

THE IMPACT OF HIGH-FAT DIET ON LIVER PARAMETERS AND ANTIOXIDANT SIGNALLING

J. Stanimirović, K. Banjac, M. Obradović and E. R. Isenović

Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (julijana@vin.bg.ac.rs)

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ABSTRACT

Due to present-day lifestyle habits, obesity is a disease of modern society. Obesity is characterized by excessive fat accumulation and lipid disturbance, which further leads to alterations in liver enzyme activity and redox signaling. We aimed to explore the effect of a high-fat (HF) diet on the concentrations of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in serum, and levels of nuclear factor erythroid 2-related factor 2 (NRF2) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) in rat liver. Our results showed a significant elevation of ALP concentration and no change in AST and ALT concentrations in serum. Additionally, protein levels of NRF2 and PGC1- α were reduced in liver of HF diet-fed rats compared to control rats. These findings indicate an increased inflammatory response and impaired antioxidant defense signaling in the liver of HF diet-fed rats, predisposing them to the development of insulin resistance.

INTRODUCTION

Obesity has emerged as one of the major public health challenges of modern society, largely driven by contemporary lifestyle habits. Excessive fat accumulation and lipid disturbance are key characteristics of obesity, which consequently lead to oxidative stress and insulin resistance [1]. Ample evidence demonstrates that high-fat (HF) diet induces intracellular and systemic lipid overload which affects liver parameters, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) [2, 3]. Furthermore, HF diet affects the hepatic antioxidant defense where nuclear factor erythroid 2-related factor 2 (NRF2) takes a central role, promoting oxidative stress and insulin resistance development [4, 5]. Additionally, obesity impairs peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1- α) in the liver, which contributes significantly to insulin resistance and non-alcoholic fatty liver disease [6, 7]. The objective of this study was to elucidate the effect of HF diet on liver parameters in serum and hepatic NRF2/PGC1- α signaling in rats.

EXPERIMENTAL

The study was conducted on adult male Wistar rats. Animals were kept in cages under standardized laboratory conditions: a controlled temperature (22 ± 2 °C), a 12-hour light/dark cycle, and *ad libitum* water and food. The animals were randomly assigned to one of two experimental groups: a control group (CONT, $n = 7$), fed a standard laboratory food, and a HF diet group (HF, $n = 7$), fed a standard laboratory food enriched with 42% sunflower oil. After 12 weeks, animals were anesthetized and euthanized, and blood samples were obtained by cardiac puncture for subsequent analyses. From each blood sample the serum was separated and preserved to further analysis. In addition, the liver was excised and stored at -80 °C until analysis. The experimental protocols were approved by the official Vinca Institute's Ethical Committee for Experimental Animals and Veterinary Directorate – No. 323-07-02710/2017-05. Serum enzyme concentrations of ALP, ALT, and AST were determined using an automated analyzer according to the manufacturer's instructions and expressed as units per liter (U/L). Furthermore, the protein expression of NRF2 and PGC1- α in rat liver was determined using Western blot analysis as we previously showed [1].

RESULTS AND DISCUSSION

To elucidate the impact of HF diet on hepatic function and redox regulation, concentrations of ALP, ALT and AST in serum and the levels of protein expression of NRF2 and PGC1- α in rat liver were analyzed.

Obesity contributes to hepatocyte damage, which can be recognized via changes in the concentrations of various biomarkers of hepatic dysfunction in circulation [2]. Previous studies have reported elevated serum concentrations of ALP, ALT, and AST enzymes in rats fed a HF diet [8, 9]. We observed that HF diet significantly increased serum ALP concentration in rats, relative to rats fed a standard diet ($p<0.001$) (Figure 1A). However, the serum ALT and AST concentrations were not significantly altered following the HF diet (Figures 1B and 1C). To investigate the impact of feeding with an HF diet on antioxidant defense signaling, we evaluated the expression of the transcriptional factor NRF2, which is also involved in lipid metabolism. Our results suggest that HF diet suppresses hepatic NRF2 protein expression ($p<0.001$), which is associated with oxidative stress and, consequently, insulin resistance (Figure 1E).

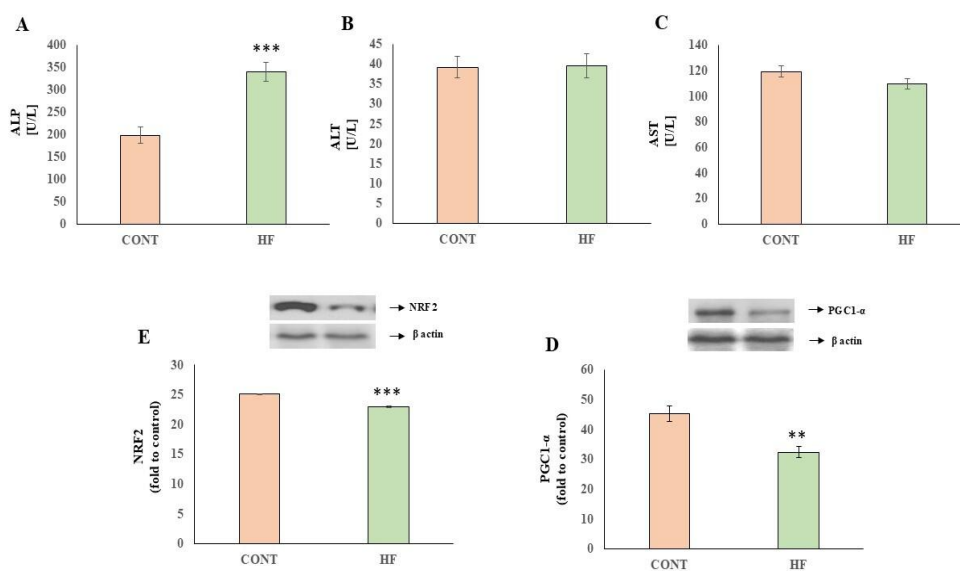


Figure 1. The ALP (A), ALT (B), and AST (C) concentrations in serum and protein expression, and representative blots of PGC1- α (D) and NRF2 (E) in the liver of control and HF rats. ALP - alkaline phosphatase, ALT - alanine aminotransferase, AST - aspartate aminotransferase, HF – high-fat, PGC1- α - peroxisome proliferator-activated receptor gamma coactivator 1-alpha, NRF2 - nuclear factor erythroid 2-related factor 2.

Consistent with our results, the literature indicates that HF diet decreases NRF2 protein expression in the liver [4, 5]. The transcriptional coactivator PGC1- α is involved in hepatic fatty acid oxidation by activating peroxisome proliferator-activated receptor target genes, thereby regulating metabolic processes under different dietary conditions. In our study obesity leads to reduction of PGC1- α protein expression ($p < 0.01$) in the liver (Figure 1D). Furthermore, results from other studies demonstrated that HF diet decreased the level of PGC1- α mRNA expression, which contributes significantly to insulin resistance and development of non-alcoholic fatty liver disease [6, 7].

CONCLUSION

These findings suggest that HF diet adversely affects hepatic function, as evidenced by significant elevation in serum ALP concentration and reduced hepatic protein expression of NRF2 and PGC1- α in the liver of obese rats relative to control rats. These findings indicate an increased inflammatory response and impaired antioxidant defense signaling in the liver of HF diet-fed rats, predisposing them to the development of insulin resistance.

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