancreatitis (CP)[12–15]; however, SAP is a rare outcome or cause of mortality in patients with CP.[16–18] Thus, the mechanisms of IPF differentially affecting the outcomes of patients with AP in the context of obesity or CP (ie, AP-on-CP) require further investigation.

AP and CP share some common etiologies such as alcohol consumption,[19] genetic mutations (eg, CFTR, PRSS1),[20–22] obstructive lesions (eg, a mass),[23–25] and metabolic causes (eg, hyperlipidemia).[26,27] Recent studies suggest AP, recurrent AP, and CP are part of a continuum.[18,19,28] However, although the first couple of attacks of AP may cause extensive pancreatic necrosis within weeks,[4,18] patients with CP have a prolonged disease course with recurrent attacks of AP and atrophy, extensive fibrosis, and fatty replacement developing over months to years. Evidence for large pancreatic areas necrotizing acutely in CP is scarce, despite the insult (eg, alcohol consumption, tumor, metabolic cause, or genetic cause) persisting over this time. Similarly, although AP may result in significant mortality over days,[29–31] mortality over the several years of the disease course of CP is rarely attributed to AP.[16–18]

The reasons for these different outcomes remain unclear. It has been variously argued that this may be due to a lower acinar cell mass in patients with CP, but studies in children

Abbreviations used in this paper: AP, acute pancreatitis; ATP, adenosine triphosphate; BMI, body mass index; CP, chronic pancreatitis; CT, computed tomography; FN, fat necrosis; IPF, intrapancreatic fat; NEFA, nonesterified fatty acid; PFAN, peri-fat acinar necrosis; PI, propidium iodide; SAP, severe acute pancreatitis; UFA, unsaturated fatty acid.

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and adolescents, who have smaller pancreata than adults older than 30 years of age[6] and lower serum amylase levels than adults[32] (which is relevant because lower serum enzyme levels in patients with CP are often attributed to lower acinar mass), experience no difference in severity compared with adults.[33–37] Rather, children with SAP have higher BMI[36] and children with a higher weight at admission are more prone to SAP.[35] Additionally, a large study of children treated for acute lymphoblastic leukemia mentioned that children "who had grade 3 or 4 pancreas/glucose toxicity during induction also had higher BMIs throughout treatment."[38] Pancreatic toxicities are based on criteria for pancreatitis. Grade 3 or 4 is severe compared with grade 1 or 2, according the Children's Cancer Group common toxicity criteria (version 2.0) used in the study (http://www.eortc.be/services/doc/ctc/ctcv20_4-30-992.pdf).

Therefore, other phenomena such as fibrosis, which increases in patients with CP,[39] may be considered to reduce the severity of AP-on-CP. We thus studied different pancreatic disease states (including controls, AP, CP, and AP-on-CP) for differences in the amount of IPF and its relationship to BMI, fibrosis, and the pancreatic parenchyma. Because the pancreas is not usually sampled in live humans without pancreatic disease and samples removed during an invasive procedure are small (eg, using fine-needle aspiration or a Tru-Cut biopsy) or altered by disease (eg, cancer removed during a Whipple procedure), we chose to examine autopsy tissue in control patients (ie, no evidence of pancreatic disease) and those with a clinical or autopsy diagnosis of AP or CP at the time of death. We have previously shown that postmortem changes, such as limited autolysis, do not significantly affect the interpretation of findings using this approach.[4]

We compared IPF and acinar injury among the various groups quantitatively and morphologically, verifying critical conclusions using external controls (eg, computed tomography [CT] scan), and mechanistically in vitro using adipocytes and acinar cells. Our findings suggest that differences in the patterns of IPF and fibrosis in patients with CP compared with obese patients may explain the differences in outcomes in these 2 groups.

Patients and Methods

The medical records of all patients in the University of Pittsburgh Medical Center/Presbyterian Hospital Autopsy Database (1998–2008) with a diagnosis of AP (n = 23), CP (n = 35), or AP-on-CP (total, n = 15; clinical AP-on-CP, n = 10) were reviewed. Pancreatic histology slides of these patients and 50 randomly selected controls were scored for IPF, fat necrosis (FN), peri-fat acinar necrosis (PFAN), and fibrotic area (as percent pancreatic area). The percentage of fat with >50% of its immediate perimeter surrounded by fibrosis was measured. For further details, please refer to Supplementary Patients and Methods.

Results

There was no significant difference in age ($F[3,119] = 0.790$, $P = .502$) or BMI ($F[3,119] = 0.845$, $P = 0.472$) (Supplementary Table 1) between the groups.

BMI Correlates Positively With IPF in Controls, Patients With AP, and Patients With AP-on-CP but not Patients With CP

Because IPF may increase with BMI[4,6] or CP,[40,41] we studied the correlation of BMI to histologically measured %IPF. Significantly positive correlations were found in controls ($r[48] = 0.592$, $P < .001$), patients with AP ($r[21] = 0.506$, $P = .014$),[4,6] and patients with AP-on-CP ($r[13] = 0.729$, $P = .002$) (Figure 1*A*–*C*) but not in patients with CP ($r[33] = 0.168$, $P = .334$) (Figure 1*A*). A sensitivity analysis after excluding a possible influential observation (a patient with a BMI >60 kg/m^2) in the CP group showed a correlation coefficient of 0.170 ($P = .337$). Two-way analysis of variance showed significant main effects for group ($F[3,115] = 3.462$, $P = .019$) and BMI ($F[1,115] = 29.548$, $P < .001$) and a significant interaction (group × BMI, $F[3,115] = 3.724$, $P = .013$) for %IPF. Subsequent post hoc tests showed that the %IPF for controls and patients with CP, AP, or AP-on-CP did not differ in the obese group; however, for those with a BMI <30 kg/m^2, patients with CP had a significantly greater IPF when compared with controls. Post hoc tests also showed that obese patients in the control, AP, and AP-on-CP groups, but not the CP group, had a significantly greater IPF than those with a BMI <30 kg/m^2 (Figure 1*C*).

Of the 35 patients with CP, 14 had CT scans with no evidence of AP in the 45 days preceding death. IPF measured on these CT scans by a blinded radiologist using either of the previously validated attenuation[4,6] or thresholding methods[4,6] showed a strong correlation with histology (Figure 1*D* and *E*). The κ value for histologic quantification of IPF by 2 independent blinded observers was 0.951, signifying strong interobserver agreement.

Patients With CP Have a Lower Amount of FN Compared With Patients With AP

Patients with AP had significantly more FN than those with CP ($P < .001$) or controls ($P < .001$) (Figure 2*A*). FN was visible as adipocytes with a bluish, amorphous look on H&E staining (Figures 3*C*, 4*C*, and 4*D*) and stained brown on von Kossa staining, suggesting saponification (Figure 4*C2* and *D2*). This FN seemed to be pathogenically relevant because nonesterified fatty acids (NEFAs) induce cell death,[4] and the FN in patients with AP was associated with surrounding parenchymal necrosis (*dotted area* in Figure 4*C* and *C2*).

Patients With AP Have More PFAN and Necrosis Than Patients With CP or AP-on-CP

We quantified parenchymal necrosis adjacent to the FN mentioned as adjacent to the FN (ie, PFAN). On H&E staining, this was notable for areas with loss of cell outlines bordering FN (*dotted area* in Figure 4*C*). On von Kossa staining, PFAN showed brown staining (Figure 4*C2*) that was most intense adjacent to the FN and decreased with increasing distance, suggesting leakage of NEFAs

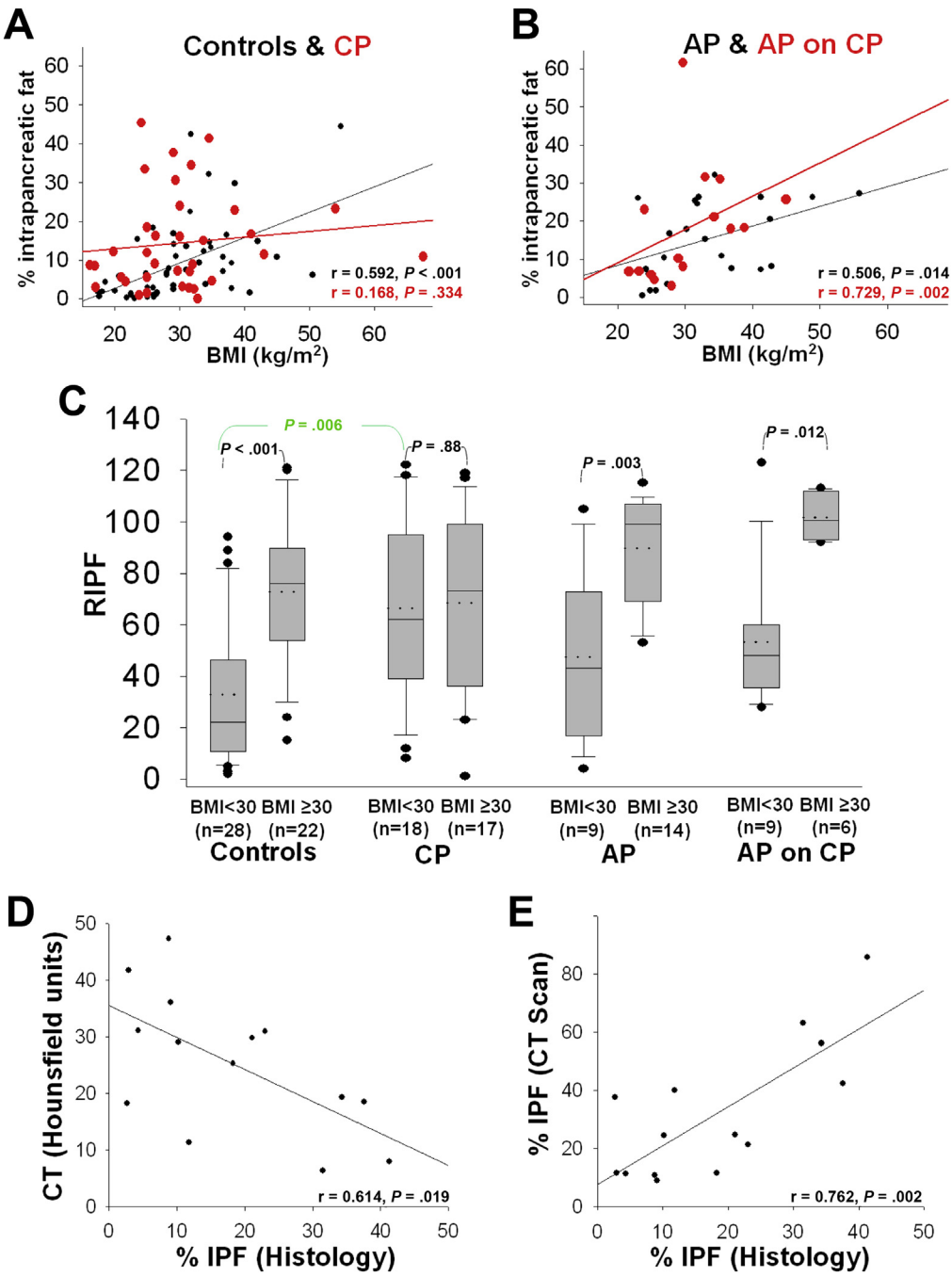

Figure 1. %IPF increases with BMI in controls, patients with AP, and patients with AP-on-CP but not in patients with CP. Scatter plot showing the association between IPF and BMI for (*A*) controls (*black dots*) and patients with CP (*red dots*) and (*B*) patients with AP (*black dots*) and patients with AP-on-CP (*red dots*). *Lines* represent the best-fit line from a linear regression analysis. Correlation analyses were calculated using the Spearman ρ correlation coefficient. Results show moderate to high correlations in all groups except in patients with CP. (*C*) The estimated marginal means from the 2-way analysis of variance that included main effects for BMI and group and their interaction (BMI × group). Rank data for %IPF (RIPF) was used for this analysis. Post hoc comparisons were performed using the Sidak adjustment method. Adjusted *P* values are shown. The 2-way analysis of variance revealed significant main effects for group and BMI and a significant interaction. Subsequent post hoc tests showed that, for the obese group, there were no statistically significant differences in IPF between the disease groups; however, for the normal group, the CP group had significantly greater IPF when compared with controls (*P* value shown in *green*). Post hoc comparisons between normal (BMI <30 kg/m^2) and obese (BMI ≥30 kg/m^2) groups are also shown (*P* values in *black*). (*D* and *E*) Linear regression analysis between %IPF measured on histology (%IPF [histology]) and Hounsfield units on CT scan or by the CT thresholding method, respectively. Spearman correlation coefficient was computed.

formed in the FN. PFAN is distinct from isolated acinar necrosis (*square*, Supplementary Figure 1), which is von Kossa negative and occurs in areas separated from FN (*circle*, Supplementary Figure 1). There was a statistically significant difference in PFAN between the groups ($F[3,123] = 47.68$, $P < .001$). Patients with AP had a much greater amount of PFAN than those with CP ($P < .001$), those with AP-on-CP ($P = .025$), or controls ($P < .001$)

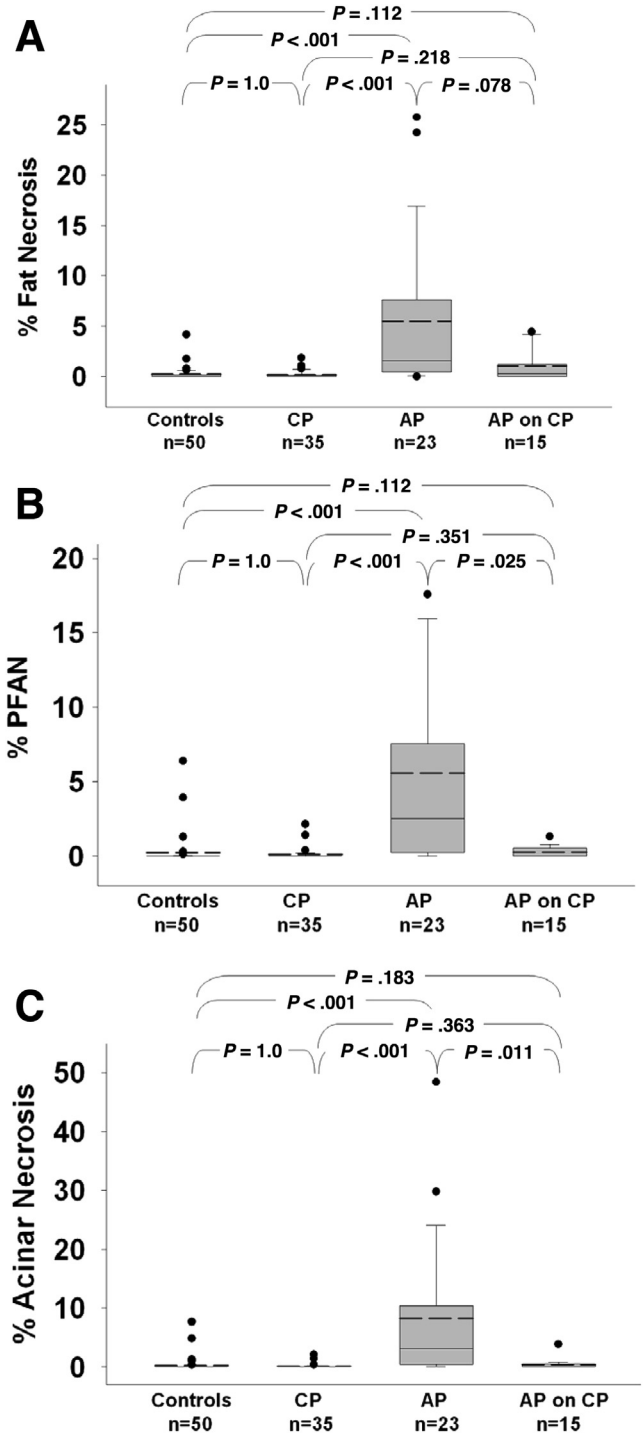

Figure 2. Histologic quantification of (*A*) FN, (*B*) PFAN, and (*C*) acinar necrosis as percentage of total area. Box plots of the mean (*dashed line*), median (*solid line*), 25th and 75th percentiles (*2 boxes*), 10th and 90th percentiles (*whiskers*), and outliers (*dots*) comparing controls, patients with CP, patients with AP, and patients with AP-on-CP for each of the parameters show these to be significantly reduced in patients with CP or AP-on-CP compared with patients with AP. Significant differences were found between the groups (Kruskal–Wallis test). Post hoc comparisons were performed using the Mann–Whitney test, and adjustment for multiple comparisons was performed using the Dunn–Sidak adjustment method. Adjusted *P* values are shown.

(Figure 2*B*). Similarly, there was a statistically significant difference in total necrosis between the groups ($F[3,123] = 50.02$, $P < .001$); patients with AP had a significantly greater amount of total necrosis when compared with the other groups (Figure 2*C*). Therefore, PFAN contributes significantly to overall parenchymal damage in AP.

IPF in a Background of CP Is Surrounded by Significantly More Fibrosis and Is Associated With Less FN and PFAN

Because %IPF in patients with CP or AP-on-CP was not significantly less when compared with AP (Supplementary Figure 2), we measured the amount and pattern of fibrosis to explain the lower PFAN and total necrosis noted in patients with CP or AP-on-CP. Patients with CP or AP-on-CP had significantly more fibrosis (Figure 3*A*) than controls or patients with AP. This fibrosis morphologically walled off large areas of IPF from the parenchyma in patients with CP or AP-on-CP, seen as bluish strands surrounding the fat on trichrome staining (*arrows*, Figure 3*C*). Quantification of %IPF surrounded by fibrosis showed this to be significantly greater in patients with CP or AP-on-CP (Figure 3*B*) compared with controls or patients with AP, in whom the fat abutted the parenchyma without intervening fibrosis (*arrowheads*, Figure 3*C*). H&E and von Kossa staining showed the areas of FN in patients with CP or AP-on-CP to be associated with minimal or no PFAN, with the FN and von Kossa–positive areas confined by surrounding pinkish, hypocellular bands (Figure 4*B*, *B2*, *D*, and *D2*). These bands were verified to be fibrosis on trichrome staining (Figure 4*B1* and *D1*), in the absence of which (*arrowhead*, Figure 4*C1*) patients with AP had more FN (Figure 4*C2*) and PFAN (Figure 4*C*). Thus, fibrosis in patients with CP or AP-on-CP prevents spillage of NEFAs into the parenchyma with associated reduction in parenchymal necrosis.

Collagen Type I Reduces Acinar Necrosis by Reducing Lipolytic Flux Between Acinar Cells and Adipocytes

To study the role of fibrosis in preventing acinar necrosis in CP, we coated Transwells (Becton Dickinson, Franklin Lakes, NJ) with collagen I, which is highly expressed in human CP,[42] and cocultured adipocytes with pancreatic acini. This system replicates the pathophysiology of AP by allowing macromolecular diffusion from the 2 cell types while preventing contamination of one cell type by the other, as described previously[4] (Supplementary Figure 3). The transfer of acinar-derived (ie, amylase and lipase) and adipocyte-derived (ie, resistin and NEFAs) macromolecules to the non-native (ie, opposite) compartment was studied in the presence and absence of collagen, as was acinar cell viability.

Levels of amylase and lipase increased in the lower (adipocyte) compartment in the absence of adipocytes (Figure 5*C* and *D*), analogous to the basal release[4] or spillage noted in pancreatitis.[43,44] However, levels of NEFAs and resistin, which are virtually absent in the upper (acinar)

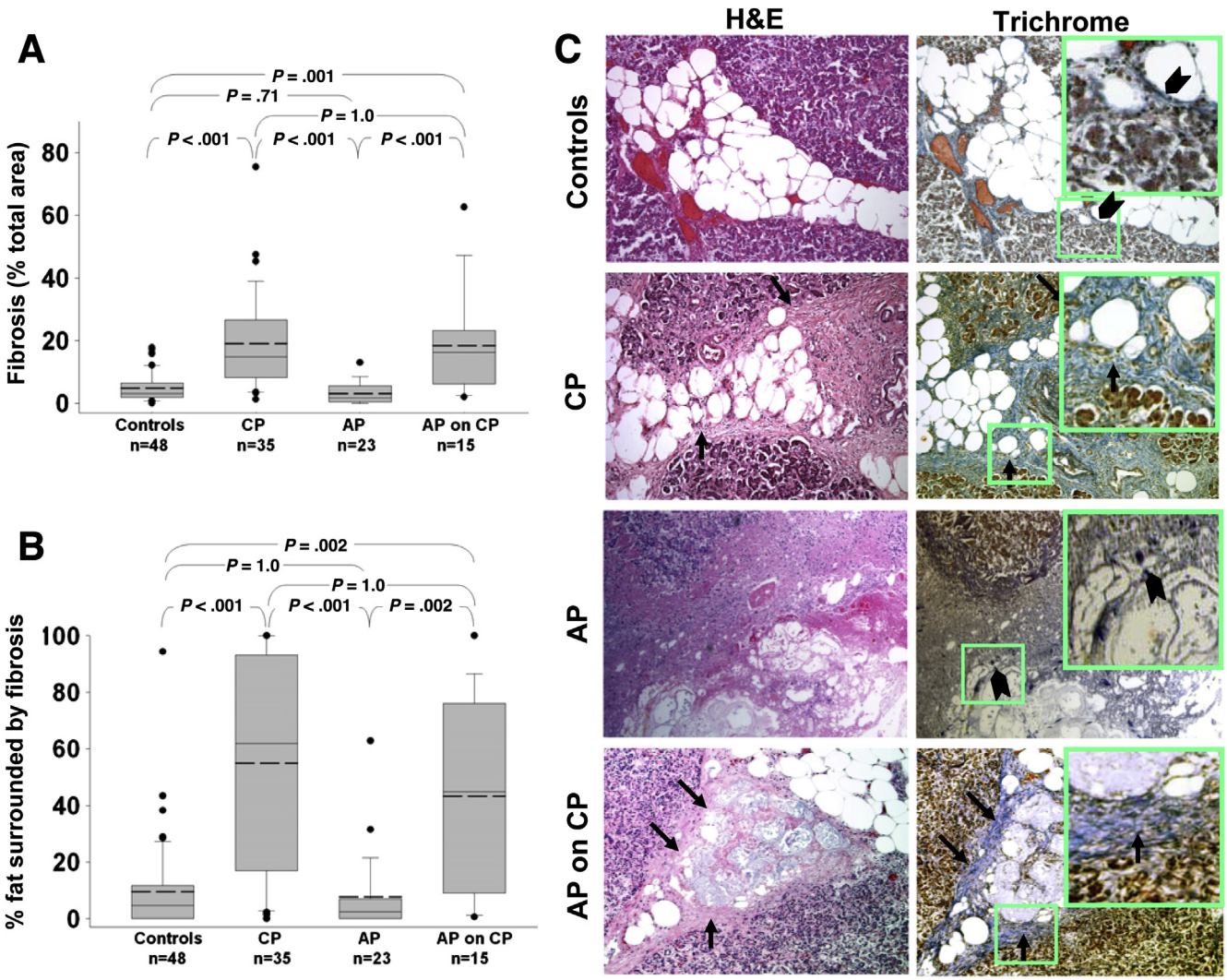

Figure 3. (*A*) Fibrotic area and (*B*) percentage of fat surrounded by fibrosis are increased in a background of CP. Box plots comparing controls, patients with CP, patients with AP, and patients with AP-on-CP for (*A*) fibrosis (as percent total area) and (*B*) percent fat surrounded by fibrosis. Significant differences were found between the groups (Kruskal–Wallis test). Post hoc comparisons were performed using the Mann–Whitney test, and adjustment for multiple comparisons was performed using the Dunn–Sidak adjustment method. Adjusted *P* values are shown. (*C*) Serial pancreatic histology sections from controls, patients with CP, patients with AP, and patients with AP-on-CP stained with H&E and Masson's trichrome (trichrome for fibrosis, *blue strands*) show fat confined by the fibrosis (*arrows*) in patients with CP or AP-on-CP. However, fat in controls and FN in patients with AP has no surrounding fibrosis (*arrowheads*).

compartment without adipocytes, significantly increased here in the presence of acini and adipocytes (T-A+A, Figure 5*A* and *B*), signifying crosstalk between the 2 compartments. Collagen I significantly prevented this coculture-dependent increase of macromolecules in the non-native compartment (C-A+A, Figure 5), supporting its role as a diffusion barrier as shown by others.[45] The increase in levels of NEFAs and resistin in the acinar compartment paralleled acinar necrosis, as noted by a large increase in trypan blue and propidium iodide (PI) uptake and a decrease in adenosine triphosphate (ATP) levels (Figure 6). This was prevented by collagen I (trypan blue: 98.9% ± 0.71% vs 17.1% ± 5.08%, $P < .001$; PI uptake: 59.3% ± 8.3% vs 16.2% ± 5.06%, $P = .004$; ATP levels: 13.9% ± 10.7% vs 96.4% ± 4.1%, $P = .0003$; ATP levels of controls: 29.2 ± 1.8 pmol/μg protein) (Figure 6). These findings show that collagen I in fibrosis reduces acinar injury in patients with AP-on-CP by reducing the flux of macromolecules between acini and adipocytes.

To study the relative contribution of NEFAs and resistin, which are increased in the serum of patients with SAP or CP,[4,46–49] we compared the toxicity of resistin and linoleic acid at concentrations relevant to those noted in the coculture system (linoleic acid, 670 ± 67 μmol/L) or in the debridement fluid of patients with SAP (linoleic acid, 1241 ± 510 μmol/L). Linoleic acid at less than half of these concentrations (300 μmol/L) resulted in a large increase in leakage of lactate dehydrogenase (83.8% ± 1.5%), cytosolic calcium (Supplementary Figure 4*A*–*C*), and PI uptake accompanied by a decrease in ATP levels (Supplementary Figure 4*D*–*F*). However, resistin at 1 μg/mL, which is >2-fold the concentration noted in the

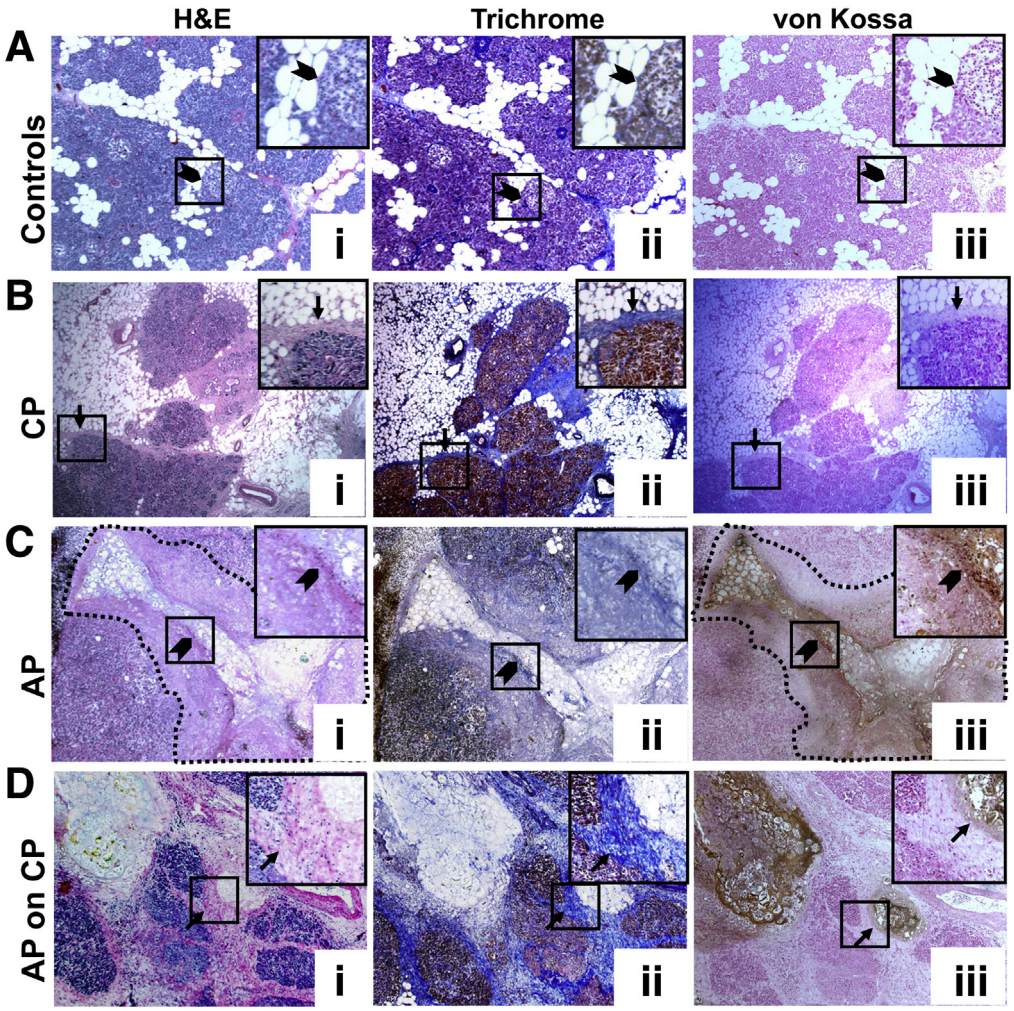

Figure 4. Morphologic analysis of pancreatic injury using serial histologic sections shows fibrosis walling off FN and restricting von Kossa staining to FN. Serial histologic sections from the pancreata of (*A–A2*) controls, (*B–B2*) patients with CP, (*C–C2*) patients with AP, and (*D–D2*) patients with AP-on-CP stained with (*A*, *B*, *C*, *D*) H&E, (*A1*, *B1*, *C1*, *D1*) Masson's trichrome (trichrome for fibrosis, *blue strands*), and (*A2*, *B2*, *C2*, *D2*) von Kossa (for saponified NEFAs stained *brown*) show PFAN around FN (*dotted shape*, *B*), which is von Kossa positive (*dotted shape*, *B2*) in patients with AP, whereas in controls there is no fibrosis surrounding the fat (*arrowheads*). However, in patients with (*B*) CP or (*D*) AP-on-CP, the fat, FN, and von Kossa–positive areas (*B2* and *D2*) are confined by the fibrosis (*arrows*, *B1* and *D1*) with preservation of the surrounding parenchyma. Controls (*A–A2*) do not have significant von Kossa–positive areas (*A2*).

coculture system (417 ± 54 ng/mL; Figure 5*A*) and >10-fold the concentration in sera of patients with SAP (87.5 ± 101.4 ng/mL),[46] did not result in any of these findings (Supplementary Figure 4*B*, *C*, and *F*). Similarly, visfatin, an adipokine increased in patients with AP, at 1 μg/mL (>100 times the serum concentration of patients with SAP[50]) did not affect cytosolic calcium levels or cause acinar injury (Supplementary Figure 4*B*, *C*, and *F*). Addition of unsaturated fatty acids (UFAs) to acini in a collagen-coated well did not protect them from UFA toxicity (Supplementary Figure 5); therefore, the protective effect of fibrosis in AP-on-CP results from it reducing the lipolytic flux between acinar cells and adipocytes.

Discussion

In this study, we note that unlike controls or patients with AP, the %IPF in patients with CP is unrelated to BMI and is higher than that in nonobese controls with a similar BMI; in addition, this IPF has significantly more fibrosis surrounding it than in controls or patients with AP. This fibrosis limits the crosstalk between adipocytes and acinar cells, thus reducing the lipolytic flux during acute exacerbations, which consequently reduces the severity of acute attacks in patients with CP. Analyses performed after excluding 5 patients in the AP-on-CP group who did not have clinical evidence of AP (n = 10) showed that differences in the variables of interest remained significant between the groups, and all post hoc comparisons were the same after excluding these patients (results not shown).

These findings correlate well with the clinical observation that patients with AP, especially those who are obese,[1–5] have a higher rate of mortality during the initial attacks[18] and worse FN, PFAN, and pancreatic necrosis.[4] However, a significant proportion of patients with AP have recurrent acute attacks[18,51] and progress to CP,[17,28] which has the hallmark of fibrosis.[39] In these patients, mortality is rarely attributed to SAP,[16–18] despite the recurrent attacks of AP and persistence of the initiating factor (eg, alcohol).

We recently provided a mechanistic rationale for the relationship of the severity of an acute attack to lipotoxicity from the NEFAs generated by lipolysis of

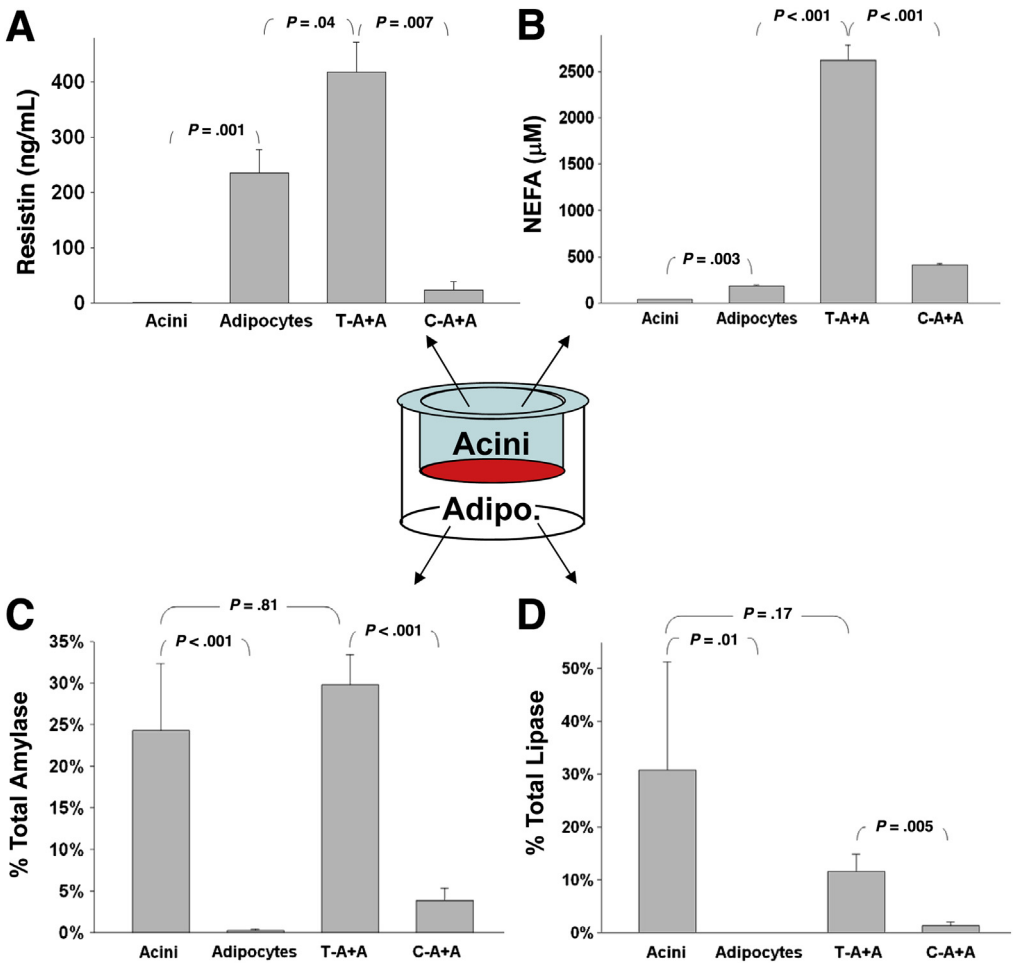

Figure 5. Collagen I reduces flux of macromolecules between acinar cells and adipocytes. (*A*) Resistin and (*B*) NEFA concentrations in the upper compartment and (*C*) amylase expressed as a percentage of total pancreatic acinar content and (*D*) lipase activity in the lower compartment at the end of 5 hours of incubation under different conditions, that is, acini alone in the upper compartment (Acini), adipocytes alone in the lower compartment (Adipocytes), acini in the upper compartment and adipocytes in the lower compartment without collagen-I (T-A+A), and with collagen I (C-A+A) coating the base of the Transwell. *P* values for comparing 2 groups were calculated using the Mann–Whitney test.

adipocyte triglyceride.[4] Patients with SAP have high NEFA concentrations in the serum[47,52] and necrosis debridement fluid.[4,53] We also noted that UFAs at relevant concentrations inhibit acinar mitochondrial complexes I and V, resulting in acinar cell necrosis.[4]

This study goes on to show that IPF in patients with CP, unlike in obese patients, is predominantly surrounded by fibrosis. This fibrosis is protective during an acute attack. In the absence of fibrosis, such as in AP, the leakage of NEFAs from the necrosed fat into the parenchyma (Figure 4*C*, *C2*), seen as PFAN,[4] is a major contributor to total necrosis. However, in the presence of fibrosis, collagen reduces the lipolytic flux between adipocytes and acinar parenchyma, PFAN, and total parenchymal necrosis. This protective role of fibrosis, despite the increased IPF in nonobese patients with CP, has implications on how IPF measured by radiologic means may be interpreted, such as for risk stratification of SAP.

Fibrosis may form up to 66% of the pancreatic area in CP.[39] We used collagen I, the major type of collagen in human CP,[42,54] to simulate this fibrosis in an acinar-adipocyte coculture model previously validated by us. The concentration (1.0%) of collagen I used by us is relevant to collagen concentrations (13.2% of total protein) noted previously in CP[39] and is in the range (up to 2%) noted to reduce macromolecular diffusion.[45] This collagen, simulating fibrosis, prevents acinar necrosis by reducing the leakage of lipase into the adipocyte compartment and reducing NEFA and resistin concentrations in the acinar compartment, but it is the UFAs, not the adipokines, that mediate the acinar damage (Supplementary Figure 4). Interestingly, UFAs and their metabolites have been previously speculated in the pathogenesis of CP in humans,[55] and diets high in UFAs with alcohol result in an AP-on-CP with acinar necrosis and FN.[56]

This study is limited by the small size, due to which comparisons between subgroups are prone to both type I and II errors. Although we adjusted the *P* values for multiple comparisons with appropriate statistical tests, it is possible that significance observed in some comparisons may be due to chance (type I error). Similarly, a lack of significance for some comparisons could have been due to limited power to detect a difference (type II error). Our observations are biologically plausible; however, because of these limitations, they should be interpreted with caution and considered preliminary.

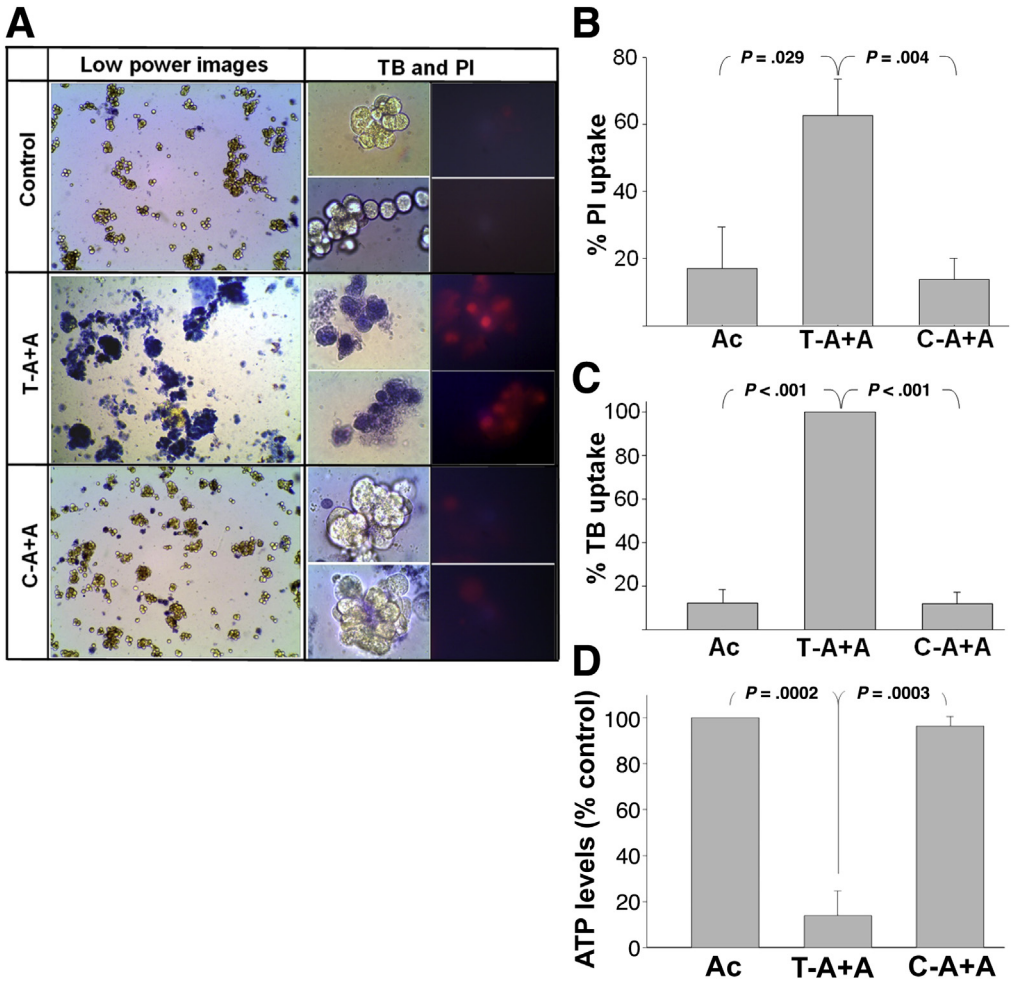

Figure 6. Collagen I reduces acinar cell death in coculture. (*A*) Images of acini (Ac) after 5 hours of incubation in the upper compartment alone (Control) or with adipocytes in the lower compartment without collagen-I (T-A+A) or with collagen-I (C-A+A) coating the base of the Transwell. The low-power images (*left column*) stained with trypan blue (TB) were taken at 4× magnification. The images on the *right* are 2 100× images showing TB and PI staining of identical areas representing each condition. Bar graphs show the percentage of acinar cell positive for (*B*) PI staining, (*C*) TB staining, and (*D*) acinar cell ATP levels as percent control (29.2 ± 1.8 pmol/μg protein) after 5 hours of incubation. *P* values for comparing 2 groups were calculated using the Mann–Whitney test.

Although a significant difference in %IPF was found between the 4 groups ($P = .016$; Supplementary Figure 2), this difference was not noted after adjustment for multiple comparisons between the groups. Combining the CP (n = 35) and AP-on-CP (n = 15) groups showed that these 50 patients have a significantly greater IPF (15.2% ± 10.1% vs 9.3% ± 10.2%; $P = .02$) compared with controls after adjusting for multiple comparisons. Because accumulation of IPF in CP is probably a chronic phenomenon that would also have occurred in patients who develop AP in the background of CP, it is reasonable to combine these groups, and the conclusion therefore is plausible. This is further supported by the fact that nonobese patients with CP have greater IPF than controls (Figure 1*C*). Our lack of knowledge of the duration of CP and %IPF before the onset of CP also affects our interpretation of why IPF increased with BMI in patients with AP-on-CP but not those with CP alone. Perhaps a shorter duration of disease preserved the correlation of BMI to % IPF in these patients and resulted in an acute attack in addition to the CP.

We are also unable to comment on how a >90% reduction in acinar mass, as occurs in patients with advanced CP and exocrine insufficiency, may contribute to AP-on-CP. However, our initial studies showed that the zymogen granule content within individual acinar cells (Supplementary Figure 6) was similar in controls and patients with CP, even while the acinar mass is reduced in CP. It is also known that serum lipase and amylase levels do not correlate with severity of AP.[57] Additionally, children and adolescents, who have a lower pancreatic mass[6] and serum amylase values[32] than adults, have similar frequency of attacks of SAP and outcomes as adults.[33–37,58] These points argue against lower acinar mass as solely responsible for the sharp decline in SAP after the second attack of AP[18] while progressing to CP.

Recent studies, in concordance with our findings, show that NEFAs released from the lipolysis of hepatic triglycerides cause hepatotoxicity and cholestasis,[59] as do NEFAs synthesized in the liver.[60] However, while the lipid droplet within the hepatocyte is a major source of NEFAs in the liver, various studies[4,10,61] suggest that NEFAs in the pancreas predominantly come from adipocytes, which contribute to PFAN in human AP[4,10,11] and are increased in obesity[7,61] or CP. Interestingly, whereas Bourbonnias et al[62] noted that fibrosis and collagen I protect

hepatocytes via down-regulating proapoptotic proteins, we note a protective effect of fibrosis by preventing NEFA-induced necrosis.[4]

In summary, although the amount of IPF is greater even at low BMIs in CP, this fat has significantly more fibrosis surrounding it. Fibrosis in turn prevents the lipolytic flux between acinar cells and adipocytes initiated during an acute attack and thus reduces acinar necrosis. As a result, fibrosis reduces the severity of acute attacks in patients with CP.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.05.012

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Conflicts of interest

The authors disclose no conflicts.

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