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Augmented resistance to oxidative stress in fatty rat livers induced by a short-term sucrose-rich diet

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Abstract

Hepatic steatosis and the accompanying oxidative stress have been associated with a variety of liver diseases. It is not known if fat accumulation per se plays a direct role in the oxidative stress of the organ. This study tested if steatosis induced by a short-term carbohydrate-rich diet results in an increased hepatic sensitivity to oxidative stress. Antioxidant status was determined in a liver perfusion system and in isolated parenchymal, endothelial and Kupffer cells from rats kept on sucrose-rich diet or on regular diet for 48 h. *t*-Butyl hydroperoxide addition (2 mM) to the perfusion fluid resulted in a release of alanine aminotransferase (ALT) in livers from controls, whereas no ALT release was observed in fatty livers. After *t*-butyl hydroperoxide addition, oxidized glutathione release was 40% less in fatty than in control livers, whereas reduced glutathione (GSH) release was not different. Sinusoidal oxidant stress was mimicked by the addition of lipopolysaccharide (LPS) from *Escherichia coli* (10 μg/ml) followed by the addition of opsonized zymosan (8 mg/ml) to the perfusion medium. LPS plus zymosan treatments resulted in the release of ALT in control but not in fatty livers. At the end of perfusion, liver glutathione content was 3-fold elevated, and the tissue content of lipid peroxidation products was approx. 40% less in fatty livers compared to controls. GSH content was doubled and glucose-6-phosphate dehydrogenase (G6PD) expression was elevated by 3- and 10-fold in sinusoidal endothelial and parenchymal cells form fatty livers compared to cells from control animals. Following H_2O_2 administration in vitro (0.2–1 mM), GSH remained elevated in endothelial and parenchymal cells from fatty livers compared to cells from controls. In contrast, G6PD activity and GSH content were similar in Kupffer cells isolated from fatty or control livers. The study shows that hepatic fat accumulation caused by a short-term sucrose diet is not accompanied by elevated hepatic lipid peroxidation, and an elevated hepatic antioxidant activity can be manifested in the presence of prominent steatosis. The diet-induced increase in G6PD expression and, thus, the efficient maintenance of reduced glutathione in endothelial and parenchymal cells are a supportive mechanism in the observed hepatic resistance against intracellular or sinusoidal oxidative stress.

Keywords: Endothelial cell; Kupffer cell; Hepatocyte; Nutrition; Glutathione; Oxidative stress; Pentose phosphate shunt; Glucose-6-phosphate dehydrogenase

1. Introduction

Hepatic steatosis is frequently present in a variety of liver diseases including alcoholism, obesity, diabetes and after excessive drug intake [1–3]. Both microvesicular and macrovesicular steatosis have been

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; MCLB, monochlorobimane; TBARS, thiobarbituric acid reactive substance; ALT, alanine aminotransferase

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shown to represent a poor prognosis and have been associated with steatohepatitis, fibrosis and cirrhosis. Oxidative stress and the accumulation of lipid peroxidation products [4–6] together with gut-derived endotoxin and hepatic cytokine effects have been suggested to play a role in the initiation of liver pathology in both alcoholic and non-alcoholic steatosis [6–11].

The causative relationship between hepatic fat accumulation and the accompanying oxidative stress in the organ, however, remains controversial. The observations that chronic alcohol, hypoxia, obesity or administration of drugs that cause oxidative stress is accompanied by hepatic steatosis, elevated lipid peroxide content, susceptibility to oxidant stress and worsening liver functions [12–14] led to the notion that the accumulation of fat per se may play an important role in the oxidative stress and subsequent development of organ dysfunction. However, evidence to the contrary also exists, suggesting that lipids do not play a direct causative role in the oxidative damage of the organ and the accumulation of lipids is an epiphenomenon [12]. The onset of hepatic steatosis and the accumulation of oxidants observed in alcoholism, obesity or after drug-induced liver damage are the result of long-term and complex metabolic, hormonal and nutritional changes [5,6,12]. Under these conditions, it is difficult to elucidate if fat itself or rather the effects of other intervening factors (i.e., vitamin status, malnutrition, endotoxemia, etc.) are the major contributors to the oxidative stress in the organ. Thus, a direct relationship between hepatic lipid accumulation and oxidative stress remains to be proven.

Using experimental conditions in which hepatic fat accumulation is achieved by a physiological challenge provides a possibility to test this relationship. We used a short-term (48 h) sucrose-rich dietary challenge which results in elevated de novo hepatic fatty acid synthesis [15–17] and the subsequent accumulation of lipids containing predominantly saturated fatty acids [18]. We tested if the sucrose-induced acute steatosis results in changes in glutathione status, accumulation of lipid peroxidation products and the manifestation of hepatic injury in the organ and in isolated cells following chemically induced or Kupffer cell-mediated oxidative challenges.

2. Materials and methods

2.1. Animals and carbohydrate-rich diet

Male Sprague-Dawley rats (300–340 g, Charles River, Wilmington, MA) were used in the study. Hepatic steatosis was induced by providing the carbohydrate-rich diet to rats for 48 h [17,19,20] which consisted of 74.5% sucrose, 20.5% protein (casein), 3.5% salt mix, 1.0% vitamin mix, 0.3% DL-methionine and 0.2% choline bitartrate (w/w), (Dyets, Bethlehem, PA, diet No. 112144, vitamin mix No. 300050, salt mix No. 200000). Control animals were fed on standard rodent diet (Purina Mills, diet No. 5001) which consisted of (w/w) 23.4% protein, 1.7% lactose, 0.3% fructose, 0.2% glucose, 3.7% sucrose, 32% starch, 5.5% fat, 2.5% salt mix, and the remaining part was ash and fibers. The standard and carbohydrate-rich diets contain the same vitamin mixture. Metabolizable energy value of standard and carbohydrate-rich pellets were 12.7 kJ/g and 15.8 kJ/g, respectively. Animals had free access to water and were kept in a 12 h dark/light cycle in a climate-controlled animal facility. The experiments were performed in accordance with NIH guidelines for the use of laboratory animals.

2.2. Cell preparation

After the dietary treatments, hepatic cell isolation was initiated in anesthetized animals (ketamine and xylazine, 90 and 9 mg/kg, i.m.). Hepatic parenchymal cells were isolated by a modified version of the collagenase perfusion, differential centrifugation method [21]. Hepatic non-parenchymal cells were isolated by subsequent pronase digestion, followed by centrifugal elutriation and gradient centrifugation methods [21]. Purity of endothelial, Kupffer and parenchymal cell preparations determined by morphology and peroxidase positivity [21] was greater than 96%, 94%, and 99%, respectively. Viability of isolated non-parenchymal and parenchymal cells, as assessed by trypan blue exclusion, was greater than 95% and 90%, respectively.

2.3. Liver perfusion and treatments

After the dietary treatments, animals were anesthe-

tized and isolated livers were perfused via the portal vein with Dulbecco's modified Eagle's medium, containing mixtures of vitamins, salts and amino acids, including precursors for glutathione synthesis, supplemented with glutamine, glucose and sodium bicarbonate before use (No. D5030, Sigma Fine Chemicals, St. Louis, MO). Liver perfusion was carried out at 37°C under constant saturation with gas (O_2:CO_2, 95:5 v/v). After 5 min of flowthrough perfusion, the livers were placed into a recirculatory perfusion apparatus (time '0') and were perfused for 180 min using 100 ml of fresh perfusion medium (12 ml/min) at 37°C under constant gas saturation.

Two modalities of oxidative stress were employed to test the sensitivity of fatty livers to oxidative stress. In order to mimic an intracellular oxidative stress *t*-butyl hydrogen peroxide (t-BOOH) was used as this organic peroxide is metabolized in all hepatic cells. In order to delineate the effect of a macrophage-mediated oxidant stress on endothelial and parenchymal cells, Kupffer cells were primed by the addition of lipopolysaccharide (LPS) into the perfusion fluid at time zero followed by the addition of opsonized zymosan. It was presumed that the latter treatment primarily affects Kupffer cells causing the release of reactive oxygen species into the sinusoidal milieu, which subsequently targets endothelial and parenchymal cells exogenously. t-Butyl hydroperoxide (t-BOOH) was added to the perfusion fluid at 2 mM final concentration at 31 min of perfusion time. In separate experiments, LPS from *Escherichia coli*, 0111:B4, 10 μg/ml (Difco Laboratories, Detroit, MI) was administered at 1 min of perfusion, followed by the addition of 0.8 mg/ml opsonized zymosan at 61 min. In order to test if reactive oxygen species are mediators of liver injury after the LPS/zymosan treatments, 200 U/ml superoxide dismutase (SOD, bovine) together with 3500 U/ml catalase (CAT, bovine) were administered at 59 min (immediately before the administration of zymosan) and also at 121 min of liver perfusion. At different times, samples were taken from the perfusion fluid and analyzed for alanine aminotransferase (ALT), oxidized (GSSG) and reduced glutathione (GSH) content. At the end of the perfusion, livers were homogenized in a teflon homogenizer and processed according to the protocols described below.

2.4. Glutathione, glucose-6-phosphate dehydrogenase (G6PD) and lipid peroxidation assays

Freshly isolated hepatic cells were suspended in 50 mmol/l Tris buffer pH 8.3 containing 100 mmol/l KCl, 0.2 mg/ml Triton X-100, 0.01 mmol/l $NADP^+$ and a cocktail of proteinase inhibitors [22]. The suspension was sonicated and samples of the $14\,000 \times g$ supernatant were analyzed for G6PD activity using a dual assay which determines the activities of both G6PD and 6-phosphogluconate dehydrogenase as described in detail earlier [22]. GSH and GSSG contents in the perfusion fluid, whole liver tissue and isolated liver cells were determined by the 5,5′-dithiobis(2-nitrobenzoate) (DTNB) assay in 3% sulfosalicylic acid extracts as described earlier [23]. Cellular GSH content in suspension cultures of hepatic cells was also assayed by the monochlorobimane method (MCLB; Molecular Probes, Eugene, OR) as previously described [24,25]. Briefly, cells were preincubated for 20 min in HEPES bicarbonate buffer containing 1% albumin, glucose (3–20 mM), 0.5 mM glutamine, 0.5 mM arginine, 1 mM lactate and 0.1 mM pyruvate at room temperature in Falcon 24-well cell culture plates. MCLB was added and cell-associated fluorescence measured at 2 min intervals using 360 nm excitation and 460 nm emission filters by an FL500 Microplate Fluorescence Reader (Bio-Tek Instruments, Winooski, VT) at room temperature. MCLB fluorescence approached a plateau after 6–8 min incubation. When cells were stimulated with freshly prepared H_2O_2, the oxidant was added 10 min prior to addition of MCLB. GSH was determined using a calibration curve.

Lipid peroxidation was assessed by the tissue levels of thiobarbituric acid reactive substance (TBARS), malondialdehyde and 4-hydroxynonenal as described [26]. ALT was determined using a commercial kit from Sigma. Values were normalized to protein content of the cell or tissue samples. Protein content was determined using a Bio-Rad protein assay kit. All chemicals and materials were obtained from Sigma (St. Louis, MO) unless otherwise noted.

2.5. Statistical analysis

Multiple comparisons were made by ANOVA, followed by the Tukey-Kramer test; for pairwise

comparisons the *t*-test was used. A *P*-value ≤ 0.05 was considered to be a statistically significant difference.

3. Results

Fig. 1 depicts the histological changes following the employed short-term carbohydrate-rich diet. Hematoxylin and eosin staining (Fig. 1A,B) indicate

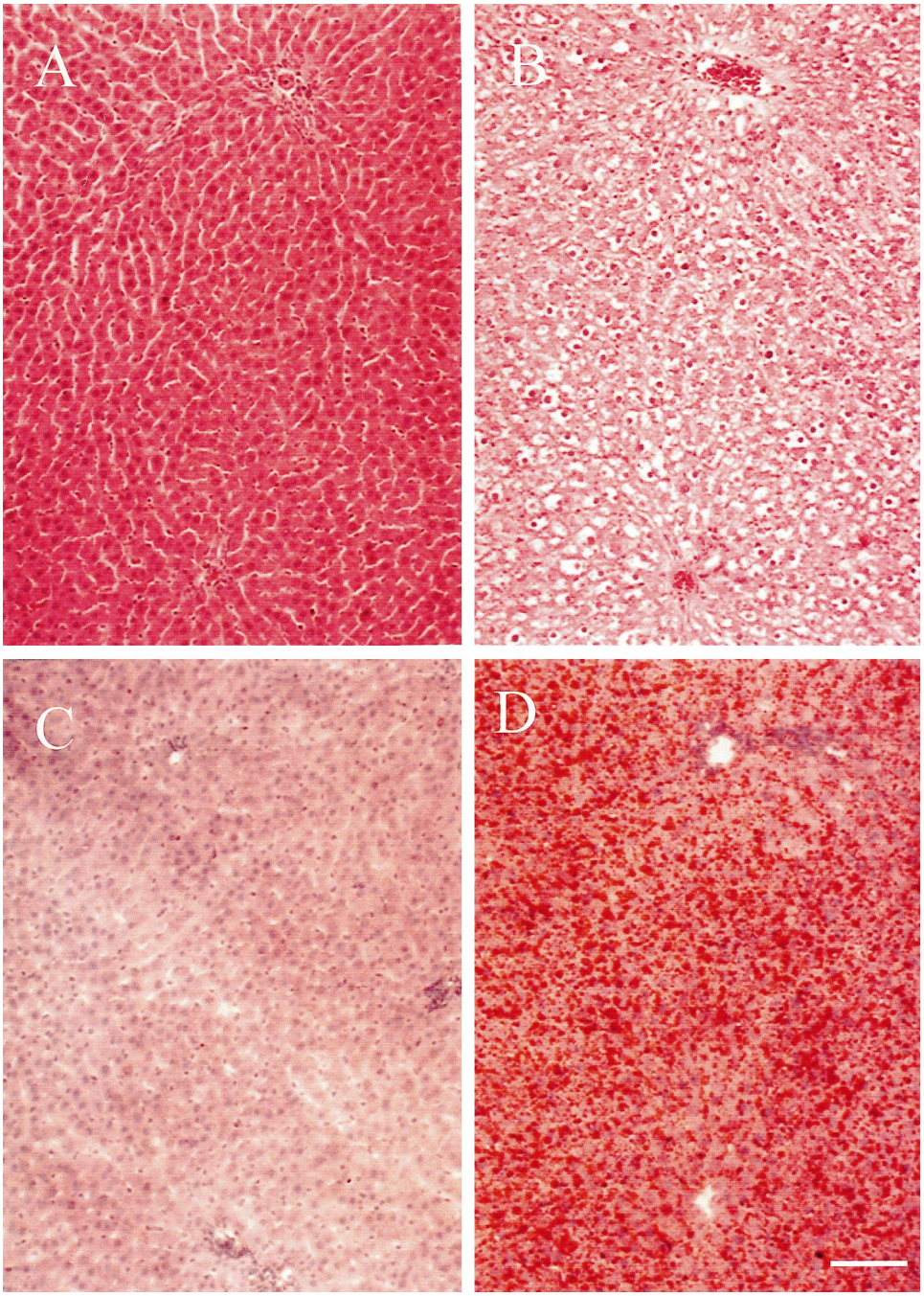

Fig. 1. Hepatic lipid accumulation following short-term carbohydrate-rich diet. Livers from control (A,C) and carbohydrate-rich diet-fed animals (B,D) are shown. Livers were fixed in 4% buffered paraformaldehyde and stained by hematoxylin and eosin (A,B) or by oil red lipid-specific stain (C,D). Bar equals 100 μm.

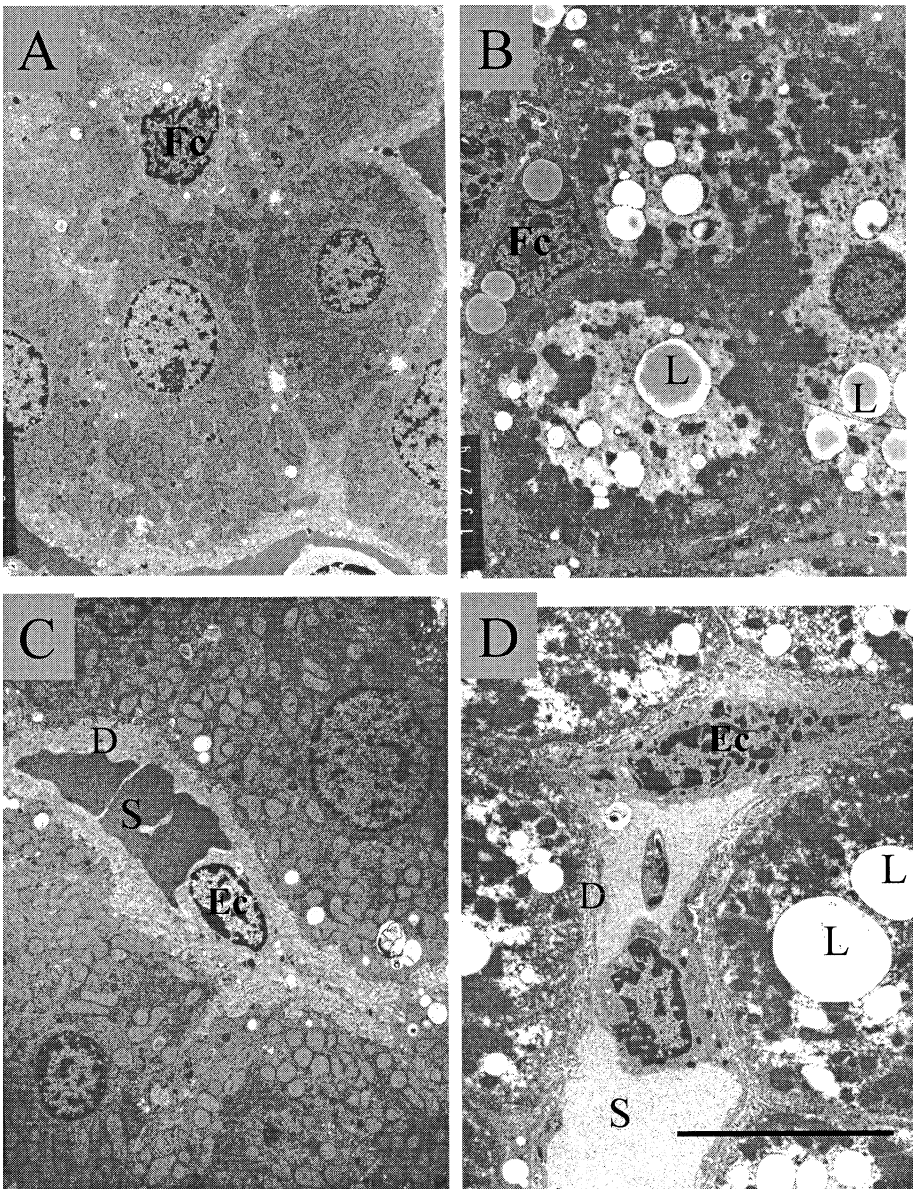

Fig. 2. Hepatic ultrastructure following short-term carbohydrate-rich diet. Livers from control (A,C) and carbohydrate-rich diet-fed animals (B,D) are shown. Livers were fixed in 3% buffered glutaraldehyde and embedded in Epon. Ultrathin sections were stained by uranyl acetate and lead citrate and examined in a Phillips 300 TEM. Bar equals 10 μm. L, lipid droplet; D, space of Disse; S, sinusoid; FC, fat storing cell.

vacuolization of parenchymal cells consistent with lipid and glycogen accumulation following the diet. Signs of inflammation or parenchymal damage are not present in fatty livers. The fat-specific staining (Fig. 1C,D, lipids stained in red) indicates large quantities of accumulated lipids after the sucrose-rich diet in the hepatic parenchyma. Within the hepatic acini, the distribution of the fat droplets is mainly mid-zonal. The electron microscopic analysis (Fig. 2) shows microvesicular distribution of fat in parenchymal cells. Lipid accumulation was not observed in sinusoidal endothelium and Kupffer cells and no remarkable morphological changes were observed in the organization of the sinusoids and the space of Disse following the diet.

t-BOOH treatment in vitro resulted in the release of ALT into the perfusion fluid in control livers indicating liver dysfunction. In contrast, ALT release

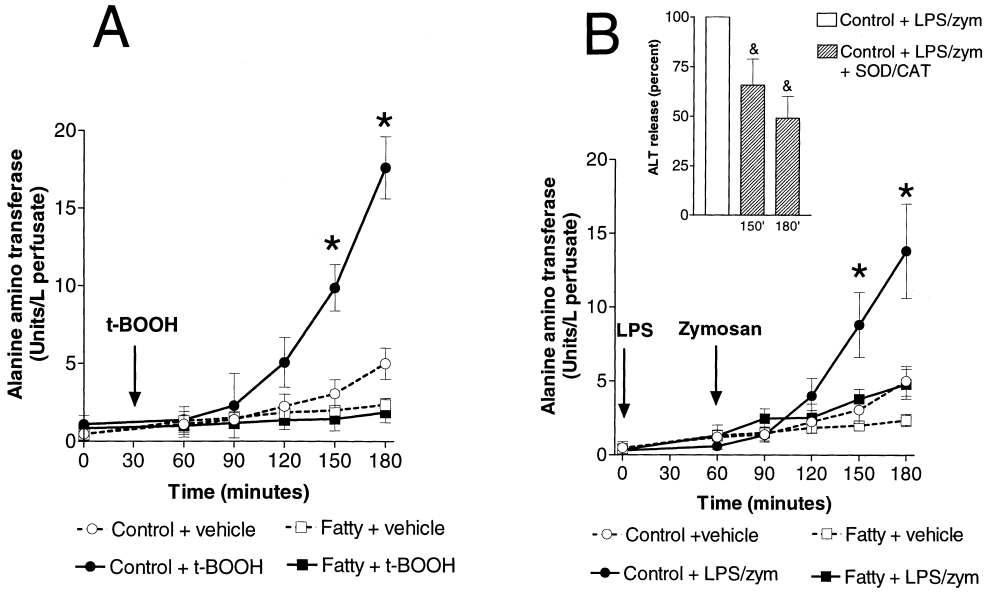

Fig. 3. Oxidant stress-induced hepatic injury. Following the dietary treatments, livers were perfused in a recirculatory perfusion apparatus and samples from the perfusion fluid were analyzed for ALT content. Livers were perfused in the presence or absence of 2 mM t-BOOH administered at 30 min (A). LPS was added to the perfusion fluid at time '0' followed by the administration of opsonized zymosan at 60 min (B). Asterisks indicate statistically significant difference between fatty and control livers. Mean ± S.E.M., $n = 6$–8 livers in each group. Inset to panel B indicates the effect of the presence of superoxide dismutase (SOD) and catalase (CAT) in the perfusion fluid. SOD together with CAT were administered immediately before the addition of zymosan (at 58 min) followed by a second administration at 120 min as described in Section 2. 100% represent values obtained after the LPS/zym challenge in the absence of CAT and SOD. '&' indicates statistically significant difference between the presence vs. absence of CAT/SOD.

was absent in fatty livers after t-BOOH treatment (Fig. 3A). LPS plus zymosan administration caused ALT release from control livers, whereas the same treatment resulted in no appreciable increase in ALT release from fatty livers (Fig. 3B). The presence of superoxide dismutase and catalase in the perfusion fluid alleviated the LPS plus zymosan-induced ALT release by approx. 50% (Fig. 3B, insert), supporting the assumption that the observed liver injury is associated, at least partially, with the release of superoxide anion and/or hydrogen peroxide into the hepatic sinusoid.

Fig. 4 depicts the accompanying changes in the hepatic release of glutathione disulfide (GSSG) and GSH following the same in vitro treatments. t-BOOH addition resulted in prompt release of GSSG into the perfusion media in both control and fatty livers. However, GSSG release was decreased in fatty livers as compared to controls (Fig. 4A). Release of reduced glutathione was not statistically different in control and fatty livers following t-BOOH (Fig. 4B). Treatment with LPS and zymosan did not result in an increased release in GSSG or GSH in either control or fatty livers (Fig. 4C,D).

At the end of the perfusion, samples of whole liver tissue were analyzed for total glutathione content and also for the tissue content of lipid peroxidation products. Fig. 5A indicates that glutathione content was approx. 3 times greater in fatty than in control livers following all in vitro treatments. t-BOOH administration depleted glutathione by approx. 50% in control livers, whereas t-BOOH treatment caused no marked decrease in glutathione content in fatty livers. LPS plus zymosan treatments did not alter hepatic glutathione content in either fatty or control livers.

Fig. 5B shows that tissue levels of lipid peroxidation products were significantly lower in fatty livers than in control livers following all in vitro treatments. t-BOOH caused no increase in the content of lipid peroxidation products in fatty livers; however, it significantly elevated hepatic content of lipid peroxidation products in control livers. Treatment by LPS plus zymosan did not significantly elevate the

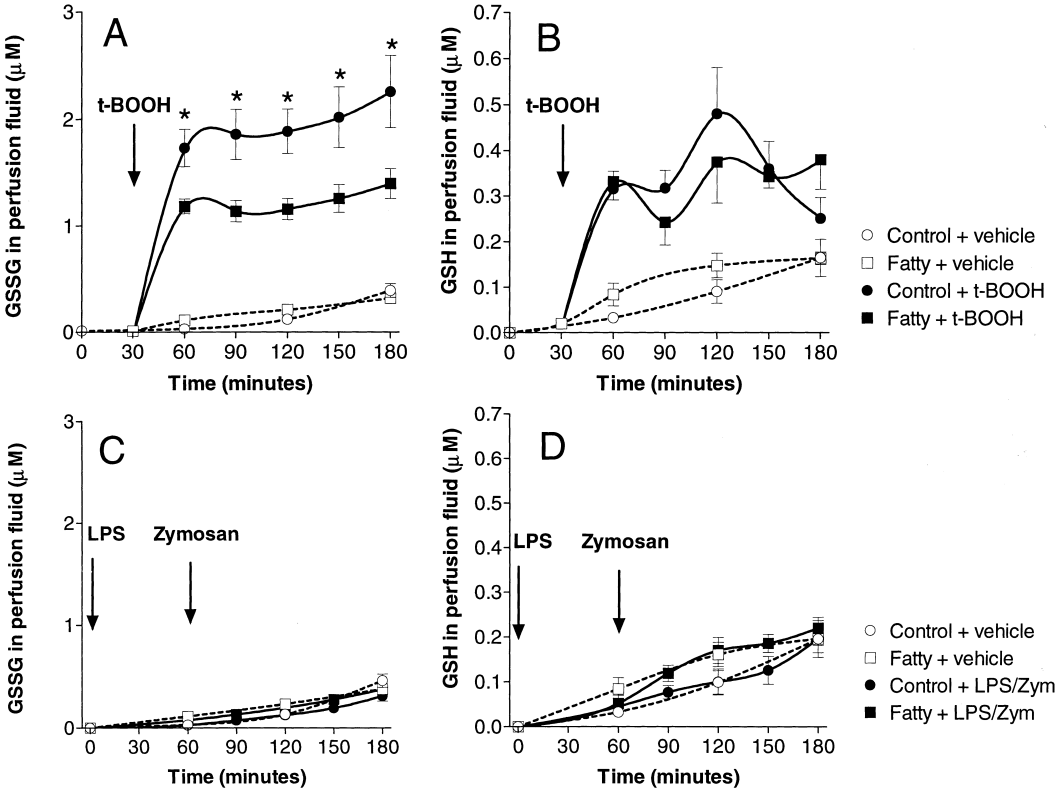

Fig. 4. Oxidative stress-induced hepatic glutathione release. Following the same treatments as described in the legend to Fig. 3, reduced (GSH) and oxidized glutathione (GSSG) were also determined in serial samples from the liver perfusion fluid. Asterisks indicate statistically significant differences between fatty and control livers. Mean ± S.E.M., $n = 6$–8 livers in each group.

tissue content of lipid peroxidation products in either control or fatty livers (Fig. 5B).

In a subsequent series of experiments we compared the glutathione status and G6PD activity in hepatic cells freshly isolated from control or fatty livers. Fig. 6A shows that G6PD activity was 3- and 10-fold greater in endothelial and parenchymal cells from fatty livers compared to cells from controls. Cellular reduced glutathione content was also doubled in endothelial and parenchymal cells isolated from fatty livers as compared to cells from control livers. G6PD activity and glutathione contents were similar in Kupffer cells isolated from fatty or control livers in accordance with previous observations [17].

In order to test if the diet-induced elevated G6PD expression in endothelial cells is accompanied by an increased resistance to oxidative stress, we compared the H_2O_2-induced glutathione depletion in the presence of low and high exogenous glucose concentration in vitro. Fig. 7A shows that 0.2 mM H_2O_2 challenge resulted in a transient decrease in cellular GSH content in sinusoidal endothelial cells from control livers. Fig. 7B indicates that the GSH depletion caused by the in vitro administration of H_2O_2 in endothelial cells from controls was dependent on glucose concentration at a physiologically relevant range. In contrast, the presence of high endogenous glucose concentration had no significant effect on the H_2O_2-induced GSH depletion in endothelial cells from fatty livers.

In Kupffer cells, the H_2O_2-induced glutathione depletion and its glucose dependence were not different in cells isolated from control or fatty livers (data not shown) in agreement with the observed unaltered glutathione status and G6PD activity in these cells. No significant decrease in glutathione content was observed after the same in vitro H_2O_2 treatments either in the absence or presence of exogenous glucose in parenchymal cells (data not shown).

4. Discussion

The study indicates that hepatic steatosis induced

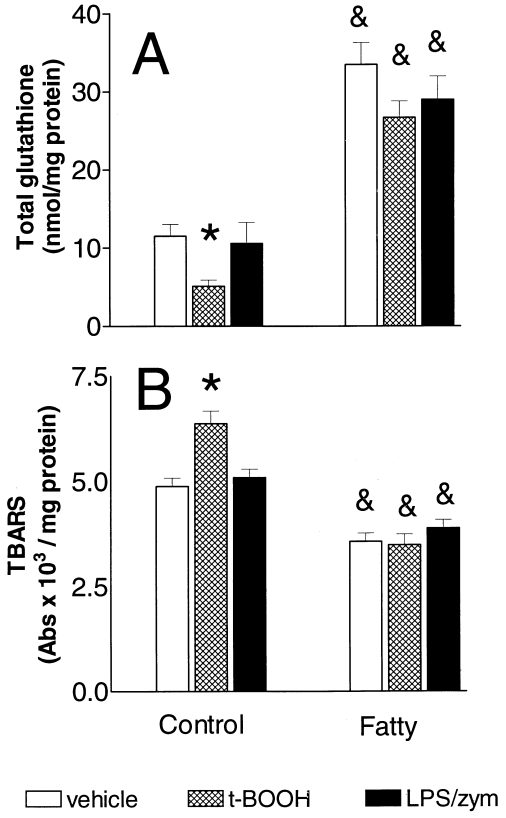

Fig. 5. Hepatic content of glutathione and lipid peroxidation products after oxidative stress. Whole liver tissue was analyzed for total glutathione content (A) and for the tissue levels of thiobarbituric acid reactive substance (TBARS; B) at the end of liver perfusion. At least 90% of total glutathione was in its reduced form after any of the employed treatments. Asterisks indicate statistically significant difference as compared to vehicle. '&' indicates statistically significant difference between fatty and control livers after the same in vitro treatments. Mean ± S.E.M., $n = 6$–8 livers in each group.

by a short-term sucrose-rich diet does not result in an oxidative stress in the organ. In contrast, fatty livers display a marked resistance against intracellular as well as extracellular oxidative stresses. Therefore, the presence of fat per se does not play a direct causative role in the initiation of oxidative stress in the organ, and the simultaneous presence of other intervening factors is required for increased lipid peroxidation and the associated liver dysfunction observed in other models of hepatic steatosis [1–3]. In agreement with this conclusion, fat-independent oxidative stress was demonstrated in transplanted livers following alcohol-induced steatosis [27]. Furthermore, our finding support the clinical observation that persistent steatosis may be present without any significant manifestation or progression of liver disease in the absence of superimposing disease [1,12]. The employed model of hepatic steatosis is potentially relevant in humans as acute haptic fat accumulation may develop after extreme sugar-rich intake such as a carbohydrate-rich parenteral nutrition [28].

Whereas there is no evidence which indicates that the employed short-term diet results in malnutrition or remarkable changes in vitamin status, it is evident this diet results in complex hormonal alterations leading to the induction of a variety of metabolic enzymes. Among these, the elevated expression of G6PD, the first and rate limiting enzyme of the hexose monophosphate shunt, may play an especially important role in the augmented hepatic antioxidant defense. Induction of G6PD in parenchymal cells supports the increased need for NADPH used for fatty acid synthesis de novo [15,18,29]. However, an elevated expression of G6PD is also important in the support of major antioxidant pathways as the gener-

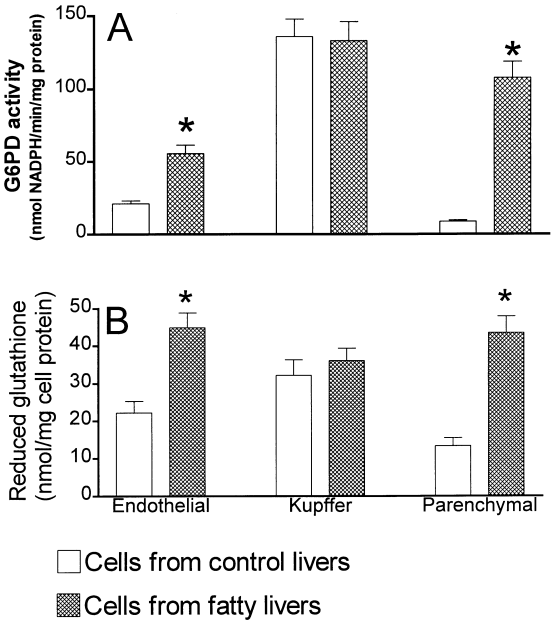

Fig. 6. G6PD activity and glutathione content in freshly isolated hepatic sinusoidal endothelial, Kupffer and parenchymal cells. G6PD activity (A) and glutathione content (B) were determined in sinusoidal endothelial, Kupffer and parenchymal cells freshly isolated from control and fatty livers. Bars represent means ± S.E.M., $n = 6$ independent cell preparations from each group. Asterisks indicate statistically significant difference as compared to cells from control animals.

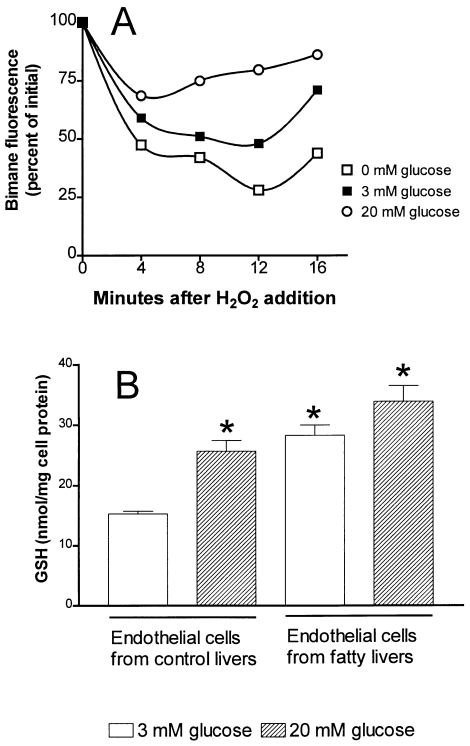

Fig. 7. Glucose dependence of reduced glutathione content following in vitro oxidative stress in sinusoidal endothelial cells. Freshly isolated endothelial cells were exposed to 0.2 mM hydrogen peroxide and the kinetics of reduced glutathione content was measured in the presence of varying glucose concentrations. Panel A depicts a typical time course. Panel B shows results obtained at 10 min after the addition of hydrogen peroxide in the presence of physiological low vs. high glucose concentrations. Asterisks indicate statistically significant difference as compared to cells from controls at 3 mM glucose. Bars show means ± S.E.M., $n = 5$ independent cell preparations.

ated NADPH is the reducing coenzyme for peroxidases (via glutathione) and it is required for the stability of catalase [29,30] The central significance of G6PD as an antioxidant enzyme has been demonstrated by several independent investigations. Eukaryotic stem cells deficient of G6PD can survive only under low oxygen tension [31], the over-expression of G6PD in cell lines increases the resistance against oxidative stress [32–34]. Deficiency of G6PD, a common human genetic polymorphism, is accompanied by elevated susceptibility of red blood cells to oxidant stress [35,36] or results in oxidative stress-induced teratogenesis [37]. In prokaryotes, G6PD is coordinately regulated with superoxide dismutase and catalase upon oxidant stress [38]. It remains to be elucidated if induction of SOD or catalase is a contributing mechanism to the augmented hepatic antioxidant capacity following a carbohydrate-rich diet.

The elevated G6PD activity and glutathione content in endothelial cells may have a particular significance in the protection against phagocyte-derived oxidative stress in fatty livers. Hepatic endothelial cells line the liver sinusoid and are in direct contact with resident macrophages and recruited neutrophils. Therefore they are the primary targets of phagocyte-derived reactive oxygen and nitrogen species [39]. The finding that the LPS plus zymosan treatments caused liver injury (ALT release) without resulting in measurable changes in hepatic glutathione release in control livers is not inconsistent with an oxidant-mediated endothelial cell injury. Endothelial and Kupffer cells represent approx. 4% and 4% of total hepatic protein [40], thus it can be estimated from data in Fig. 6 that approx. 5% of the total hepatic glutathione is present in endothelial cells, 7% in Kupffer cells and 88% in parenchymal cells. Therefore, the basal release of oxidized glutathione from the parenchymal cells could mask even a total loss of glutathione from endothelial cells following the Kupffer cell-mediated oxidative injury.

Superoxide anion and nitric oxide production by macrophages and polymorphonuclear phagocytes is dependent on NADPH and, thus, on the activity of G6PD. The presence of activated phagocytes in the hepatic sinusoid has been shown to mediate liver injury after a variety of conditions including endotoxemia and ischemia-reperfusion injury [41]. Induction of G6PD in hepatic macrophages supports a primed oxidative burst under these conditions. The observation that G6PD expression is similar in Kupffer cells from fatty and control livers suggests that the macrophage is not primed to an increased prooxidant response after a carbohydrate-rich diet. An unaltered or decreased oxidative burst activity in Kupffer cells from fatty livers, together with the increased expression of G6PD and elevated glutathione content in endothelial and parenchymal cells, indicate a dominance of antioxidant mechanisms in the sinusoidal intercellular oxidant/antioxidant balance [39] following a short-term carbohydrate-rich diet.

Hepatic glutathione metabolism is under nutrition-

al and hormonal control [42,43]. The observed diet-induced increase in hepatic glutathione is presumably a reflection of in vivo stimulated synthesis. However, an elevated hepatic glutathione alone does not sufficiently protect against sustained oxidative stimulus unless the reconversion of the reduced glutathione from GSSG is also accelerated. Upon oxidative stress, the accumulating GSSG is released from the cells, a process which eventually results in a decrease in the antioxidant potential of the cell [44–46]. The elevated G6PD activity in sinusoidal endothelial and parenchymal cells from fatty livers can support a more efficient conversion of cellular GSSG to GSH upon oxidative stress which alleviates the accumulation oxidized glutathione and subsequent loss of hepatic glutathione.

Taken together, these studies indicate that an elevated hepatic antioxidant capacity can be manifested in the presence of prominent hepatic steatosis induced by a short-term carbohydrate-rich diet. Fat per se does not initiate an oxidative stress in the organ, unless other superimposing stresses (i.e., malnutrition, changes in vitamin status, direct oxidative effects of drugs or ethanol, gut-derived endotoxin) are also present simultaneously [2,3,12]. The elevated G6PD expression in hepatic endothelial and parenchymal cells in sucrose-induced fatty livers supports a more efficient maintenance of hepatic glutathione upon oxidative stress which may be an important mechanism in the elevated antioxidant activity in the organ.

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