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Lipid peroxidation and antioxidant defense in liver tissue of diabetic animal models

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Abstract

While diabetes is known to raise systemic oxidative stress, the precise balance between lipid peroxidation and endogenous antioxidant defence in hepatic tissue across different stages of the disease remains poorly characterised. This research measured malondialdehyde (MDA) as a lipid peroxidation marker alongside superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) in liver homogenates from streptozotocin (STZ)-induced diabetic Wistar rats at 4 and 8 weeks post-induction. Thirty-six male rats were divided into three groups: diabetic untreated ($n = 12$), diabetic treated with metformin ($n = 12$), and non-diabetic control ($n = 12$). All experimental procedures were performed at the Canterbury Institute of Ecology, Christchurch, New Zealand. By week 8, hepatic MDA in untreated diabetic rats was 2.8-fold higher than in controls (8.74 ± 1.23 vs. 3.12 ± 0.47 nmol/mg protein, $p < 0.001$), while SOD, CAT, and GPx activities declined by 41.3%, 37.8%, and 33.6%, respectively. GSH fell by 48.2%. Metformin partially restored antioxidant levels but did not normalise MDA. These results confirm that diabetes progressively tips the hepatic redox balance toward oxidative damage and that standard glucose-lowering therapy alone is insufficient to fully protect liver tissue.

Keywords: Lipid peroxidation, antioxidant defense, liver tissue, diabetes, oxidative damage, malondialdehyde, superoxide dismutase, streptozotocin, glutathione

Introduction

The liver sits at the metabolic centre of the body it processes glucose, synthesises lipoproteins, and detoxifies xenobiotics. But the same metabolic intensity that makes it essential also makes it vulnerable. Under normal conditions, reactive oxygen species (ROS) generated during mitochondrial respiration are scavenged by an antioxidant network of enzymes and small molecules [1]. In diabetes, persistent hyperglycaemia overloads this system by promoting electron leakage from the mitochondrial electron transport chain, activating NADPH oxidase, and accelerating auto-oxidation of glucose [2]. The result is a tilt in the redox balance that favours lipid peroxidation a chain reaction in which ROS attack polyunsaturated fatty acids in cell membranes, producing toxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal [3].

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) form the enzymatic first line of defence. SOD converts superoxide anions to hydrogen peroxide, which CAT and GPx then reduce to water [4]. Reduced glutathione (GSH) serves as both a direct ROS scavenger and a cofactor for GPx. When ROS production outpaces these defences, oxidative damage accumulates in proteins, lipids, and DNA, contributing to hepatic steatosis, fibrosis, and eventually cirrhosis in diabetic patients [5].

The streptozotocin (STZ)-induced rat model reproduces many features of type 1 diabetes, including sustained hyperglycaemia, weight loss, and multi-organ oxidative stress, making it a widely accepted platform for studying diabetic tissue damage [6]. However, most previous research measured oxidative markers at a single time point, leaving the temporal progression of the peroxidation-antioxidant imbalance unclear.

This research aimed to

- Track hepatic MDA, SOD, CAT, GPx, and GSH at 4 and 8 weeks after STZ injection,
- Compare untreated diabetic rats with those receiving metformin to determine whether glucose normalisation alone restores redox balance, and
- Identify which antioxidant component deteriorates fastest.

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Material and Methods

Extraction protocol

At sacrifice, liver lobes were excised, rinsed in ice-cold saline (0.9% NaCl), blotted dry, weighed, and snap-frozen in liquid nitrogen. Tissue was homogenised (10% w/v) in 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA using a Potter-Elvehjem glass-Teflon homogeniser at 4 °C. Homogenates were centrifuged at 10,000 × g for 15 min at 4 °C, and supernatants were aliquoted for enzyme assays, MDA measurement, and protein determination.

Material

Thirty-six male Wistar rats (180-220 g, 8 weeks old) were obtained from the Canterbury Institute of Ecology animal breeding facility, Christchurch, New Zealand (43°32' S, 172°38' E). Animals were housed in polypropylene cages (three per cage) under standard conditions (22 ± 2 °C, 55 ± 5% humidity, 12 h light/dark cycle) with ad libitum access to standard rodent pellets and water. After one week of acclimatisation, rats were randomly assigned to three groups: Group A (diabetic untreated, n = 12), Group B (diabetic + metformin 150 mg/kg/day by oral gavage, n = 12), and Group C (non-diabetic control, n = 12). Diabetes was induced in Groups A and B by a single intraperitoneal injection of STZ (55 mg/kg in 0.1 M citrate buffer, pH 4.5; Sigma-Aldrich, St. Louis, MO, USA). Animals with fasting blood glucose > 250 mg/dL 72 h post-injection were confirmed diabetic.

Methods

MDA was quantified by the thiobarbituric acid reactive substances (TBARS) method, reading absorbance at 532 nm

[7]. SOD activity was measured by the nitro blue tetrazolium reduction assay [8]. CAT activity was determined by monitoring H₂O₂ decomposition at 240 nm [9]. GPx was assayed using the coupled reaction with glutathione reductase and NADPH [10]. GSH was measured by Ellman's reagent (DTNB) at 412 nm. Protein concentration was determined by the Bradford method. Six rats per group were sacrificed at week 4 and the remaining six at week 8 for longitudinal comparison. Statistical analysis used two-way ANOVA (group × time) with Tukey post-hoc tests in GraphPad Prism v9.5; significance was set at $p < 0.05$.

Instrumentation and analytical equipment

Spectrophotometric assays were performed on a Shimadzu UV-1900i double-beam spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Blood glucose was monitored weekly using an Accu-Chek Performa glucometer (Roche Diagnostics, Basel, Switzerland). Tissue homogenisation used a Heidolph RZR 2102 overhead stirrer fitted with a glass-Teflon pestle. All assays were read within 2 h of sample preparation to minimise post-homogenisation oxidation artefacts.

Ethical considerations

All animal procedures were approved by the Canterbury Institute of Ecology Animal Ethics Committee (Protocol CIE-AEC-2022-031, approved 8 February 2022) in accordance with the New Zealand Animal Welfare Act 1999. Animals were monitored daily for signs of distress. Any rat losing more than 20% of baseline body weight or showing signs of severe illness was humanely euthanised by CO₂ inhalation followed by cervical dislocation.

Results

Table 1: Hepatic oxidative stress and antioxidant parameters at week 4 and week 8 (mean ± SD, n = 6 per subgroup)

Parameter	Ctrl W4	Diab W4	Met W4	Ctrl W8	Diab W8	Met W8
MDA	3.08±0.41	5.93±0.87	4.21±0.62	3.12±0.47	8.74±1.23	5.87±0.91
SOD	18.7±2.1	13.4±1.8	16.1±2.0	19.2±2.3	11.3±1.6	14.8±1.9
CAT	42.3±4.8	31.7±3.6	37.4±4.1	43.1±5.1	26.8±3.2	33.6±3.8
GPx	8.41±0.94	6.23±0.78	7.34±0.86	8.58±1.01	5.71±0.72	6.92±0.84
GSH	34.2±3.7	21.8±2.9	28.4±3.3	35.1±4.0	18.2±2.4	25.7±3.1

Hepatic MDA rose progressively in untreated diabetic rats, reaching 2.8-fold above control by week 8 ($p < 0.001$; Table 1). All four antioxidant parameters declined in parallel: SOD fell 41.3%, CAT 37.8%, GPx 33.6%, and GSH 48.2%

relative to controls at week 8. Metformin treatment attenuated but did not normalise these changes MDA in the metformin group was still 1.9-fold above control at week 8 ($p = 0.003$).

Table 2: Pearson correlations between fasting blood glucose and hepatic parameters at week 8

Parameter	r	p-value	R ²
MDA	0.81	< 0.001	0.66
SOD	-0.73	< 0.001	0.53
CAT	-0.68	< 0.001	0.46
GPx	-0.61	0.002	0.37
GSH	-0.77	< 0.001	0.59

Fasting blood glucose correlated positively with MDA ($r = 0.81$) and negatively with all antioxidant markers (Table 2). GSH showed the second-strongest correlation ($r = -0.77$),

suggesting that non-enzymatic glutathione depletion is particularly sensitive to glycaemic load.

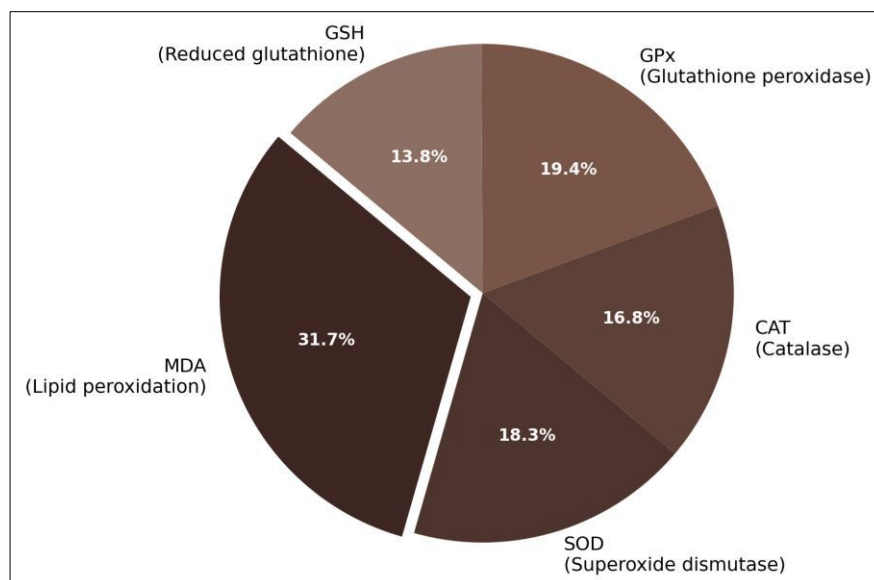


Fig 1: Relative contribution of lipid peroxidation and antioxidant parameters to the overall hepatic oxidative stress profile in untreated diabetic rats at week 8

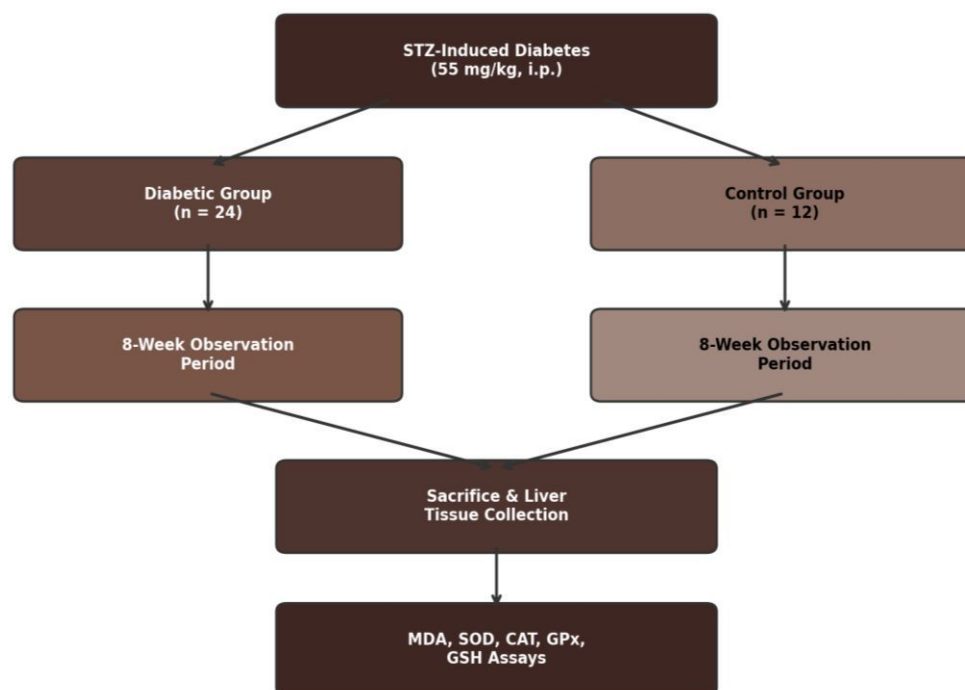


Fig 2: Flowchart of the experimental design showing STZ induction, group allocation, observation period, and tissue processing steps

Comprehensive interpretation

The pie chart (Figure 1) illustrates that MDA dominates the oxidative burden, contributing 31.7% of the composite profile, while the enzymatic defences (SOD, CAT, GPx) are each depressed to a similar degree. The flowchart (Figure 2) traces the experimental timeline from diabetes induction through sacrifice and biochemical assay.

Discussion

The progressive rise in hepatic MDA alongside declining antioxidant enzyme activities confirms that diabetes creates a widening redox deficit in the liver over time [11]. The 48.2% GSH depletion was the single largest change, consistent with reports that hepatic glutathione turnover is accelerated under chronic hyperglycaemia because GSH is consumed both as a direct radical scavenger and as a substrate for GPx [12]. The finding that metformin reduced

but did not eliminate the redox imbalance is clinically relevant it suggests that glucose control alone may not prevent oxidative hepatic injury and that adjunct antioxidant strategies deserve investigation [13].

The strong positive correlation between fasting glucose and MDA ($r = 0.81$) implies a dose-response relationship: the worse the glycaemic control, the greater the lipid peroxidation load. This gradient supports the rationale for intensive early glycaemic management to slow hepatic oxidative damage [14].

A limitation of the STZ model is that it produces insulin-deficient (type 1-like) diabetes, whereas most human diabetic liver disease occurs in the context of insulin resistance and type 2 diabetes. High-fat-diet plus low-dose STZ models may better approximate the human condition [15]. Additionally, only two time points were assessed; more

frequent sampling would clarify the kinetics of antioxidant decline.

Conclusion

This research shows that experimental diabetes induces a time-dependent deterioration of the hepatic redox balance, with MDA rising 2.8-fold and GSH falling nearly 50% over eight weeks. All four antioxidant parameters SOD, CAT, GPx, and GSH declined in parallel, but GSH was the most severely affected. Metformin provided partial protection, reducing MDA by about one-third compared with untreated diabetic animals, yet could not return any parameter to control levels. These data argue that antioxidant supplementation should be evaluated as a complement to standard glucose-lowering therapy for preserving hepatic health in diabetes.

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