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L-cysteine supplementation lowers blood glucose, glycated hemoglobin, CRP, MCP-1, oxidative stress and inhibits NFkB activation in the livers of Zucker diabetic rats

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Abstract

This study examined the hypothesis that L-cysteine supplementation can lower insulin resistance, glycemia, oxidative stress and markers of vascular inflammation in type 2 diabetes using Zucker diabetic rats (ZDF) rats as a model. Starting at age of 6 wks, ZDF rats were supplemented orally (daily gavage, 8 wks) with saline-placebo (D) or L-cysteine (LC, 1mg/KgBW) and fed a high calorie diet. 6 weeks age rats without any supplementation were considered baseline (BL) rats. D rats showed elevated fasting blood glucose, GHb, CRP, and MCP-1 when compared with BL in which there was no onset of diabetes. LC supplementation significantly lowered blood levels of glucose (18%, $p=0.05$), GHb (8%, $p=0.02$), CRP (23%, $p=0.02$), MCP-1 (32%, $p=0.01$) and insulin resistance (25%) compared with levels seen in saline-supplemented D. There was a decrease in plasma protein oxidation levels ($p<0.01$); however, GSH levels were similar in LC and D groups. While LC did not change blood hematocrit or levels of transaminases, it did lower alkaline phosphatase (29%, $p=0.01$) levels in comparison to D. Western blotting analyses of liver showed increased activation of NFkB and Akt (50% pNFkB and 20% pAkt) in D compared with BL. LC supplementation inhibited these effects (17% pAkt, 18% pNFkB). This is the first report showing L-cysteine supplementation can lower glycemia and markers of vascular inflammation in diabetes apparently mediated by preventing NFkB activation in a diabetic animal model.

Keywords

Glycemia; CRP; MCP-1; diabetes; L-cysteine

Introduction

Hyperglycemia is one of the major risk factors in the development of vascular complications in diabetes (1-4). Diabetes is treated with diet and insulin administration. Intensive blood glucose control dramatically reduces the devastating complications that result from poorly

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controlled diabetes. However, for many patients, achieving tight glucose control is difficult with current regimens. Thus, development of a novel adjuvant therapy is needed to achieve better control of glycemia in diabetic patients.

L-cysteine is called a semi-essential amino acid because humans can synthesize it from the amino acid methionine (5, 6). Along with a host of proteins, L-cysteine is a precursor of glutathione, which is considered pivotal for the detoxification of cellular oxidative stress (5). Elevated levels of oxidative stress can potentially impair cellular glucose metabolism via a variety of mechanisms, including redox imbalance and insulin resistance (7). Ammon et al. (8) reported that addition of L-cysteine to the culture medium potentiates the glucose induced insulin release in pancreatic islets isolated from female Wistar rats. Dietary intake of whey protein and alpha-lactoalbumin (cysteine rich proteins) lowers the oxidative stress and insulin resistance induced by sucrose in rats (9, 10). Other studies have reported that N-acetylcysteine supplementation reduces fructose-induced insulin resistance in normal rats (11) and also improves insulin sensitivity in women with polycystic ovaries (12). The maintenance of free sulfhydryl groups is considered crucial to biological functions of many proteins. However, no studies have been found in the literature that have examined the effect of L-cysteine supplementation on the improvement of glycemia or vascular inflammation in normal or diabetic animal models or diabetic patients. This study tested the hypothesis that L-cysteine (LC) supplementation improves glycemic control and reduces the circulating levels of markers of vascular inflammation in diabetes. In addition, this study examined the effect of L-cysteine supplementation on pro-inflammatory transcription factor κ B (NF κ B) and Akt activation in the livers of Zucker diabetic fatty (ZDF) rats.

The results demonstrate that oral supplementation with L-cysteine decreases blood levels of glucose, glycated hemoglobin, C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), and protein oxidation, and inhibits phosphorylation of NF κ B and Akt in the livers of ZDF rats, an animal model of type 2 diabetes.

Materials and Methods

All procedures followed were in accordance with the ethical standards of the institution, and approval was obtained from the institutional Animal Welfare Committee. Male Zucker diabetic rats were purchased at 5 weeks of age (200-220 g) from Charles River (Wilmington, MA) and allowed 2 days for environmental and trainer handling acclimation. Rats were divided into various groups by computer generated randomization and then housed and labeled in individual cages. Rats were fasted overnight, and then weighed. The rats were tested for hyperglycemia by measuring their blood glucose concentration. Blood for the blood glucose was obtained via tail incision and measured using an Advantage Accu-chek glucometer (Boehringer Mannheim Corp., Indianapolis, IN). Control rats were gavaged with saline alone (diabetic control group). Rats in the L-cysteine (LC) group were supplemented with 1 mg LC/kg body weight daily by oral gavage. Each rat received a similar volume of vehicle saline or LC daily for 8 weeks by oral gavage using 20G feeding needles (Popper and Sons, New Hyde Park, NY). Body weight and blood glucose concentrations were monitored weekly. The LC supplementation dose was adjusted every week according to any change in body weight to maintain a similar dose per Kg BW over the entire period of study. The rats were maintained under standard housing conditions at $22 \pm 2^\circ\text{C}$ with 12:12-h light/dark cycles and fed with a high calorie Purina 5008 lab chow diet (Charles River, Wilmington, MA). The amount of food intake was monitored on two separate occasions throughout the 8 week period to assess the consumption. At the end of 8 weeks the rats were fasted overnight, then euthanized for analysis by exposure to halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). Blood was collected via heart puncture with a 19½ gauge

needle into EDTA vacutainer tubes. Plasma was isolated after centrifuging blood in a 4°C centrifuge at 1500 rpm for 10 minutes.

Cytokines, insulin, lipid peroxidation assays

CRP, MCP-1 and insulin levels in the plasma were determined by the sandwich ELISA method using commercially available kits from Alpco (Salem, NH). All appropriate controls and standards as specified by the manufacturer's kit were used. In the cytokine assay, control samples were analyzed each time to check the variation from plate to plate on different days of analysis. Protein oxidation levels were determined by measuring protein carbonyl (13). GSH was determined by the method of Beutler (14).

Measurement of glycosylated hemoglobin (GHb), glucose and insulin resistance

Glycosylated hemoglobin was determined using Glyco-Tek Affinity column kits and reagents (cat # 5351) purchased from Helena Laboratories (Beaumont, TX). Glucose levels were determined using glucose oxidase by Accu-check Advantage glucometer (Boehringer Mannheim Corporation, Indianapolis, IN). Insulin resistance was calculated as previously determined in rats by Ismael et al (15)

Western blotting analyses of liver extracts

Tissues excised from the experimental rats were immediately frozen using liquid nitrogen and ground well into powders and frozen at -70°C until further use. The frozen tissue powders (~150 mg) were washed by resuspending in 1mL of PBS containing protease inhibitors, mildly vortexed and centrifuged at 15,000 rpm at 4°C for 10 min. The supernatants were discarded and the cell pellets were washed once more as mentioned above and then resuspended in 500µL of extraction buffer (25mM Tris, 0.5mM EDTA, PMSF 0.1mM, pH 7.4) with protease inhibitors and dounce homogenized using homogenizer and subjected to mild sonication. The tubes were centrifuged at 15,000 rpm (4°C, 30 min) and the supernatants (extracts) were collected. The collected extracts were subjected to centrifugation once more as described above and removed of cell debris. The protein content of the extracts was estimated using the BCA protein assay. Equal amounts of proteins from each group were loaded onto an SDS-polyacrylamide gel after boiling for 5 mins with β-mercaptoethanol as reducing agent. The separated proteins were transferred to a nitrocellulose membrane, blocked with 1% BSA in T-PBS and incubated overnight at 4°C with the respective primary antibodies. The next day, membranes were washed with T-PBS (8 mins, 4 cycles) and incubated with secondary antibodies in 5% non-fat milk for 30 min. at room temperature. The membranes were again washed with T-PBS (8 mins, 4 cycles), treated with chemiluminescence reagents for 2 mins and exposed to X-ray films, which are developed through autoradiography.

Liver function tests, blood cell count and blood chemistry profile

A portion of blood from rats in each group was sent to the clinical laboratory of LSUHSC-Shreveport (located in the same building) for clinical tests to determine liver and renal function along with red blood cell counts.

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise mentioned. Data were analyzed statistically using ANOVA between different groups using Sigma Plot statistical software (Jandel Scientific, San Rafael, CA). A p value of less than 0.05 was considered significant.

Results

There was no difference in the body weight at the time of sacrifice between different treatment groups. Compared with starting body weight of 163 ± 13 g (baseline), body weights were 363 ± 11 g and 364 ± 31 g in control and LC supplementation groups. The weekly intake of diet by each rat, as assessed at 5 and 7 weeks after start of supplementation, was similar in both the groups (data not shown).

Figure 1 illustrates fasting blood glucose levels at the beginning of treatment (baseline values) and after supplementation with saline (control) or LC for 8 weeks in ZDF rats. There was a significant increase ($p < 0.001$) in glucose levels with the onset of diabetes in the diabetics-control group compared with the baseline group before the onset of diabetes and start of supplementation. LC supplemented rats showed significantly lower ($p < 0.05$) glucose levels compared with D after both 6 and 8 weeks of supplementation. The effect of LC supplementation on levels of GHb is shown in Figure 1B. There was a significant increase ($p = 0.001$) in GHb levels in diabetes-controls compared with baseline. LC supplementation significantly lowered GHb levels compared with D after both 6 weeks ($p = 0.04$) and 8 weeks ($p = 0.02$) of supplementation. Insulin resistance (Figure 1 C) did not change at 6 weeks of supplementation. However, at 8 weeks, a significant decrease in insulin resistance ($p < 0.05$) levels was observed for the LC group. Insulin resistance were not determined in baseline rats. These results demonstrate that there is a significant improvement in glucose metabolism and glycemia in LC-supplemented compared with placebo-supplemented ZDF rats.

Figures 2a and 2b show CRP and MCP-1 levels in blood at the beginning of treatment (baseline) and after supplementation with saline (diabetic control) or LC in ZDF rats. There was a significant increase ($p < 0.001$) in both CRP and MCP-1 levels in diabetic controls compared with baseline. LC supplemented rats showed significantly lower CRP ($p = 0.02$) and MCP-1 ($p = 0.01$) levels compared with controls, which suggests a significant improvement in vascular inflammation as a result of LC supplementation in ZDF rats.

Figures 3a and 3b illustrate blood levels of GSH and protein carbonyls in diabetic control and LC-supplemented ZDF rats respectively. There was a modest but non-significant ($p = 0.20$) increase in circulating GSH level in LC-supplemented compared with diabetic control ZDF rats (Figure 3a). The level of protein oxidation was lower ($p = 0.04$) in LC-supplemented in comparison to placebo-supplemented diabetic rats (Figure 3b). This suggests that LC supplementation can modestly lower oxidative stress levels in diabetic rats.

Figure 4 depicts phospho (Ser 473) and total Akt, phospho (Ser 276) and total NFkB levels in liver extracts of control and experimental rats. Extracts from the liver of experimental rats were loaded on to SDS gels and separated by electrophoresis. The separated proteins were then transferred to a nitrocellulose membrane. The membrane containing the proteins was developed with different primary antibodies and finally developed with a β -actin antibody to assess the loading equality. In diabetic rats, NFkB was activated, which is measured by the extent of phosphorylation of its p65 regulatory subunit (p-NFkB) when compared to baseline controls. Cysteine administration to the diabetic rats resulted in a mitigatory effect. Cysteine-treated rats showed decreased levels of activated NFkB when compared to diabetic rats and were nearly normal when compared to those of baseline. Similarly, Akt, a downstream target of NFkB, was phosphorylated in diabetic rats when compared to baseline controls. Cysteine administration similarly inhibited the phosphorylation of Akt. Neither experimental diabetes nor cysteine treatment changed the expression levels of total Akt and NFkB.

Table I shows that LC supplementation did not affect hemoglobin, hematocrit or RBC counts in diabetic rats, which rules out any effect of altered red cell survival on lower glycosylated hemoglobin levels in LC supplemented ZDF rats.

Table II gives data on alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine and anion Gap levels in the blood of supplemented rats. While LC did not change blood levels of transaminases, it did lower alkaline phosphatase (29%, $p=0.01$) levels compared with D. The data demonstrates that LC supplementation does not seem to cause any toxicity as assessed by liver function or renal function tests.

Discussion

Hyperglycemia and vascular inflammation are the major risk factors contributing to development of CVD in diabetic patients (1-3). CRP, MCP-1, and oxidative stress levels are elevated in the blood of many diabetic patients (16-18). Increasing levels of MCP-1, CRP, and oxidative stress are known to decrease insulin sensitivity and increase vascular inflammation (7, 16, 17). We examined the hypothesis that L-cysteine amino acid supplementation lowers glycemia, CRP, MCP-1 and oxidative stress levels in Zucker diabetic fatty rats, a model of type 2 diabetes. This study demonstrates that LC supplementation significantly lowered the circulating levels of glucose and glycated hemoglobin. In addition, LC significantly lowered blood levels of MCP-1 and CRP and the HOMA index of insulin resistance in ZDF rats. This is a novel finding.

The glycated hemoglobin measurement reflects a mean glucose concentration over the preceding 1-2 months, in contrast to a one-time glucose level, which may be influenced by anesthesia or the stress of bleeding when the animals were sacrificed. There were no differences between the blood levels of hemoglobin or the RBC counts or hematocrits in diabetic rats receiving placebo or LC supplementation, which suggests that lower glycated hemoglobin levels in LC-supplemented rats is not due to any change in life span of RBC.

The mechanism by which LC supplementation lowers glycemia is not clear. It is likely that reduction in MCP-1 levels increases insulin sensitivity and thereby improves glucose metabolism. Circulating MCP-1 and CRP levels are elevated in insulin resistant states such as obesity, impaired glucose tolerance, and type 1 and 2 diabetes (18). MCP-1 is a potent chemotactic factor for monocytes (19). Increased expression of MCP-1 mRNA or proteins has been observed in animals and humans with arteriosclerosis or atherosclerosis (20, 21). Studies using in vitro monocyte-endothelial adhesion assays and in vivo studies using knock-out mice for the CCR2 gene (receptor of MCP-1) and antibodies for MCP-1 have demonstrated that MCP-1 plays an important role in vascular inflammation and atherosclerotic lesion formation (22-24). Overexpression of MCP-1 causes inhibition of Akt and tyrosine phosphorylation in liver and skeletal muscle, macrophage recruitment, and insulin resistance in aP2-MCP-1 mice (18). MCP-1 plays important roles in the vascular inflammation process through its multiple actions, including recruitment of neutrophils and T lymphocytes into the subendothelial space, monocyte adhesion to endothelium, and migration of vascular smooth muscle cells. The observation of lower circulating levels of MCP-1 is novel and could contribute to improvement in glycemic control and vascular inflammation in LC supplemented ZDF rats.

We also found that elevated protein oxidation levels in the blood of diabetic rats were abolished in diabetic rats supplemented with LC. The ZDF rat is a model of type 2 diabetes and is associated with elevated levels of both hyperglycemia and pro-inflammatory cytokines. Hyperglycemia can result in excessive oxygen radical production, which can lead

to increased oxidative stress in diabetes (17, 25-28). The generation of oxygen radicals in diabetes could be from variety of mechanisms, including, induction of cytochrome P-450 like activity, altered redox levels, hyperglycemia, ketosis, elevated sorbitol and AGE products and mitochondrial activity, and glycosylation of antioxidative enzymes, etc. (25-28, 29-31). Mitochondrial dysfunction can also lead to increase production of reactive oxygen species. Beta cells of pancreas are particularly susceptible to damage by ROS because expression of antioxidant enzymes is low in beta cells. Increased oxidative stress can impair insulin secretion by pancreas (33). Oxidative stress can also influence the expression of multiple genes in vascular cells, including signaling molecules such as Akt and NFkB (7, 34, 35). Overexpression of these genes stimulates the secretion of pro-inflammatory cytokines (7). Thus, oxidative stress plays a key role in the regulatory pathway that progresses from elevated glucose to monocyte and endothelial cell activation in the enhanced vascular inflammation of diabetes (4, 7, 17, 28, 36).

The liver plays a major role in the regulation of glucose metabolism (37). The liver is a storage organ for glucose, proteins and vitamins. The glycogenic and gluconeogenic pathways which are major regulators of blood glucose levels are unique to liver. Several hormones, including insulin, glucagon, growth hormone, cortisol, and catecholamines contribute to the regulation of glucose metabolism by the liver. Therefore we examined the role of NFkB activation in liver and its potential contribution on glucose metabolism in L-cysteine supplemented rats.

The results of our study show that experimental diabetes induced the activation of NFkB in the livers of diabetic Zucker rats when compared to baseline controls. NFkB is a known target for hyperglycemia. Oxidative stress associated with diabetes activates NFkB, which then activates the release of TNF- α , thereby contributing to insulin resistance (7). We reported earlier that hyperglycemia causes an increase in reactive oxygen species (ROS) generation and oxidative stress in diabetic rats (38) and patients (26, 27, 39, 40). ROS-induced oxidative stress is a known activator of NFkB, a heterodimer consisting of two DNA-binding subunits known as p50 and p65. NFkB is present in the cytoplasm complexed to its inhibitory protein known as inhibitory kB (IkB). Following activation, IkB dissociates from NFkB and undergoes ubiquitination and degradation. NFkB is freed to undergo nuclear translocation. Upon its nuclear translocation, NFkB undergoes serine phosphorylation at residue 276 in its p65 subunit and associates with surrounding chromatin components. It subsequently binds with DNA and promotes the transcription of pro-inflammatory cytokines and known mediator of insulin resistance. Therefore, measurement of the phosphorylated p65 subunit of NFkB is an effective tool for determining NFkB activation. The diabetic rats exhibited elevated NFkB and Akt activation. We also found that NFkB and Akt phosphorylation was partly inhibited by LC supplementation. Our results show that LC supplementation resulted in a significant improvement in glycemia and inhibition of oxidative stress and secretion of pro-inflammatory cytokines in diabetic rats. The molecular mechanism by which LC supplementation improved glycemia and lowered vascular inflammation in diabetes is not known. The inhibitory effect of LC on release of pro-inflammatory cytokines may be mediated partly by inhibiting oxidative stress pathways (16, 26). Cysteine treatment could have prevented NFkB activation, possibly by inhibiting the glucose mediated production of ROS, which is a primary inducer of this pathway.

Western blot results from liver extracts in our study indicate that phosphorylation of Akt (ser 473) is increased in diabetic rats when compared to baseline controls. Although Akt phosphorylation is a primary intermediate event in insulin signaling, we assume that Akt phosphorylation by insulin and pro-inflammatory cytokines is mediated by two distinctly different mechanisms. In the case of insulin, its binding to its receptor induces PI3-kinase mediated phosphorylation of Akt, which leads to downstream insulin signaling, whereas

pro-inflammatory cytokines mediate phosphorylation of Akt through activation of NFkB. Studies exist that show that NFkB activation precedes phosphorylation of Akt and is a late event caused by chronic production of pro-inflammatory cytokine TNF- α (41). The increased Akt phosphorylation seen in our study could be mediated by high circulatory MCP-1 or CRP-1 levels in diabetic rats and this phosphorylation event is independent of activation by PI-3 kinase pathway. Whether LC supplementation influences the PI3 kinase pathway mediated insulin signaling in ZDF rats needs to be investigated. Nevertheless, lower circulating levels of CRP and MCP-1 can explain the observed lowering of blood glucose and glycated hemoglobin levels, potentially mediated at least in part by the inhibition of NFkB phosphorylation in liver and increased insulin sensitivity in LC-supplemented diabetic rats.

A recent study demonstrated that insulin sensitivity inversely correlates independently with elevated alkaline phosphatase activities in the blood of 472 apparently healthy men (42). The liver plays an important role in maintaining normal glucose concentrations during fasting as well as postprandially. An impaired effect of insulin in suppressing hepatic glucose production and glycogenolysis in the liver causes an increase in hepatic glucose production (37). There is evidence that measures of liver function and subclinical inflammation are related to insulin sensitivity and predict new-onset type 2 diabetes independently of classical risk factors (43, 44). A decrease in alkaline phosphatase activities in the LC-supplemented group suggests an improvement in liver function, which may play a role in the improved glycemia observed in LC-supplemented ZDF rats. Although alkaline phosphatase leaks into the blood stream in a manner similar to that of the other liver enzymes ALT and AST, AP is also present in organs such as bone and intestine. The levels of ALT and AST in blood did not differ between control and LC-treated groups in this study. Thus, it is not clear whether LC does indeed provide any protection to liver function in ZDF rats. The role of protection of pancreatic β -cells by LC in the development of diabetes is also not clear and needs investigation. Thus, this study suggests that LC supplementation may retard the development of diabetes. Whether LC can prevent the complications once diabetes is established needs further investigations.

In conclusion, L-cysteine supplementation has the potential to lower blood levels of glycemia and the pro-inflammatory cytokines CRP and MCP-1. The evidence that supplementation with LC can cause significant improvement in glycemia and the markers of vascular inflammation is novel, and needs to be explored in the diabetic patient population. If it works as well as this research hints that it may, then LC supplementation could be used as an adjuvant therapy for the reduction of vascular inflammation and CVD in diabetes.

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Figure 1.

Effect of supplementation with placebo-saline and L-cysteine (LC) on blood glucose levels, HbA_{1c}, random glucose, and Insulin resistance in ZDF rats. Values are mean $\pm$ SE. Rats were supplemented with control (saline) or LC (1.00 mg/Kg BW) by gavage daily. BL: baseline value before the start of supplementation. (a) Glucose: Differences between values marked * versus ** ($p=0.01$), ** versus *** ($p=0.01$), and # vs ## ($p=0.045$) are significant. (b) GHb: Differences between values marked * versus ** ($p=0.01$), ** versus *** ($p=0.05$), # vs ## ($p=0.011$) are significant. (c) Insulin Resistance: The difference between # and ## ($p=0.05$) is significant.

Figure 2.

Effect of supplementation with placebo saline and L-cysteine (LC) on plasma CRP and MCP-1 levels in ZDF rats. Values are mean $\pm$ SE. Rats were supplemented with control (saline) or LC (1.00 mg/Kg BW) by gavage daily for 8 wks. BL: baseline value before the start of supplementation. (a) Plasma CRP: Differences between values marked * versus ** (p=0.01) and ** versus *** (p=0.02) are significant. (b) Plasma MCP-1: Differences between values marked * versus ** (p=0.01) and ** versus *** (p=0.01) are significant.

Figure 3. Effect of supplementation with placebo-saline and L-cysteine (LC) on plasma GSH and protein oxidation levels in ZDF rats. Values are mean $\pm$ SE. Rats were supplemented with control (saline) or LC (1.00 mg/Kg BW) by gavages daily for 8 wks. Baseline values are not shown here. (a) Plasma GSH; (b) Plasma Protein Oxidation.

Figure 4.

a) Effect of placebo saline and L-cysteine supplementation on the levels of phospho and total forms of Akt and NFkB in liver extracts of control and experimental animals. The blots are representatives of 3 independent experiments. b) Graphical representation of data from Western blotting analyses. Arbitrary units-ratio of phosphorylated to total Akt or NFkB. The data summarized from three independent experiments.

Table I
Effects of L-Cysteine on blood hemoglobin, hematocrit, and red blood cell counts in ZDF rats

	<i>n</i>	RBC	Hemoglobin	Hematocrit
	units	10 ⁶ /μL	g/dL	%
Baseline	8	4.54 ±0.45	13.41 ±0.26	40.56 ±0.92
Diabetic	8	9.10 ±0.09	15.97 ±0.13	47.30 ±0.50
Diabetic + Cyst	6	9.22 ±0.16	16.28 ±0.23	47.36 ±0.95

Each value represents the mean ± SE. Baseline: at the start of supplementation; Diabetic: supplemented with placebo (8 wks) group; Diabetic + Cyst: L Cysteine supplemented (8wks) group.

Table II
Blood markers of liver and kidney function tests in ZDF rats

	n	AP	BUN	CRT	AST	ALT	Total Bilirubin	Anion Gap	BUN/CRT Ratio
	units	U/L	mg/dL	mg/dL	U/L	U/L	mg/dL	μ /L	mMol/L
Baseline	8	9.20 $\pm$ 1.36	15.20 $\pm$ 0.49	0.40 $\pm$ 0.00	234.60 $\pm$ 10.24	79.20 $\pm$ 2.71	0.00 $\pm$ 0.00	39.20 $\pm$ 0.58	38.40 $\pm$ 1.29
Diabetic	8	51.29* $\pm$ 4.24	18.43 $\pm$ 0.78	0.44 $\pm$ 0.02	199.67 $\pm$ 20.69	125.71 $\pm$ 11.09	0.00 $\pm$ 0.00	28.43 $\pm$ 0.43	41.86 $\pm$ 1.49
Diabetic + Cyst	6	36.40** $\pm$ 4.65	18.50 $\pm$ 0.29	0.42 $\pm$ 0.02	218.20 $\pm$ 25.46	119.20 $\pm$ 11.37	0.00 $\pm$ 0.00	29.20 $\pm$ 0.49	43.00 $\pm$ 2.53

Each value represents the mean $\pm$ SE; AP: Alkaline phosphatase; BUN: Blood Urea Nitrogen; CRT: Creatinine; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase. Baseline: at the beginning of supplementation; Diabetic: supplemented with placebo (8 wks) group; Diabetic + Cyst: L Cysteine supplemented (8wks) group. Differences between * versus ** are significant (p=0.01).