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Liver regeneration is suppressed in alcoholic cirrhosis: Correlation with decreased STAT3 activation

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Abstract

Liver regeneration is suppressed in alcoholic patients; however, the underlying mechanisms are not fully understood. We examined liver regeneration and STAT3 activation (an important signal for liver regeneration) in cirrhotic livers from alcoholics, HCV infection, and alcoholic plus HCV infection. Liver regeneration and STAT3 activation were determined by immunohistochemistry analysis of Ki67 and STAT3 phosphorylation, respectively, in 20 alcoholic cirrhosis, 13 HCV cirrhosis, 13 alcoholic+HCV cirrhosis. Alcoholic or alcoholic plus HCV cirrhotic livers had significantly lower Ki67⁺ and pSTAT3⁺ hepatocytes and bile duct cells than HCV cirrhotic livers. The pSTAT3 positive staining did not correlate with liver injury (elevation of serum levels of aspartate transaminase (ALT) and alkaline phosphatase (ALP) but correlated positively with cell proliferation (Ki67 positive staining). In conclusion: Liver regeneration is suppressed in alcoholic cirrhotic livers, which may be partly due to decreased STAT3 activation.

Keywords

alcohol; STAT3; liver regeneration; cirrhosis; HCV; cell proliferation

Introduction

Chronic alcohol consumption is one of the major causes of liver disease worldwide (Williams, 2006). In the last several decades, cellular and molecular mechanisms underlying alcoholic liver injury have been extensively investigated. It is generally believed that the hepatotoxicity induced by alcohol is mediated by complex mechanisms including induction of oxidative stress and lipid peroxidation, alteration of methionine metabolism, immunogenic processes initiated by formation of protein adducts of acetaldehyde, and increasing endotoxin sensitivity (Arteel, 2003; Dey and Cederbaum, 2006; Lumeng and Crabb, 2001; Purohit and Brenner, 2006; Tsukamoto and Lu, 2001). In addition, impaired liver regeneration is also an important mechanism contributing to the pathogenesis of alcoholic liver disease. Chronic ethanol consumption has been shown to inhibit liver regeneration after partial hepatectomy in rodents (Diehl et al., 1988; Koteish et al., 2002; Orrego et al., 1981; Wands et al., 1979; Zhang et al.,

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1996). Clinical studies have showed that proliferating hepatocytes in alcoholic liver diseases (not cirrhotic) or alcohol plus HCV-mediate diseases (not cirrhotic) were lower than in viral liver damage (not cirrhotic), suggesting that liver regeneration is also suppressed in noncirrhotic patients with alcoholic liver diseases (Cardin et al., 2002; Farinati et al., 1996; Farinati et al., 2001).

The liver has the ability to regenerate after loss of tissue or injury via activation of a wide array of signaling and transcriptional factors (Fausto et al., 2006; Michalopoulos and DeFrances, 2005). Among them, signal transducer and activator of transcription 3 (STAT3), mainly activated by IL-6 and IL-22 in the liver, has been shown to play an important role in liver regeneration (Haga et al., 2003; Li et al., 2002), while STAT1, mainly activated by IFN- γ , inhibits liver fibrosis and regeneration (Jeong et al., 2006; Sun et al., 2006). IL-6 activation of STAT3 in the liver is also involved in induction of acute phase proteins and in protection of the liver against a wide variety of insults including ethanol (Gao, 2005; Hong et al., 2002; Hong et al., 2004; Sun et al., 2003). Although activation of STAT3 by IL-6 protects against ethanol-induced liver injury, both acute and chronic ethanol consumption have been shown to inhibit IL-6 activation of STAT3 in rodent livers. We have previously demonstrated that acute ethanol exposure inhibits IL-6 activation of STAT3 in freshly isolated rat hepatocytes (Chen et al., 2001; Chen et al., 1999) and rat livers (Chen et al., 1997), and chronic ethanol consumption attenuates IL-6 activation of STAT3 in rat hepatocytes (Chen et al., 1997) and rat sinusoidal endothelial cells (Gao, 2004). Therefore, ethanol inhibition of liver regeneration in rodents is mediated at least in part by impairing IL-6 activation of STAT3 in the liver. Starkel et al (2005) showed that STAT3 expression and phosphorylation was not altered in HCV and alcoholic cirrhosis, while STAT3 DNA-binding was not detected in all alcoholic and most HCV samples. Since whole liver tissues were used in their study, it was not possible to distinguish the cell type associated with altered STAT3 in these cirrhotic livers.

In the current paper, we examined the liver regeneration in alcoholic, HCV, alcoholic+HCV cirrhotic liver samples by immunostaining with Ki67 antibodies. STAT3 activation was also investigated in these samples by immunostaining with anti-phospho-STAT3 antibodies. Since atypical ductular cell proliferation also contributes to liver regeneration in cirrhotic livers (Fausto, 2004), both hepatocyte and ductular cell proliferation were examined and compared in all cirrhotic samples. Our results show that hepatocyte and bile ductular cell proliferation were suppressed in alcoholic cirrhotic or alcoholic+HCV cirrhotic livers compared to HCV cirrhotic livers, and this correlated with decreased STAT3 activation in alcoholic cirrhosis.

Materials and Methods

Materials

Anti-phospho-STAT3 (Tyr⁷⁰¹) antibody was purchased from Cell Signaling Technology (Beverly, MA). Anti-CK19 antibody was obtained from DAKO (Produktionsvej, Denmark). Anti-Ki67 antibody was purchased from Zymed Laboratories (San Francisco, CA).

Cirrhotic liver tissues and normal healthy liver controls

The Liver Tissue Procurement Distribution System (LTPDS, the Division of Pediatric Gastroenterology and Nutrition, University of Minnesota, Minneapolis, MN) provided 46 liver samples from patients with end-stage cirrhosis who received liver transplantation. These include 20 alcoholic cirrhosis, 13 HCV cirrhosis, and 13 alcoholic+HCV cirrhosis. The characteristics of these patients are listed in Table 1. Livers from patients with alcoholic or alcoholic +HCV cirrhosis, abstained from alcohol drinking for at least 3 months prior to sample collection. Alcoholic cirrhosis was diagnosed by LTPDS and based on a history of alcohol drinking and liver histology. Liver pathology of these specimens showed bridging fibrosis and

cirrhosis. Chronic HCV infection was confirmed histopathologically and serologically. Patient histories indicate that specimens were from patients who had not received IFN- α therapy in the 2 years prior to collection of the specimens. LTPDS also provided 16 normal healthy liver specimens obtained from human donor livers not used for transplantation. The serum levels of aspartate transaminase (AST) and alkaline phosphatase (ALP) were also provided by the LTPDS. The LTPDS was funded by NIH Contract #N01-DK-9-2310. The protocol for using these liver samples has been approved by the Liver Tissue Procurement and Distribution System of the University of Minnesota and the National Institutes of Health.

Immunohistochemical staining with pSTAT3, Ki67 and doublestaining with pSTAT3 and CK19 antibodies

Immunostaining for pSTAT3 or Ki67 was performed on formalin-fixed, paraffin-embedded sections using anti-pSTAT3 antibody or anti-Ki67 antibody. For pSTAT3 immunostaining, antigen retrieval was performed by microwave for 10 minutes in 10 mmol/L sodium citrate buffer and subsequent cooling at 25°C for 30 min. Phospho-STAT3 antibody was incubated at a dilution of 1:25 overnight at 4°C and immunodetection was performed using the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC-Elite kit; Vector Laboratories, Burlingame, CA) with 3,3'-diaminobenzidine tetrahydrochloride (DAB). For Ki67 immunostaining, the procedure was the same as pSTAT3 staining except for antigen retrieval that was performed by boiling the sections at 98°C for 20 minutes in 10 mmol/L sodium citrate buffer. For double immunostaining with pSTAT3 and CK19 antibodies, paraffin sections were immunostained with anti-pSTAT3 antibody as described above, followed by incubation with anti-CK19 primary antibody at a dilution of 1:50 overnight at 4°C and developed with DAB (nickel). To evaluate labeling index (percentage of positive nuclei), 3-5 fields per slide were randomly selected and determined by counting 500 hepatocyte nuclei and 300 bile duct nuclei in sequential high-powered fields (x400).

Statistical analysis

Data are expressed as means $\pm$ SEM. To compare values obtained from these groups, statistical analyses were performed using parametric (Student's *t* test) and non-parametric (Kruskal-Wallis and Mann-Whitney *U*) tests. All *p* values were two tailed and were considered significant if the associated value was less than 0.05. The correlations between variables were assessed by the Spearman rank order test. Statistical significance was taken at the *P* < 0.05 level.

Results

Liver regeneration is suppressed in alcoholic cirrhosis and alcoholic plus HCV cirrhosis compared to HCV cirrhosis

Table 1 shows that serum levels of AST are elevated in all of cirrhotic patients compared to normal healthy individuals (0-40 IU/L). Among cirrhotic patients, serum levels of AST are slightly higher in the patients with HCV plus alcoholic cirrhosis compared to the patients with alcoholic cirrhosis or HCV cirrhosis, but the difference did not reach statistical significance.

Liver regeneration in cirrhotic samples was determined by immunohistochemistry analysis of Ki67 staining, a marker of cell proliferation (Crary and Albrecht, 1998). As shown in Fig. 1A and Fig. 1B, the percentage of Ki67⁺ hepatocytes was higher in cirrhotic livers compared to normal healthy livers. HCV cirrhotic livers had the highest percentage Ki67⁺ hepatocytes when compared to alcoholic cirrhosis or HCV+alcoholic cirrhosis.

The Ki67⁺ bile duct cells were also compared in liver samples. As shown in Fig. 2, Ki67⁺ bile duct cells were detected more frequently in all cirrhotic livers compared to normal healthy

controls. HCV cirrhotic livers had the highest percentage of Ki67⁺ bile duct cells compared to alcoholic cirrhosis or alcoholic plus HCV cirrhosis.

Activation of STAT3 in human cirrhotic livers: suppression in alcoholic cirrhosis

The above data suggest that liver regeneration is suppressed in alcoholic cirrhosis. To understand the underlying mechanisms, we compared STAT3 activation in these cirrhotic liver samples by immunohistochemistry analysis of phospho-STAT3 (p-STAT3). As shown in Fig. 3A, pSTAT3 positive staining was rarely detected in normal healthy livers. In contrast, significant pSTAT3 positive staining was observed in all cirrhotic livers with staining in hepatocytes, bile duct cells, nonparenchymal cells, and inflammatory cells. pSTAT3⁺ hepatocytes were observed frequently around the inflammatory region. Fig. 3B shows that the percentage of pSTAT3⁺ hepatocytes in all 3 forms of cirrhosis was significantly higher than in normal healthy livers. Compared to HCV cirrhotic livers, alcoholic cirrhotic or alcoholic plus HCV cirrhotic livers had significantly lower percentage of pSTAT3⁺ hepatocytes.

We also examined the STAT3 phosphorylation in bile duct cells in liver samples. As shown in Fig. 4A, pSTAT3 was rarely detected in bile duct cells of normal healthy livers, whereas significant pSTAT3 staining was detected in the bile duct cells of HCV, alcoholic and HCV +alcoholic cirrhotic liver tissues. The pSTAT3 in bile duct like cells was also confirmed by double staining with anti-pSTAT3 and anti-CK19 antibodies. As shown in Fig. 4B, the left panel shows representative bile duct cells with pSTAT3 negative staining (CK19⁺pSTAT3⁻), and the right panel shows the representative bile duct like cells with pSTAT3 positive staining (CK19⁺pSTAT3⁺). The percentage of pSTAT3⁺ duct cells in all 3 forms of cirrhosis was significantly higher than in normal healthy livers, and that HCV cirrhotic livers had more pSTAT3⁺ bile duct cells than alcoholic cirrhosis and alcoholic plus HCV cirrhosis (Fig. 4C).

A positive correlation between pSTAT3⁺ and Ki67⁺ hepatocytes or Ki67⁺ bile duct cells

The hepatoprotective function of STAT3 has been well documented (Gao, 2005), and STAT3 α mRNA has been shown to correlate inversely with serum levels of alanine transaminase (Larrea et al., 2006). Next, we asked whether pSTAT3 in these samples had any correlation with liver injury (serum levels of AST) and cell proliferation (Ki67⁺ hepatocytes). As shown in Fig. 5A, pSTAT3⁺ hepatocytes had no correlation with serum levels of AST. In contrast, pSTAT3⁺ hepatocytes exhibited a positive correlation with Ki67⁺ hepatocytes (Fig. 5B, $p=0.002$).

STAT3 activation has also been implicated in bile duct cell survival and growth (Isomoto et al., 2005; Isomoto et al., 2007). Thus, we asked whether pSTAT3 had any correlation with the numbers of Ki67⁺ bile duct cells or serum levels of alkaline phosphatase (ALP) in cirrhotic livers. As shown in Fig. 6, the pSTAT3⁺ bile duct like cells exhibited a positive correlation with the number of Ki67⁺ bile duct cells but not with serum levels of ALP.

Discussion

Liver regeneration is suppressed in human alcoholic patients; however, the underlying mechanisms are not fully understood. In this investigation, we compared hepatocyte and bile ductal cell proliferation in end-stage of liver cirrhosis. Our findings show that cell proliferation is higher in all cirrhotic livers compared to normal healthy livers, suggesting that cirrhotic livers have the ability to regenerate. Although our current findings do not explain whether liver regeneration is suppressed in cirrhotic livers compared to the noncirrhotic livers with similar infections or alcohol abuse. Previous clinical studies have demonstrated that hepatocyte proliferation decreases with the severity of cirrhosis (Clouston et al., 2005; Delhay et al., 1996; Lunz et al., 2005), indicating that liver regeneration is suppressed in cirrhosis compared

to non-cirrhotic injured livers. In this study we observed that liver regeneration was suppressed in alcoholic cirrhosis or alcoholic plus HCV cirrhosis compared to HCV cirrhosis, and this correlates with decreased STAT3 activation. These findings suggest that impaired liver regeneration in alcoholic cirrhosis may, in part be due to decreased STAT3 activation.

Previously, Farinati and colleagues have reported that detection of proliferating hepatocytes were less frequent in noncirrhotic alcoholic or noncirrhotic alcoholic plus HCV than in noncirrhotic viral hepatitis (Cardin et al., 2002; Farinati et al., 1996; Farinati et al., 2001). In the present study, we demonstrate that the percentage of Ki67⁺ hepatocytes and Ki67⁺ bile duct cells were significantly lower in alcoholic cirrhosis or alcoholic cirrhosis plus HCV than in HCV cirrhosis. Together, these findings suggest that liver regeneration is suppressed not only in early stage but also in end-stage of alcoholic liver disease. However, the mechanisms underlying alcohol suppression of liver regeneration in alcoholic patients are not poorly understood.

Our results show that cirrhotic livers exhibited increased pSTAT3 staining in hepatocytes, bile duct like cells, nonparenchymal cells, and inflammatory cells. Most pSTAT3⁺ hepatocytes were located around inflammatory regions, suggesting that activation of STAT3 in hepatocytes in these cirrhotic livers is likely induced by cytokines released by the inflammatory cells. IL-6 has been shown to activate STAT3 in the liver (Gao, 2005), and serum levels of IL-6 are elevated in cirrhotic patients (Lee et al., 1996). Thus elevated IL-6 likely contributes to STAT3 activation in the cirrhotic livers. Other cytokines (such as IL-22) and other factors (such as HCV proteins) may also contribute to STAT3 activation in cirrhotic livers. IL-22 is mainly produced by activated T cells and NK cells and plays an important role in acute phase response and hepatoprotection via activation of STAT3 in hepatocytes (Pan et al., 2004; Radaeva et al., 2004; Wolk and Sabat, 2006). Since T and NK cells are activated during chronic liver diseases, it is likely that these cells can produce IL-22, followed by activation of STAT3 in hepatocytes in cirrhotic livers. Investigations of HCV activation of STAT3 have been controversial. It has been reported that HCV core protein and NS5A protein activate STAT3 in hepatic cells (Gong et al., 2001; Kawamura et al., 2006), whereas other studies show that HCV replication in Huh7 cells inhibit STAT3 signaling (Larrea et al., 2006). Thus, whether HCV contributes to STAT3 activation in these HCV cirrhotic livers requires further study.

Alcoholic cirrhotic livers had significantly less pSTAT3⁺ hepatocytes and pSTAT3⁺ bile duct cells compared to HCV cirrhotic liver tissues. Interestingly, pSTAT3 positive staining was significantly lower in cirrhotic livers from patients with HCV infection plus a history of alcohol drinking compared to those with HCV infection alone. These findings suggest that STAT3 activation is suppressed in alcoholic cirrhosis compared to HCV cirrhosis. In contrast, strong nuclear staining of pSTAT3 in hepatocytes has been detected in a single patient with alcoholic hepatitis (Larrea et al., 2006). The difference between this study and ours may represent a difference in the stage of liver disease, early-stage alcoholic hepatitis liver (Larrea et al., 2006) and end-stage alcoholic cirrhotic liver (our study). However, more samples are required to confirm the elevated STAT3 activation in the early stage of alcoholic liver injury. The underlying mechanisms by which STAT3 activation is suppressed in alcoholic cirrhosis compared to HCV cirrhosis are not clear and may be related to a direct inhibition by ethanol of STAT3 (Chen et al., 1997; Chen et al., 2001; Chen et al., 1999; Gao, 2004) and elevation of PIAS3 (an inhibitor for STAT3) expression in alcoholic cirrhosis (Starkel et al., 2005).

Studies from animal models have shown that STAT3 plays an important role in cell proliferation, survival, and transformation in the liver (Gao, 2005); however, the role of STAT3 in human liver disease is less clear. STAT3 α mRNA has been shown to correlate inversely with serum ALT levels in patients with chronic HCV infection (Larrea et al., 2006). In contrast, our results show that pSTAT3⁺ hepatocytes and bile duct like cells do not correlate with serum

AST levels and ALP levels in these cirrhotic livers, respectively. This difference may be due to the end-stage cirrhotic liver samples used in our study whereas, noncirrhotic HCV liver samples were used by Larrea et al (Larrea et al., 2006). These findings may also suggest that STAT3 is involved in the protection against liver injury in early stage of liver injury in patients with HCV infection but not in end-stage of liver cirrhosis. STAT3 has also been demonstrated to contribute to hepatocyte proliferation and liver regeneration as disruption of the STAT3 gene suppressed liver regeneration after partial hepatectomy (Haga et al., 2003; Li et al., 2002). In the present study the correlation of pSTAT3 staining with cell proliferation, as determined by Ki67 staining, suggests that STAT3 may also be involved in liver regeneration. Furthermore, the percentage of Ki67⁺ staining was significantly lower in alcoholic cirrhosis and alcoholic plus HCV cirrhosis compared to HCV cirrhosis, suggesting that liver regeneration is suppressed in alcoholic cirrhosis. Thus decreased liver regeneration in alcoholic cirrhotic livers may in part be due to downregulation of STAT3 activation.

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Abbreviations

JAK-STATs, Janus kinase-signal transducers and activators of transcription; HCV, hepatitis C virus; STAT3, signal transducer and activator of transcription 3; AST, aspartate transaminase; ALP, alkaline phosphatase.

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Fig 1.

Ki67 staining in hepatocytes of end-stage liver cirrhosis. (A) Panel A shows the representative positive immunostaining with anti-Ki67 antibody in hepatocytes in normal healthy livers (normal) and end-stage human cirrhotic livers including HCV cirrhosis (HCV), alcoholic cirrhosis (ALD), and HCV plus alcoholic cirrhosis (HCV+ALD). (B) Panel B summarizes the percentage of Ki67⁺ hepatocytes. Values are shown as means $\pm$ SEM from 13 to 20 patients. The *P* value is shown in panel B.

Fig 2.

Ki67 staining in bile duct cells in cirrhotic livers. (A) Panel A shows the representative positive immunostaining with Ki67 antibody in bile duct cells in normal healthy livers (normal) and cirrhotic livers. (B) Panel B summarizes the percentage of Ki67⁺ bile duct cells. Values are shown as means $\pm$ SEM from 13 to 20 patients. The *P* value is shown in panel B. N.S: not significant.

Fig 3. pSTAT3 staining in hepatocytes of end-stage liver cirrhosis. (A) Panel A shows the representative positive immunostaining with anti-phospho-STAT3 antibody in hepatocytes in normal healthy livers (normal) and cirrhotic livers. Arrows indicate pSTAT3⁺ hepatocytes. (B) Panel B summarizes the percentage of pSTAT3⁺ hepatocytes. Values are shown as means $\pm$ SEM from 13 to 20 patients. The *P* value is shown in panel B. N.S: not significant.

Fig 4.

Phosphorylation of STAT3 in bile duct like cells of end-stage liver cirrhosis. Panel A shows the representative immunostaining with anti-phospho-STAT3 antibody in normal healthy livers and end-stage human cirrhotic livers. Panel B shows the representative double immunostaining with anti-pSTAT3 and anti-CK19 antibodies. pSTAT3⁺ bile duct cells were stained in nuclei (brown), while CK19⁺, a marker for bile duct cells, were stained in cytoplasm (nickel). Panel C summarizes the percentage of pSTAT3⁺ bile duct like cells. Values are shown as means $\pm$ SEM from 13 to 20 patients. The *P* value is shown in panel C. N.S: not significant.

Fig 5.

Correlation between pSTAT3⁺ and Ki67⁺ hepatocytes in end-stage liver cirrhosis. (A) No correlation was observed between pSTAT3⁺ hepatocytes and serum levels of AST in end-stage liver cirrhosis. (B) A positive correlation was observed between pSTAT3⁺ and Ki67⁺ hepatocytes in end-stage liver cirrhosis. N.S: not significant.

Fig 6.

Correlation between pSTAT3⁺ and Ki67⁺ bile duct like cells in end-stage liver cirrhosis. (A) No correlation was observed between pSTAT3⁺ bile duct like cells and serum levels of ALP in end-stage liver cirrhosis. (B) A positive correlation was observed between pSTAT3⁺ and Ki67⁺ bile duct like cells in end-stage liver cirrhosis. N.S: not significant.

Table 1

Patient Profile

[[characteristic]]	ALD Group (n= 20)	HCV Group (n=13)	HCV/ALD Group (n=13)	[[p value]]
Age, years	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Mean ± SEM]]	[[53.4 ± 2.3]]	[[52.3 ± 2.0]]	[[47.7 ± 1.2]]	[[N.S.]]
[[Median]]	[[54.5]]	[[51]]	[[48]]	[[[]]]
Range (min - max)	[[33 - 67]]	[[43 - 68]]	[[40 - 54]]	[[[]]]
Sex	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Male (%)]]	[[16 (80)]]	[[10 (77)]]	[[10 (77)]]	[[N.S.]]
[[Female (%)]]	[[4 (20)]]	[[3 (23)]]	[[3 (23)]]	[[[]]]
AST (UI / L)	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Mean ± SEM]]	[[72.2 ± 11.2]]	[[121.0 ± 18.0]]	[[128.0 ± 15.6]]	[[N.S.]]
[[Median]]	[[68]]	[[76]]	[[84]]	[[[]]]
[[Range]]	[[34 - 440]]	[[42 - 704]]	[[46 - 406]]	[[[]]]
Bililubin (mg/dl)	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Mean ± SEM]]	[[5.6 ± 1.2]]	[[4.6 ± 0.8]]	[[6.2 ± 1.7]]	[[N.S.]]
[[Median]]	[[3.4]]	[[3.3]]	[[4.3]]	[[[]]]
Range (min - max)	[[0.6 - 18.9]]	[[0.6 - 10.2]]	[[0.6 - 21.4]]	[[[]]]
Alkaline phosphatase (UI/L)	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Mean ± SEM]]	[[166.2 ± 19.1]]	[[139.4 ± 12.0]]	[[178.8 ± 19.7]]	[[N.S.]]
[[Median]]	[[142]]	[[136]]	[[158]]	[[[]]]
[[Range]]	[[82 - 580]]	[[77 - 354]]	[[74 - 366]]	[[[]]]
Albumin (g/dl)	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Mean ± SEM]]	[[2.9 ± 0.1]]	[[2.6 ± 0.1]]	[[2.4 ± 0.2]]	[[N.S.]]
[[Median]]	[[2.9]]	[[2.6]]	[[2.4]]	[[[]]]
Range (min - max)	[[1.7 - 3.6]]	[[2 - 3.3]]	[[1.7 - 3.4]]	[[[]]]
Creatinine (mg/dl)	[[[]]]	[[[]]]	[[[]]]	[[[]]]
[[Mean ± SEM]]	[[2.4 ± 0.3]]	[[1.5 ± 0.3]]	[[2.0 ± 0.4]]	[[N.S.]]
[[Median]]	[[1.5]]	[[1.2]]	[[1.3]]	[[[]]]
Range (min - max)	[[0.8 - 8.8]]	[[0.7-4.8]]	[[0.5 - 6.7]]	[[[]]]