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Contribution of Angiotensin II to alcohol-induced pancreatic fibrosis in rats

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Abbreviations: α-SMA; α-smooth muscle actin, AgII; angiotensin II, ACE; angiotensin converting enzyme, MIF; macrophage migration inhibitory factor, TGFβ; transforming growth factor β, TNFα; tumor necrosis factor α

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Abstract

The mechanisms by which alcohol causes pancreatic fibrosis remain unknown. Recent studies have demonstrated that angiotensin II contributes to the development of fibrosis in liver, kidney, and heart injury. Here, the effects of angiotensin converting enzyme inhibitor (captopril) and angiotensin II receptor antagonist (losartan) on alcohol-induced pancreatic fibrosis were examined in an intragastric ethanol feeding model. Male rats were fed a high-fat liquid diet with either ethanol (16–20 g/kg/day) or isocaloric maltose-dextrin (control) for 4 weeks. Subgroups daily received captopril (60 mg/kg/day), losartan (3 mg/kg/day) or no additional agent included in liquid diets. Mean urine alcohol concentrations in all groups fed ethanol were more than 270 mg/dl and not significantly different. Dietary alcohol caused diffuse gland atrophy and interlobular and intralobular fibrosis with mild structural distortion in the pancreas, effect which were blunted by captopril or losartan treatment. Alcohol also increased the number of α-smooth muscle actin positive cells and transforming growth factor β mRNA expression in the pancreas. These increases were blunted significantly by captopril or losartan treatment. These data suggest that angiotensin II contributes to the development of chronic alcohol-induced pancreatic fibrosis through its stimulation of transforming growth factor β expression. (199 words)

A relationship between chronic alcohol abuse and pancreatitis is well described in numerous clinical studies, and alcoholism is now reported to be the dominant cause of chronic pancreatitis (Johnson and Hosking, 1991; Cavallini et al., 1994). Histological changes of alcohol-induced pancreatitis are characterized by acinar cell loss, islet cell loss, inflammatory cell infiltration and irregular fibrosis (Etemad and Whitcomb, 2001). Of these changes, the severity of pancreatic fibrosis appears to a major determinant of the prognosis of chronic alcoholic pancreatitis, because of its irreversibility (Ammann and Muellhaupt, 1999). There are currently no approved therapeutic options designed to delay or reverse the progression of pancreatic fibrosis because the mechanisms of pathophysiology are still largely unknown.

Angiotensin II (AgII), generated by endothelial and circulating angiotensin converting enzyme (ACE), is a classic endocrine hormone that plays a central role in the regulation of blood pressure and sodium homeostasis (Gavras and Gavras, 2002). AgII is also known to have a number of blood pressure-independent actions including mitogenic and trophic effects on cell growth. Indeed, the profound protection observed with inhibitors of the renin-angiotensin system (RAS) in the Heart Outcomes Prevention Evaluation (HOPE) study, appeared to mediated more by direct prevention of cardiac remodeling, than by their blood pressure lowering effects (Yusuf et al., 2000; Sleight et al., 2001). Recent in vivo studies have demonstrated that components of local renin-angiotensin systems may be involved in remodeling in other organs. For example, remodeling (i.e., fibrosis) in liver, kidney, lung, and skin have also been linked to AgII activity in experimental animal models and/or in human studies (Molteni et al., 1985; Jonsson et al., 2001; Brown et al., 2002; Kawaguchi et al., 2004). It has been shown that AgII contributed to the development of fibrosis by enhancing the production of transforming growth factor (TGFβ), a potent fibrogenic cytokine via AT_1 receptors (Ardaillou, 1999). Therefore,

local expression of the renin-angiotensin system appears to play key roles in tissue remodeling and scarring, especially after injury.

In addition to the organs mentioned above, previous studies have shown that key elements of the renin-angiotensin system (e.g., angiotensinogen and renin) are also present in the pancreas [see (Leung, 2001), for review]. Further, these factors are upregulated in experimental models of pancreatitis (Tsang et al., 2004). The activation of the pancreatic renin-angiotensin system could play a role in the pathophysiology of the pancreatitis, especially fibrosis (Leung and Chappell, 2003; Kuno et al., 2003). Indeed, AT_1 receptor knockout mice were recently shown to be protected against caerulein-induced pancreatic fibrosis (Nagashio et al., 2004). On this basis, it was hypothesized that AgII is involved in alcohol-induced pancreatic fibrosis via induction of profibrotic cytokines (e.g., TGFβ) through the AT_1 receptor. In the present study, the long-term intragastric ethanol feeding protocol was used with modifications to increase the volume of ethanol administered to develop a model of pancreatic fibrosis (Kono et al., 2001). To investigate the role of AgII in alcohol-induced pancreatic fibrosis, the effects of ACE inhibitor (captopril) or an AT_1 receptor antagonist (losartan) on the development of alcohol-induced pancreatic fibrosis were examined.

Methods

Animals and treatments

Male Wistar rats (220-240g) were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care. All animals received humane care in compliance with institutional guidelines. Rats were fed a high-fat liquid diet with or without ethanol continuously for 4 weeks using the intragastric enteral feeding protocol developed by Tsukamoto and French with modification to increase the volume of ethanol administered (Tsukamoto et al., 1984; French et al., 1986). Behavior was assessed using a 0-3 scoring system (0, normal; 1, sluggish movement; 2, loss of movement but still moving if stimulated; 3, loss of consciousness). Based on this score, alcohol administration was then carefully adjusted to prevent overdosing. Ethanol initially was delivered at 16 g/kg/day and was increased 1 g/kg/3 days until the end of the second week, then it was maintained near 20 g/kg/day for the last two week of the experiment. A liquid diet described by Thompson and Reitz (Thompson and Reitz, 1978) supplemented with lipotropes as described by Morimoto et al. (Morimoto et al., 1994) was used. It contained corn oil as fat (37% of total calories), protein (23%), carbohydrate (5%), minerals and vitamins, plus ethanol (35 to 40 % of total calories) or isocaloric dextrose (control diet) as described elsewhere (Tsukamoto et al., 1990).

After surgery for intragastric tubing, rats were assigned to 1 of the 3 following treatment groups: (1) no additional agents: (2) captopril (60 mg/kg/day) added to the liquid diet beginning 3 days before initiation of liquid diet; (3) losartan (3 mg/kg/day) added to the liquid diet beginning 3 days before initiation of liquid diet. Treatment group sizes were n=4 and n=6 for those receiving high-fat control and ethanol-containing diets, respectively. The doses of

captopril and losartan were selected in pilot dose-finding studies and had no noticeable deleterious effects on behavior or general health.

Clinical chemistry

Ethanol concentrations in urine, which are representative of blood alcohol levels (Badger et al., 1993), were measured daily. Rats were housed in metabolic cages that separated urine from feces, and urine was collected over 24 hours in bottles containing mineral oil to prevent evaporation. Each day at 9 A.M., urine collection bottles were changed and a 1 mL sample was stored at -20°C in a microtube for later analysis. Ethanol concentration was determined by measuring absorbance at 366 nm resulting from the reduction of NAD^+ to NADH by alcohol dehydrogenase (Bergmeyer, 1988). Blood was collected via the aorta after 4 weeks enteral feeding under anesthesia with sodium pentobarbital (75 mg/kg i.p.) just prior to sacrifice (by exanguination) and centrifuged. Serum was stored at -80°C until it was assayed for amylase using analytical kits (Sigma Chemical, St. Louis, MO).

Histological analysis

Tissues were collected at sacrifice after 4 weeks of enteral feeding. Portions of pancreatic tissues were fixed in 10 % formalin, snap frozen in liquid nitrogen and stored at -80°C, or immediately extracted for RNA analyses (see below). Formalin fixed, paraffin embedded sections were cut at 6 μm and mounted on glass slides. Sections were deparaffinized and stained with hematoxylin-eosin. Pathologic changes were assessed in blinded manner. Collagen accumulation in pancreatic sections was determined by staining for Masson's trichrome and Sirius red-fast green (Lopez-De Leon and Rojkind, 1985).

Immunohistochemistry for α-smooth muscle actin (α-SMA) was performed on 6 μm sections of formalin-fixed, paraffin-embedded pancreas according to supplier's instructions.

Sections were deparaffinized in xylene, rehydrated in a series of graded alcohol concentrations and placed in phosphate buffered saline with 1% Tween 20. Staining was performed with a monoclonal primary mouse anti-α-smooth muscle actin antibody (Dako, Carpinteria, CA) and followed by peroxidase labeling using EnVision kit (Dako, Carpinteria, CA). The primary antibody was diluted 1:200 with 1% bovine albumin (Sigma, St. Louis, MO) in PBS. After immunohistochemistry, samples were lightly counterstained with hematoxylin.

Sirius red staining and α-SMA immunohistochemistry were quantitated by image analysis. Specifically, a Universal Imaging Corp. Image-1/AT image acquisition and analysis system (Chester, PA) incorporating an Axioskop 50 microscope (Carl Zeiss, Inc., Thornwood, NY) was used to capture and analyze 10 non-overlapping fields per section at 200× (for Sirius red) or 400× (αSMA) final magnification. Image analysis was performed using modifications of techniques described previously (McKim et al., 2003). Detection thresholds were set for the red (Sirius red) or brown (αSMA) color based on an intensely labeled point and a default color threshold. The degree of labeling in each section was determined from the area within the color range divided by the total area.

RNase Protection Assay.

Total RNA was isolated from liver tissue using RNA STAT 60 (Tel-Test). RNase protection assays were performed using the RiboQuant multiprobe assay system (Pharmingen) or individual probes. In short, using multiprobe template sets for TNFα and TGFβ (rCK-3) or a single probe template for collagen I, [^{32}P] RNA probes were transcribed with T7- or SP6-polymerase followed by phenol:chloroform extraction and ethanol precipitation. Twenty micrograms of total RNA per sample was hybridized to 3.4 x 10^5 cpm of probe overnight at 56°C and digested with RNase followed by proteinase K treatment and phenol: chloroform extraction, ethanol and

ammonium acetate precipitation. Samples were then resolved on 5% acrylamide-bisacrylamide (19:1) urea gels and visualized by autoradiography after drying. Bands corresponding to the protected labeled fragment were quantitated by scanning densitometry software (Image Quant 5.0, Molecular Dynamics, CA), and where statistical data are given, normalized to the level of L32 as the housekeeping gene.

Statistics

Data are presented as mean ± SEM. Two-way ANOVA with Bonferroni's post-hoc test was used for the determination of statistical significance between treatment groups. A *p* value less than 0.05 was selected before the study as the level of significance.

Results

Body weights.

All animals survived surgery, and liquid diets were initiated after 1 week to allow for complete recovery. Steady weight gains were observed during 4 weeks of continuous enteral feeding of liquid diets with or without ethanol, indicating adequate nutrition (Table I). There were no significant differences in weight gains among the groups studied. There were no apparent adverse side effects of captopril or losartan.

Urine alcohol concentration.

In animals fed high-fat control diet, inclusion of either captopril or losartan in the diet had a diuretic effect, as expected; daily urine output increased from ~15 ml/d to ~50 ml/d. Inclusion of ethanol alone in the diet also increased urine output to ~50 ml/d; captopril or losartan did not noticeably increase urine output in the presence of ethanol. Representative plots of daily urine alcohol concentrations in rats fed ethanol are depicted in Figure 1. As reported in previous studies (Tsukamoto et al., 1985; Badger, Crouch, Irby, and Shahare, 1993), urine alcohol levels fluctuated in a cyclic pattern during enteral ethanol feeding and similar patterns were observed in all groups (Figure 1). Mean urine alcohol concentrations over the course of the 4 week study period were >270 mg/dl and not significantly different between the groups (Table I).

Serum enzymes and histology.

In rats fed a high-fat control diet for 4 weeks, values of serum α-amylase were similar with those of rats fed chow (Table I). Although there was a slight increase in amylase in animals fed ethanol alone, there were no significant differences in serum α-amylase among the groups studied (Table I). There were no pathological changes seen in rats fed high fat control diet (Figure 2A). Mild acinar steatosis was observed in rats from all groups fed ethanol (Figure 2B,

C and D). Diffuse gland atrophy and distortion of pancreatic structure were observed in rats fed ethanol for 4 weeks (Figure 2B). These changes were prevented by captopril or losartan treatment (Figure 2C and D). In agreement with serum enzymes, neither hemorrhage nor massive infiltration of inflammatory cells was observed in rats from any groups studied, indicating that inflammation and necrosis were mild under the conditions tested.

Fibrogenesis in the pancreas.

Figure 3 shows representative photomicrographs of pancreata stained with Masson's Trichrome (Panels A-D) and Sirius red (Panels E-H) as indices of collagen deposition in the pancreas. In the pancreata of rats fed high fat control diet (Figure 3A and E), no staining was only detected around vessels and pancreatic ducts; inclusion of captopril or losartan in the diet had no significant effect under these conditions (not shown). In contrast, inclusion of ethanol in the diet increased both intralobular and interlobular staining changes after 4 weeks of enteral feeding (Figure 3B and F). Captopril (Figure 3C and G) or losartan (Figure 3D and H) treatment blunted these changes caused by alcohol; pancreata under these conditions appeared similar to animals fed high-fat control diet to levels similar to animals fed control diet. To evaluate fibrogenic change, Sirius red staining was quantitated with image-analysis (Figure 5A). The amount of Sirius-red positive staining increased ~7-fold in pancreata of rats fed ethanol diet for 4 weeks (Figure 5A); this effect was blunted significantly by concomitant captopril or losartan treatment (Figure 5A).

Immunohistochemical staining for α-smooth muscle actin.

Fibroblast-like cells immunoreactive for α-SMA are considered to be activated myofibroblasts. Pancreatic tissue from rats fed high fat diet contained no interstitial α-SMA positive staining cell except faint staining of vascular smooth muscle cells (Figure 4A). In

contrast, dietary ethanol increased the number of α-SMA positive staining cells and intensity of staining in the periacinar space (Figure 4B) and in the islets of Langerhans (Figure 4C). Periacinar α-SMA immunoreactive cells (myofibroblasts) displayed an elongated shape (Figure 4B). However, captopril or losartan treatment reduced significantly both the number of α-SMA positive staining cells and intensity of staining (Figure 4D and E). These changes were also quantitated with image-analysis (Figure 5B). The amount of α-SMA positive staining increased ~7-fold in pancreata of rats fed ethanol diet for 4 weeks (Figure 5B) and was blunted significantly by concomitant captopril or losartan treatment (Figure 5B).

Collagen I and cytokine mRNA in the pancreas.

Collagen I mRNA levels in the pancreas were similar in all groups fed high-fat control diets after 4 weeks (Figure 6). In rats fed ethanol for 4 weeks, collagen I mRNA expression was increased significantly over high-fat controls (Figure 6; lanes 4-6). This increase was blunted by ~80% (lanes 7-9) and ~60% (lanes 10-12) by concomitant captopril or losartan treatment, respectively. Levels of TGF-β, tumor necrosis factor α (TNFα) and macrophage migration inhibitory factor (MIF) mRNA in the pancreas were similar in groups fed high-fat control diets after for 4 weeks (Figure 7A and B). In rats fed ethanol for 4 weeks, TGF-β mRNA expression was increased significantly over high-fat controls (Figure 7). Levels of TNFα and MIF were also elevated under these conditions; these effects of ethanol were all blunted significantly in captopril or losartan treatment (Figure 7).

Discussion

Enteral alcohol causes fibrosis in rat pancreas

Therapies for alcohol-induced chronic pancreatitis are limited, due in part to the lack of understanding of the mechanism(s) leading to this disease. The previous lack of an appropriate animal model to study this disease has contributed to this problem. Previously, Tsukamoto et al. (Tsukamoto et al., 1988) employed an intragastric enteral feeding protocol in the rat to develop a model of alcohol-induced pancreatitis. In that study, enteral ethanol (8-15 g/kg/day) with high-fat diet caused atrophy and apoptosis in pancreatic acinar cells; however, fibrosis was present in only about 30% of animals given ethanol chronically for 30-160 days. The severity of clinical alcohol-induced pancreatitis and fibrosis is known to be related to the dose- and time-dependent alcohol consumption in man (Durkec and Sarles, 1978). Kono et al. (2001) demonstrated the pathological changes characteristic of the initial stages of chronic alcoholic pancreatitis in humans as early as 4 weeks after enteral ethanol with modification of this model to deliver more ethanol (10-18 g/kg/day). Here, the dose of alcohol was progressively increased from 16 to 20 g/kg/day in the first 2 weeks by careful monitoring of intoxication and maintained at 20 g/kg/day for the rest of the experiment. This high dose of enteral ethanol induced diffuse gland atrophy and mild interlobular and intralobular fibrosis with mild structural distortion in the pancreas at four weeks (Figure 2B, 3B, and 3F). Since weight gain was similar in all groups, the observed pancreatic changes are unlikely to be due to morbidity of the alcohol doses employed.

In the current study, ethanol induced profibrotic changes and activation of myofibroblasts occurred in the absence of any significant inflammatory reaction. Indeed, the observed changes occurred without a dramatic increase in serum amylase activity (Table I) often associated with clinical expression and models of acute pancreatitis (Pandol et al., 1999). During chronic

alcoholic pancreatitis, patients often do not now show elevated serum enzymes, unless the disease is complicated with bouts of acute gland damage (Etemad and Whitcomb, 2001). It is therefore likely that this model represents the chronic alcoholic pancreatitis, which is predominantly characterized by fibrosis (Etemad and Whitcomb, 2001).

Role of TGFβ and pancreatic stellate cell activation in alcohol-induced pancreatic fibrosis.

Myofibroblasts are phenotypically fibroblast-like cells that express α-smooth muscle actin (α-SMA) and extracellular matrix such as collagen (Desmouliere, 1995). In the liver, transformation of stellate cells into myofibroblasts represents a critical step in the progression of hepatic fibrosis (Bissell, 2001). Recent studies have indicated that pancreatic stellate cells also undergo a similar transformation to a myofibroblast phenotype during pancreatic fibrosis (Bachem et al., 1998). When activated, the pancreatic myofibroblasts proliferate and generate a large amount of extracellular matrix materials including fibril-forming collagens, fibronectin, and αSMA (Vaquero et al., 1999; Masamune et al., 2003). TGFβ plays a dominant role in the development of fibrosis in a number of organs and directly stimulates myofibroblast transformation (e.g., Overall et al., 1989).

Previous studies reported that TGFβ induces fibroblast proliferation and gland atrophy in the pancreas and promotes development of fibrosis after repeated courses of acute pancreatitis in mice (Lee et al., 1995; van Laethem et al., 1996). Moreover, administration of a neutralizing antibody against TGFβ reduces fibronectin expression in the pancreas from rats with caerulein induced pancreatitis (Saotome et al., 1997). In the current study, dietary ethanol increased significantly expression of TGFβ mRNA in the pancreas (Figure 7). Taken together these data indicate that chronic enteral alcohol feeding activates pancreatic stellate cells, leading to their

transformation to profibrotic myofibroblast cells, most likely via AngII stimulation of TGFβ production.

Inhibition of renin-angiotensin system attenuates alcohol-induced pancreatic fibrosis

As mentioned above, it has been shown that AgII plays an important role in cardiac, renal, and hepatic fibrosis in certain pathological states in association with tissue repair. A similar role of AgII has been shown in spontaneous pancreatic fibrosis in Wistar Bonn/Kobori rats (Yamada et al., 2003; Kuno et al., 2003). In the current study, pharmacologic blockade of the renin-angiotensin system prevented significantly the progression of alcohol-induced pancreatic fibrosis (Figure 3, 4), indicating that AgII is also a key mediators of fibrogenesis due to alcohol in the pancreas. Previous in vitro studies have shown that AgII can directly stimulate transcription as well as bioactivation of TGFβ1 in vascular smooth muscle cells, fibroblasts, and endothelial cells (Weber et al., 1999). Furthermore, in various in vivo models, AgII-mediated TGFβ1 induction has been shown to contribute to progression of fibrotic disease through activation and proliferation of myofibroblasts (Mezzano et al., 2001; Williams, 2001). Importantly, Nagashio et al. (2004) recently showed that expression of αSMA and AT_1 receptor expression colocalized in pancreata of mice during caerulein-induced pancreatic fibrosis. Furthermore, it was shown in that study that AT_1 receptor-deficient mice were protected against experimental pancreatic fibrosis. Thus, similar to other organs, there are accumulating data implicating the RAS, via activation of the AT_1 receptors, in remodeling/fibrosis in pancreas.

In this study, the number of α-SMA positive staining cells increased by ethanol were blunted significantly by ACE inhibition (captopril) or AgII receptor blockade (losartan). Furthermore, TGFβ mRNA levels were strikingly reduced in the pancreata of rats treated with captopril or losartan (Figure 7). Similar protective effects of these drugs have been observed in other models

of fibrosis [e.g., lung and heart, (Kanda et al., 1995; Uhal et al., 2003)] and are in line with previous studies with RAS inhibitors in other models of pancreatic fibrosis (Kuno et al., 2003; Yamada et al., 2003). These data suggest that blockade of renin angiotensin system prevents alcohol-induced pancreatic fibrosis by inhibition of activation and proliferation of pancreatic myofibroblasts and by reduction of TGFβ production, analogous to previous findings in other organs. Previous work may suggest a mechanistic link between alcohol and the pancreatic renin-angiotensin system. Specifically, Ip et al. (2002) demonstrated that chronic global hypoxia leads to upregulation of the pancreatic renin-angiotensin system. Previous work from this group has shown that alcohol causes a similar level of chronic hypoxia in pancreas as does global hypoxia (McKim et al., 2003). Therefore, it is possible that the hypoxia caused by ethanol is mediating the effects observed here.

In conclusion, the animal model presented here is the first to demonstrate the prevention of the progression of alcohol-induced pancreatic fibrosis by blockade of the renin-angiotensin system. The implications of these findings are compelling since captopril and losartan have been used in the clinics for several years and have been proven to be safe drugs with a low incidence of serious side effects. These data indicate that pharmacological regulation of the renin-angiotensin system may be useful in the treatment of chronic alcoholic pancreatic fibrosis.

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Footnotes

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Figure legends

Figure 1. Representative plots of daily urine alcohol concentrations from rats fed ethanol. Urine alcohol concentrations were measured daily as described in Methods. Typical urine alcohol concentrations in rats fed high-fat liquid diet with ethanol **(A)**, ethanol plus captopril **(B)**, and ethanol plus losartan **(C)** are shown.

Figure 2. Representative photomicrographs of pancreata from rats after 4 weeks of liquid diets. Rats were treated as described in Methods. Representative photomicrographs (Hematoxylin and Eosin stain; 200×) of pancreata from rats with different treatments are shown. (**A**), high-fat control diet; **(B)** high-fat ethanol diet; **(C)** high-fat ethanol diet with captopril treatment; (D) high-fat ethanol diet with losartan treatment. Panel (E) is the same treatment as panel (B), but at higher magnification (400×).

Figure 3. Trichrome or Sirius-red staining of pancreata from rats after 4 weeks of liquid diets. Pancreatic tissues were stained with trichrome **(A, C, E, G)** or Sirius-red and fast-green **(B, D, F, H)** as described in Methods. Representative photomicrographs (200×) of pancreata from rats with different treatments are shown. (**A, B**), high-fat control diet; **(C, D)** high-fat ethanol diet; **(E, F)** high-fat ethanol diet with captopril treatment; **(G, H)** high-fat ethanol diet with losartan treatment.

Figure 4. Immunohisochemical detection of α-smooth muscle actin (αSMA) in liver. Immunohistochemical detection of αSMA protein was performed as described in Methods. Representative photomicrographs (40×) of pancreata from rats with different treatments are shown. (**A**), high-fat control diet; **(B, C)** high-fat ethanol diet; **(D)** high-fat ethanol diet with captopril treatment; **(E)** high-fat ethanol diet with losartan treatment.

Figure 5. Quantitation of Sirius red and αSMA in the pancreas from rats after 4 weeks of liquid diets. Sirius-red- (panel A) and αSMA- (panel B) positive areas were determined by image analysis as described in Methods. Values are mean ± SEM (n = 4-6). [a], $p < 0.05$ compared with rats fed high-fat control diet; [b], $p < 0.05$ compared with rats fed ethanol with no additional agents by two-way ANOVA with Bonferroni's post-hoc test.

Figure 6. Collagen α1(I) mRNA expression in the pancreas from rats after 4 weeks of liquid diets. Pancreatic tissues were assayed for fibrillar collagen α1(I) mRNA using an RNase protection assay with L32 as the housekeeping gene as described in Methods. Lane1, high-fat control diet; lane2, high-fat control diet with captopril treatment; lane 3, high-fat control diet with losartan treatment, lane 4-6, high-fat ethanol diet; lane 7-9, high-fat ethanol diet with captopril treatment; lane 10-12, high-fat ethanol diet with losartan treatment.

Figure 7. TGF-β mRNA expression in the pancreas from rats after 4 weeks of liquid diets. Pancreatic tissues were assayed for TGF-β, tumor necrosis factor α (TNFα) and macrophage migration inhibitory factor (MIF) mRNA by an RNase protection assay using a cytokine template set as described in Methods (Panel A). Lane1, high-fat control diet; lane2, high-fat control diet with captopril treatment; lane 3, high-fat control diet with losartan treatment, lane 4-6, high-fat ethanol diet; lane 7-9, high-fat ethanol diet with captopril treatment; lane 10-12, high-fat ethanol diet with losartan treatment. Representative autoradiogram. Specific bands were quantitated by scanning densitometry and normalized to the signal of L32 (Panel B). Data presented are mean ± SEM as % of control (n = 4-6). [a], $p < 0.05$ compared with rats fed high-fat

control diet; [b], $p < 0.05$ compared with rats fed ethanol with no additional agents by two-way ANOVA with Bonferroni's post-hoc test.

Table I. Effect of chronic ethanol on routine parameters in rats

	High-fat control			High-fat ethanol		
	No agent	**Captopril**	**Losartan**	**No agent**	**Captopril**	**Losartan**
Weight gain (g/day)	3.2 ± 0.1	3.2 ±0.2	3.1 ± 0.1	2.9 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
[Urine alcohol] (mg/dL)	N/A	N/A	N/A	274 ± 8	277 ± 6	286 ± 4
Serum α-Amylase (IU/ml)	828 ±63	886 ± 21	1027 ± 37	1381 ± 208	920 ± 76	1130 ± 56

Urine alcohol concentrations were measured daily as described in Methods. Serum α-amylase was measured as described in Materials and Methods using blood collected via the aorta at 4 weeks. Values are means ± SEM. High-fat control; n=4, high-fat ethanol; n=6.

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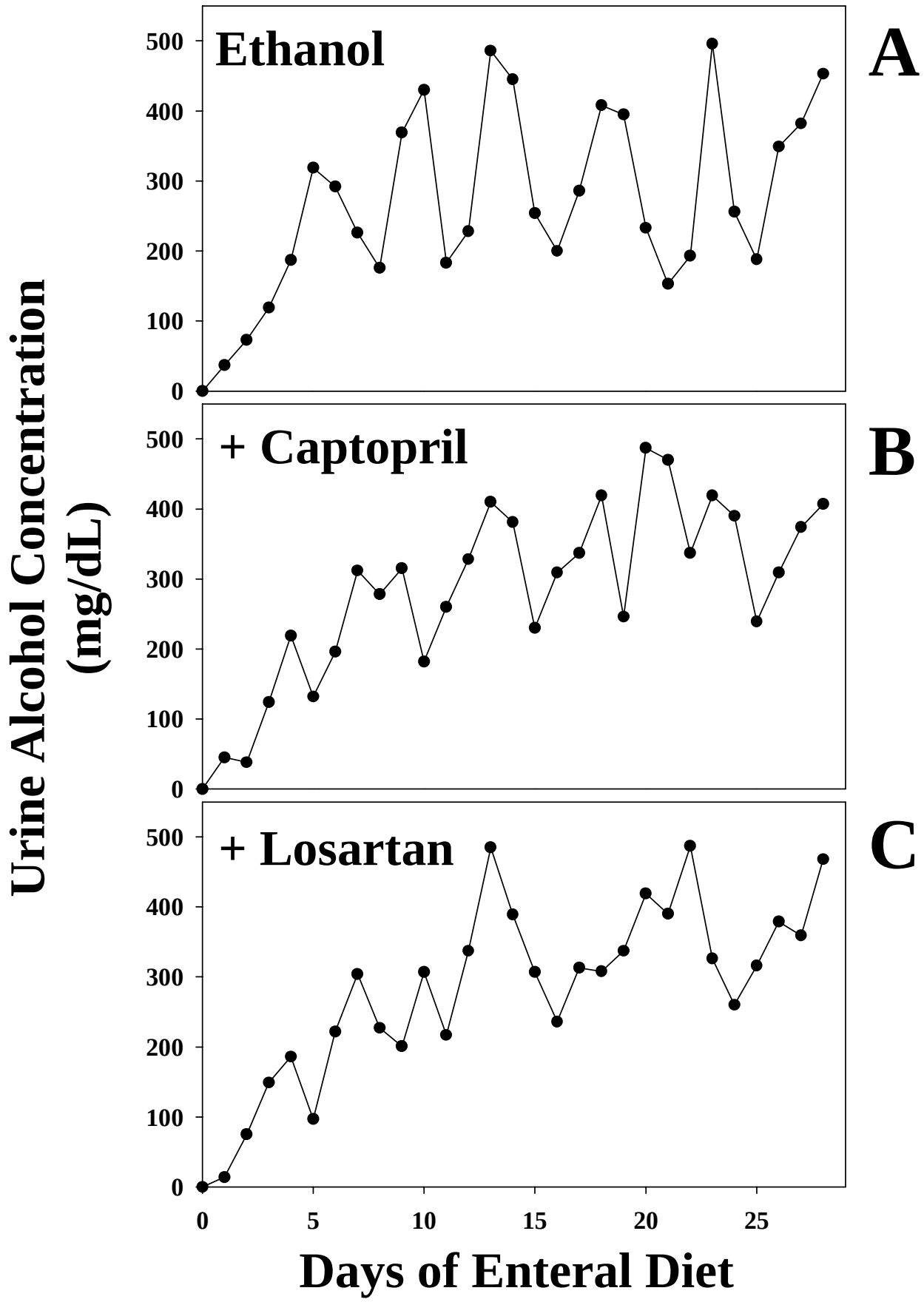

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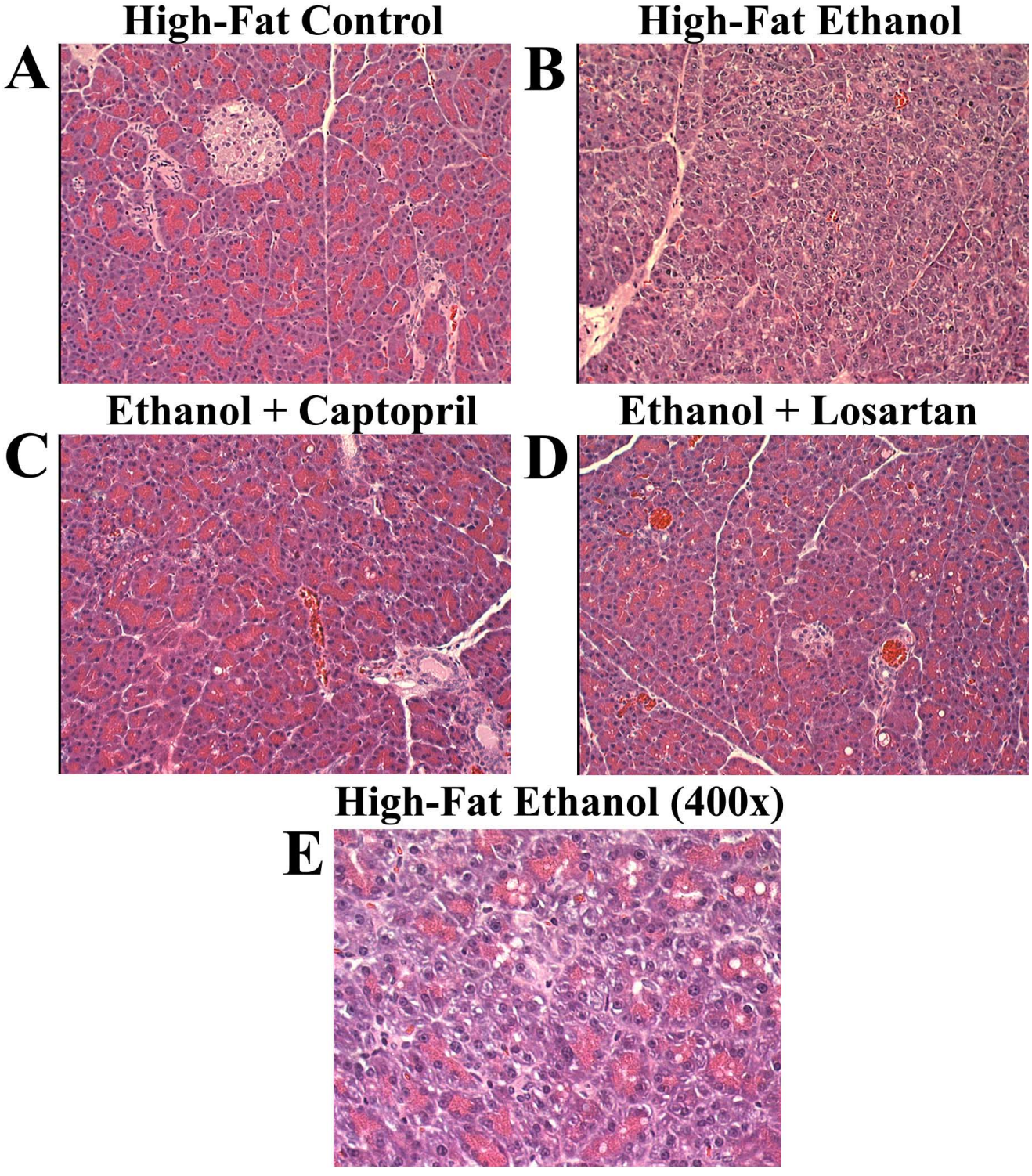

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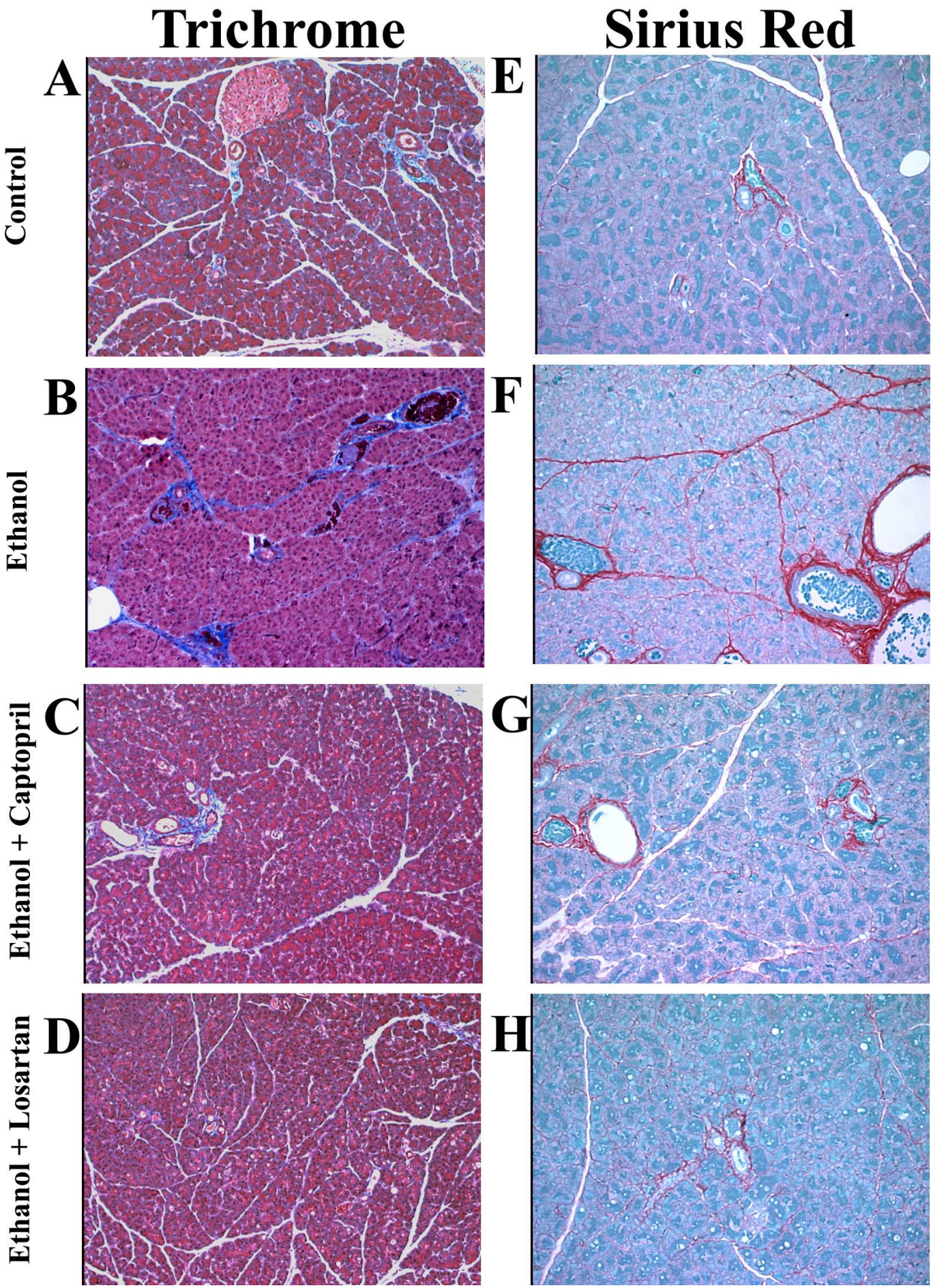

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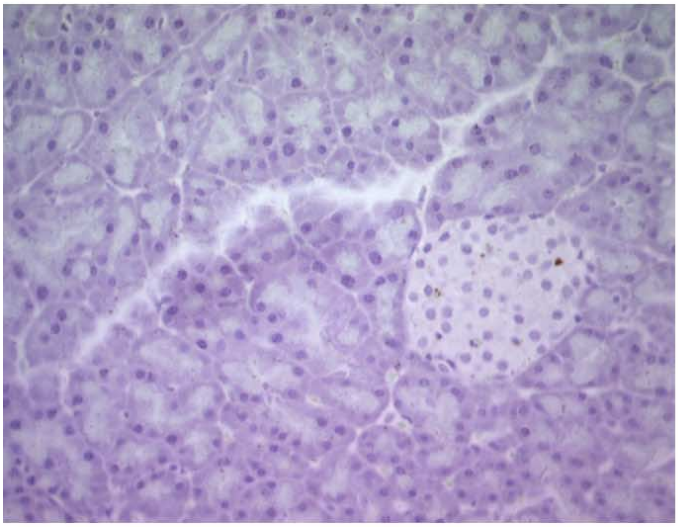
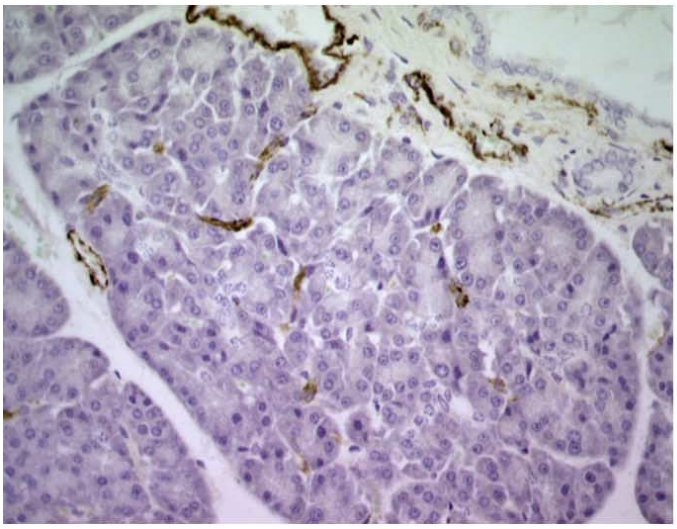
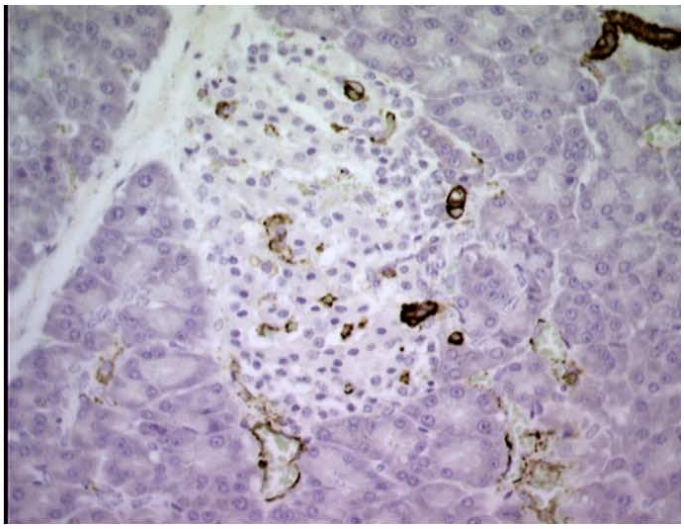
A) High-fat control



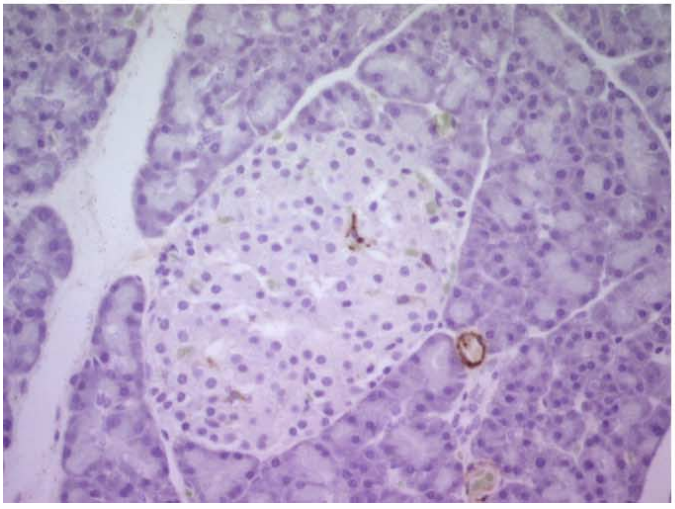
B) High-fat ethanol (Acinar)



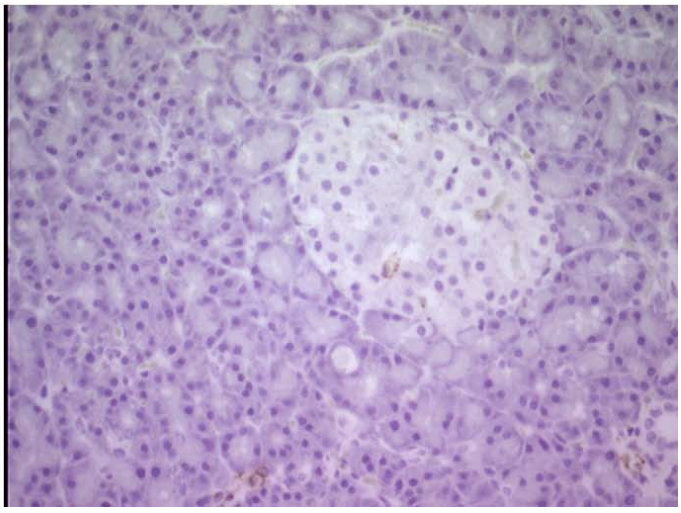
C) High-fat ethanol (Islet)



D) Ethanol + Captopril



E) Ethanol + Losartan



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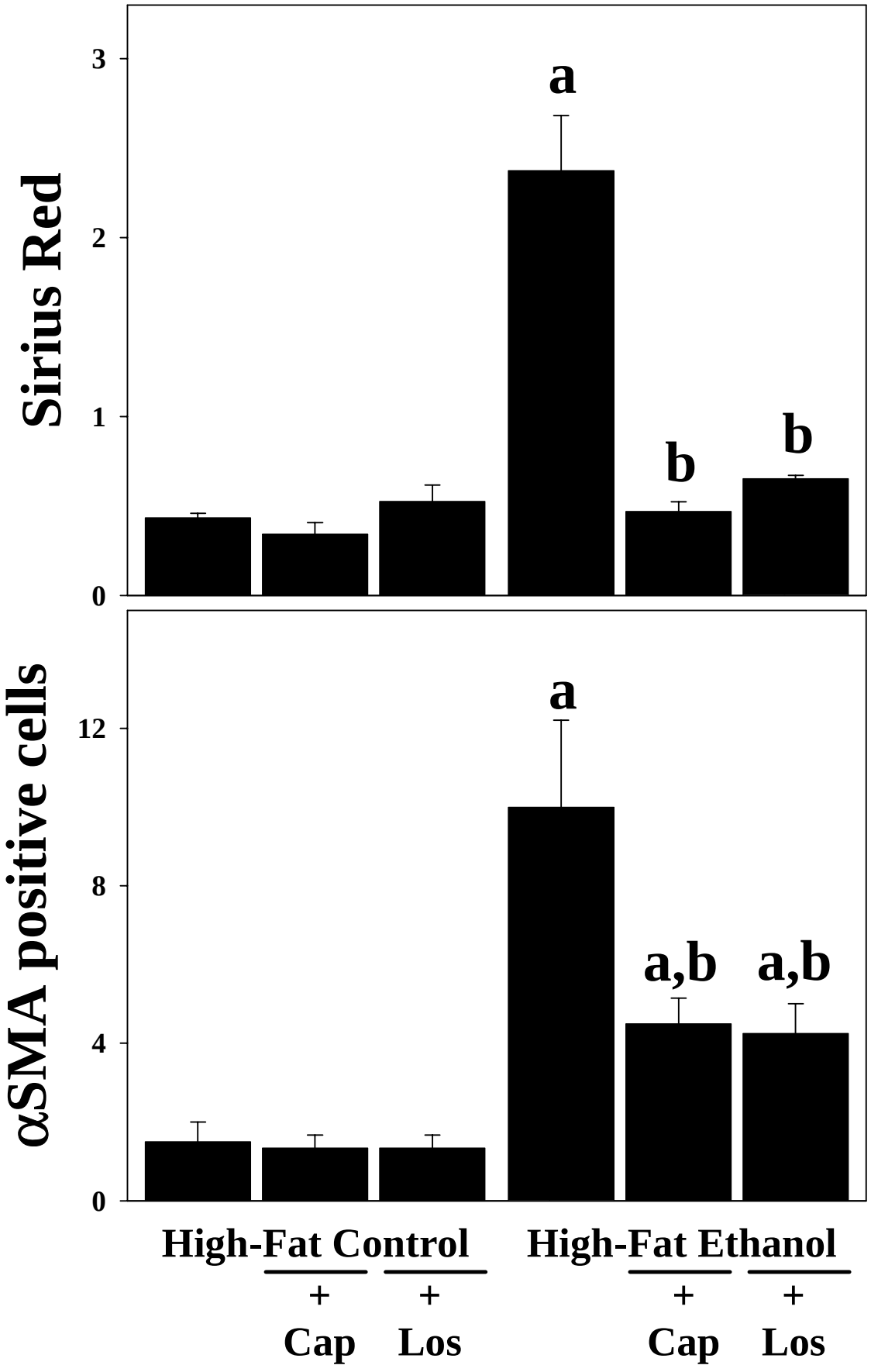

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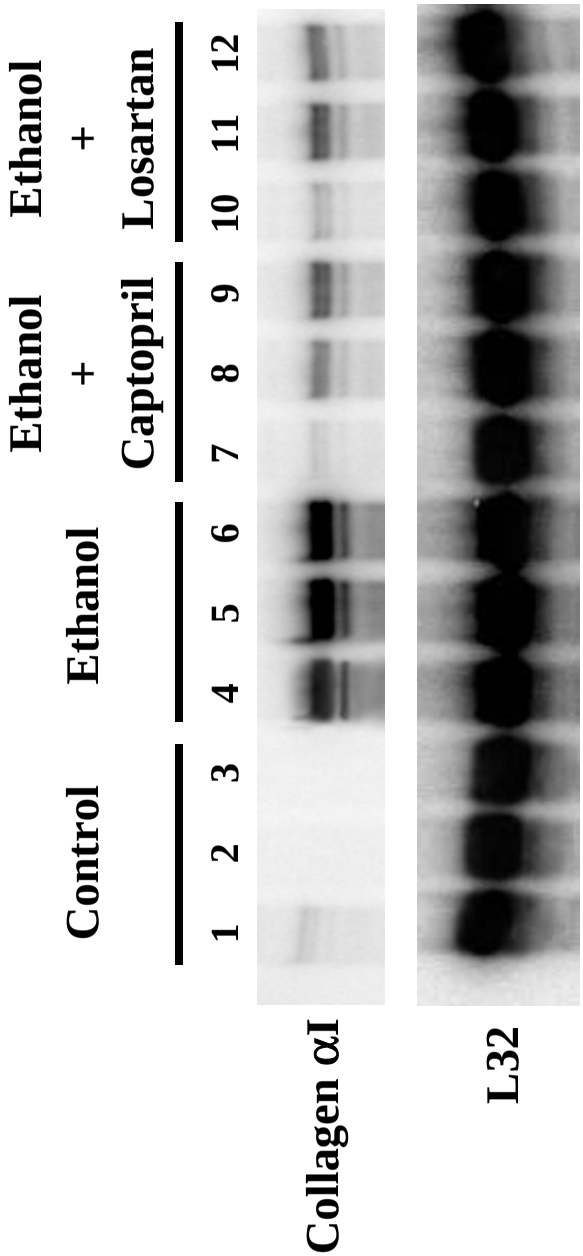

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