Role of Hyperketonemia in Inducing Oxidative Stress and Cellular Damage in Cultured Hepatocytes and Type 1 Diabetic Rat Liver

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Key Words
ROS • GSH • Hyperketonemia • Ketones • Liver • Type 1 diabetes

Abstract

Background/Aims: Type 1 diabetic (T1D) patients have a higher incidence of liver disease. T1D patients frequently experience elevated plasma ketone levels along with hyperglycemia. However, no study has examined whether hyperketonemia per se has any role in excess liver damage in T1D. This study investigates the hypothesis that hyperketonemia can induce oxidative stress and cellular dysfunction. Methods: STZ treated diabetic rats, FL83B hepatocytes, and GCLC knocked down (GSH deficient) hepatocytes were used. Results: The blood levels of ALT and AST, biomarkers of liver damage, and ketones were elevated in T1D rats. An increase in NOX4 and ROS along with a reduction in GSH and GCLC levels was observed in T1D rat livers in comparison to those seen in non-diabetic control or type 2 diabetic rats. MCP-1 and ICAM-1 were also elevated in T1D rat livers and ketone treated hepatocytes. Macrophage markers CCR2 and CD11A that interact with MCP-1, and ICAM-1 respectively, were also elevated in the T1D liver, indicating macrophage infiltration. Additionally, activated macrophages increased hepatocyte damage with ketone treatment, which was similar to that seen in GCLC knockdown hepatocytes without ketones. Conclusion: Hyperketonemia per se can induce macrophage mediated damage to hepatocytes and the liver, caused by GSH depletion and oxidative stress up regulation in T1D.

Introduction

Hyperketonemia (elevated blood ketones) is associated with type 1 and type 2 diabetes. Severe insulin resistance and worsening of hyperglycemia are reported in type 2 diabetic (T2D) patients with elevated ketone levels [1, 2]. Frequency of elevated ketones is found...
much higher in type 1 diabetic (T1D) patients compared to T2D. Ketone bodies acetoacetate (AA), 3-β-hydroxybutyrate (BHB), and acetone can be detected along with high glucose in the plasma and urine samples of T1D patients [3-6].

Oxidative stress has been suggested to contribute both to the onset and progression of higher incidence of liver disease in diabetic patients [7, 8]. The role of ketones in elevating oxidative stress leading to liver injury is not known. Studies have shown that elevated ketones can increase the generation of hydrogen peroxide, thereby increasing oxidative stress in hyperketonemic diabetic (HKD) patients [9-12]. It was recently reported that NADPH oxidases, enzymes involved in the reactive oxygen species production, were up regulated in endothelial cells treated with ketones [13]. Studies have also shown that hyperketonemia can induce the expression of cytokines and adhesion molecules along with the up regulation of the oxidative stress [10, 14-19]. HKD patients also show reduced GSH levels [10]. Oxidative burden within the cells can cause the up regulation of several pathways leading to the elevation in the adhesion molecules and cytokines [13, 14]. This can result in the activation and recruitment of blood monocytes or macrophages, which once recruited could result in the injury and even death of liver cells [20].

Liver damage and injury is more commonly seen in T1D patients [21, 22]. Elevated transaminases such as, alanine amino transferase (ALT) and aspartate amino transferase (AST) are indicative of the leakage of intracellular hepatic enzymes into the circulation and are used to determine the extent of hepatic injury [7]. Serum aminotransferases found abnormally elevated in diabetics, are used routinely as biomarkers in clinical practice to screen for liver diseases [23]. Liver function parameters were especially found to be deteriorated in diabetic patients with elevated blood ketones [24]. These studies suggest that elevated ketones can interfere with normal functioning of the liver in T1D. Factors such as elevated liver enzymes, oxidative stress, increased expression of adhesion molecules on the liver cells, and macrophage activation and infiltration into the liver potentiate liver damage and dysfunction [23-25]. Impairment in liver function could lead to dysregulation in glucose homeostasis further worsening hyperglycemia in diabetic patients [24]. There is no study that has investigated the effect of hyperketonemia in inducing liver damage in T1D. This study tested the hypothesis that elevated ketones in T1D rats are associated with increased liver damage compared to T2D rats that show no ketone elevation. The results from this study demonstrate for the first time that both in vivo and in vitro elevated levels of ketones are associated with excess liver/hepatocyte damage in T1D.

Materials and Methods

**Type 1 diabetic animals**

All procedures used were in accordance with the ethical standards of the institution, and prior approval was obtained from the LSUHSC Animal Welfare Committee. 5 week old male Sprague Dawley (SD) rats weighing about 200–220 g were purchased from Charles River (Wilmington, MA) and allowed 2 days for environmental and trainer handling acclimation. The rats were fasted overnight and injected with streptozotocin (STZ) intraperitoneally at a dose of 65 mg/kg body weight in citrate buffer (pH 4.5). Control rats received only the citrate buffer injections. Blood glucose concentrations were tested for hyperglycemia (>300 mg/dL) 3 and 7 days post STZ injections. The rats were maintained under standard housing conditions at 22 ± 2°C with 12/12-h light/dark cycles and fed a standard 8640 lab chow diet (Harlan, Indianapolis, IN). At the age of 14 weeks, the rats were euthanized, after an overnight fast, by exposure to isoflurane (Webster Veterinary Supply Inc., Devens, MA).

**Type 2 diabetic animals**

5 week old male Zucker Diabetic Fatty (ZDF) rats weighing about 200–220 g were purchased from Charles River (Wilmington, MA) and allowed 2 days for environmental and trainer handling acclimation. The rats were maintained under standard housing conditions at 22 ± 2°C with 12/12-h light/dark cycles and fed a high-calorie Purina 5008 lab chow diet (Charles River). At 14 weeks of age the ZDF rats became
diabetic (blood glucose > 300 mg/dL) (T2D) after which they were fasted overnight and euthanized by exposure to isoflurane (Webster Veterinary Supply Inc., Devens, MA).

In both T1D and T2D animals blood glucose levels were assessed from blood obtained via tail incision using an Advantage Accu-Chek glucometer (Boehringer Mannheim Corp., Indianapolis, IN). After the experiment blood was collected via heart puncture with a 19gauge needle into EDTA Vacutainer tubes. Plasma was isolated after centrifuging the blood in a 4°C centrifuge at 1500 rpm for 10 min. An aliquot of blood from rats in each group was sent to the clinical laboratory of our LSUHSC-Shreveport facility for clinical tests to determine blood glucose levels, liver function, and kidney function.

Preparation of liver lysates
Liver tissues excised from the experimental rats were immediately perfused with cold saline to remove leftover blood, immediately frozen using liquid nitrogen, and stored at -80°C until further use. The frozen liver tissue (~50 mg) was homogenized in 500 μL radioimmuno precipitation assay (RIPA) buffer (50 mM Tris pH 8, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS) supplemented with protease and phosphatase inhibitors (1 mM PMSF, 5 μg/mL leupeptin, 2 μg/mL aprotinin, 1 mM EDTA, 10 mM NaF, and 1 mM Na3VO4), using a Dounce homogenizer. The tubes were centrifuged at 15,000 rpm (4°C, 30 min) and the supernatants (extracts) were collected. The protein content was estimated using the BCA protein assay kit (Pierce/Thermo Scientific, Rockford, IL).

Hepatocytes, Monocytes and Monocyte-macrophage differentiation
FL83B cells and Human THP-1 monocytes were purchased from American Type Culture Collection (ATCC, Manassas, VA). Hepatocytes were cultured according to the vendor’s instructions in F12K media supplemented with 10% (v/v) heat-inactivated fetal bovine serum and maintained at 37°C in a humidified atmosphere containing 5% CO2. Monocytes were cultured in RPMI 1640 medium and the cell number was maintained close to one million per mL of media [13]. Monocyte-macrophage differentiation was done by incubating monocytes with 200 nM phorbol 12-myristate 13-acetate (PMA) at 37°C for 48 h [26]; then cells were washed once with PBS and allowed to rest for 48 h in complete RPMI medium before treatment.

Ketone and high glucose treatment
Cells were treated with acetoacetate (AA, 0-4 mM), DL-β-hydroxybutyrate (BHB, 0-12 mM), and/or HG (25 mM) for 24 h. Concentrations used here are similar to those found in blood of patients [3], and similar to those used in previous studies[13]. Keto-butyric acid and mannitol were used as osmolarity controls. Cell viability was determined in all treatments to rule out cell death.

Ketones/ROS/NADPH oxidase activity/GSH/MCP-1/ALT assays
Plasma AA and BHB were determined as described before [10, 11]. ROS were detected in cells treated with ketones and HG as previously described [13], using an aliquot of 20 μM oxidant-sensitive probe dichlorodihydrofluorescein diacetate (H2DCFDA). ROS in liver lysates (20 μg) was measured by incubating in 20 μM H2DCFDA for 5 min at 37°C in the dark and reading fluorescence every minute for 15 min. Fluorescence was calculated as mean fluorescence intensity (MFI)/mg of protein. NOX activity of the cell and liver lysates was determined using 50 μg protein as previously described [13]. Luminescence was measured using a Synergy HT microplate reader. GSH analysis was carried out using HPLC [27, 28]. ALT assay was performed in cell supernatants/media that were collected after 24 h treatments using ALT kit.

Western blotting
After treatment the cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors. Lysates were then centrifuged for 15 min at 13,000 rpm at 4°C. Supernatants were collected and the protein concentrations were determined using a BCA assay kit (Thermo Scientific, Rockford, IL). An aliquot of 20 μg protein from each sample (cell or liver lysates) was prepared in SDS buffer and run on 10% Tris-SDS acrylamide gels. Nitrocellulose membranes were used for transfer. The blots were then blocked in 1% BSA and incubated in a primary antibody solution overnight followed by 1 h incubation in

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HRP-conjugated secondary antibody. ECL detection reagents (PerkinElmer, Boston, MA) were used to detect protein bands that were exposed on blue X-ray film (Phenix Research Products, Candler, NC).

**siRNA knockdown in hepatocytes**

Transfection was carried out using GCLC siRNA (Santa Cruz Biotech. Dallas, TX) as previously described [13] using serum free transfection medium and an aliquot of 5 µL transfection reagent (Lipofectamine Invitrogen) mixed with siRNA (200 nM). Confluent cells were used in co-culture experiments with macrophages.

All chemicals were purchased from Sigma Chemical Co. (St. Louise, MO) unless otherwise indicated. Data was analyzed with Sigma Plot statistical software using one way analysis of variance (ANOVA, SPSS, Chicago, IL, USA). Values are expressed as Mean ± SE. A p value of 0.05 or less was considered significant.

**Results**

Table 1 shows the levels of glucose, ketones, and the biomarkers of liver injury: ALT (alanine transaminase) and AST (aspartate transaminase) in the blood of control, T1D, and T2D rats. Results show that STZ-treated T1D rats have similar levels of blood glucose compared to T2D rats but they vary greatly in their plasma ketone levels. T1D rats have elevated ketones and a greater anion gap compared to the other two groups. In addition, T1D rats show higher levels of ALT and AST compared to T2D rats. In terms of body weight T1D weighed less than the other two groups. The levels of adhesion molecule ICAM-1 and cytokine MCP-1 were significantly elevated in T1D rats compared to those in control rats. The liver triglyceride levels were also significantly (p < 0.05) elevated in T1D rats compared to that of controls. The concentration of triglycerides was 0.78 ± 0.15, 2.02 ± 0.30, and 1.39 ± 0.19 for control, T1D, and T2D respectively.

**ROS production, GSH down regulation, and NADPH oxidase up regulation in T1D rat livers**

The level of ROS was significantly higher in both groups of diabetic animals (T1D and T2D) in comparison to non-diabetic controls, but the level of ROS increase seen in T1D was much higher than that observed in T2D (Fig. 1A). Elevated ROS can lead to the depletion of antioxidant molecules such as glutathione (GSH). A noticeable decrease in GSH levels in T1D rats was observed, which was not seen in other groups (Fig. 1B).

Glutamate cysteine ligase (GCL) is a key determinant in GSH synthesis and is composed of a catalytic (GCLC) and a modifier (GCLM) subunit [29]. The expression levels of both GCLC and GCLM were significantly (p < 0.05) reduced in T1D compared to that of control and T2D (Fig. 1C and Table 1.

**Table 1.** Various blood parameters of control, T1D, and T2D rats. Values are Mean ± SE (n = 6). Differences between values marked * vs. †, and † vs. ‡ are statistically significant (p ≤ 0.05)
1D), the quantified values (n = 6) of GCLC expression were 0.7 ± 0.1, 0.4 ± 0.1, and 0.8 ± 0.2 and GCLM expression were 1.7 ± 0.4, 0.5 ± 0.2, and 1.5 ± 0.2 for control, T1D, and T2D respectively.

NOX4 activation plays a critical role in diabetes involved oxidative stress, which has been shown to be up regulated in diabetic liver and kidney [30]. However, there is no study that has investigated the effect of ketones on the expression or the activity of NOXs in the liver. NOX4 expression in these T1D animals was significantly (p < 0.05) up regulated (as seen in Fig. 1E), compared to that of control and T2D, the quantified values (n = 6) of NOX4 expression were 0.4 ± 0.1, 1.3 ± 0.1, and 0.5 ± 0.1 for control, T1D, and T2D respectively. The level of NOX2 was only found to be up regulated in T2D (Fig. 1F), the quantified values (n = 6) of NOX2 expression were 0.9 ± 0.3, 0.9 ± 0.3, and 2.0 ± 0.7 for control, T1D and T2D respectively. There was no change in expression of other forms of NOX (data not shown).

NOX activity was higher in T1D rats compared to that in T2D rats or controls (Fig. 1G).

**ICAM-1, MCP-1, and macrophage marker up regulation in T1D rats**

MCP-1 levels were found to be elevated in the blood of T1D rats (Table 1). The primary role of MCP-1 is to recruit monocytes and macrophages via CCR2 receptor. The level of MCP-1 mRNA was also significantly (p < 0.05) high in the T1D livers compared to that of control or T2D (values of MCP-1 mRNA fold induction in control, T1D, and T2D rat livers were 0.8 ± 0.6, 17.7 ± 7.3, and 3.2 ± 3.4 respectively, n = 6), suggesting a possible role of liver in the secretion of MCP-1. As MCP-1 levels rise in the liver and get into the circulation, this causes the activation and migration of macrophages towards the liver. Expression of TNF-α and IL-8 did not significantly change within the groups (data not shown).

ICAM-1 is considered a key player in mediating adhesion events leading to macrophage and leucocyte infiltration in the liver [20]. Rats in the T1D group show a significant (p <0.05) increase in ICAM-1 expression compared to those of controls or T2D rats (Fig. 2A), the quantified values (n = 6) of ICAM-1 were 0.1 ± 0.1, 0.9 ± 0.2, and 0.1 ± 0.0 for control, T1D, and T2D respectively. The trend observed in ICAM-1 up regulation correlates well with...
the circulating ICAM-1 (sICAM-1) levels in the plasma of these rats (Table 1). Elevation in the expression of adhesion molecules on the liver cells can mediate the adherence of blood monocytes or macrophages. The expression of various monocyte/macrophage markers was determined to test whether the liver had any signs of macrophage infiltration. CD11A (LFA-1) is a ligand that enables monocytes or macrophages to adhere to cells expressing ICAM-1 while; CCR2 binds to MCP-1. The expression of CCR2 (Fig. 2B), and CD11A (Fig. 2C) that are important in macrophage mediated injury were found to be elevated in T1D livers suggesting the infiltration of monocytes/macrophages. The quantified values (n = 6) of CCR2 expression were 0.7 ± 0.2, 1.3 ± 0.1, and 1.0 ± 0.2, and of CD11A were 0.3 ± 0.1, 1.6 ± 0.3, and 0.9 ± 0.1 for control, T1D, and T2D respectively.

**NOX4 and ICAM-1 up regulation along with GSH and GCLC downregulation in hepatocytes treated with ketones**

The up regulation in the markers of oxidative stress was mainly seen in T1D rats which also showed an elevation in ketones. To link ketones with oxidative stress and liver injury found in T1D it was tested whether ketones when added to hepatocytes can bring about
similar responses as seen in the liver of T1D rats. Hepatocytes were treated with ketones in the presence or absence of high glucose to mimic T1D conditions. The action of ketones appears to be more enhanced in the presence of high glucose suggesting that elevated ketones when present along with high glucose can bring about a much severe outcome.

Expression of NOX4 when hepatocytes were treated with both ketones and high glucose (HK+HG) together was found to be upregulated (Fig. 3A), the quantified values (n = 3, mean ± SE) of NOX4 were 0.6 ± 0.1 and 1.0 ± 0.2 for control and HK+HG respectively, but NOX2 expression did not change with the treatment (data not shown). There was a similar down regulation in GCLC expression (Fig. 3B) in hepatocytes treated with HK+HG as seen in T1D livers, the quantified values (n = 3) of GCLC were 0.7 ± 0.2 and 0.3 ± 0.1 for control and HK+HG respectively. The elevated oxidative stress resulting from NOX4 up regulation reduced GSH levels in hepatocytes treated with ketones (Fig. 3D). The expression of adhesion molecule ICAM-1 (Fig. 3C) and MCP-1 (Fig. 3E) secretion was also up regulated in response to HK+HG. The quantified values (n = 3) of ICAM-1 were 0.2 ± 0.1 and 0.4 ± 0.1 for control and HK+HG respectively.

Macrophase induced hepatocyte injury with ketone treatment

The expression of CCR2 and CD11A (Fig. 4A) in THP-1 monocytes increased with ketone treatment, the quantified values (n = 3) of CCR2 were 0.2 ± 0.0 and 0.4 ± 0.1, and that of CD11A were 0.9 ± 0.2 and 1.5 ± 0.1 for control and HK+HG respectively. This suggests that ketone treatment increases adhesion molecules on both (monocytes and hepatocytes) cells enabling their attachment leading to monocyte/macrophage mediated hepatocyte injury. To address the role of ketones in macrophage induced hepatocyte injury, THP-1 monocytes were differentiated into macrophages with PMA treatment and cultured with hepatocytes. This approach is commonly used to detect the influence of macrophages on hepatocyte function [31]. If there is any damage or injury to hepatocytes then ALT within the cells would leak out and can be detected. Hence, the cell culture media/supernatant after various treatments was collected and the ALT content in them was determined. Upon ketone treatment there was an increase in the leakage of ALT from hepatocytes into the supernatant. The combination of ketones with high glucose increased the ALT levels in the cell supernatant even more suggesting a higher degree of hepatocyte damage (Fig. 4B).

To understand if the effects of ketones are mediated by a state of heightened oxidative stress, GSH deficiency was induced in hepatocytes by knocking down GCLC. These hepatocytes were co-cultured with macrophages. The hepatocyte damage in the presence of macrophages and in the absence of GSH was comparable to the level of damage seen with ketone treatment (Fig. 4C). L-cysteine, a precursor of GSH synthesis, before ketone treatment significantly inhibited ALT leakage suggesting that it is indeed oxidative stress that is required for the initiation and progression of hepatocyte damage. The efficiency of GCLC siRNA in knocking down GCLC protein expression is shown in Fig. 4D.

Discussion

In addition to hyperglycemia, hyperketonemia is also present in T1D. In uncontrolled diabetes blood levels of ketones are elevated to above normal levels [3, 32, 33]. Individuals with T1D have a higher incidence of organ damage and dysfunction compared to healthy subjects. Heightened oxidative stress found in diabetic patients can activate transcription factors, induce pro-inflammatory cytokines, and increase adhesion molecule expression, which in turn can cause cellular injury and contribute to the onset of complications associated with diabetes [30]. This study demonstrates for the first time that ketones per se can contribute to the increased cellular injury in T1D liver.

Frequent episodes of ketosis significantly increased the risk of hepatic glycogen overload in diabetic patients [34]. On the other hand hepatic infarction was also reported to be associated with ketonemia [35]. It was shown that ketogenic diet-fed mice develop nonalcoholic fatty liver disease, systemic glucose intolerance, and their livers exhibit ER
stress, steatosis, cellular injury, and macrophage accumulation [36, 37]. Cell culture studies have also shown that the ketone body AA can activate ERK1/2 and p38MAP kinase in hepatocytes, endothelial cells, and monocytes [38-40].

Analyses of the blood of experimental rats in this study show that both T1D and T2D rats have elevated liver enzymes, but the higher AST and ALT values in T1D rats in comparison to T2D implicate much severe liver damage in T1D group. This led us to analyze the liver tissues of these animals for any potential injury. T1D animal livers not only showed an up regulation of NOX4 (a mediator of ROS production) but also were deficient in their GSH status. These two appear to be the main players in inducing oxidative stress burden in the liver. Along with the up regulation of the oxidative stress there has also been reports showing decreased levels of GSH in diabetic patients suggesting an inadequate antioxidant status [20]. High ketone levels were reported to be associated with reduced GSH and increased oxidative stress in T1D patients and in cell models [10, 11, 41, 42]. GSH is the most abundant redox molecule in cells and alterations in the redox balance observed in GSH deficient conditions can mediate hepatic injury [43]. GSH deficiency in diabetics increases their susceptibility to infections [44]. Therefore, maintenance of hepatic GSH levels is important in the prevention of ROS mediated injury. Impaired synthesis due to the deficiency of the rate limiting enzyme GCLc and increased utilization due to the heightened oxidative stress seem to be responsible in depleting GSH levels in T1D liver compared to those of T2D or controls. Similar to our results, others have also shown a pronounced GSH loss in T1D rats [45].

The increased expression of the cytokine MCP-1 observed in the plasma and the liver of T1D rats could play a pivotal role in the recruitment of blood macrophages via CCR2 to this site. The expression of intercellular adhesion molecule-1 (ICAM-1) was also found to be significantly up regulated in the livers of T1D rats in comparison to that in T2D. ICAM-1 is a cell surface glycoprotein expressed on cells in response to inflammatory stimuli that allows the macrophages to adhere. ICAM-1 also plays a pivotal role in the liver and is known to be expressed on sinusoidal endothelial cells, Kupffer cells, and hepatocytes under ischemic stress conditions [46]. Elevation in ICAM-1 levels is a known biomarker for cardiovascular diseases [47]. Liver biopsy samples of patients with various clinical syndromes also reveal ICAM-1 expression [48]. The elevated ICAM-1 expression on the liver cells allows the MCP-1 recruited macrophages to adhere to these cells via their LFA-1 ligand. The presence and/or up regulation of macrophage/monocyte markers (CCR2 and CD11A) in the T1D liver suggest that the infiltration of blood monocytes and macrophages was in a MCP-1/CCR2 mediated and an ICAM-1/LFA-1 dependent manner. Macrophages recruited this way along with the liver resident Kupffer cells cause significant damage to the liver [49] and the abundance of liver enzymes found in T1D rat blood suggests that liver damage might be induced by the infiltrating macrophages.

As the extent of liver damage in T1D rats with hyperketonemia was more compared to that of T2D, this raises the question that could the elevated ketone levels in these animals be contributing to the additional damage seen in T1D. Elevated ketone levels occur mostly coupled with other metabolic and endocrine changes, and the effects seen in these animals cannot be exclusively ascribed to the elevated ketone levels. Nevertheless, based on the blood glucose values, which are comparable between T1D and T2D rats, the role of hyperglycemia can to some extent be ignored as its effect in these two groups would essentially be approximately the same. To demonstrate the effects due exclusively to ketones, hepatocytes were treated with ketones. Ketone treatment increased NOX4 and decreased GSH and GCLc protein expressions. An upregulation observed in NOX4, ICAM-1, MCP-1 and the down regulation of GCLc and GSH in hepatocytes exposed to ketones was far greater when ketones were present along with HG. When THP-1 derived macrophages were co-cultured with hepatocytes in the presence of ketones there was an increase in the ALT levels in the supernatant suggesting macrophage mediated hepatocyte cell injury. The ALT levels in the supernatant were induced several fold when ketones were treated along with HG. The depletion of GSH levels in hepatocytes, achieved by GCLc knockdown, increased ALT levels similar to that of ketone treatment in the supernatant. Thus, suggesting that it is the depletion
of GSH level, caused by ketones, is mediating all of these effects leading to hepatocyte injury. Depletion in cellular GSH pools can cause an imbalance in the redox potential of the cell and result in dysregulating signaling and damage. These results indicate that ketones are damaging due to their pro-oxidant activity. If this elevated oxidative stress can be controlled (in this case with L-cysteine) it could help in alleviating ketone mediated damage. Previous studies have reported that L-cysteine supplementation can reduce oxidative stress in the diabetic rat livers [50]. L-cysteine was also shown to reduce ketone and high glucose induced ROS, and ICAM-1 up regulation in cell culture studies [15, 51]. L-cysteine levels positively correlate with GSH levels and L-cysteine supplementation in diabetic patients restores GSH levels [52]. Therefore, L-cysteine supplementation is considered beneficial in reducing diabetes associated complications.

Figure 5 summarizes the proposed mechanism by which hyperketonemia can contribute to the elevation of oxidative stress and injury in T1D liver and can therefore be considered as a risk factor in the development of pathophysiologies associated with T1D.

Acknowledgements

The authors are supported by grants from NCCAM of the NIH (RO1 AT007442), the Malcolm Feist Endowed Chair in Diabetes, and by a fellowship from the Malcolm Feist Cardiovascular Research Endowment from LSUHSC, Shreveport. The authors thank Georgia Morgan for excellent editing of this manuscript.

Disclosure Statement

The authors have declared that no conflict of interest exists.

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