Role of Taurine on the Actions of Alcohol Against Systemic and Cardiac Biochemical Changes in the Diabetic Rat

Mitul C. Parikh, Sanket N. Patel, Yuanjun Shen, and Cesar A. Lau-Cam

Abbreviations

EtOH	Ethanol
TAU	Taurine
STZ	Streptozotocin
AST	Aspartate transaminase
CK	Creatine kinase
LDH	Lactate dehydrogenase
FA	Fatty acid
MDA	Malondialdehyde
GSH	Reduced glutathione
GSSG	Glutathione disulfide
CAT	Catalase
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
IL-1β	Interleukin-1β
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor-α
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate

M.C. Parikh • S.N. Patel • Y. Shen • C.A. Lau-Cam (✉)
Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Jamaica, NY, USA
e-mail: claucam@usa.net

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1 Introduction

Diabetic cardiomyopathy is a form of diabetes disease characterized by changes at the cellular and functional levels of cardiomyocytes and capable of progressing to structural, morphological and functional cardiac abnormalities that can lead to heart failure and arrhythmias (Battiprolu et al. 2010). The etiology of diabetic cardiomyopathy is not completely understood, but hyperglycemia and oxidative stress in cardiomyocytes are considered to play an important role in the pathogenesis. A unified mechanism that includes glucose autoxidation, increased production of advanced glycosylation end products, overproduction of superoxide anion radical ($O_2^{-\bullet}$) by the mitochondrial electron transport chain (ETC), and activation of the polyol and hexosamine pathways has been proposed to explain hyperglycemia-induced oxidative stress in cardiomyocytes (Brownlee 2005; Wang et al. 2013). Oxidative stress is thought to contribute to the initiation and progression of cardiac dysfunction and remodeling of the extracellular matrix in the heart.

Some of the mechanisms responsible for the cardiac dysfunction of this diabetic disease are increased expression of proinflammatory cytokines, accelerated myocardial injury, and metabolic changes such as increased lipolysis in adipocytes, increased free fatty acids (FFAs) in the circulation and cardiomyocytes, increased myocardial FA oxidation, and decreased myocardial glucose uptake and utilization (Abel 2005; Battiprolu et al. 2010). In addition there is impaired mitochondrial function, depletion of reduced glutathione (GSH), and altered mitochondrial morphology and integrity (Shen et al. 2004). With the help of chemical and transgenic animal models of type 1 and type 2 diabetes, it has been possible to demonstrate that myocardial insulin resistance is at the center of the mitochondrial dysfunction and the reduced ability of the heart to oxidize both glucose and FAs (Belke et al. 2002), with FAs serving as a substrate for building the intracardiac pool of triacylglycerols and ceramides, which together with unmetabolized FAs contribute to insulin resistance, cardiac lipotoxicity and the development of diabetic cardiomyopathy (Battiprolu et al. 2010; Ruberg 2007). In diabetes, an excessive accumulation of nonesterified FAs is detrimental to the cardiomyocyte in part by increasing oxygen demand, by contributing to mitochondrial uncoupling and the generation of reactive oxygen species (ROS) and the development of oxidative stress, one of the mechanisms responsible for mitochondrial dysfunction and decreased ATP synthesis (Abel 2005; Battiprolu et al. 2010).

Epidemiological studies suggest that a light to moderate ethanol (EtOH) consumption reduces the risk of developing cardiovascular diseases such as coronary artery disease, angina pectoris and myocardial infarction (Chen et al. 2003), improves survival after myocardial infarction, and can render the heart more tolerant to myocardial ischemia-reperfusion injury (Miyamae et al. 2010; Wang et al. 2007). Proposed mechanisms of cardioprotection by EtOH consumption have included the activation of adenosine A1 receptors, G-protein coupled α_1-adrenoceptors, protein kinase C isozymes, endothelial and inducible nitric oxide synthases (NOS), ATP-dependent potassium channels, decreased platelet aggregation, increases in high density lipoprotein cholesterol accompanied by decreases in

low density lipoprotein cholesterol, increased fibrinolytic activity, suppression of inflammatory cytokines, increases in insulin sensitivity and modifiable cardiac risks such as lipids and blood pressure, and activation of antioxidant enzymes such as catalase and superoxide dismutase (Miyamae et al. 2010).

Taurine (TAU) is an abundant endogenous product of the metabolism of methionine and cysteine endowed with cytoprotective activity in diabetes in general and in the diabetic heart in particular. There is experimental evidence to indicate that this sulfur-containing amino acid could be of benefit in diabetes by reducing the levels of blood glucose, glycated hemoglobin and plasma lipids, tissue lipid peroxidation and nitrosative injury, the loss of reduced glutathione, and the intracellular accumulation of Ca^{2+}, free radicals and other ROS produced during inflammatory responses (Roysommuti et al. 2003). In addition, TAU can decrease the formation of proinflammatory cytokines and of advanced glycation end products, the expression of chemokines, and the levels of neutrophil-generated hypochlorous acid (HClO) through formation of the less toxic and more stable taurine chloramine (TAU-Cl), a regulator of the severity of inflammatory responses and the activation of apoptotic pathways (Ito and Schaffer 2012; Roysommuti et al. 2003). There are also reports indicating that TAU can modulate mitochondrial Ca^{2+} handling, and prevent FA-decreased glucose-stimulated islet cell insulin secretion both in vivo and ex vivo, probably through an antioxidant action (Oprescu et al. 2007). On the other hand, in rats made diabetic with streptozotocin, the daily feeding of TAU, before and after the induction of the diabetes, was found to attenuate diabetes-induced alterations in cardiac function and cellular damage but without affecting the plasma levels of glucose, total cholesterol, triglycerides and high-density lipoprotein cholesterol as well as the myocardial accumulation of glycogen and lipids after a 11-week treatment period (Tappia et al. 2011). Moreover, the significant increase in cardiac TAU levels observed in diabetic cardiomyopathy may reflect a modulatory role for this amino acid on glycolysis (Militante et al. 2000).

Taking into account the known protective actions of EtOH and TAU on diabetes and/or the heart, this study was undertaken in a chemical rat model of diabetes to compare the abilities of these two agents in preventing diabetes-induced alterations of indices of glucose and lipid metabolism and of myocardial indicators of cell membrane damage, oxidative stress, inflammation and energy status. An additional purpose was to determine whether a combined treatment with EtOH and TAU can enhance the protective actions attained with the individual treatments.

2 Methods

2.1 Animals and Treatments

All the experiments were conducted with male Sprague-Dawley rats, 310–340 g in weight, randomly assigned to groups of six. Diabetes was induced with a single, 60 mg/kg, intraperitoneal dose of streptozotocin (STZ), dissolved in 10 mM citrate

buffer pH 4.5, on day 15. The diabetic rats were allowed to drink an EtOH solution (5 %, v/v, sweetened with Splenda® and flavored with a commercial sugar-free powdered lemonade product) in place of the drinking water from day 1 until day 28, with or without a concurrent treatment with TAU (2.4 mM/kg, by oral gavage). Control groups received only 10 mM citrate buffer pH 4.5, only STZ, only 5 % EtOH, only TAU or only 5 % EtOH plus TAU. All the treatments were terminated on day 28. The progress of the diabetes was monitored periodically by measuring the concentration of glucose in a drop of tail vein blood using a commercial glucometer. (TRUEtrack™ and test strips, Nipro Diagnostics, Fort Lauderdale, FL).

2.2 Samples

All the rats were sacrificed on day 29 of the study by cardiac puncture under isoflurane anesthesia. Blood samples were collected in heparinized tubes and centrifuged within 24 h at 700×*g* and 4 °C for 10 min to obtain their plasma fractions. Immediately thereafter, the animals were cut open and their hearts exposed and surgically excised using the freeze-clamp technique, and kept frozen in liquid nitrogen. A 0.5 g portion of frozen heart tissue was cut into small pieces with the help of a razor blade, placed in a polyethylene tube, mixed with ice-cold phosphate buffered saline (PBS) solution of pH 7.4, and homogenized using a hand-held electric tissue homogenizer (Tissue Tearor™, BioSpec Products, Inc., Bartlesville, OK). After bringing the volume to 10 mL with sufficient iced-cold PBS, the suspension was centrifuged at 3,000 rpm and 4° C for 30 min to obtain a clear supernatant which was withdrawn and kept on ice until needed.

2.3 Assays

2.3.1 Plasma Glucose

The plasma glucose was measured with a commercially available colorimetric assay kit (procedure No. 510, Sigma-Aldrich, St. Louis, MO) based on the method of Raabo and Terkildsen (1960). In the assay, the plasma sample was mixed with glucose oxidase, peroxidase and *ortho*-dianisidine, and the reaction is allowed to proceed at 37 °C for 30 min. The intensity of the resulting brown color was measured at 450 nm on a spectrophotometer. The results are expressed in mg/dL of plasma.

2.3.2 Plasma Insulin

An ELISA assay kit (Insulin ELISA, Calbiotech Inc., Spring Valley, CA) was used to measure the plasma insulin (INS) level. The results are expressed as μU/mL of plasma.

2.3.3 Plasma Cholesterol and Triglycerides

The total plasma cholesterol was measured by means of a commercial enzymatic-colorimetric cholesterol assay kit (Cholesterol LiquiColor®, Catalog No. 1010-430, Stanbio Laboratory, Boerne, TX) that is based on the method of Allain et al. (1974). The concentration of total (the sum of the esterified plus preformed free) cholesterol in the sample is expressed in mg/dL of plasma.

The levels of plasma and heart triglycerides were measured by means of commercial enzymatic-colorimetric assay kit (Enzymatic Triglycerides Procedure No. 2100 from Stanbio Laboratory, Boerne, TX) based on method of Fredrickson et al. (1967). The concentration of triglycerides in the sample is expressed in mg/dL of plasma.

2.3.4 Plasma Enzymatic Indices of Myocardial Damage

The plasma aspartate aminotransferase (AST) activity was measured according to the method of Bergmeyer et al. (1978) which is based on a set of two coupled reaction catalyzed by AST and malic dehydrogenase, respectively, and leading to the oxidation of NADH to NAD^+, which can be monitored spectrophotometrically at 340 nm. The results are expressed as U/L of plasma. The plasma lactate dehydrogenase (LDH) activity was measured by the method of Buhl and Jackson (1978), in which LDH catalyzes the oxidation of lactate to pyruvate with the concurrent reduction of NAD^+ to NADH. The rate of formation of NADH was followed spectrophotometrically by measuring the increase in absorbance at 340 nm. The results are expressed in U/L of plasma. The plasma creatine kinase (CK) activity was measured using a commercially available assay kit (CK Liqui-UV® Test catalog no. 2910-430, Stanbio Laboratory, Boerne, TX) based on a modification of the spectrophotometric method of Szasz et al. (1976) and in which *N*-acetyl-L-cysteine (NAC) is used to reactivate CK. The method determines the increase in absorbance at 340 nm due to the reduction of $NADP^+$ to NADPH. The results are expressed as U/L of plasma.

2.3.5 Plasma FFAs

The plasma concentration of FFAs was measured using a commercial microplate assay kit (Free Fatty Acid Quantification Colorimetric/Fluorometric Kit, BioVision Inc., Mountain View, CA). In the assay, the FFAs are converted to their coenzyme A derivatives which are subsequently oxidized with the concomitant generation of a color that can be quantified on a spectrophotometer at 570 nm. The results are expressed as nmol/mL of plasma.

2.3.6 Plasma and Heart Nonenzymatic Indices of Oxidative Stress

The extent of lipid peroxidation (LPO) in the plasma and heart was assessed by measuring the levels of malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS) using the colorimetric end-point method of Buege and Aust

(1978). In this method, MDA is reacted with TBA to yield a pink chromophore whose absorbance can be measured spectrophotometrically at 535 nm. The results are expressed as nM/mL of plasma or as nM/g of tissue.

The content of reduced (GSH) and disulfide (GSSG) glutathione in the plasma and heart was measured with the fluorometric method of Hissin and Hilf (1976), which is based on the reaction of *ortho*-phthalaldehyde with GSH at pH 8 and with GSSG at pH 12.0. Prior to the measurement of GSSG, any interfering GSH is complexed with *N*-ethylmaleimide using the method of Güntherberg and Rost (1966). The concentrations of GSH and GSSG are expressed as μM/mL of plasma or as μM/g of tissue.

2.3.7 Plasma and Heart Enzymatic Indices of Oxidative Stress

The plasma and heart activities of catalase (CAT), glutathione peroxidase (GPx) and Cu, Zn superoxide dismutase (SOD) were measured according to the methods of Aebi (1984), Günzler and Flohé (1985) and Misra and Fridovich (1972), respectively. The results are expressed as U/min/mL of plasma or as U/min/g of tissue.

2.3.8 Heart Cytokines

The levels of heart interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured using commercial microplate assay kits for rat tissue (Rat IL-1β ELISA Kit, Catalog No. ELR-IL/1β-001c, Rat IL-6 ELISA Kit, Catalog No. ELR-IL6-001c and Rat TNF-α ELISA Kit, Catalog No. ELR-TNF alpha-001/001c, obtained from RayBiotech Inc, Norcross, GA). A sample for each assay was prepared by pulverizing 50 mg of frozen heart tissue with the help of a mortar and pestle precooled in liquid nitrogen. The powder was immediately mixed with 1 mL of the sample diluent buffer included in the assay kit, and the suspension centrifuged at 3,000 rpm and 4 °C for 30 min. A 100 μL volume of heart homogenate was used in each assay as instructed by the manufacturer of the assay kit. The results are expressed as pg/g of tissue.

2.3.9 Heart ATP/ADP Ratio

The contents of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in the heart were measured using commercially available assay microplate assay kits (ATP Colorimetric/Fluorometric Assay Kit, Catalog No. K354-100 and ADP Colorimetric/Fluorometric Assay Kit, Catalog No. K355-100, both obtained from BioVision, Mountain View, CA). A lysate for the assay of ATP was homogenizing a 10 mg portion of heart tissue, kept on an ice bath, with 100 μL of ATP assay buffer with the help of a hand held tissue homogenizer, and subjecting the resulting suspension go centrifugation at 3,000 rpm and 4 °C for 10 min to obtain a clear

supernatant suitable for analysis. A sample for the assay of ADP was prepared in identical manner but using 100 μL of ADP assay buffer. A 50 μL volume of lysate was used in the assay. The samples were read on a microplate reader set at 570 nm after they were processed as suggested by the manufacturer of the kits. The concentrations of ATP and ADP are expressed in μM/g of tissue.

2.4 Statistical Analysis of Data

The experimental results are reported as mean ± standard error of the mean (SEM) for $n = 6$ rats. The results were analyzed for statistical significance using unpaired Student's *t*-test followed by one-way analysis of variance (ANOVA) and Tukey's *post hoc* test. Intergroup differences were considered to be statistically significant when $p \leq 0.05$.

3 Results

3.1 Plasma Glucose Levels

Figure 1 shows the changes in plasma glucose levels of treated and untreated diabetic rats. Diabetic rats exhibited a plasma glucose level that was more than fourfold higher ($p < 0.001$) than that of naive rats (127.03 ± 7.57 mg/dL). In contrast, the plasma glucose of diabetic rats drinking EtOH before the induction of diabetes was significantly lower (by 34 %, $p < 0.01$) than that of diabetic rats drinking plain water. A similar, although slightly greater, effect was observed in the presence of TAU (−42 %, $p < 0.001$ vs. diabetes). A further enhancement of this hypoglycemic action was possible by providing EtOH and TAU concurrently (−50 %, $p < 0.001$ vs. diabetes). Neither EtOH nor TAU altered the baseline plasma glucose to a significant extent.

3.2 Plasma INS Levels

As seen in Fig. 2, in diabetic rats the increase in plasma glucose was accompanied by a parallel significant decrease (−67 %, $p < 0.001$) of the plasma INS seen in normal rats (6.9 ± 1.2 μU/mL). The daily consumption of EtOH led to an effective attenuation of this effect (only −46 %, $p < 0.001$), which became $p < 0.01$. Allowing diabetic rats to consume EtOH in the presence of TAU led to a further reduction of the decrease in plasma INS brought about by diabetes (−26 %, $p < 0.01$) than was possible with the individual treatments. Neither EtOH nor TAU altered the baseline plasma INS to a significant extent.

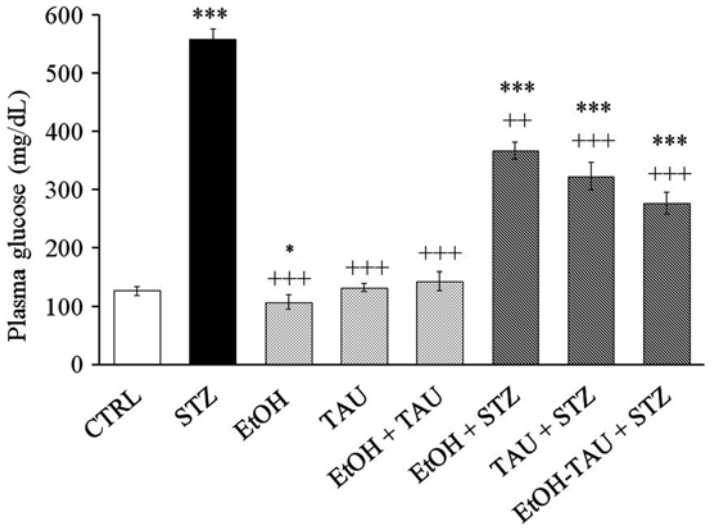


Fig. 1 The effects of EtOH, TAU and TAU-EtOH on the plasma glucose levels of diabetic rats. Results are shown as mean ± SEM for $n=6$. Differences were significant vs. control (CTRL) at $^{*}p<0.05$ and $^{***}p<0.001$; and vs. diabetes (STZ) at $^{++}p<0.01$ and $^{+++}p<0.001$

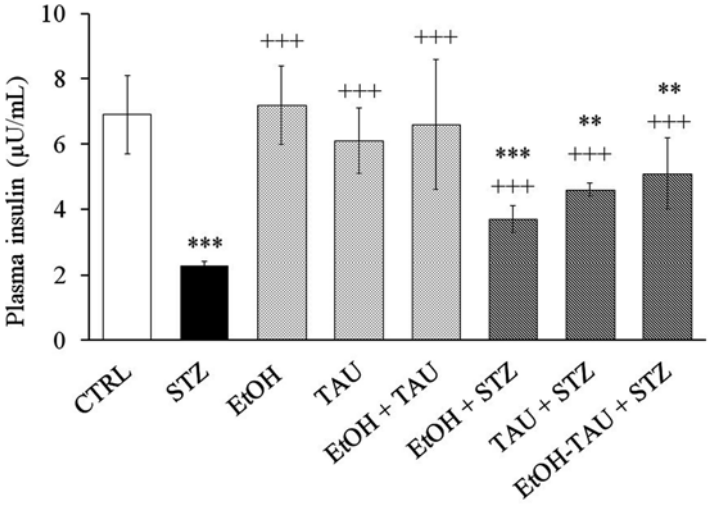


Fig. 2 The effects of EtOH, TAU and TAU-EtOH on the plasma INS levels of diabetic rats. Results are shown as mean ± SEM for $n=6$. Differences were significant vs. control (CTRL) at $^{**}p<0.01$ and $^{***}p<0.001$; and vs. diabetes (STZ) at $^{+++}p<0.001$

3.3 Plasma Cholesterol and Triglycerides Levels

The results for the plasma concentrations of cholesterol and triglycerides for the various experimental groups are displayed in Fig. 3a, b, respectively. Diabetes markedly elevated the circulating cholesterol level (+61 %, $p < 0.001$) over the

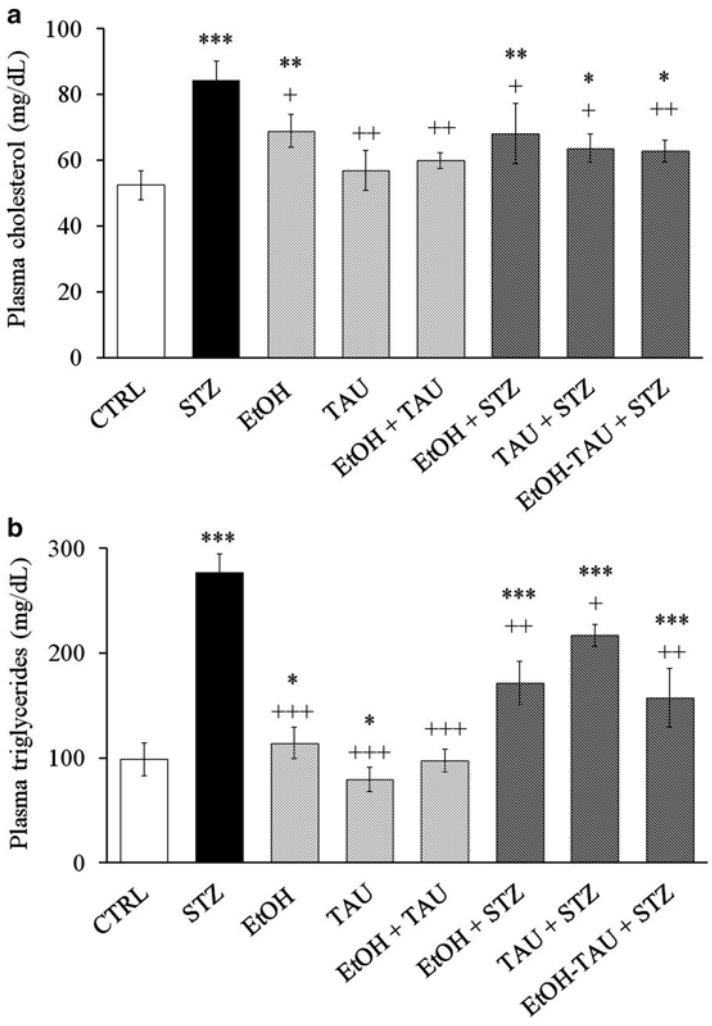


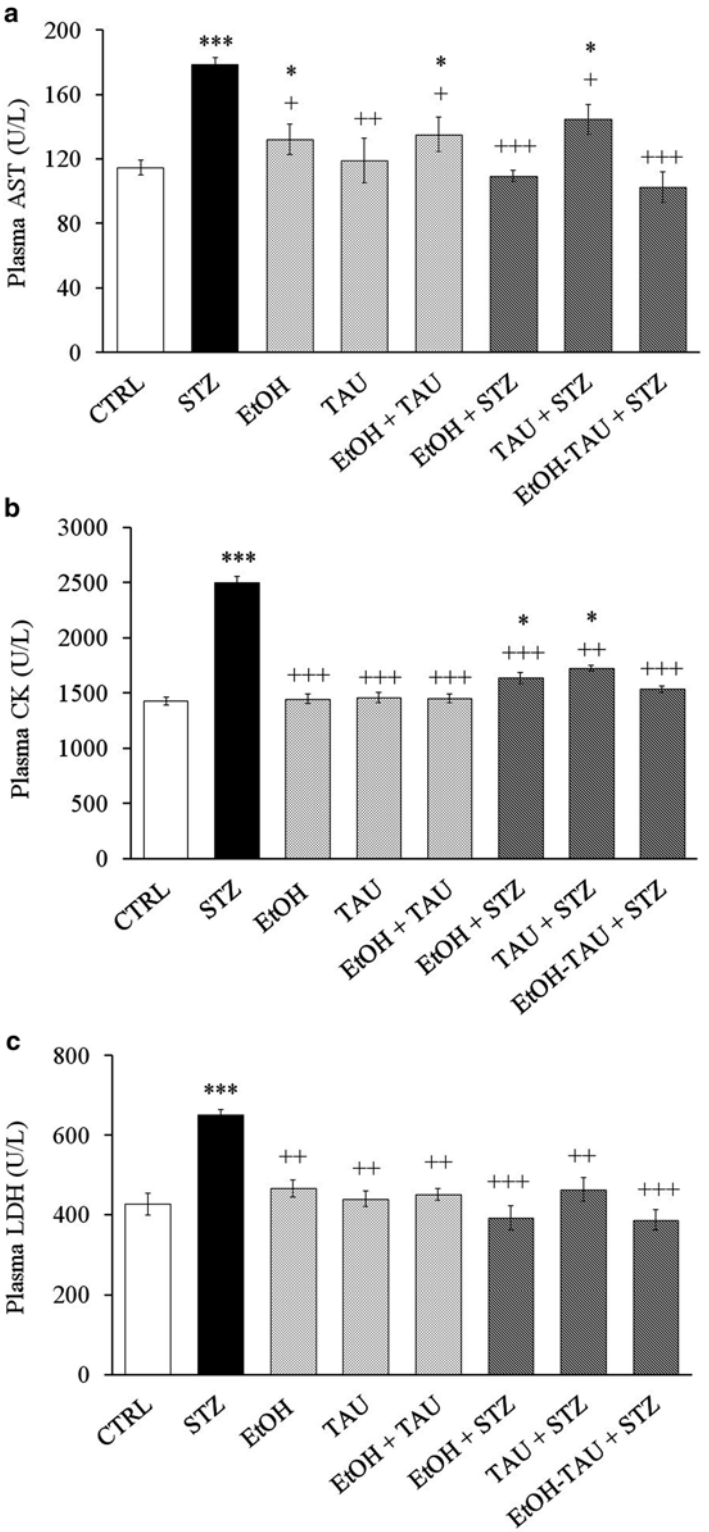
Fig. 3 The effects of EtOH, TAU and TAU-EtOH on the plasma levels of (**a**) cholesterol and (**b**) triglycerides of diabetic rats. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$

control value (84.22 ± 5.89 mg/dL) (Fig. 3a). This effect was significantly attenuated in rats consuming EtOH (+30 %, $p < 0.01$) or receiving TAU (+22 %, $p < 0.05$) daily. On the other hand a combined treatment with EtOH plus TAU with equipotent to a treatment with TAU alone. The plasma triglyceride levels (Fig. 3b) followed a similar trend to those seen with the cholesterol levels. In diabetic rats the plasma triglycerides was greatly (+180 %, $p < 0.001$) relative to control rats (98.87 ± 15.74 mg/dL). Allowing the rats to start consuming EtOH before the induction of diabetes lead to a significant attenuation of this effect (−38 %, $p < 0.001$ vs. diabetes). A similar preconditioning with TAU also reduced the increase but to a lower extent (−22 %, $p < 0.05$ vs. diabetes); and one with EtOH plus TAU was not significantly different from one with EtOH alone (−43 %, $p < 0.001$ vs. diabetes) (Fig. 3b).

3.4 Plasma AST, CK and LDH Activities

The occurrence of cardiac injury was investigated by measuring the activities of AST, CK and LDH in the plasma. As shown in Fig. 4a, the diabetic value for plasma AST rose significantly (by ~55 %, $p < 0.001$) over the control value (114.54 ± 4.59 U/L). This change was virtually abolished by the intake of EtOH (only +4 %), and significantly reduced by a treatment with TAU (+26 %, $p < 0.01$). A combined treatment with EtOH plus TAU reduced the elevation to a value not significant different from the control value (+11 %). Similarly, the data presented in Fig. 4b indicates that diabetes caused a massive increase in plasma CK (+75 %, $p < 0.001$) compared to the value for control rats (1,424.30 ± 35.76 U/L). The intake of EtOH or a treatment with TAU resulted in a strong reducing effect on this increase (only +15 % and +21 %, respectively, both at $p < 0.05$ vs. control), an effect that was accentuated when these two agents were provided alongside. Diabetes also promoted the release of LDH into the circulation (+53 %, $p < 0.001$) although to a lesser extent than those seen with AST and CK (Fig. 4c). EtOH and TAU, alone or in combination, reduced the release (<10 %) to values not significant different from the control value (426.22 ± 26.64 U/L). Except for a small increase in the plasma AST activity by EtOH (+18 %, $p < 0.05$), neither EtOH nor TAU, alone or together, had a significant elevating effect on the baseline values of CK and LDH.

Fig. 4 The effects of EtOH, TAU and TAU-EtOH on the plasma activities of (**a**) AST, (**b**) CK and (**c**) LDH of diabetic rats. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$ and $^{***}p < 0.001$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$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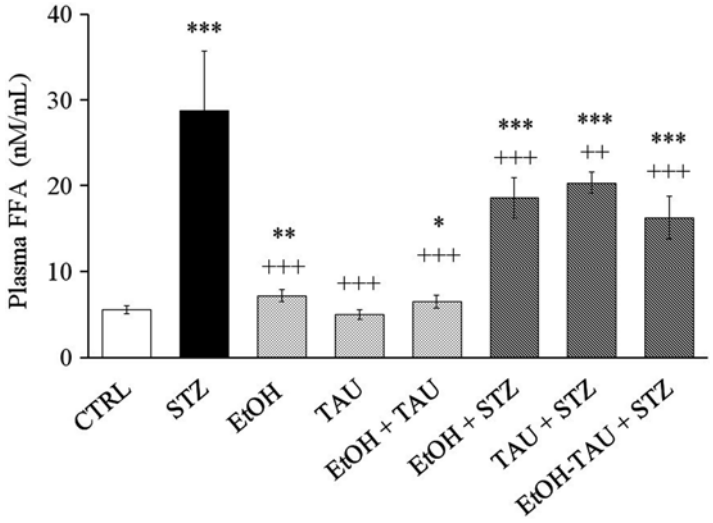

Fig. 5 The effects of EtOH, TAU and TAU-EtOH on the plasma FFAs levels of diabetic rats. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$

3.5 Plasma FFAs Levels

As shown in Fig. 5, the plasma of diabetic rats contained much higher levels of plasma FFAs levels (>410 %, $p < 0.001$) than the plasma from control rats (5.46 ± 0.44 nM/mL). This increase was reduced significantly in diabetic rats consuming EtOH (+235 %) or receiving TAU (+265 %) on a daily basis (both at $p < 0.001$ vs. diabetes). The same figure also indicates that a conditioning with EtOH plus TAU was more effective than either agent alone (only +190 %, $p < 0.001$ vs. diabetes). In naive rats, EtOH, alone or in the presence of TAU, showed a significant elevating effect on the plasma FFAs (≥18 %, $p \leq 0.05$ vs. control).

3.6 Plasma and Heart MDA Levels

As shown in Fig. 6a, the concentration of MDA was significantly higher in the plasma and heart of diabetic rats (both by 29 %, $p < 0.01$) than in control rats (11.24 ± 0.18 nM/mL and 136.50 ± 2.36 nM/g, respectively). In rats receiving either EtOH or TAU, the formation of MDA was significantly lower than in diabetic rats both in the plasma (by 19 % and 22 %, respectively, both at $p < 0.05$ diabetes) and in the heart (by 16 %, $p < 0.05$, and 32 %, $p < 0.01$, respectively). A combined treatment of with EOH and TAU, on the other hand, reduced the diabetic concentrations

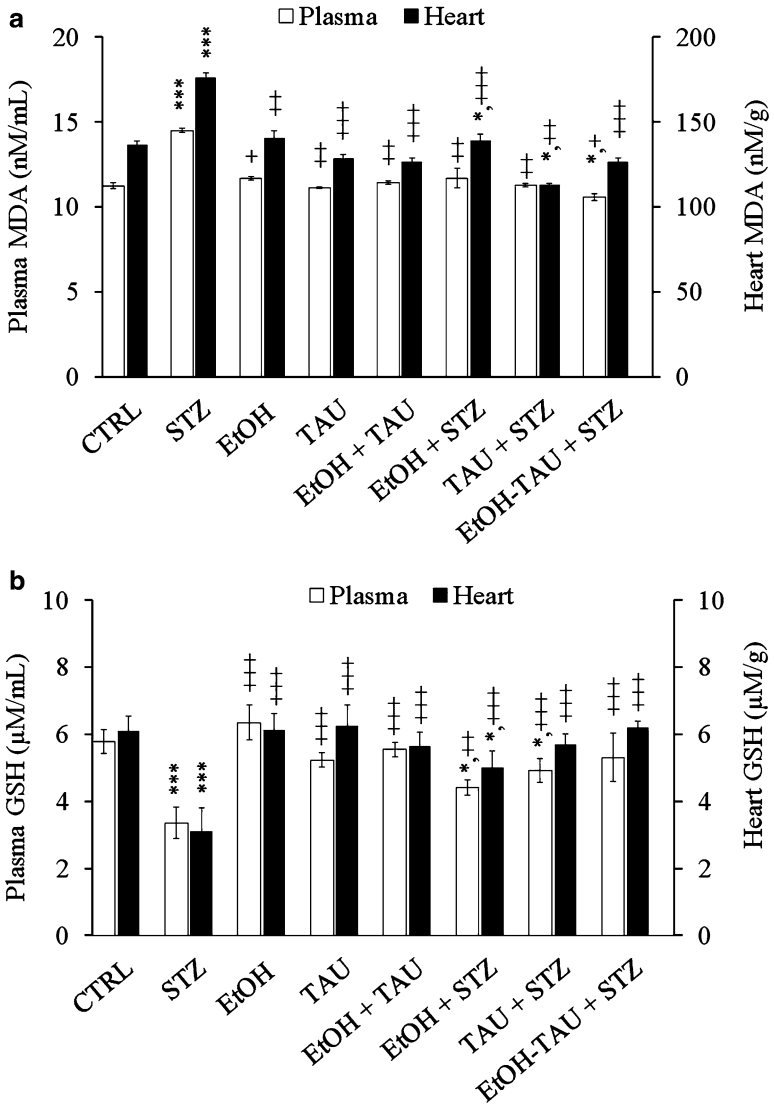

Fig. 6 The effects of EtOH, TAU and TAU-EtOH on the plasma and heart levels of MDA, GSH and GSSG. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$

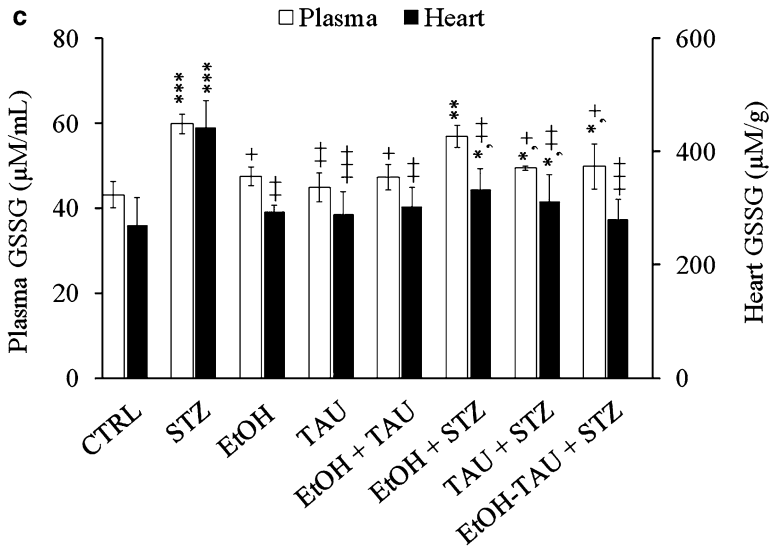

Fig. 6 (continued)

of MDA in the plasma (by 27 %, $p<0.01$) and heart (by 24 %, $p<0.05$) to values that were closer to those seen with TAU than with EtOH.

3.7 Plasma and Heart GSH and GSSG Levels

The results for the assays of the plasma and heart GSH are shown in Fig. 6b. Diabetic rats showed lower plasma and heart GSH levels (by 42 % and 49 %, respectively, $p<0.001$) than corresponding control values (5.78 ± 0.36 μM/mL and 6.09 ± 0.44 μM/g, respectively). The intake of EtOH by diabetic rats lowered the losses of GSH in the plasma and heart significantly (only −24 % and −18 %, respectively, both at $p<0.05$ vs. controls). In both instances, a daily treatment with TAU appeared more potent than one with EtOH (−15 % and +4 %, respectively). On the other hand, making EtOH and TAU concurrently available to the diabetic rats reduced the losses in plasma and heart GSH to values not different from control values. In naïve rats, neither EtOH nor TAU was found to exert an appreciable effect on the baseline GSH values.

From the results presented in Fig. 6c, it is apparent that diabetes caused an elevation of the plasma (by ~40 %, $p<0.001$) and heart (by 64 %, $p<0.001$) GSSG contents seen in control normal rats (43.16 ± 3.14 μM/mL and 269.61 ± 48.74 μM/g, respectively). Allowing the diabetic rats to drink EtOH reduced both increases to different extents (+32 %, $p<0.01$, and +23 %, $p<0.05$, respectively). The administration of TAU in place of EtOH resulted in a small gain in attenuating action (+15 % and +16 %, respectively); and a co-treatment with EtOH plus TAU led to a plasma

GSSG similar to that seen in the presence of TAU alone and a heart GSSG level similar to control. None of the treatment agents demonstrated a significant positive effect on the plasma and heart GSH and GSSG levels (Fig. 6c).

3.8 Plasma and Heart CAT, GPx and SOD Activities

A further insight into the development of oxidative stress was obtained by measuring the activities of the antioxidant enzymes CAT, GPx and SOD in the plasma and heart of diabetic rats. The data presented in Fig. 7a–c indicate that diabetes reduced the activities of the three enzymes in both samples to a significant extent. In diabetic rats, the plasma and heart CAT activities were much lower (−50 %, $p<0.001$, and 41 %, $p<0.001$, respectively) than corresponding control values (91.28 ± 2.18 U/min/mL and 7.46 ± 0.38 U/min/mg, respectively) (Fig. 7a). These effects were attenuated appreciably in the presence of a daily intake of EtOH (−33 %, and −31 %, respectively, both at $p<0.01$ vs. control). A similar treatment schedule with TAU afforded a greater protection (−10 % and −15 %, respectively); but one with EtOH plus TAU was only slightly better than one with TAU alone (8 % and 2 % decreases).

Diabetes also lowered the activity of GPx both in the plasma (by 36 %, $p<0.01$) and heart (by 54 %, $p<0.001$) (Fig. 7b). As observed with CAT, the intake of EtOH by diabetic rats lessened the decreases in activity (by only 19 %, $p<0.05$, and 13 %, respectively). While a similar degree of protection was obtained derived from a treatment with TAU (−12 % and −17 %, $p<0.05$), one with EtOH plus TAU was no different in potency than one with TAU alone.

The data reported in Fig. 7c is also consistent with a lowering by diabetes of the plasma and heart activities of SOD (by 38 % in both, $p<0.001$) of control animals. The daily consumption of EtOH by the diabetic rats resulted in a significant attenuation of these decreases (−7 % and −20 %, $p<0.05$, respectively, vs. controls). Providing TAU in place of EtOH also resulted in effective attenuation of the SOD activity loss, particularly in the heart (−6 % and −8 %, respectively), but which was not significant different from the effects of EtOH. On the other hand, a co-treatment with EtOH and TAU was no different in potency from one with TAU alone (−4 % and −8 %, respectively).

Except for a small decrease in heart CAT activity (−18 %, $p<0.05$) cause by EtOH, none of the treatment agents or their combination demonstrated a significant effect on the baseline activities of the antioxidant enzymes in the plasma and heart (Fig. 7a–c).

3.9 Heart ATP and ADP Levels and ATP/ADP Ratios

The existence of an abnormal myocardial energy metabolism as a result of diabetes was investigated by measuring the cardiac levels of ATP and ADP. From the results presented in Fig. 8, it is evident that the heart of diabetic rats contained less ATP

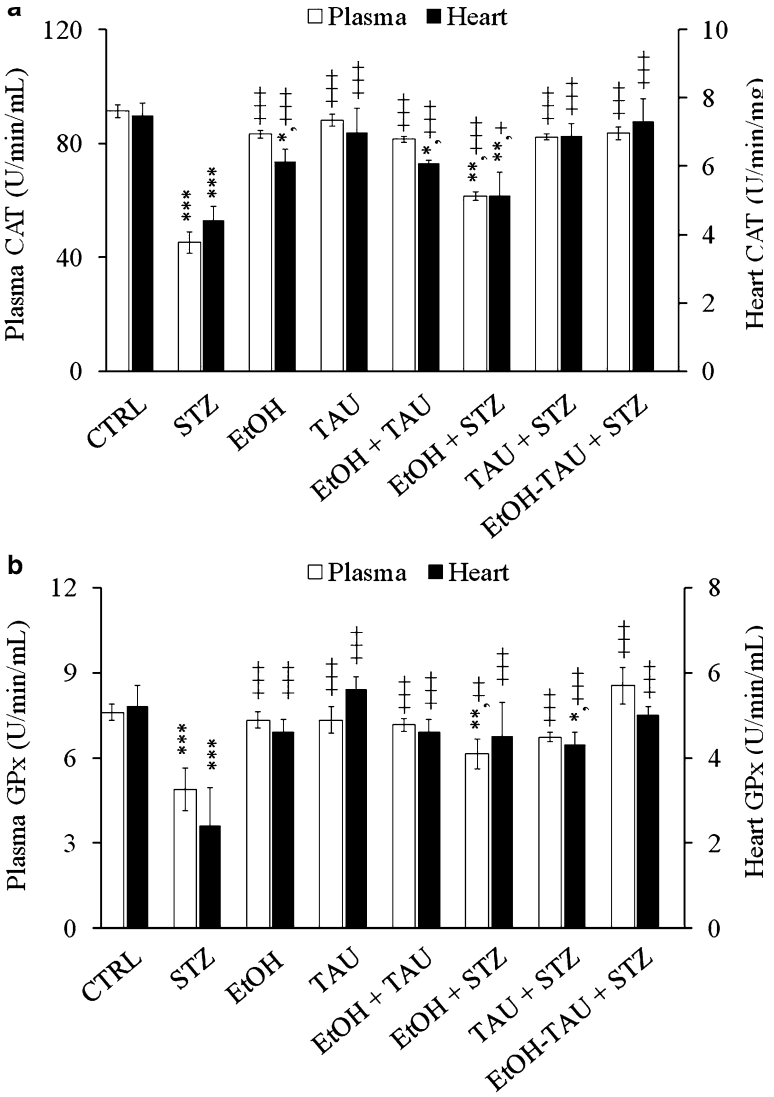

Fig. 7 The effects of EtOH, TAU and TAU-EtOH on the plasma and heart activities of (**a**) CAT, (**b**) GPx and (**c**) SOD of diabetic rats. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.01$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$

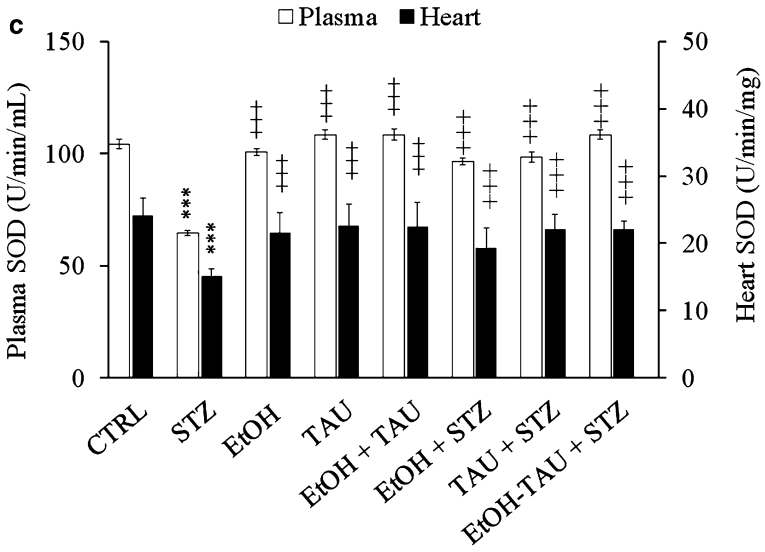

Fig. 7 (continued)

(−24 %, $p < 0.05$), more ADP (+40 %, $p < 0.001$) than hearts of naive rats (40.91 ± 1.55 μM/g and 20.55 ± 1.01 μM/g, respectively) and, hence, a lower ATP/ADP ratio (1.08 vs. 1.99). Allowing the diabetic rats to drink EtOH in place of plain water led to a marked reduction in the loss of ATP (only −14 %), gain of ADP (+6 %), and ensuing decrease in the ATP/ADP ratio (−19 %, $p < 0.05$). In this regard, treating the diabetic rats with TAU rather than with EtOH led to a reversal of the effect of diabetes on ATP (+6 %), a lower ADP accumulation (+17 %, $p < 0.05$), and a higher ATP/ADP ratio than untreated diabetic rats (1.80). On the other hand, the effect of a combined treatment of diabetic rats with EtOH plus TAU on the myocardial ATP (−3 %), ADP (+17 %, $p < 0.05$) and ATP/ADP ratio (1.7) was rather similar to one with TAU. Neither EtOH nor TAU, alone or in combination, altered the myocardial ATP/ADP ratio significantly (Fig. 8).

3.10 Heart Proinflammatory Cytokines

The occurrence of inflammation in the diabetic heart was investigated on the basis of the cardiac levels of proinflammatory cytokines known to be associated with coronary heart disease, namely IL-1β, IL-6 and TNF-α (Yudkin et al. 2000). From the results shown in Fig. 9a–c it is evident that the heart of diabetic rats contained higher levels of IL-1β (+100 %), IL-6 (+77 %) and TNF-α (+135 %) than normal hearts (1,593.85 ± 42.09 pg/g, 1,622.12 ± 175.21 pg/g and 1,314.67 ± 143.33 pg/g,

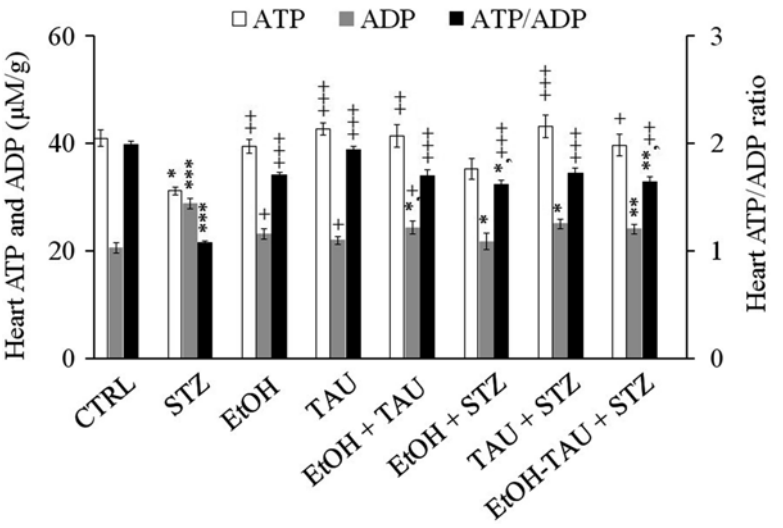

Fig. 8 The effects of EtOH, TAU and TAU-EtOH on the plasma and heart (**a**) ATP and (**b**) ADP levels and on (**c**) ATP/ADP ratio of diabetic rats. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$

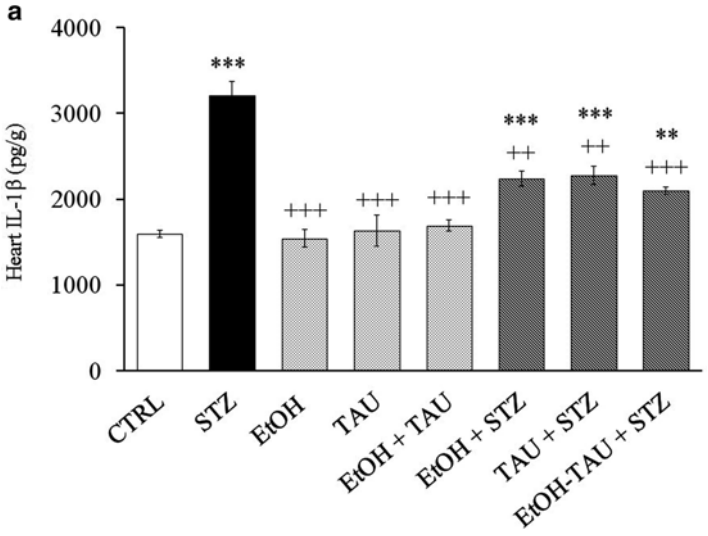

Fig. 9 The effects of EtOH, TAU and TAU-EtOH on the heart levels of (**a**) IL-1β, (**b**) IL-6 and (**c**) TNF-α of diabetic rats. Results are shown as mean ± SEM for $n = 6$. Differences were significant vs. control (CTRL) at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and vs. diabetes (STZ) at $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$

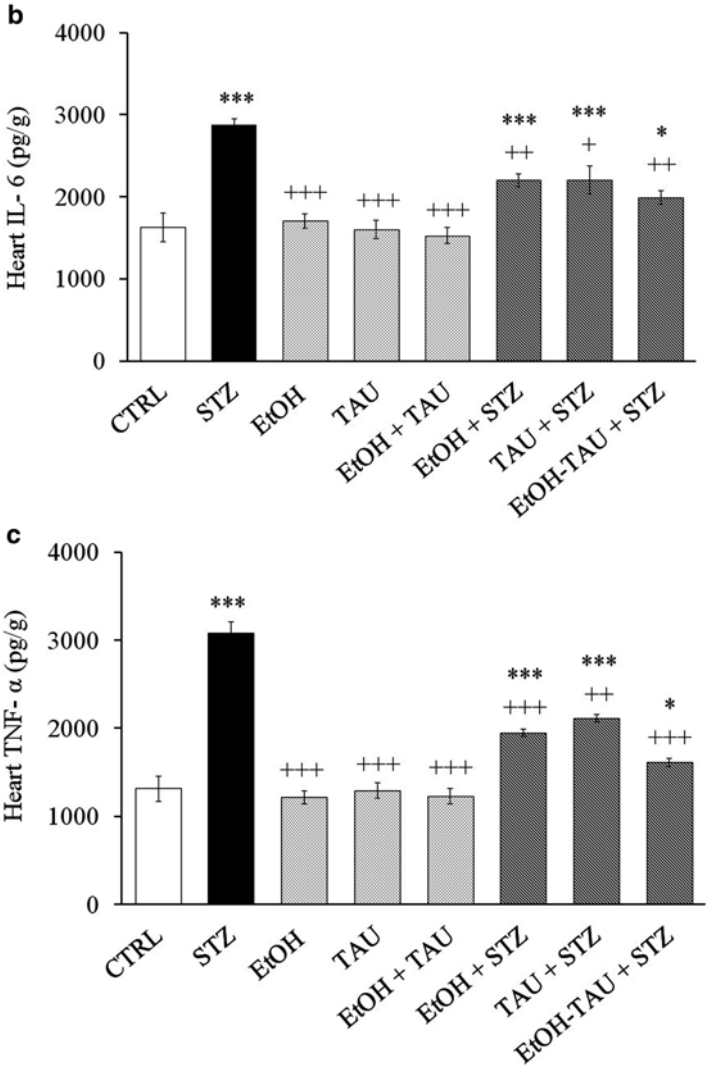

Fig. 9 (continued)

respectively, all at $p<0.001$). The same figures also indicate that the daily consumption of a low concentration of EtOH was effective in reducing these elevations (increases of only 41 %, 36 % and 48 %, respectively, $p<0.001$ vs. controls). In comparison with EtOH, a similar treatment schedule with TAU was somewhat less effective against the increase in IL-6 (+36 %, $p<0.001$) and TNF-α (+60 %, $p<0.001$) and about equipotent against the increase in IL-1β (+43 %, $p<0.001$). However, providing TAU and EtOH concurrently led to greater attenuating effect

against increases of all three cytokines (increases of 32 %, 23 % and 23 %, respectively, $p \leq 0.05$ vs. controls).

4 Discussion

Preconditioning is an endogenous adaptive protective response to a deleterious insult by cells, tissues or organs following a brief exposure to a physicochemical stress (Kuhlmann et al. 1997), to a low to moderate concentration of an exogenous substance (Collins et al. 2009) or as a result of an appropriate environmental adjustment (Wang et al. 2013). Among preconditioning agents, EtOH is probably the most widely used in situations where cell injury such as ischemic-reperfusion cardiac injury (Chen et al. 2003; Miyamae et al. 1997; Collins et al. 2009), renal injury (Fan et al. 2012) or inflammation (Sierksma et al. 2002) are of concern. In the present study, preconditioning was carried out by continuously making EtOH available in the drinking water and starting at 2 weeks prior to the induction of diabetes with STZ. The concentration of the EtOH solution used (5 % v/v) is one reported to improve survival and to reduce cardiomyocyte damage in guinea pigs later developing a myocardial infarction (Miyamae et al. 1997). Using the same treatment schedule, TAU was provided by oral gavage at a dose (2.4 mM/kg) known in this laboratory to be effective in curtailing metabolic and biochemical changes associated with STZ-induced diabetes in the rat (Pandya et al. 2010).

Irrespective of the metabolic parameter examined, both EtOH and TAU were able to attenuate the hyperglycemia, hypoinsulinemia, dyslipidemia and increased lipolysis seen in diabetic rats. The effects were found to be not only invariably statistically significant but to follow a common pattern. However, when comparing the individual potencies, the attenuating effect was in all instances, but on lipolysis, greater with TAU than with EtOH. The possibility that EtOH and TAU could be exerting their protective effects through different mechanism was suggested by the results of experiments in which these agents were used concurrently and which found the combination to be more effective than the individual treatments. Moderate ingestion of EtOH in type 2 diabetics are associated with reduced fasting INS level and improved INS sensitivity, which also translates into reduced dyslipidemia (Nogueira et al. 2014). In high concentrations EtOH is known to cause hypoglycemia by inhibiting hepatic gluconeogenesis, which may be of concern in the absence of adequate dietary intake (Badawy 1977). The lowering of the plasma FFAs by 5 % EtOH confirms the report that consumption of low dose EtOH by young healthy male volunteers leads to a modest activating action on hepatic de novo lipogenesis and hypertiglyceridemia, and that its hepatic metabolism generates acetate for transport to peripheral tissues where it inhibits lipolysis (Feinman and Lieber 1999). The beneficial effects of TAU on parameters of glucose metabolism are consistent with its reported protective effects against diabetes-induced β-cell damage (Chang and Kwon 2000) and apoptosis (Lin et al. 2013), against FA-mediated decrease in

glucose-stimulated INS secretion and islet ROS production (Oprescu et al. 2007), and ability to enhance peripheral glucose uptake in INS-resistance states (Haber et al. 2003).

The significance increases in plasma AST, total CK and LDH activity values is taken as an indication that diabetes can cause myocardial injury. In this case, the trend of the increases (i.e. total CK>AST>LDH) is akin with that reported for human patients experiencing chest pains during a myocardial infarct (Moss et al. 1986). Preconditioning with either EtOH or TAU reduced these elevations to values that were not very difference from those of naïve animals. As a result, protection by EtOH and TAU was about equal and protection by their combination was only insignificantly better than the individual treatments. Since in type 2 diabetes, myocardial injury is regarded as a consequence of oxidative stress arising directly or indirectly from hyperglycemia, hyperlipidemia, hyperinsulinemia, and INS resistance, alone or in combination (Ansley and Wang 2013), the protective role of EtOH and TAU may be tied up, at least in part, to their inherent antioxidant activities (Ito and Schaffer 2012) and, in terms of TAU, also to a membrane stabilizing effect (Roysommuti et al. 2003). At low concentrations, the metabolism of EtOH generates nicotinamide adenine dinucleotide for creating a reductive environment that decreases oxidative stress and the secondary production of aldehydes through LPO, and for maintaining the intracellular cysteine and GSH in their reduced state (Vasdev et al. 2006). Indeed, in the present work and that of other laboratories (Ansley and Wang 2013; Kumawat et al. 2013; Maritim et al. 2003), the development of diabetes is found to be accompanied by increases in MDA, a marker of lipid peroxidation (LPO), in the plasma and heart tissue, by a drop in the corresponding GSH/GSSG ratios, and by lower circulating and cardiac activities of antioxidant enzymes participating in the removal of hydrogen peroxide, organic peroxides and superoxide anion radicals ($O_2^{-\bullet}$). A direct lowering of free radicals and other ROS by TAU has been a subject of a long debate since its molecule lacks a readily oxidizable functionality and antiradical activity in cell-free radical-generating systems has been weak to nil (Aruoma et al. 1998; Tadolini et al. 1995). An indirect antioxidant role seems to underline some of the actions of TAU, which includes the enhancement of expression and activities of antioxidant enzymes (Jang et al. 2009) and the binding of reactive aldehydes including glucose (Ogasawara et al. 1993). In the diabetic heart in particular, the occurrence of oxidative stress has been correlated with increases in $O_2^{-\bullet}$ levels, LPO and activity of NADPH oxidase (Ansley and Wang 2013), with decreases in antioxidant enzyme defenses, and with the leakage of electron and $O_2^{-\bullet}$ from a dysfunctional myocardial mitochondrion (Ansley and Wang 2013). Hyperglycemia is the driving factor in $O_2^{-\bullet}$ overproduction in the mitochondrion by causing the disruption of the ETC, activating NADPH oxidase and uncoupling NOS (Giacco and Brownlee 2010). While a depletion of TAU by hyperglycemia may contribute to mitochondrial dysfunction (Hansen et al. 2010) and apoptotic myocardial cell death (Jong et al. 2011), a supplementation with it is found to reduced mitochondrial LPO and $O_2^{-\bullet}$ generation in the oxidatively stressed heart under diabetic (Schaffer et al. 2009) and nondiabetic (Parvez et al. 2008) conditions.

The role of inflammation in the pathogenesis and progression of myocardial injury in chronic diabetes is well established. The injurious effect of chronic diabetes in the myocardium could result from either a persistent inflammatory signaling directly to the heart or from the dysregulation of antiinflammatory signaling systems (Drimal et al. 2008). Irrespective of its etiology, an inflammatory component of diabetes leading to cardiac damage and dysfunction is the release of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α and of chemoattractants from inflammatory cells in chemically-induced diabetes in mice (Chao et al. 2009) and rats (Drimal et al. 2008; Jain et al. 2007). In the present work, the cardiac levels of these three cytokines were dramatically increased relative to levels recorded for nondiabetic rats, with those of TNF-β predominating over those of IL-1β and Il-6 in that order. Both EtOH and TAU drastically suppressed these elevations, especially when provided alongside, with the degree of the effect varying inversely to the degree of proinflammatory cytokine elevation. The present effects of EtOH, representing a light and short consumption, contrast markedly with those observed during prolonged heavy consumption and in which it can trigger the recruitment and activation of inflammatory cells (Szabo et al. 2007). In contrast, results of in vitro studies in which monocytes were exposed to moderate concentration of EtOH found IL-6 release to be decreased via a transient decrease in the activity of the transcription factor nuclear factor-kappa B (NF-κB) (Mandrekar et al. 1997); and human intervention studies with moderate EtOH intake have verified a decrease inflammatory response via an increase in circulating adinopectin, an adipocyte protein that enhances insulin sensitivity by increasing FA oxidation and inhibiting hepatic glucose production (Lihn et al. 2005). Moreover, EtOH is able to induce the antiinflammatory cytokine IL-10 that can cause long lasting disruption of proinflammatory, cytokine-yielding, cascades (Qin et al. 2008). The characteristic natural abundance of TAU in cells with a high capacity for generating oxidants (i.e., neutrophils, macrophages) heralds an important protective role in oxidative stress-related inflammation. However, its antioxidant action is exerted only after conversion by neutrophil myeloperoxidase to TAU-Cl upon condensation with HOCl (Marcinkiewicz and Kontny 2014). TAU-Cl is a strong modulator of the immune system, capable of down regulating the production of proinflammatory cytokines in human and rodent leukocytes by inhibiting the activation of NF-κB, a potent signal transducer for proinflammatory cytokine expression (Schuller-Levis and Park 2004).

To be able to sustain its physiological functions, basal metabolic functions and ion-modulating actions, the heart needs a continuous supply of energy in the form of ATP, most of which is derived from mitochondrial β-oxidation of FA and the oxidative phosphorylating activity of the ETC, with the rest coming from glycolysis and guanosine triphosphate formed in the citric acid cycle (Lopaschuk et al. 2010). In the present study, the energy status of the diabetic heart was assessed by measuring the changes in ATP and ADP concentrations and calculating the corresponding ratios. Evidence of mitochondrial dysfunction was suggested by the extensive decrease in the value of the ATP/ADP ratio. Both EtOH and TAU were found to be effective in limiting this decrease, making the value barely significant when compared to the baseline value. Moreover, the effect of a co-treatment with these agents

was not significantly different from that attained with each agent alone. In the case of EtOH, the concentration of the EtOH solution seems to be a critical determining factor of the trend of changes in adenosine nucleotides in the heart. In this study, free intake of a low (5 %) concentration of EtOH had only a small decreasing effect on the heart ATP/ADP ratio, solely as a result of a small increase in ADP. On the other hand, the oral intake of 7.5 % EtOH (equal to 1 g/kg) was without effect on ATP, ADP and inorganic phosphate levels in the rat liver (Lindros and Stowell 1982). In contrast; treating rats with an intraperitoneal 1.5 g/k dose of EtOH elicited a slight increase in the ATP level 1 h later but without altering the levels of other adenine nucleotides (Pösö and Forsander 1976). In high doses and a long exposure, however, EtOH can reduce mitochondrial GSH, a cofactor for enzymes reducing hydrogen peroxide and organic peroxides, thus making this organelle more susceptible to oxidative damage and the development of LPO, and unable to carry out oxidative phosphorylation and ATP synthesis (Fernández-Checa and Kaplowitz 2005). In this context, TAU has proven of benefit in preventing excessive generation of ROS by the ETC (Schaffer et al. 2014), and in suppressing LPO and enhancing antioxidant enzyme activity in the myocardium (Wang et al. 2013). At present, the exact mechanism whereby diabetes causes mitochondrial dysfunction in the heart is incompletely understood since more than one factor seems to be involved. In addition to oxidative stress, hyperglycemia, increased uptake of FFAs, hyperinsulinemia and insulin resistance may all play a role (Abel 2005; Ansley and Wang 2013). Hyperglycemia is found to disrupt the ETC, to activate NADPH oxidase to produce $O_2^{-\bullet}$, and to uncouple NOS, a problem that compounds the oxidative stress since more $O_2^{-\bullet}$ and less NO is produced (Ansley and Wang 2013). Increased FFAs not only stimulates NADPH oxidase and decreases intracellular GSH but also contributes to the formation of di- and triacylglycerols and ceramides, the accumulation of which has been implicated in the development of INS resistance, cardiac dysfunction and cardiac failure (Lopaschuk et al. 2010). In turn, INS resistance can increase myocardial oxygen utilization and FA utilization and oxidation to ROS for eventual oxidative stress and cell damage. $O_2^{-\bullet}$ is of particular importance in cardiac energetics since it can uncouple ATP synthesis from oxidative metabolism directly by activating uncoupling proteins and indirectly via formation of LPO products (Lopaschuk et al. 2010). In addition, the use of proteomics has established a correlation between a deficiency of INS in diabetes with increased nitration of mitochondrial proteins and with downregulation of genes encoding for specific protein components of the ETC (Abel 2005).

5 Conclusions

Preconditioning with either a low-concentration EtOH solution or TAU was found to effectively protect the diabetic rat heart in a similar manner against diabetes-related biochemical alterations relevant to the pathogenesis of diabetic cardiomyopathy. Although differences in potency between the two treatment agents were

apparent, they were generally nonsignificant. However, providing EtOH and TAU together generally resulted in a greater protection than that attained with the individual treatments.

References

Abel ED (2005) Metabolic perturbations in the diabetic heart: mechanisms of molecular target. Drug Discov Today Dis Med 2:115–122

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20:470–475

Ansley DM, Wang B (2013) Oxidative stress and myocardial injury in the diabetic rat. J Pathol 229:232–241

Aruoma OI, Halliwell B, Hoey BM, Butler J (1998) The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J 256:251–255

Badawy AAB (1977) A review of the effect of alcohol on carbohydrate metabolism. Br J Alcohol Alcohol 12:30–42

Battiprolu PK, Gillette TG, Wang ZV, Lavandero S, Hill JA (2010) Diabetic cardiomyopathy: mechanisms and therapeutic targets. Drug Discov Today Dis Mech 7:e135–e147

Belke DD, Larsen TS, Gibbs EM, Severson DL (2002) Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice. Am J Physiol Endocrinol Metab 279:E1104–E1113

Bergmeyer HU, Scheibe P, Wahlefeld AW (1978) Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem 24:58–73

Brownlee M (2005) The pathobiology of diabetic complications. A unifying mechanism. Diabetes 54:1615–1625

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Buhl SN, Jackson KY (1978) Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 °C. Clin Chem 24:828–831

Chang KJ, Kwon W (2000) Immunohistochemical localization of insulin in pancreatic beta-cells of taurine-supplemented or taurine-depleted diabetic rats. Adv Exp Med Biol 483:579–587

Chao P, Hsu C, Yin M (2009) Anti-inflammatory and anti-coagulatory activities of caffeic acid and ellagic acid in cardiac tissue of diabetic mice. Nutr Metab 6:33

Chen Y, Davis-Gorman G, Watson RR, McDonagh PF (2003) Chronic ethanol consumption modulates myocardial ischaemia-reperfusion injury in murine AIDS. Alcohol Alcohol 38:18–24

Collins MA, Neafsey EJ, Mukamal KJ, Gray MO, Parks DA, Das DK, Korthuis RJ (2009) Alcohol in moderation, cardioprotection and neuroprotection: epidemiological considerations and mechanistic studies. Alcohol Clin Exp Res 33:206–219

Drimal J, Knezi V, Navarova J, Nedelcevova J, Paulovicova E, Sotnikova R, Snirc V, Drimal D (2008) Role of inflammatory cytokines and chemoattractants in the rat model of streptozotocin-induced diabetic heart failure. Endocr Regul 42:129–135

Fan LH, He L, Cao ZQ, Xiang B, Liu L (2012) Effect of ischemia preconditioning on renal ischemia/reperfusion injury in rats. Int Braz J Urol 38:842–854

Feinman L, Lieber CS (1999) Ethanol and lipid metabolism. Am J Clin Nutr 70:791–792

Fernández-Checa JC, Kaplowitz N (2005) Hepatic mitochondrial glutathione: transport and role in disease and toxicity. Toxicol Appl Pharmacol 204:263–273

Fredrickson DS, Levy RI, Lees RS (1987) Fat transport in lipoproteins: an integrated approach to medicine and disorders. N Engl J Med 276:34–42

Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. Circ Res 107:1058–1070
Güntherberg H, Rost J (1966) The true oxidized glutathione content of red blood cells obtained by new enzymic and paper chromatographic methods. Anal Biochem 15:205–210
Günzler WA, Flohé L (1985) Glutathione peroxidase. In: Greenwald RA (ed) CRC handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL, pp 285–290
Haber CA, Lam TK, Yu Z, Gupta N, Oh T, Bogdanovic E, Giacca A, Fantus IG (2003) N-acetylcysteine (NAC) and taurine prevent hyperglycemia-induced insulin resistance in vivo: possible role of oxidative stress. Am J Physiol Endocrinol Metab 285:E744–E753
Hansen SH, Andersen ML, Grunnet N (2010) A role for taurine in mitochondrial function. J Biomed Sci 17(Suppl 1):S23
Hissin PJ, Hilf R (1976) A fluorometric method for the determination of oxidized and reduced glutathione in tissue. Anal Biochem 74:214–226
Ito T, Schaffer SW (2012) The potential usefulness of taurine on diabetes mellitus and its complications. Amino Acids 42:1529–1539
Jain SK, Rains J, Croad J (2007) Chromium-niacinate (Cr-N) supplementation (suppl) lowers proinflammatory cytokines and lipid peroxidation (LP) in cultured human monocytes exposed to high-glucose (HG) and in streptozotocin (STZ)-treated diabetic rats (D). Diabetes 56(Suppl 1):pA450
Jang JS, Piao S, Cha YN, Kim C (2009) Taurine chloramine activates Nrf2, increases HO-1 expression and protects cells from death caused by hydrogen peroxide. J Clin Biochem Nutr 45:37–43
Jong CJ, Azuma J, Schaffer SW (2011) Role of mitochondrial permeability transition in taurine deficiency-induced apoptosis. Exp Clin Cardiol 16:125–128
Kuhlmann MK, Betz R, Hanselmann R, Köhler H (1997) Heat-preconditioning confers protection from Ca2+-mediated cell toxicity in renal tubular epithelial cells (BSC-1). Cell Stress Chaperones 2:175–179
Kumawat M, Sharma TK, Singh I, Singh N, Ghalaut VS, Vardey SK, Shankaer V (2013) Antioxidant enzymes and lipid peroxidation in type 2 diabetes mellitus patients with and without nephropathy. N Am J Med Sci 5:213–219
Lihn L, Pedersen SB, Richelsen B (2005) Adinopectin: action, regulation and association to insulin sensitivity. Obes Rev 6:13–21
Lin S, Yang J, Wu G, Liu M, Ly Q, Yang Q, Hu J (2013) Inhibitory effects of taurine on STZ-induced apoptosis of pancreatic islet cells. Adv Exp Med Biol 775:287–297
Lindros KO, Stowell A (1982) Effects of ethanol-derived acetaldehyde on the phosphorylation potential and on the intramitochondrial redox state in intact rat liver. Arch Biochem Biophys 218:429–437
Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS, Stanley WC (2010) Myocardial fatty acid metabolism in health and disease. Physiol Rev 90:207–258
Mandrekar P, Catalano D, Szabo G (1997) Alcohol-induced regulation of nuclear regulatory factor-κB in human monocytes. Alcohol Clin Exp Res 21:988–994
Marcinkiewicz J, Kontny E (2014) Taurine and inflammatory diseases. Amino Acids 46:7–20
Maritim AC, Sanders RA, Watkins JB 3rd (2003) Diabetes, oxidative stress and antioxidants: a review. J Biochem Mol Toxicol 17:24–38
Militante JD, Lombardini JB, Schaffer SW (2000) The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. Cardiovasc Res 46:393–402
Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175
Miyamae M, Diamond I, Weiner MW, Camacho SA, Figueredo VM (1997) Regular alcohol consumption mimics cardiac preconditioning by protecting against ischemia-reperfusion injury. Proc Natl Acad Sci U S A 94:325–3239
Miyamae M, Kaneda K, Domae N, Fiqueredo VM (2010) Cardioprotection by regular ethanol consumption: mechanisms and clinical application. Curr Drug Abuse Rev 3:39–48

Moss DW, Henderson AR, Kachmar JF (1986) Enzymes. In: Tietz NW (ed) Textbook of clinical chemistry. W.B. Saunders Co., Philadelphia, PA, pp 698–700
Nogueira LC, Couri S, Trugo NF, Lollo PCB (2014) The effect of different alcoholic beverages on blood alcohol levels, plasma insulin and plasma glucose in humans. Food Chem 158:527–533
Ogasawara M, Nakamura T, Koyama I, Nemoto M, Yoshida T (1993) Reactivity of taurine with aldehydes and its physiological role. Chem Pharm Bull 41:2172–2173
Oprescu AI, Bikopoulos G, Naassan A, Allister EM, Tang C et al (2007) Free fatty acid-induced reduction in glucose-stimulated insulin secretion: evidence for a role of oxidative stress in vitro and in vivo. Diabetes 56:2927–2937
Pandya KG, Patel MR, Lau-Cam CA (2010) Comparative study of the binding characteristics to and inhibitory potencies towards PARP and in vivo antidiabetogenic potencies of taurine, 3-aminobenzamide and nicotinamide. J Biomed Sci 17(Suppl 1):S16
Parvez S, Tabassum H, Banerjee BD, Raisuddin S (2008) Taurine prevents tamoxifen-induced mitochondrial oxidative damage in mice. Basic Clin Pharmacol Toxicol 102:382–387
Pösö AR, Forsander OA (1976) Influence of ethanol oxidation rate on the lactate/pyruvate ratio and phosphorylation state of the liver in fed rats. Acta Chem Scand B 30:801–806
Qin L, He J, Hanes RN, Pluzarev O, Hong JS, Crews FT (2008) Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. J Neuroinflammation 5:10
Raabo E, Terkildsen TC (1960) On the enzymatic determination of blood glucose. Scand J Clin Lab Invest 12:402–407
Roysommuti S, Azuma J, Takahashi K, Schaffer S (2003) Taurine cytoprotection: from cell to system. J Physiol Sci 16:17–27
Ruberg FL (2007) Myocardial lipid accumulation in the diabetic heart. Circulation 116:110–1112
Schaffer SW, Azuma J, Mozaffari M (2009) Role of antioxidant activity of taurine in diabetes. Can J Physiol Pharmacol 87:91–99
Schaffer SW, Jong CJ, Ito T, Azuma J (2014) Effect of taurine on ischemia-reperfusion injury. Amino Acids 46:21–30
Schuller-Levis GB, Park E (2004) Taurine and its chloramines: modulators of immunity. Neurochem Res 29:117–126
Shen X, Zheng S, Thongboonkerd V, Xu M, Pierce WM Jr et al (2004) Cardiac mitochondrial damage and biogenesis in a chronic model of type 1 diabetes. Am J Physiol Endocrinol Metab 287:E896–E905
Sierksma A, Gaag MS, van Tol A, James RW, Hendriks HF (2002) Kinetics of HDL cholesterol and paraoxonase activity in moderate alcohol consumers. Alcohol Clin Exp Res 26:1430–1435
Szabo G, Madrekar P, Oak S, Mayerle J (2007) Effect of ethanol on inflammatory responses. Pancreatology 7:115–123
Szasz G, Gruber N, Bernt E (1976) Creatine kinase in serum: 1. Determination of optimum reactions conditions. Clin Chem 22:650–656
Tadolini B, Pintus G, Pinna GG, Bennardini F, Franconi F (1995) Effects of taurine and hypotaurine on lipid peroxidation. Biochem Biophys Res Commun 213:820–826
Tappia PS, Thliveris J, Dhalla NS (2011) Effects of amino acid supplementation on myocardial cell damage and cardiac function in diabetes. Exp Clin Cardiol 16:e17–e22
Vasdev S, Gill V, Singal PK (2006) Beneficial effect of low ethanol intake on the cardiovascular system: possible biochemical mechanisms. Vasc Health Risk Manag 2:263–276
Wang Q, Sun AY, Simonyi A (2007) Ethanol preconditioning protects against ischemia/reperfusion-induced brain damage: role of NADP oxidase-derived ROS. Free Radic Biol Med 43:10461060
Wang WN, Li W, Lu X, Zhao X, Xu L (2013) Taurine attenuates oxidative stress and alleviates cardiac failure in type I diabetic rats. Croat Med J 54:171–179
Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V (2000) Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148:209–214