Published in final edited form as:

Gastroenterology. 2013 August ; 145(2): 466–475. doi:10.1053/j.gastro.2013.05.012.

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

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Abstract

BACKGROUND & AIMS—Acute pancreatitis (AP) and chronic pancreatitis (CP) share etiologies, but AP can be more severe and has higher mortality. We investigated features of CP that protect against severity. The amount of intra-pancreatic fat (IPF) is increased in obesity and fibrosis is increased in 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 Autopsy database (1998–2008) for patients with diagnosed 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 co-culture system, with or without collagen Type-I (collagen-I), was used to study the effects of fibrosis on acinar-adipocyte interactions. We studied the effects of non-esterified fatty acids (NEFA) and adipokines on acinar cells in culture.

RESULTS—Levels of IPF were significantly higher among non-obese patients with CP than non-obese Controls. In CP or AP-on-CP, areas of IPF were surrounded by significantly more fibrosis than in Controls or patients with AP. Fat necrosis (FN)-associated peri-fat acinar necrosis

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Author Contributions: V.P.S and S.N. designed, performed research, obtained funding, supervised the study, and wrote the manuscript. C.A., R.A.C., D. J., P.N., J.P. D, A. F., J.M.K., K.P., V. M., C.D., performed experiments. K.B. & C.J. B supervised the radiologic and calcium studies. B. L. R-D performed statistical analysis. D.Y. analyzed clinical data.

Disclosure: No conflicts of interest exist.

(PFAN, indicated by NEFA spillage) contributed to most of the necrosis observed in AP samples; however, PFAN and total necrosis were significantly lower in samples from patients with CP and AP-on-CP. Fibrosis appeared to wall off the FN and limit PFAN, 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, NEFA, but not adipokines, caused acinar-cell necrosis.

CONCLUSIONS—Based on analysis of pancreatic samples from patients with CP, AP and 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

INTRODUCTION

Several studies show the risk for severe AP (SAP) and mortality increases in obese patients¹⁻⁵ (BMI ≥ 30 Kg/m²). Additionally, various studies^{4, 6, 7} show visceral fat depots (i.e. IPF) to increase with body weight or body mass index (BMI) in children^{8, 9} and adults, and that fat which is abutting acinar cells is involved in parenchymal damage during AP^{4, 10, 11}. Fatty pancreas replacement may also occur in CP¹²⁻¹⁵; however, SAP is a rare outcome or cause of mortality in CP¹⁶⁻¹⁸. Thus, the mechanisms of IPF differentially affecting the outcomes of AP in the context of obesity or CP (i.e., AP-on-CP) need further investigation.

AP and CP share some common etiologies like alcohol¹⁹, genetic mutations (e.g., CFTR, PRSS1)²⁰⁻²², obstructive lesion (e.g., a mass)²³⁻²⁵, and metabolic causes (e.g., hyperlipidemia)^{26, 27}. Recent studies suggest acute, recurrent acute, and chronic pancreatitis to be a continuum^{18, 19, 28}. However, while AP in the first one or two attacks may cause extensive pancreatic necrosis within weeks^{4, 18}, patients with CP have a prolonged course with recurrent AP attacks 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 (e.g., alcohol, tumor, metabolic or genetic cause) persisting over this time. Similarly, while AP may result in significant mortality over days²⁹⁻³¹, mortality over the several years of CP is rarely attributed to AP¹⁶⁻¹⁸.

The reasons for these different outcomes have remained unclear. While it has been variously argued that this may be due to a lower acinar cell mass in CP, studies in children and adolescents, who have smaller pancreata than adults >30 years of age⁶ and a lower serum amylase than adults³² (which is relevant since lower serum enzymes in CP are often attributed to lower acinar mass), experience no difference in severity compared with adults³³⁻³⁷. Rather, children with SAP have higher BMI³⁶ and children with a higher weight at admission are more prone to SAP³⁵. Additionally, a large study of children treated for acute lymphoblastic leukemia mentions that children “who had grade 3 or 4 pancreas/ Glucose toxicity during induction also had higher BMIs throughout treatment”³⁸. Pancreas toxicities are based on criteria for pancreatitis. Grades 3 or 4 are severe vs. grades 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 CP³⁹, may be considered to reduce the severity of AP-on-CP. We thus studied different pancreatic disease states – Controls, AP, CP, and AP-on-CP – for differences in the amount of IPF and its relation to BMI, fibrosis and the pancreatic parenchyma. Since the pancreas is not usually sampled in

live humans without pancreatic disease, and samples removed during an invasive procedure are small (e.g., using Fine needle aspiration or a Trucut biopsy) or altered by disease (e.g., cancer removed at a Whipple procedure), we chose to examine autopsy tissue in control patients (i.e., 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 post mortem changes, such as limited autolysis, do not significantly affect the interpretation of findings using this approach⁴.

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

METHODS

Medical records of all patients in the UPMC-Presbyterian Hospital Autopsy database (1998-2008) with AP (n=23), CP (n=35), or a diagnosis of AP-on-CP (total; n=15, clinical AP-on-CP; n=10) were reviewed. Pancreas slides of these and 50 randomly selected Controls were scored for IPF, FN, PFAN and fibrotic area (as % pancreatic area). Percentage of fat with >50% of its immediate perimeter surrounded by fibrosis was measured. For further details, please refer to the supplementary methods.

RESULTS

There was no significant difference in age ($F(3,119)=0.790$, $P=0.502$) or BMI ($F(3,119)=0.845$, $P=0.472$) (Supplementary Table1) between the groups.

BMI correlates positively with IPF in Controls, AP patients, and AP-on-CP patients but not in CP patients

Since IPF may increase with BMI^{4,6} or CP^{40,41}, we studied the correlation of BMI to histologically measured %IPF. Significant positive correlations were found in Controls ($r(48)=0.592$, $P<0.001$), AP ($r(21)=0.506$, $P=0.014$)^{4,6}, and AP-on-CP groups ($r(13)=0.729$, $P=0.002$, (Figures 1A-C), but not in the CP group ($r(33)=0.168$, $P=0.334$, Figure 1A). A sensitivity analysis after excluding a possible influential observation (patient with a BMI >60 Kg/m²) in the CP group showed a correlation coefficient of 0.170 ($P=0.337$). The two-way ANOVA showed significant main effects for group ($F(3,115)=3.462$, $P=0.019$) and BMI ($F(1,115)=29.548$, $P<0.001$), and a significant interaction (group $\times$ BMI, $F(3,115)=3.724$, $P=0.013$) for %IPF. Subsequent post-hoc tests showed that, for the obese group, Controls, CP, AP and AP-on-CP did not differ; however, for the <30 BMI group, the CP group had significantly greater IPF when compared to the Controls. Post-hoc tests also showed that obese patients in Controls, AP and AP-on-CP, but not the CP groups, had significantly greater IPF than the BMI <30 group (Figure 1C).

Of the 35 CP patients, 14 had CT scans with no evidence of AP in the 45 days preceding death. IPF measured on the CT scans of these by a blinded radiologist using either of the previously validated attenuation^{4,6} or thresholding methods^{4,6} showed a strong correlation with histology (Figures 1D-E). The kappa value for histological IPF quantification by 2 independent blinded observers was 0.951, signifying strong inter-observer agreement.

CP patients have a lower amount of FN compared to AP patients

Patients with AP had significantly more FN than CP ($P<0.001$) or Controls ($P<0.001$) (Figure 2A). FN was visible as adipocytes with a bluish, cheesy look on H&E staining (Figure 3C, 4C, D) and stained brown on von Kossa, suggesting saponification (Figure 4C2, D2). This FN seemed to be pathogenically relevant since the NEFA so formed induce cell death⁴, and the FN in AP was associated with surrounding parenchymal necrosis (dotted area Figure 4C, C2).

AP patients have more PFAN and necrosis than CP, AP-on-CP patients

We quantified parenchymal necrosis adjacent to the FN mentioned above PFAN. On H&E staining this was notable for areas with loss of cell outlines bordering FN (dotted area, Figure 4C). On von Kossa staining PFAN showed brown staining (Figure 4C2) that was most intense adjacent to the FN and decreased with increasing distance, suggesting leakage of NEFAs formed in the FN. PFAN is distinct from isolated acinar necrosis (square, supplementary Figure 2) which is von Kossa negative and occurs in areas separated from FN (circle, supplementary Figure 2). There was a statistically significant difference in PFAN between the groups ($F(3,123)=47.68$, $P<0.001$). AP patients had a much greater amount of PFAN than CP ($P<0.001$), AP-on-CP patients ($P=0.025$), or Controls ($P<0.001$) (Figure 2B). Similarly, there was a statistically significant difference in total necrosis between the groups ($F(3,123)=50.02$, $P<0.001$), where AP patients had a significantly greater amount of total necrosis when compared to the other groups (Figures 2C). 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

Since % IPF in CP or AP-on-CP patients was not significantly lesser when compared to AP (Supplementary Figure 1), we measured the amount and pattern of fibrosis to explain the lower PFAN and total necrosis noted in the CP, AP-on-CP patients. CP and AP-on-CP patients had significantly more fibrosis (Figure 3A) than Controls or AP. This fibrosis morphologically walled off large areas of IPF from the parenchyma in CP and AP-on-CP, seen as bluish strands surrounding the fat on trichrome staining (arrows, Figure 3C). Quantification of % IPF surrounded by fibrosis showed this to be significantly more in CP and AP-on-CP patients (Figure 3B) compared to Controls or AP patients in whom the fat abutted the parenchyma without intervening fibrosis (Arrowheads Figure 3C). 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 being confined by surrounding pinkish, hypocellular bands (Figure 4B, B2 and D, D2). These bands were verified to be fibrosis on trichrome staining (Figure 4B1, D1), in the absence of which (arrowhead figure 4C1) AP patients had more FN (figure 4C2) and PFAN (Figure 4C). Thus, fibrosis in CP and AP-on-CP prevents spillage of NEFAs into the parenchyma with associated reduction in parenchymal necrosis.

Collagen-I reduces acinar necrosis by reducing lipolytic flux between acinar cells and adipocytes

In order to study the role of fibrosis in preventing acinar necrosis in CP, we coated transwells with collagen-I, which is highly expressed in human CP⁴², and co-cultured adipocytes with pancreatic acini. This system replicates the pathophysiology of AP by allowing macromolecular diffusion from the two cell types while preventing contamination of one cell type by the other, as described previously⁴ (Supplementary Figure 3). The transfer of acinar derived (i.e. amylase and lipase) and adipocyte derived (i.e. resistin and

NEFA) macromolecules to the non-native (i.e. opposite) compartment was studied in the presence and absence of collagen, as was acinar cell viability.

Amylase and lipase increased in the lower (adipocyte) compartment in the absence of adipocytes (Figure 5 C, D), analogous to the basal release⁴ or spillage noted in pancreatitis^{43, 44}. However, NEFA and resistin, which are virtually absent in the upper (acinar) compartment without adipocytes, significantly increased here in the presence of acini and adipocytes (T-A+A, Figure 5 A, B), signifying crosstalk between the two compartments. Collagen-I significantly prevented this co-culture dependent increase of macromolecules in the non-native compartment (C-A+A, Figure 5), supporting its role as a diffusion barrier as shown by others⁴⁵. The increase in NEFA and resistin in the acinar compartment paralleled acinar necrosis, as noted by a large increase in trypan blue and PI uptake and a drop in ATP levels (Figure 6). This was prevented by collagen-I (trypan blue $98.9 \pm 0.71\%$ vs. $17.1 \pm 5.08\%$, $P < 0.001$, PI uptake $59.3 \pm 8.3\%$ vs. $16.2 \pm 5.06\%$, $P = 0.004$, ATP levels $13.9 \pm 10.7\%$ vs. $96.4 \pm 4.1\%$, $P = 0.0003$, Controls ATP concentration 29.2 ± 1.8 pmol/ μ g protein, Figure 6). These findings show that collagen-I in fibrosis reduces acinar injury in AP-on-CP by reducing the flux of macromolecules between acini and adipocytes.

To study the relative contribution of NEFA 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 (LA) at concentrations relevant to those noted in the co-culture system (LA; $670 \pm 67 \mu$ M) or in the debridement fluid of patients with SAP (LA; $1241 \pm 510 \mu$ M). LA at less than half of these concentrations (300μ M) resulted in a large increase in LDH leakage ($83.8 \pm 1.5\%$), cytosolic calcium (Supplementary Figure 4A-C), and PI uptake accompanied by a decrease in ATP levels (Supplementary Figure 4D-F). However, resistin at 1μ g/ml, which is >2 fold the concentration noted in the co-culture system (417 ± 54 ng/ml, Figure 5A) 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 (Supplementary Figure 4 B, C, F). Similarly, visfatin, an adipokine increased in AP, at 1μ g/ml (>100 times the serum concentration of SAP patients⁵⁰) did not affect cytosolic calcium or cause acinar injury (Supplementary Figure 4 B, C, F). Addition of 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 pancreatitis 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 CP is unrelated to BMI, being higher than non-obese Controls with similar BMI, and this IPF has significantly more fibrosis surrounding it than in Controls or AP patients. This fibrosis limits the crosstalk between adipocytes and acinar cell, thus reducing the lipolytic flux during acute exacerbations, which consequently reduces the severity of acute attacks in CP. Analyses performed after excluding five 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).

The above findings correlate well with the clinical observation that patients with AP, especially those who are obese¹⁻⁵, have a higher mortality during the initial attacks¹⁸ and worse FN, PFAN, and pancreatic necrosis⁴. However, a significant proportion of AP patients have recurrent acute attacks^{18, 51} and progress to CP^{17, 28}, which has the hallmark of fibrosis³⁹. In these patients, mortality is rarely attributed to SAP¹⁶⁻¹⁸, despite the recurrent attacks of AP and persistence of the initiating factor (e.g. alcohol).

We recently provided mechanistic rationale for the relationship of the severity of an acute attack to lipotoxicity from the NEFAs generated by lipolysis of adipocyte triglyceride⁴. 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 necrosis⁴.

This study goes on to show that IPF in CP, unlike in obesity 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 NEFA from the necrosed fat into the parenchyma (Figure 4C-C2), seen as PFAN⁴, 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 non-obese 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 pancreatic area in CP³⁹. We used collagen-I, the major type of collagen in human CP^{42, 54}, to simulate this fibrosis in an acinar-adipocyte co-culture 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 CP³⁹ and is in the range (up to 2%) noted to reduce macromolecular diffusion⁴⁵. 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 UFA, 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 humans⁵⁵, and high UFA diets with alcohol result in an AP-on-CP with acinar and FN⁵⁶.

This study is limited by 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, due to above limitations, should be interpreted with caution and considered preliminary.

While a significant difference in %IPF was found between the 4 groups ($P=0.016$, Supplementary Figure 1), this difference was not noted after adjustment for multiple comparisons between the groups. Combining CP ($n=35$) along with the AP-on-CP group ($n=15$), showed these 50 patients have a significantly greater IPF ($15.2\pm 10.1\%$ vs. $9.3\pm 10.2\%$, $P=0.02$) compared to Controls after adjusting for multiple comparisons. Since IPF accumulation in CP is probably a chronic phenomenon which 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 non-obese CP patients having greater IPF than Controls (Figure 1C). Our lack of knowledge of the duration of CP and % IPF present prior to the onset of CP also affects our interpretation of why IPF increased with BMI in the AP-on-CP patients but not those with CP alone. Perhaps a shorter duration of the disease may have preserved the BMI to %IPF correlation in these patients and resulted in an acute attack on top of 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 CP patients, even while

the acinar mass is reduced in CP. It is also known that serum lipase and amylase do not correlate with severity of AP⁵⁷. Additionally, children and adolescents, who have a lower pancreatic mass⁶ and serum amylase values³² than adults, have a similar frequency of severe AP attacks and outcomes as adults^{33-37, 58}. These points argue against lower acinar mass being solely responsible for the sharp decline of SAP after the second attack of AP¹⁸ while progressing to CP.

Recent studies, in concordance with our findings, show that NEFA released from the lipolysis of hepatic triglycerides cause hepatotoxicity and cholestasis⁵⁹, as do NEFA synthesized in the liver⁶⁰. However, while the lipid droplet within the hepatocyte is a major source of NEFA in the liver, various studies^{4, 10, 61} suggest NEFA in the pancreas predominantly come from adipocytes, which contribute to PFAN in human AP^{410, 11} and are increased in obesity^{7, 61} or CP. Interestingly, while Bourbonnias et al⁶² noted that fibrosis and collagen-I protect hepatocytes via down-regulating pro-apoptotic proteins, we note a protective effect of fibrosis by preventing NEFA induced necrosis⁴.

In summary, while 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 during CP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Michelle Kienholz, Drs. Sohail Husain and Mark Lowe for a critical appraisal of this manuscript.

Grant support: The project described was supported by Grant Number RO1DK092460 (V.P.S.) and UL1 RR024153 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research (V.P.S, S.N), and the Clinical Translational Science Institute (CTSI) supported by the National Institutes of Health through Grant Numbers UL1 RR024153 and UL1TR000005. The contents of the manuscript are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Information on NCRR is available at <http://www.ncrr.nih.gov/>. Information on Re-engineering the Clinical Research Enterprise can be obtained from <http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp>. Funding was also provided by a startup package from the University of Pittsburgh Department of Medicine (VPS).

Abbreviations

UFA	Unsaturated fatty acids
SFA	saturated fatty acids
NEFA	Non-esterified fatty acid
MSOF	Multisystem organ failure
IPF	Intrapancreatic fat
AP	acute pancreatitis
CP	chronic pancreatitis
SAP	Severe acute pancreatitis
H&E	Hematoxylin and eosin

PFAN	Peri-fat acinar necrosis
ATP	adenosine triphosphate
BMI	Body mass index
LDH	Lactate dehydrogenase
DMSO	Dimethyl sulfoxide
PI	propidium iodide
CFTR	cystic fibrosis transmembrane receptor

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Figure 1. %IPF increases with BMI in Controls, AP patients and AP-on-CP patients but not in CP

Scatter plot showing the association between IPF and BMI for (A) Controls (black) and CP patients (red) and (B) AP patients (black) and AP-on-CP patients (red). Lines represent the best-fit line from a linear regression analysis. Correlation analyses were calculated using the Spearman's rho correlation coefficient. Results show moderate to high correlations in all groups except in CP patients. Figure C shows the estimated marginal means from the two-way ANOVA that included main effects for BMI and group, and their interaction (BMI $\times$ 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 two-way

ANOVA 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 to Controls (*P* value shown in green). Post-hoc comparisons between normal (BMI < 30) and obese (BMI ≥ 30) groups are also shown (*P* values in black). Figures D and E show linear regression analysis between %IPF measured on histology [%IPF (histology)] and Hounsfield units on CT scan or by the CT thresholding method, respectively. Spearman's correlation coefficient was computed.

Figure 2. Histological quantification of FN (A), PFAN (B), acinar necrosis (C) as percentage of total area

Box plots showing the mean (dashed line), the median (solid line), the 25th and 75th percentiles (2 boxes), the 10th and 90th percentile (whiskers) and the outliers (dots), comparing Controls, CP, AP and AP-on-CP patients for each of the above parameters show these to be significantly reduced in CP and AP-on-CP compared to AP patients. 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 3. Fibrotic area (A) and percentage of fat surrounded by fibrosis (B) are increased in a background of CP

Box plots comparing Controls, CP, AP and AP-on-CP patients for (A) fibrosis (as % total area) and (B) % 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 pancreas histologic sections from Controls, CP, AP and AP-on-CP patients stained with H&E, Masson's Trichrome (Trichrome for fibrosis-blue strands;) show fat confined by the fibrosis (arrows) in CP and AP-on-CP. However, fat in Controls and FN in AP has no surrounding fibrosis (arrowheads).

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 Controls (A-A2), CP (B-B2), AP, (C-C2) patients and those with AP-on-CP (D-D2) stained with H&E (A, B, C, D), Masson's Trichrome (Trichrome for fibrosis-blue strands; A1,B1, C1, D1) and von Kossa (for saponified NEFA stained brown, A2, B2, C2, D2) show PFAN around FN (dotted shape, B), which is von Kossa positive (dotted shape B2) in AP, where like in Controls there is no fibrosis surrounding the fat (Arrow heads). However, in CP (B) and AP-on-CP (D) the fat, FN and von Kossa positive areas (B2, D2) are confined by the fibrosis (arrows, B1, D1) with

preservation of the surrounding parenchyma. Controls (A-A2) do not have significant von Kossa positive areas (A2).

Figure 5. Collagen-I reduces flux of macromolecules between acinar cells and adipocytes Resistin (A) and NEFA concentrations (B) in the upper compartment and Amylase (C) expressed as a % of total pancreatic acinar content, lipase activity (D) in the lower compartment at the end of five hours incubation under different conditions; i.e. acini alone in the upper compartment (Acini), adipocytes alone in the lower compartment (Adipocytes), Acini in the upper, 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 two groups were calculated using the Mann-Whitney test.

Figure 6. Collagen-I reduces acinar cell death in co-culture

A: Images of acini (Ac) after 5 hours 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 $\times$. The images on the right are two 100 $\times$ images showing TB and PI staining of identical areas representing each condition. Bar graphs showing percentage of acinar cell positive for PI staining (B), TB staining (C) and acinar cell ATP levels as % Control (29.2 ± 1.8 pmol/ μ g protein) (D) after 5 hours incubation. *P* values for comparing two groups were calculated using the Mann-Whitney test.