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Short Communication

Validation of a mouse model with Apoe gene knockout

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Abstract

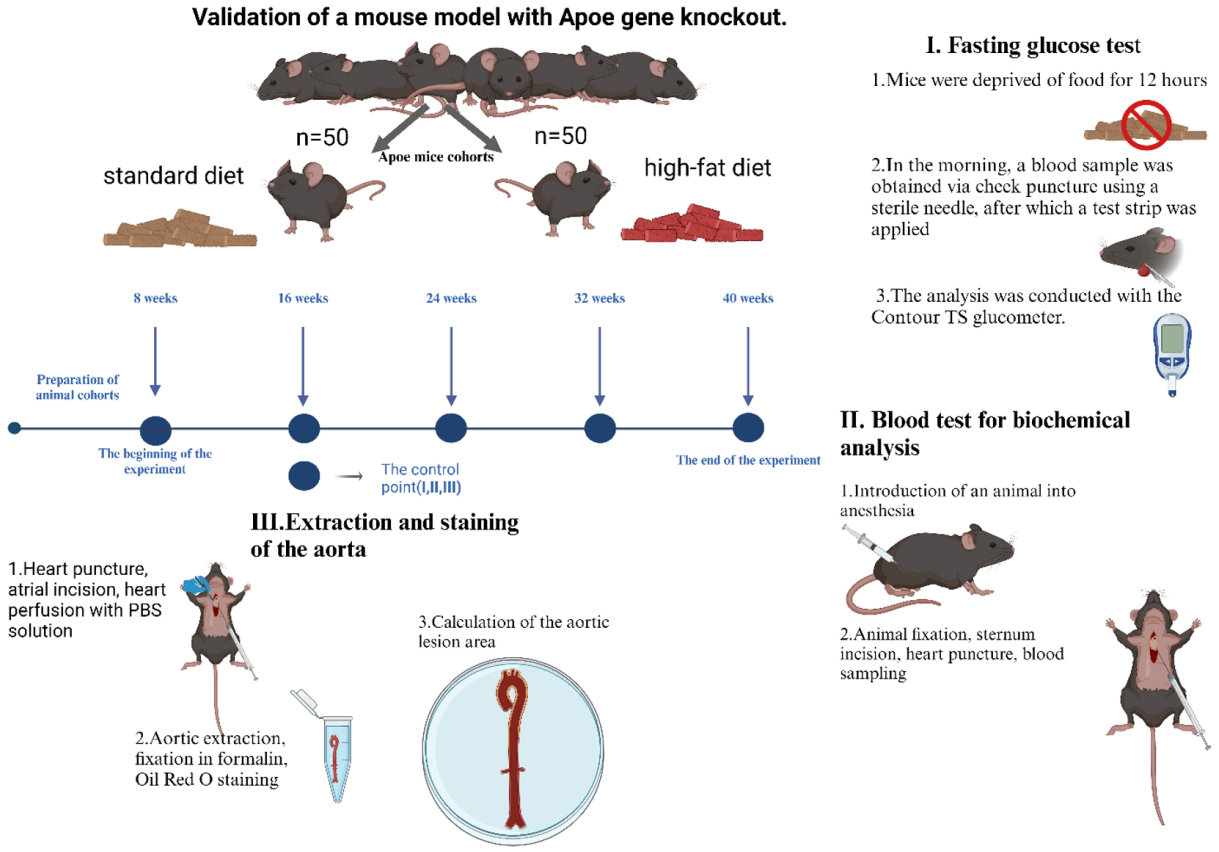
Introduction: The research focuses on the ApoE-/- transgenic mouse model. This model enhances the understanding of the mechanisms underlying disease progression and allows for the testing of new therapeutic strategies.

Materials and Methods: The study employed ApoE (-/-) knockout mice, which were bred to create cohorts of male mice. These males were divided into two equal groups, receiving either a standard diet or a high-fat diet. Analyses included lipid profile assessments, fasting glucose levels, and evaluation of aortic lesion area.

Results and Discussion: The findings revealed statistically significant differences in the ages at which disease symptoms appeared between the groups. The high-fat diet induced hyperlipidemia, leading to accelerated atherosclerosis in the aorta, aligning with existing literature.

Conclusion: ApoE knockout mice represent a promising genetic model for assessing therapeutic approaches to treat cardiovascular diseases. This model exhibits clear phenotypic manifestations of the disease and can be used for pharmacological correction of endothelial dysfunction.

Graphical abstract



Keywords

cardiovascular diseases, endothelial dysfunction, atherosclerotic lesions, lipid profile, high-fat diet

Introduction

Currently, cardiovascular diseases (CVDs) represent one of the most significant and serious health issues. Pathological conditions associated with endothelial damage (Tabarov et al. 2012), such as endothelial dysfunction (Shcheblykin et al. 2022), lead to atherosclerotic changes (Fan and Watanabe 2022) in blood vessels, arising under the influence of various factors (Lechner et al. 2020), which, in turn, are accompanied by the progression of several comorbid diseases.

For adequate prediction of human responses to new drugs, relevant animal models must be utilized. ApoE knockout mice exhibit pronounced endothelial dysfunction and a predisposition to develop atherosclerosis (Ilyas et al. 2022), making them suitable for scientific research aimed at understanding the pathogenesis of cardiovascular diseases and developing effective pharmacological interventions. Understanding the relationship between vascular damage and age in ApoE -/- mice is crucial, as the degree of damage may influence the choice of pharmacological correction. Thus, validating this model is essential for a detailed study of these relationships and the development of effective treatment strategies for cardiovascular and related pathologies (Korokina 2023).

The aim of research is to establish the optimal age and maintenance conditions for ApoE -/- mouse models to evaluate pharmacological correction in cases of endothelial dysfunction (ED).

Materials and Methods

Animals

The animals were housed under SPF conditions at Belgorod State National Research University (BelSU), with artificially regulated light cycles (12 hours of

darkness and 12 hours of light) at a temperature range of +22 to +26°C, with free access to food and water. The study adhered to ethical principles regarding laboratory animals in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 170). All painful procedures on animals were conducted following regulatory standards: Directive 2010/63/EU of the European Parliament and Council on the Protection of Animals Used for Scientific Purposes, dated September 22, 2010. The research was approved by the Animal Care and Use Committee at BelSU, expert conclusion No. 01-06i/24 dated June 3, 2024.

ApoE (-/-) animal groups were obtained by crossing homozygous animals to create synchronized cohorts. Mice were divided into a control group on standard feed and a group receiving a high-fat diet. The sample size consisted of 50 animals in each group. Euthanasia was performed at 8, 16, 24, 32, and 40 weeks; fasting glucose measurements were taken; blood was collected post-mortem for biochemical analysis, and aorta perfusion and extraction were conducted.

Statistical analysis

Statistical processing was carried out using GraphPad Prism Software 8.0 (GraphPad Software Inc, USA). Two-way ANOVA was applied to assess the significance of differences between multiple data sample groups. In post hoc analysis, an unpaired Student's t-test or Mann-Whitney test was used. Results were considered statistically significant at $p \leq 0.05$.

Results and Discussion

Fasting glucose results

The results of fasting glucose analysis are presented in Figure 1.

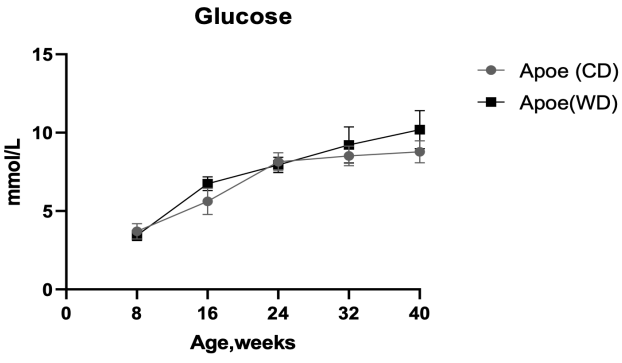


Figure 1. Results of fasting glucose analysis in ApoE(-/-) mice at 8, 16, 24, 32, and 40 weeks. ApoE (CD) represents the dynamics of blood glucose concentration in the control group of mice, while ApoE (WD) represents the dynamics of blood glucose concentration in mice receiving a high-fat diet.

According to the study results, a statistically significant difference between the groups was observed at weeks 16, 32, and 40 ($p \leq 0.01$). Throughout the period from 8 to 40 weeks, both groups exhibited a statistically significant increase in blood glucose levels ($p \leq 0.01$). This indicates that with age, both groups experience pronounced hyperglycemia, which, in turn, exerts toxic effects on the vascular endothelium, leading to increased vasoconstriction, vascular remodeling, and subsequent atherosclerosis.

Results of lipid profile analysis

Figure 2 shows the dynamics of changes in the lipid profile of ApoE(-/-) mice.

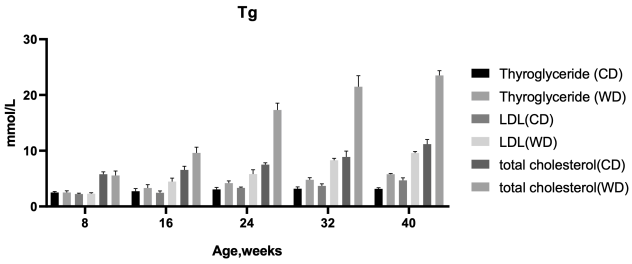


Figure 2. The results of the lipid profile analysis in ApoE (-/-) mice at 8, 16, 24, 32, and 40 weeks.

ApoE (CD) indicates the dynamics of triglycerides, LDL, and total cholesterol concentrations in the blood of control group mice, while ApoE (WD) represents these parameters in mice fed a high-fat diet.

At the first checkpoint, triglyceride, LDL, and total cholesterol levels did not significantly differ between the standard and high-fat diet groups ($p > 0.05$). From 8 to 40 weeks, both groups exhibited a statistically significant increase in these parameters ($p \leq 0.05$). The most pronounced increase was observed in the group of animals receiving the high-fat diet, particularly in LDL and total cholesterol levels.

The study of the lipid profile indicates that with age, there is a more substantial increase in blood levels of LDL and total cholesterol. This demonstrates that ApoE (-/-) animals develop hypercholesterolemia regardless of dietary composition; however, lipid spectrum indicators will be significantly higher in animals consuming a high-fat diet. Consequently, manifestations of endothelial dysfunction and subsequent associated pathologies will appear earlier in the high-fat diet group.

The results of the macroscopic analysis of aortic lesions

In Figure 3 presents findings from tissues stained with Oil Red O dye.

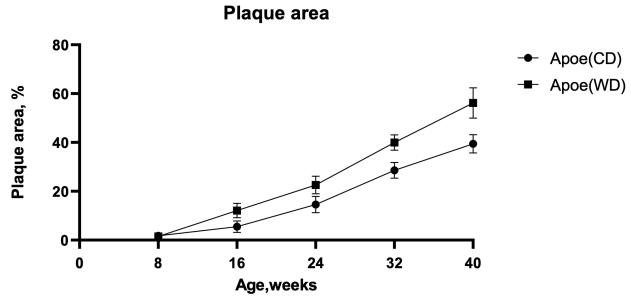


Figure 3. The dynamics of aortic lesion area growth in ApoE mice were studied, comparing the standard diet group (ApoE (CD)) and the high-fat diet group (ApoE (WD)).

At 8 weeks, no significant differences were observed between the experimental groups. However, by 16 weeks, a significant difference in the accumulation of atherosclerotic plaques on vessel walls was noted between the standard and high-fat diet groups ($p \leq 0.01$), and this difference persisted until the final checkpoint at 40 weeks.

These results emphasize the importance of age and dietary factors in the pathogenesis of atherosclerotic lesions and associated endothelial dysfunction (ED).

Conclusion

The findings underscore the significance of using ApoE knockout mice as a promising model for studying endothelial dysfunction, atherosclerosis, and related cardiovascular diseases. It is essential to consider that the choice of diet and age of the animal significantly affects research outcomes. Selecting the appropriate timing for pharmacological intervention will yield the most reliable results. Future research should expand on identifying diagnostic features of endothelial dysfunction, phenotypic manifestations of pathology, and optimizing therapeutic strategies using this model. This approach will enhance understanding of endothelial dysfunction and aid in the search for new effective treatment methods.

Conflict of interest

The authors declare the absence of a conflict of interests.

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The authors have no support to report.

Data availability

All of the data that support the findings of this study are available in the main text.

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