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Composition of free fatty acid and triglyceride fractions in human necrotic pancreatic tissue

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SUMMARY

Background: *Lipolytic enzymes, such as lipase, phospholipase A_2, lipoprotein lipase, and hormone-sensitive adipocyte lipase are probably implicated in the pathogenesis of acute pancreatitis. The increased activity of these enzymes can cause specific changes in fatty acid composition, both to free fatty acid (FFA) and triglyceride (TG) fractions in necrotically changed pancreatic tissue.*
Material and methods: *Specimens of necrotically changed pancreatic tissue taken intraoperatively from 13 patients operated for serious necrotic acute pancreatitis were analysed. The Fölsch extraction method and separation of lipids by thin-layer chromatography was used, and the final fatty acid composition was determined after methylation by gas liquid chromatography.*
Results: *The changes in the FFA fraction are more definite than those for the triglyceride fraction and refer mostly to the increased percentage distribution of unsaturated fatty acids.*
Conclusion: *These studies lead to the hypothesis that fatty acids released in the process of lipolysis play a key role in the pathogenesis of acute pancreatitis.*

BACKGROUND

In 1882, Balser described fat necrosis, which he considered to be a thermal disease, but he failed to recognize the relationship between acute pancreatitis and fat necrosis. The results of studies performed by Fitz and Langerhans documented that relationship [1].

Fat necrosis can be seen as the 'ghost' remains of adipocytes in the tissue surrounding the pancreas, and within the gland [2]. The earliest fat necrosis in humans involves peripancreatic adipose tissue, while the intrapancreatic fat is involved later [2]. Morphologically, the differentiation of acute pancreatitis into mild and severe depends on the extent and site of fat necrosis. Fat necrosis may occur in the absence of necrosis of pancreatic parenchyma [2]. Parenchymal necrosis, however, is frequently observed adjacent to fat necrosis [3,4]. According to Klöppel, in the mild edematous form of acute pancreatitis, small disseminated necrotic foci involve the fatty tissue surrounding the pancreas; Bockman, however, claims that they occur in the connective tissue septa between lobules within the gland, and some necrotic acinar cells may be present [2–4]. In cases of severe acute pancreatitis, necrosis affects large parts of the peripancreatic adipose tissue and within the pancreas, involving vessels, small ducts, and parenchyma. Disseminated foci of fat necrosis can usually be detected in the bursa omentalis, the omentum, the root of the intestinal mesentery, and the retroperitoneum [4]. Based on results from experimental studies and observations from humans, it would appear that fat necrosis plays a pivotal role in the development of

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acute pancreatitis [4–6]. The mechanism of the development of acute pancreatitis is damage to acinar cells adjacent to fat necrosis by fatty acids released from adipocytes [4,5].

Fat necrosis associated with acute pancreatitis is most probably caused by the liberation of lipase and phospholipase A_2 from pancreatic tissue. Other lipolytic enzymes, such as lipoprotein lipase and hormone-sensitive adipocyte lipase, are probably involved as well. In the course of acute pancreatitis, the increased activity of these enzymes can cause specific changes in fatty acid composition in the necrotically changed pancreatic tissue, both to free fatty acid (FFA) and triglyceride (TG) fractions.

MATERIAL AND METHODS

Specimens of necrotically changed pancreatic tissue taken intraoperatively from 13 patients operated for serious necrotic acute pancreatitis were analysed. After the tissue samples were placed in physiological saline solution, they were homogenised, and subsequently subjected to extraction according to the procedure of Fölsch et al. [7]. Extracts from the lipid fractions obtained from the tissue specimens were dehydrated using anhydrous sodium sulphate, and subsequently evaporated to dry mass in a nitrogen atmosphere at a temperature of 37°C. The individual lipid fractions were separated using thin-layer chromatography performed according to the method of Chedid et al. [8]. Glass plates were coated with silica gel (Kieselgel 60G – Merck) with the addition of rhodamine G facilitating the identification of lipid fractions. Chromatograms were developed in two other mixtures with the following composition:

a) petroleum ether, butanone, and glacial acetic acid (85:10:1,5 v/v)
b) petroleum ether and diethyl ether (97:3 v/v)

Standard solutions of palmitic acid, oleic acid, triolein (all standards of Sigma Comp.) were used to identify the fractions under study: . Additionally, the accuracy of the free fatty acids separation from other lipid fractions was controlled by adding palmitic acid labelled with ^{14}C to the standard mixture directly after coating the plate. The retrieved activity from a sample containing free fatty acids was approximately 85% of the amount used on the plate. The activity of palmitic acid labelled with ^{14}C was measured by liquid scintillation counter (Beckman).

Separation of fatty acids using gas-liquid chromatography

Of the two lipid fractions (FFA and TG) obtained by using the method described above, the triglyceride fraction was subjected to saponification to release fatty acids. Subsequently, fatty acids from both fractions were methylated by boron trifluoride in methanol (Sigma) according to the Metcalfe and Schmitz method [9]. The internal standard, pentadekanoic acid (C15, Sigma), was added in a specified amount to each analyzed sample. The methyl esters of fatty acids thus obtained were extracted using heptane and dehydrated with anhydrous sodium sulphate. The methyl esters of fatty acids were separated in a gas chromatograph (Hewlett-Packard, model 5890) using a 30 m-long capillary column (manufactured by Supelco, type SP-2330) with an internal diameter of 0.25 mm, and applying helium as the carrier gas. Simultaneously, two other standard solutions by Sigma were analyzed, the first of which contained methyl esters of caprylic, capric, lauric, myristic and palmitic acid, and the second contained methyl esters of palmitic, stearic, oleic, linoleic, linolenic, and arachidonic acid. The area of the analyzed and identified peaks for the individual fatty acids was calculated by using an automatic integrator (Hewlett-Packard, model 3392).

Since for ethical reasons it is impossible to collect tissue from normal pancreas or even from patients with mild pancreatitis, the results of the present study were compared with the data published by Durand et al, who used the same procedure [10].

RESULTS

The percentage distribution of the individual fatty acids in the necrotically changed pancreas was calculated and compared with the results obtained from normal human pancreas in the studies conducted by Durand et al. [10].

Table 1 presents our results and those of Durand et al. [10]

The data shown in table 1 indicate that only the percentages of lauric and myristic acids in the FFA fraction in the necrotically changed pancreas were comparable with the values obtained for healthy persons. The percentages of saturated acids in the FFA fraction – palmitic (C 16:0) and stearic (C 18:0) – were clearly lower (two- and three-fold, respectively). The percentages of unsaturated acids

Table 1. Percentage distribution of the individual fatty acids in the specimens of necrotically changed pancreas

Acid	FFA	TG*	Values obtained in normal pancreas**	
	X±SD	X±SD	FFA	TG
C 12 : 0 (lauric)	0.85±0.8	1.0±1.0	0.91	Undeterminable
C 14 : 0 (myristic)	2.8±0.8	2.9±0.6	3.19	1.04
C 16 : 0 (palmitic)	23.8±6.8	26.6±5.8	48.57	27.51
C16:1(palmitooleic)	6.4±2.0	6.2±2.0	1.0	2.30
C 18 : 0 (stearic)	6.2±1.7	6.5±2.4	19.64	11.30
C 18 : 1 (oleic)	52.6±9.8	49.9±5.6	6.1	46.21
C 18 : 2 (linoleic)	7.1±2.6	7.3±2.8	0.66	3.74
C 18 : 3 (linolenic)	0.6±0.09	0.2+0.2	Undeterminable	Undeterminable
C20:4 (arachidonic)	1.3±0.6	Undeterminable	Undeterminable	3.0

* TG - triglycerides, ** according to Durand et al. [10]

– palmitooleic (C 16:1), oleic (C 18:1) and linoleic (C 18:2) – were 6-11-fold higher as compared to those in normal pancreas. The percentages of linoleic and arachidonic acids occurring in the FFA fraction, undeterminable according to Durand et al, were 0.5%, and 1.3%, respectively.

The triglyceride fraction demonstrated an almost 3--fold increase in the percentages of myristic (C 14:0) and palmitooleic acid (C 16:1), and double the amount of linoleic acid (C 18:2). The percentage of stearic acid (C 18:0) was proportionally lowered. Linolenic acid (C 18:3), undeterminable according to Durand et al, accounted for 0.2% of this fraction. The percentages of palmitic (C 16:0) and oleic acid (C 18:1) were comparable with those reported by Durand et al. in healthy persons. Arachidonic acid was not detected in this fraction in necrotically changed pancreas.

In summary, the differences between necrotic and normal tissue are more definite in the FFA fraction than in the triglyceride fraction, and involve mostly the increased percentage distribution of unsaturated acids, notably linoleic, linolenic and arachidonic acids, occurring in much higher percentages in necrotically changed pancreas than in normal tissue.

DISCUSSION

The percentage distribution of fatty acids in the analyzed lipid fractions indirectly reflects the significant role of some lipolytic enzymes in the inflammatory process in pancreas, notably phospholipase $\dot{A}_2$ and lipase [11]. Our results do not support the hypothesis of Durand at al. that the triglycerides in human pancreas contain mostly unsaturated fatty acids, while phospholipids contain saturated acids [10]. The results we obtained are also contrary to the observation of the same author regarding the low level of linoleic acid in pancreatic parenchyma, especially with regard to phospholipids. The several-times-increased level of unsaturated fatty acid in the FFA fraction was most likely the effect of phospholipase $\dot{A}_2$ activity, resulting in the release of free fatty acids from the phospholipids of the necrotically changed cell membranes of acinar cells, as well as fat cells [12]. The changes in the percentage profile of fatty acids in the triglyceride fraction were comparable with those observed in the FFA fraction; however, their expression was substantially lower. Phospholipase $\dot{A}_2$ occurs in significant amount in pancreatic cells, and has been implicated in the pathophysiology of acute pancreatitis [11,13–18]. The phospholipase $\dot{A}_2$ substrates lecithin and cephalin are the main structural components of cell membranes, and their products lysolecithin and lysocephalin are cytotoxic substances [11]. Some authors claim that phospholipase $\dot{A}_2$ is responsible for damage to fat cell membranes, allowing for the penetration of pancreatic lipase and the hydrolysis of intracellular triglycerides [13]. Lecithin may also be released from cell membranes under the toxic influence of fatty acids, which are products of triglyceride breakdown by lipase [19]. This enzyme also occurs in the macrophages and polynuclear neutrophils which infiltrate pancreatic parenchyma in the course of acute pancreatitis [13,18,20–22].

The experiments conducted by Lee revealed not only the effects of pancreatic lipase in inducing fat necrosis, but also of colipase [1]. Colipase facilitates the access of pancreatic lipase to fat cells, leading to their necrosis. Some authors claim that lipase also occurs in the pancreatic interstitial space [23]. Decreased blood circulation in pancreas and

disturbed lymphatic drainage in the course of acute pancreatitis prolongs lipase contact with fat cells and increases the release of fatty acids. Schmitz--Moormann and Boger, based on the outcomes of their studies, have advanced the hypothesis that the morphological changes in necrotic pancreatitis are mainly produced by the release of fatty acids by active lipase [5]. Those acids which are not linked with albumins have a direct cytotoxic effect on cell membranes, and not only in the pancreas. They are also responsible for vessel necrosis and clot formation, and in consequence gland ischemia [24]. Schmitz-Moormann and Boger postulate that these fatty acids cause similar damages in other organs as well, such as liver and kidneys.

Still other authors believe that hormone-sensitive adipocyte lipase preferentially releases polyunsaturated fatty acids from triglicerides [25]. Our observations do not support this opinion, with the exception of linoleic acid. This may be associated with its high content in pancreatic triglycerides [10,26]. The almost twofold increase in the linoleic acid content in the specimens of necrotically changed pancreas may support this observation.

Based on our studies, it may be concluded that as a result of the primary necrosis of the fat surrounding the pancreas and within it, which is the result of the action of lipolytic enzymes, the local concentration of the released fatty acids may be sufficiently high to cause the destruction of acinar cells. It would appear that the resulting damage to the acinar cell membrane may induce necrosis. The consequence of this is the release of the enzyme into the interstitial space, which further exacerbates the complex processes of autodigestion in the course of acute necrotic pancreatitis, and probably proteolytic as well.

CONCLUSION

Our results indicate that the fatty acids released in the process of lipolysis play a key role in the formation of necrotic lesions of adipose and pancreatic tissue characteristically present in acute pancreatitis.

REFERENCES:

1. *Lee PC, Howard JM: Fat necrosis. Surg Gynecol Obstet, 1979; 148: 785-789*
2. *Bockman DE: Pathology of edematous (interstitial) pancreatitis. Bradley III EL ed. Acute pancreatitis. Diagnosis and therapy. Raven Press, Ltd, New York 1994; 241-247*
3. *Klöppel G, von Gerkan R, Dreyer T: Pathomorphology of acute pancreatitis: analysis of 367 autopsy cases and 3 surgical specimens. In: Gyr KE, Singer MV, Sarles H. ed. Pancreatitis: concepts and classification. Amsterdam Excerpta Medica, 1984; 29-35*
4. *Kloppel G: Pathology of severe acute pancreatitis. In: Bradley III EL ed. Acute Pancreatitis. Diagnosis and therapy. Raven Press, Ltd, New York 1994; 35-46*
5. *Schmitz-Morgan P, Boger A: Tissue damage by fatty acids released by lipolysis. Path Res Pract 1981; 171: 303-313*
6. *Storck G: Fat necrosis in acute pancreatitis. Acta Chir Scand (Suppl) 1971; 417: 1-36*
7. *Fölsch J, Lees M, Sloane SGH: Simple method for isolation and purification of total lipids from animal tissue. J Biol Chem, 1957; 226: 497-509*
8. *Chedid A, Haux P, Natelson S. Use of thin layer chromatography on silica gel for serum lipid fractionation and measurement in the routine clinical laboratory. Clin Chem, 1972; 18: 348-390*
9. *Metchalfe LD, Schmitz AA: The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal Chem, 1961; 33: 363-372*
10. *Durand S, Estival A, Vieu C et al: Lipid content of human and rat pancreas. Pancreas, 1987; 2: 326-332*
11. *Niederau C, Fronhoffs K, Klonowski H et al: Active pancreatic digestive enzymes show striking differences in their potential to damage isolated rat pancreatic acinar cells. J Lab Clin Med, 1995; 125: 265-275*
12. *Dennis EA: Phospholipase A_2 mechanism: inhibition and role in arachidonic acid release. Drug Dev Res, 1987; 10: 205-220*
13. *Nevalainen TJ: Phospholipase A_2 in acute pancreatitis. Scand J Gastroenterol, 1988; 23: 897-904*
14. *Buchler M, Malfertheiner P, Shädlich H et al: Role of phospholipase A_2 in human acute pancreatitis. Gastroenterology, 1989; 97: 1521-1526*
15. *Gronroos JM, Nevalainen TJ: Increased concentrations of synovial-type phospholipase A_2 in serum and pulmonary and renal complications in acute pancreatitis. Digestion, 1992; 52: 232-236*
16. *Nevalainen TJ, Gronroos JM, Kortesuo PT: Pancreatic and synovial type phospholipase A_2 in serum samples from patients with severe acute pancreatitis. Gut, 1993; 34: 1133-1136*
17. *Keppainen E, Hietaranta A, Puolakkainen P et al: Bactericidal/permeability-increasing protein and group I and II phospholipase A2 during the induction phase of human acute pancreatitis. Pancreas, 1999; 18: 21-27*
18. *Yoshikawa T, Naruse S, Kitagawa M et al: Effect of a new inhibitor of type II phospholipase A_2 on experimental acute pancreatitis in rats. Pancreas, 1999; 19: 193-198*
19. *Borgstrom B: Phosphatidylcholine as substrate for human pancreatic phospholipase A_2. Importance of the physical state of the substrate. Lipids, 1993; 28: 371-375*
20. *Kimura W, Secknus R, Fischbach W et al: Role of phospholipase A_2 in pancreatic acinar cell damage and possibilities of inhibition: studies with isolated rat pancreatic acini. Pancreas, 1993; 8: 70-79*
21. *Smith DM, Waite M: Phosphatidylinositol hydrolysis by phospholipase A_2 and C activities in human peripheral blood neutrophils. J Leukoc Biol, 1992; 52: 670-678*
22. *Rae D, Beechey-Newman N, Burditt L et al: Activation of human granulocyte type 1-prophospholipase A_2. Scand J Gastroenterol, 1996; 32 (219): 24-27*

23. *Saharia P, Margolis S, Zuidema GD: Acute pancreatitis with hyperlipemia: studies with an isolated perfused canine pancreas. Surgery, 1977; 82: 60-67*
24. *Kimura T, Zuidema GD, Cameron JL: Acute pancreatitis. Experimental evaluation of steroid, albumin and trasylol therapy. Am J Surg, 1980; 140: 403-408*
25. *Gavino VC, Gavino GR: Adipose hormone-sensitive lipase preferentially releases polyunsaturated fatty acids from triglycerides. Lipids, 1992; 27: 950-954*
26. *Paye F, Chariot J, Molas G et al: Nonesterified fatty acids in acute cerulein-induced pancreatitis in the rat. Are they really deleterious in vivo? Dig Dis Sci, 1995; 40: 540-545*