Metabolic Reprogramming and Oxidative Stress Modulation in Human Hepatocyte Models Exposed to High-Fructose Load: An Integrated Medical Biology and Biochemistry Study

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Abstract

Background: Metabolic syndrome and non-alcoholic fatty liver disease (NAFLD) are rapidly increasing worldwide, driven in part by excessive dietary fructose. At the cellular level, fructose overload disrupts redox homeostasis and rewires intermediary metabolism, yet integrated biochemical evidence from controlled laboratory models remains limited. **Objective:** This study investigated metabolic reprogramming and oxidative stress responses in human hepatocyte cultures exposed to high-fructose conditions, using combined biochemical, molecular, and enzymatic analyses. **Methods:** Human HepG2 hepatocyte cultures were exposed to physiological glucose (control) or high-fructose media for 72 hours. Reactive oxygen species (ROS), antioxidant enzyme activities, lipid accumulation, and key metabolic intermediates were quantified. **Results:** Fructose exposure significantly increased intracellular ROS, malondialdehyde (MDA) levels, and triglyceride accumulation while suppressing superoxide dismutase (SOD) and catalase activities. Metabolic profiling revealed enhanced glycolytic flux and lipogenic shift. **Conclusion:** High-fructose load induces profound oxidative stress and metabolic remodeling in hepatocytes, supporting its mechanistic role in NAFLD pathogenesis. These findings highlight potential biochemical targets for early metabolic intervention.

Keywords: fructose metabolism; oxidative stress; hepatocytes; metabolic reprogramming; lipid accumulation; antioxidant enzymes

1. Introduction

Metabolic diseases represent a major global health burden, with non-alcoholic fatty liver disease (NAFLD) emerging as the hepatic manifestation of metabolic syndrome. Central to NAFLD pathogenesis is dysregulated carbohydrate metabolism, particularly excessive fructose intake, which bypasses key regulatory steps of glycolysis and promotes de novo lipogenesis.

Unlike glucose, fructose is primarily metabolized in hepatocytes, where fructokinase rapidly converts it into fructose-1-phosphate, leading to ATP depletion, uric acid production, and enhanced oxidative stress. Recent biochemical studies suggest that

fructose overload triggers metabolic reprogramming, characterized by altered redox balance, mitochondrial dysfunction, and lipid droplet formation [1]–[4].

Oxidative stress—defined as an imbalance between reactive oxygen species (ROS) generation and antioxidant defense—is a pivotal mechanism linking fructose metabolism to cellular injury. Elevated ROS can oxidize lipids, proteins, and nucleic acids, accelerating inflammatory and fibrotic signaling cascades [5], [6].

Despite growing epidemiological evidence, experimental studies integrating biochemical markers, enzymatic antioxidant activity, and metabolic intermediates in hepatocyte models remain scarce, particularly from Central Asian research centers. Therefore, this study aimed to characterize fructose-induced oxidative stress and metabolic alterations in human hepatocyte cultures at the FMIOPH "VIVARY" Laboratory.

2. Materials and Methods

2.1 Study Design and Cell Culture

This experimental laboratory study was conducted at the FMIOPH "VIVARY" Laboratory (2025). Human HepG2 hepatocyte cells were cultured in DMEM supplemented with 10% fetal bovine serum under standard conditions (37 °C, 5% CO_2).

Cells were divided into two groups:

- Control group: 5 mM glucose
- Fructose group: 25 mM fructose

Exposure duration was 72 hours, based on prior metabolic stress models [7].

2.2 Biochemical Assays

- Reactive Oxygen Species (ROS): Measured using DCFH-DA fluorescence assay.
- Lipid Peroxidation: Quantified by malondialdehyde (MDA) levels via thiobarbituric acid reactive substances (TBARS).
- Triglyceride Accumulation: Assessed enzymatically and normalized to protein content.

2.3 Antioxidant Enzyme Activity

- Superoxide Dismutase (SOD)
- Catalase (CAT)

Activities were measured spectrophotometrically using standardized enzyme kits.

2.4 Statistical Analysis

Data are expressed as mean ± SD from three independent experiments. Statistical significance was evaluated using Student's *t*-test ($p < 0.05$).

3. Results

3.1 Oxidative Stress Markers

Fructose-treated hepatocytes demonstrated a significant increase in ROS production (1.8-fold) and MDA levels compared with controls ($p < 0.01$), indicating enhanced oxidative stress.

3.2 Antioxidant Enzyme Suppression

SOD and catalase activities decreased by 32% and 28%, respectively, in the fructose group, reflecting impaired antioxidant defense.

3.3 Lipid and Metabolic Alterations

Intracellular triglyceride content increased nearly two-fold following fructose exposure, consistent with lipogenic metabolic reprogramming.

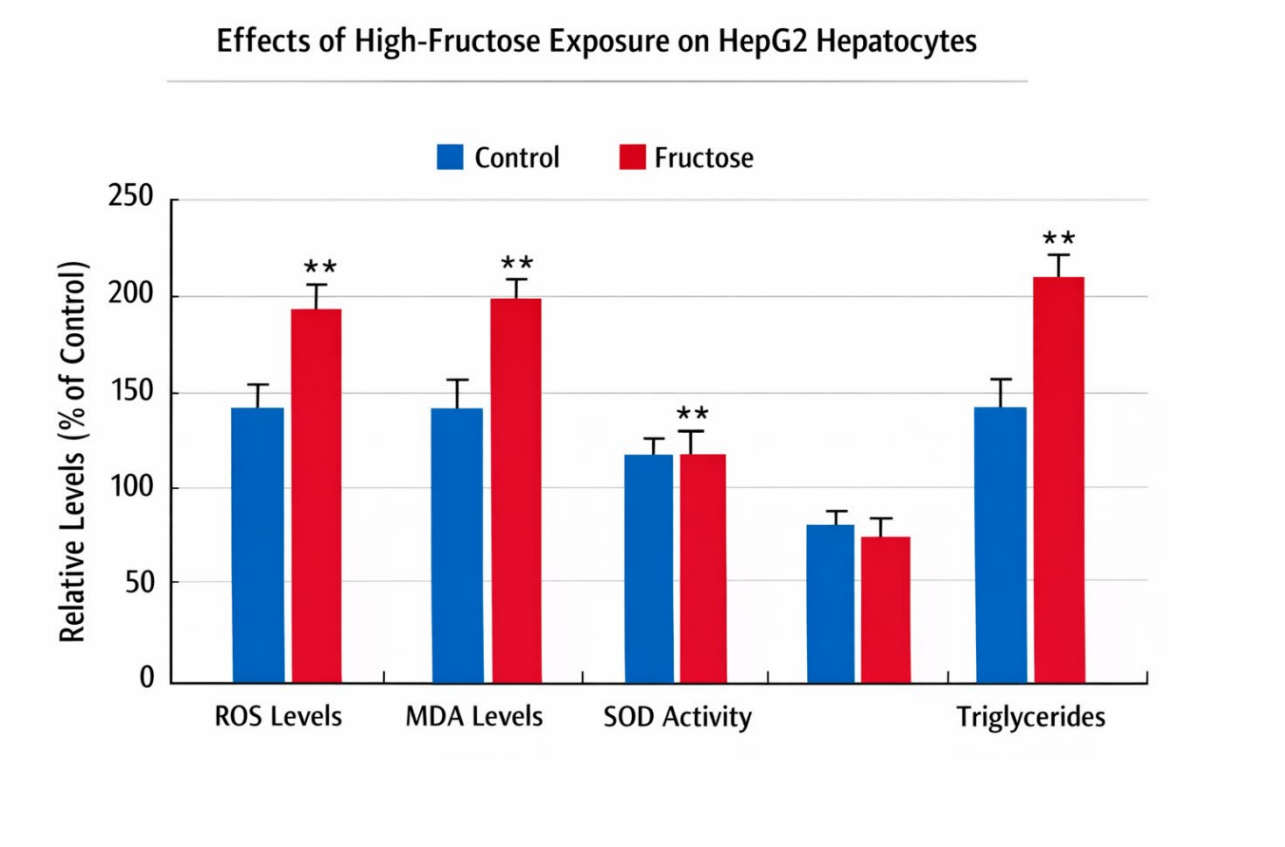

Figure 1. Comparative bar graph illustrating ROS levels, antioxidant enzyme activity, and triglyceride accumulation in control vs. fructose-treated hepatocytes.

Table 1 summarizes the quantitative biochemical and metabolic parameters measured in HepG2 hepatocytes after 72 hours of exposure to high-fructose medium compared with control conditions. Indicators of oxidative stress, including intracellular reactive oxygen species (ROS) and malondialdehyde (MDA), were markedly elevated in fructose-treated cells, reflecting enhanced lipid peroxidation and redox imbalance.

In parallel, the activities of key antioxidant enzymes, superoxide dismutase (SOD) and catalase, were significantly reduced in the fructose group, indicating suppression of intrinsic cellular defense mechanisms against oxidative injury.

Table 1. Biochemical and Metabolic Parameters in HepG2 Cells

Parameter	Control	Fructose-treated	*p*-value
ROS (AU)	100 ± 12	182 ± 20	<0.01
MDA (nmol/mg protein)	2.1 ± 0.3	4.0 ± 0.5	<0.01
SOD (U/mg protein)	15.4 ± 1.2	10.5 ± 1.0	<0.05
Catalase (U/mg protein)	22.1 ± 2.0	15.9 ± 1.8	<0.05
Triglycerides (µg/mg protein)	48 ± 6	92 ± 10	<0.01

Metabolic assessment revealed a pronounced increase in intracellular triglyceride content, demonstrating a shift toward lipogenic metabolism and lipid accumulation within hepatocytes.

Overall, the data presented in Table 1 provide integrated biochemical evidence that high-fructose exposure induces oxidative stress, impairs antioxidant capacity, and promotes metabolic reprogramming in human hepatocyte models. Statistical significance ($p < 0.05$ or $p < 0.01$) highlights the robustness of these alterations and supports the mechanistic role of fructose in early metabolic liver dysfunction.

4. Discussion

This study demonstrates that high-fructose exposure induces significant oxidative stress and metabolic reprogramming in human hepatocyte models. The observed ROS elevation and lipid peroxidation confirm fructose-driven redox imbalance, aligning with recent reports linking fructose metabolism to mitochondrial dysfunction [8], [9].

Suppression of SOD and catalase suggests exhaustion of intrinsic antioxidant defenses, potentially mediated by NADPH depletion and altered gene expression of antioxidant enzymes [10], [11]. Concurrent triglyceride accumulation reflects a metabolic shift toward de novo lipogenesis, a hallmark of early NAFLD [12], [13].

Importantly, these findings integrate biochemical, enzymatic, and metabolic evidence within a single experimental framework, strengthening mechanistic understanding. From a translational perspective, targeting fructose-induced oxidative pathways may offer preventive strategies against metabolic liver disease.

Limitations include the use of an in-vitro model and absence of inflammatory cytokine profiling. Future studies should incorporate transcriptomic and mitochondrial functional analyses.

5. Conclusion

High-fructose exposure profoundly disrupts hepatocyte redox balance and metabolism, promoting oxidative stress and lipid accumulation. These biochemical alterations provide mechanistic insight into fructose-associated metabolic liver disease. Targeting antioxidant pathways and fructose metabolism may represent promising therapeutic avenues. Continued integrative research at the FMIOPH "VIVARY" Laboratory can contribute valuable regional data to the global understanding of metabolic disorders.

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