Date: 9th August-2026

IMPORTANCE OF A 2×2 FACTORIAL EXPERIMENTAL DESIGN IN ASSESSING THE INTERACTION OF A HIGH-FAT DIET AND ANTIBIOTIC THERAPY ON THE GUT–LIVER AXIS

Irisqulov B. U. Musayev X.A.
Tashkent State Medical University, Department of Normal and Pathological Physiology

Background. Fatty liver disease is a complex process accompanied by simultaneous changes in metabolic factors, the gut microbiota, and the immune-inflammatory response. While a high-fat diet promotes triglyceride accumulation in hepatocytes, antibiotics rapidly alter the composition and metabolic activity of the gut microbiota. However, the final direction of the antibiotic effect is not always the same: under some conditions, a reduction in the microbial load may alleviate endotoxemia and steatosis, whereas in other situations, persistent dysbiosis may weaken the epithelial barrier and aggravate hepatic inflammation. Therefore, comparison of intact animals only with a "high-fat diet + antibiotic" group makes it difficult to determine which factor is predominant. An experimental design that separates the effects of diet, antibiotic therapy, and their interaction is necessary for an objective assessment of gut–liver relationships.

Objective. To substantiate the methodological advantages of a 2×2 factorial experimental design for assessing the independent and combined effects of a high-fat diet and antibiotic therapy on fatty liver disease and indicators of the gut–liver axis.

Materials and Methods. Our experiment is based on the principle of establishing four parallel groups of laboratory rats: a control group receiving a standard diet without antibiotics; a high-fat diet group; an antibiotic group receiving a standard diet; and a group receiving a combination of a high-fat diet and antibiotics. The animals are stratified according to baseline body weight and then randomly allocated. During follow-up, body weight, food and water intake, fasting glucose, insulin, HOMA-IR, triglycerides, total cholesterol, lipoprotein fractions, ALT, and AST are assessed. To evaluate the gut–liver axis, fecal microbiota, short-chain fatty acids, intestinal permeability using FITC-dextran, and blood levels of lipopolysaccharide, zonulin, TNF-α, and IL-6 are selected as indicators. Hepatic triglycerides, lipid peroxidation, antioxidant enzymes, and the degree of steatosis and lobular inflammation are determined in liver tissue. An individual animal is considered the statistical unit. The effects of the diet factor, the antibiotic factor, and the "diet × antibiotic" interaction are evaluated using two-way analysis of variance or an appropriate regression model.

Results and Discussion. The proposed design, unlike a simple intergroup comparison, makes it possible to distinguish the source of the biological phenomenon. If steatosis increases in the high-fat diet group while the antibiotic group shows no changes in liver parameters, the main effect can be attributed to the diet. If microbiota diversity and intestinal permeability change in the separate antibiotic group, the independent contribution of the drug factor can be identified. If the result in the combination group

differs significantly from the sum of the two separate effects, a statistical interaction can be inferred. For example, an antibiotic-induced increase in endotoxemia and hepatic inflammation beyond the expected level against the background of a high-fat diet would indicate a synergistic effect, whereas attenuation of steatosis would indicate an antagonistic effect. This conclusion prevents the antibiotic from being interpreted in advance as exclusively harmful or protective. In addition, collection of fecal, blood, and tissue samples at identical time points helps analyze the sequence linking microbiota changes, epithelial barrier disruption, and liver injury. A statistical model that accounts for cages, food intake, and repeated measurements reduces the risk of pseudoreplication.

Conclusion. The proposed four-group 2×2 factorial design is methodologically appropriate for accurately evaluating the role of antibiotic therapy in fatty liver disease. It distinguishes the main effect of a high-fat diet, the independent effect of antibiotics, and their interaction within a single experiment. Combined investigation of the gut microbiota, epithelial barrier, endotoxemia, metabolic indicators, and liver morphology enables cause-oriented interpretation of the findings and provides a reliable experimental platform for subsequent corrective studies.

Keywords: fatty liver disease, high-fat diet, antibiotic therapy, gut–liver axis, factorial design, dysbiosis, endotoxemia.