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Comparison of Etomoxir, a Lipid Metabolism Blocker, and Interferon-β Treatment on Antibody Recognition of Brain Proteins in Multiple Sclerosis



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Anne Skøttrup Mørkholt¹, Kenneth Kastaniegaard¹, Michael Trabjerg¹, Wanda Niganze¹,
Gopana Gopalasingam¹, Agnete Larsen², Allan Stensballe¹, Søren Nielsen¹ and John Dirk Nieland¹

¹Department of Health Science and Technology, Aalborg University, Denmark

²Department of Biomedicine, Aarhus University, Denmark

Correspondence: jdn@hst.aau.dk



Abstract

Background: The pathogenesis of Multiple Sclerosis (MS) involves a new hypothesis concerning mitochondrial dysfunction, dysregulated lipid metabolism and inflammation. Carnitine palmitoyl transferase 1a (CPT1a) is a key molecule involved in lipid metabolism, which is necessary for transport of lipids into mitochondria. Lipids are important for the central nervous system as it constitute myelin sheaths and thereby shield proteins, e.g. myelin basic protein (MBP), for the immune system. Dysregulated lipid metabolism results in changes in concentration and composition of lipids and down regulates glucose metabolism, which is the main energy source of the brain. Furthermore, an upregulated lipid metabolism results in prostaglandin E2 (PGE2) production, thereby inducing an inflammatory B and T cell response. It has been found that B cells start generating antibodies to brain proteins after immunization with MBP suggesting a correlation between MS induction and brain antigens. Blocking CPT1a by Etomoxir favours glucose metabolism rather than lipid metabolism, thus normalizing lipid levels in the brain, as well as downregulating PGE2 production and B cell response. This suggests Etomoxir as an innovative treatment strategy for MS.

Methods: Experimental autoimmune encephalomyelitis (EAE) models of MS were established to test the efficacy of the CPT1 blocker, Etomoxir. Western Blotting was executed in order to test antibody response against brain proteins in EAE animal sera. Antigens recognized by autoantibodies were immunoprecipitated and analyzed by mass spectrometry with the purpose to identify these antigens, and thereby identifying a correlation between MS and brain antigens.

Results: EAE models of both mice and rats showed 50 % and 25 % healthy animals after treatment with Etomoxir. We identified autoantibodies in sera from the EAE animals by an altered antibody response between different treatment groups and also isotype of the antibody response. After Etomoxir treatment, antibodies to antigens such as apolipoprotein-E, clusterin and serum amyloid P component are i.e. recognized and involved in downregulation of the immune system.

Conclusions: Alteration in metabolic pathways is pivotal regarding the pathogenesis of MS, as Etomoxir treatment is efficient in treating MS by downregulating lipid metabolism and inflammation. Moreover, autoantibodies against brain antigens were identified suggesting a role of these antigens in the progression of MS with the potential of using these as biomarkers for MS.

Background

New Hypothesis

- Multiple Sclerosis is a systemic disease caused by dysfunction of lipid metabolism and consequently induction of an inflammatory and autoimmune response.

Metabolic Pathways

- Normally, the brain utilizes both glucose and fatty acids for cellular energy production (glycolysis and beta-oxidation), whereas in conditions with stress the metabolism shifts to lipid utilization (Figure 1).
- CPT-1 is a key molecule involved in lipid metabolism.
- Conversion of acyl-CoA to acyl-carnitine by CPT-1 serves as a central regulator of the metabolism of the cell.

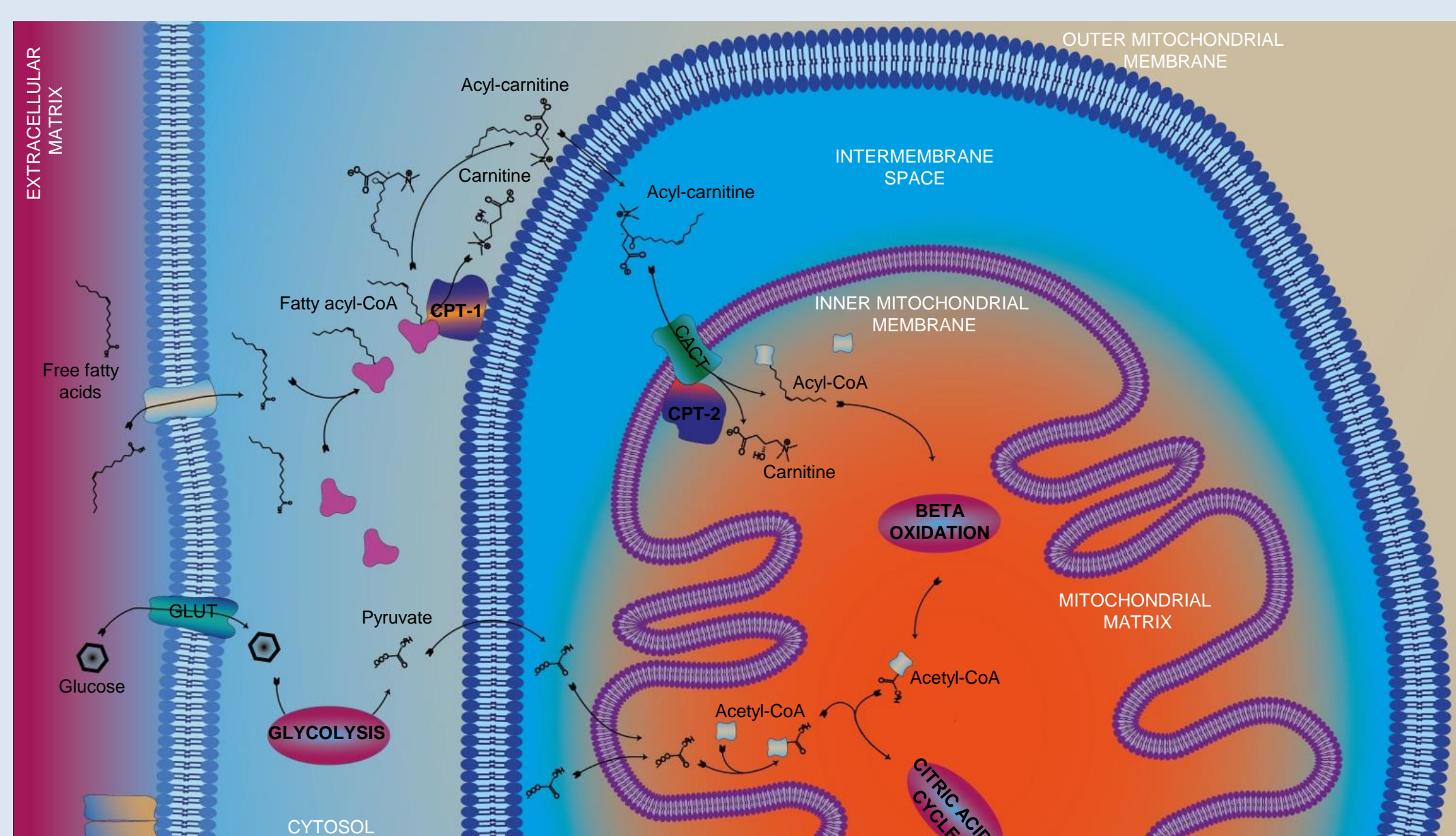


Figure 1. Metabolic pathways in the mitochondria.

Dysregulated Lipid Metabolism

- Demyelination of neurons leading to exposure of proteins such as Myelin Basic Protein (MBP) to immune cells.
- Recruiting inflammatory cells, which start production of prostaglandin E2 and antibodies.
- Generation of autoantibodies to self-antigens leading to autoimmunity.

Conclusions

- Blockage of lipid metabolism resulted in 50 % healthy mice and 25 % healthy rats.
- Recognition of brain antigens against autoantibodies revealed:
 - Identification of 35 autoantigens.
 - Identification of modulated autoantibodies against clusterin and serum amyloid P component and others after Etomoxir treatment compared to placebo.
 - Identification of modulated autoantibodies against complement component C9 and gelsolin and others after Interferon-β treatment compared to placebo.

Acknowledgements

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Methods

Project I

- Experimental Autoimmune Encephalomyelitis Mice Model: Female C57BL/6 mice were immunized with MBP in Freund's adjuvant. The mice were weighed and scored daily. At day 10 the mice received either Etomoxir (n=21) or placebo (n=21).

Project II

- Experimental Autoimmune Encephalomyelitis Rat Model: Female Lewis rats were immunized with MBP in Freund's adjuvant. The rats were weighed and scored daily. At day 1 or day 5 the rats were treated with either Etomoxir or Interferon-β (n=10 in each of the five treatment groups).

Project III:

- Normal rat brain proteins were stained with EAE-induced rat sera by Western Blotting.
- Antigens were immunoprecipitated by antibodies in sera from EAE-induced rats and subsequently antigens recognized by autoantibodies were identified by mass spectrometry (Figure 2).

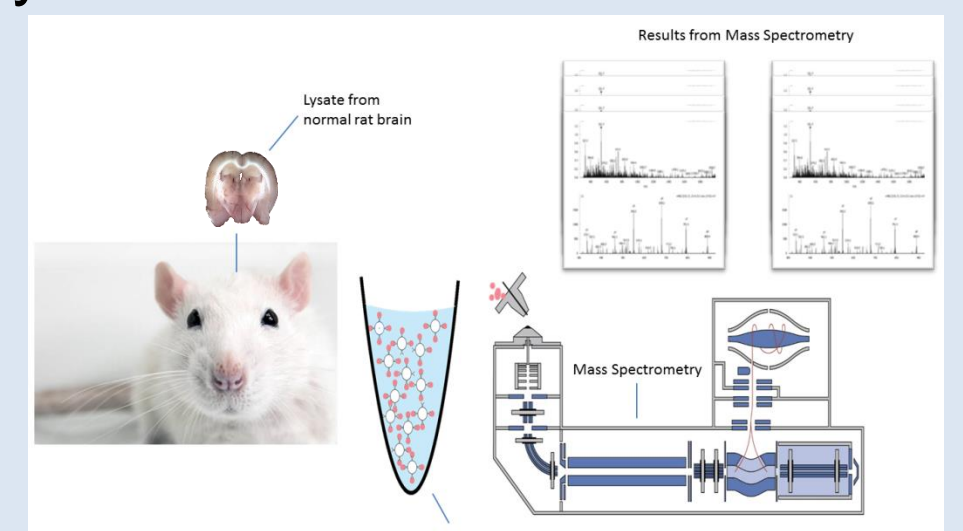


Figure 2. Experimental setup for project III.

Project I and II

Results

Etomoxir Blocks Lipid Metabolism in EAE-induced Animals

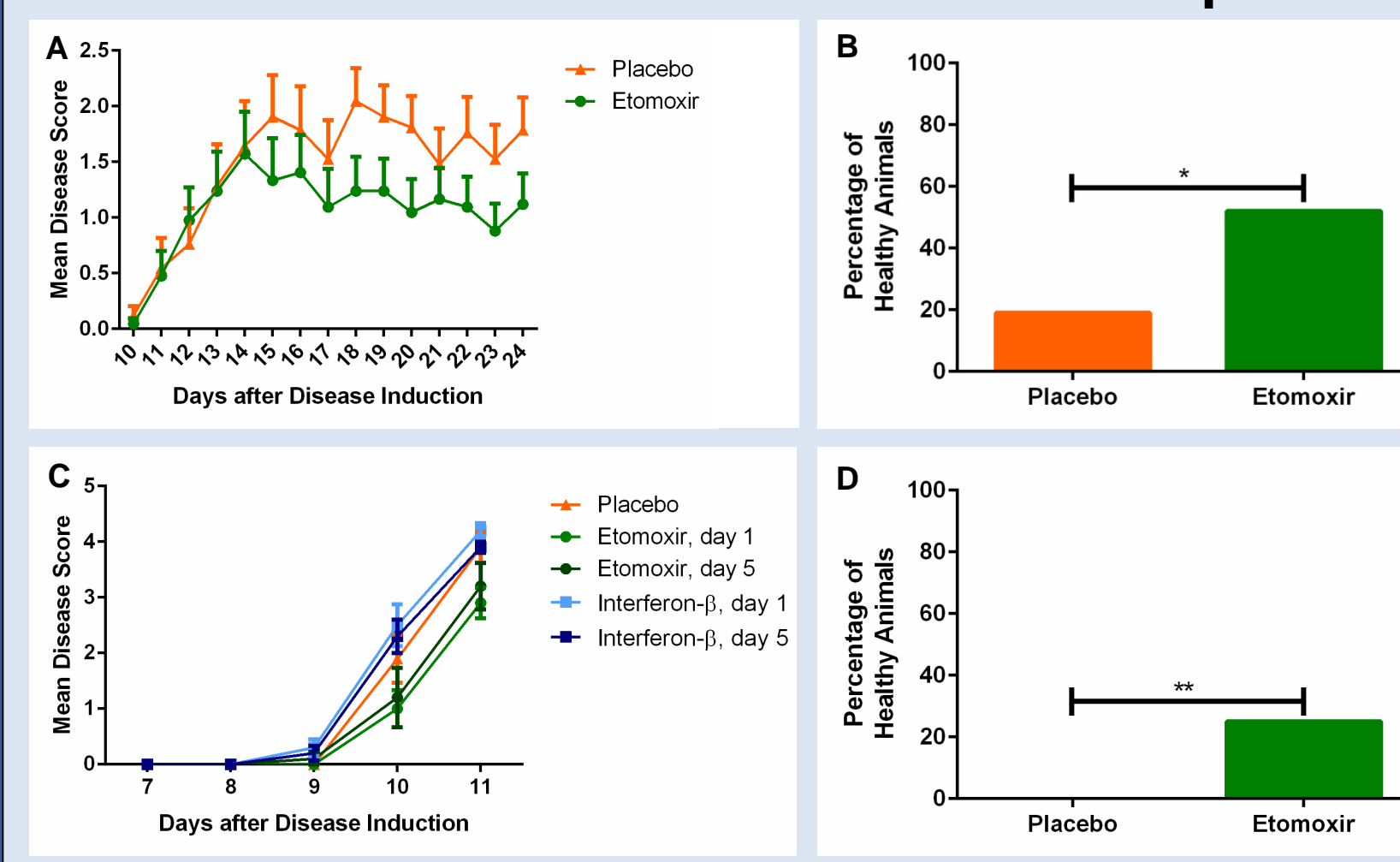


Figure 3. Results from EAE models in mice and rats. A-B) EAE mice model. Mean disease score of day 10-24 and percentage of healthy animals at day 24.

C-D) EAE rat model. Mean disease score of day 7-11 and percentage of healthy animals at day 11.

The statistical analyses applied are two-way RM-ANOVA tests and Chi-square tests. Results are presented as mean ± SEM. Number of asterisks indicates level of statistical significance (*p=0.01-0.05 and **p=0.001-0.01).

Project III

Results

Recognition of Autoantibodies in EAE-induced Animals

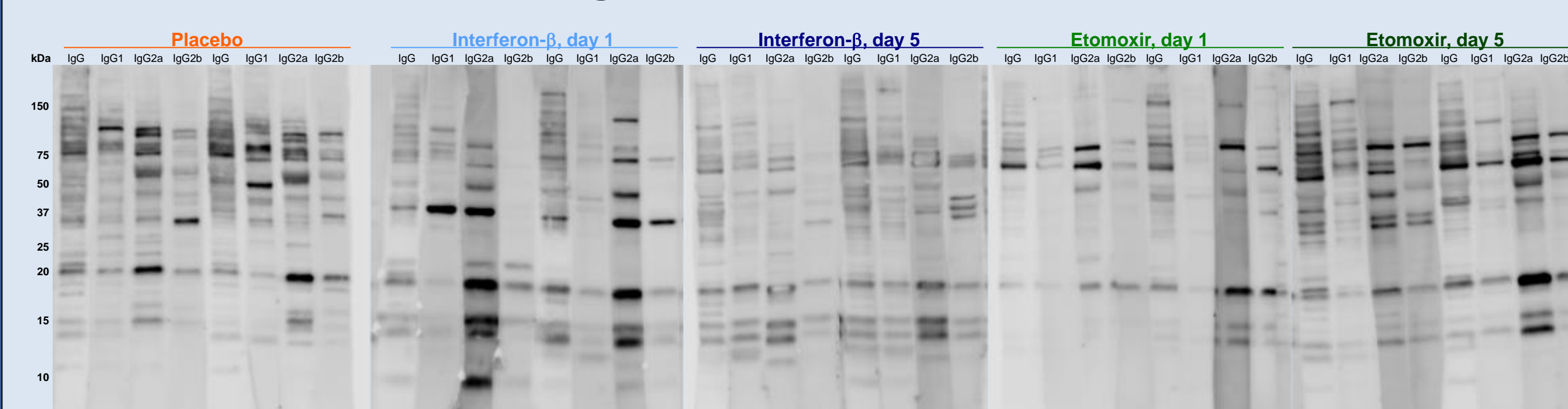


Figure 4. Recognition of autoantibodies. Results from Western Blotting showing recognition of autoantibodies in EAE-induced rat sera. Each treatment group (Placebo, Interferon-β day 1 and 5, and Etomoxir day 1 and 5) represents two animals each of which are tested for IgG, IgG1, IgG2a and IgG2b.

Project III

Results

Examples of Antigens Involved in Inflammation

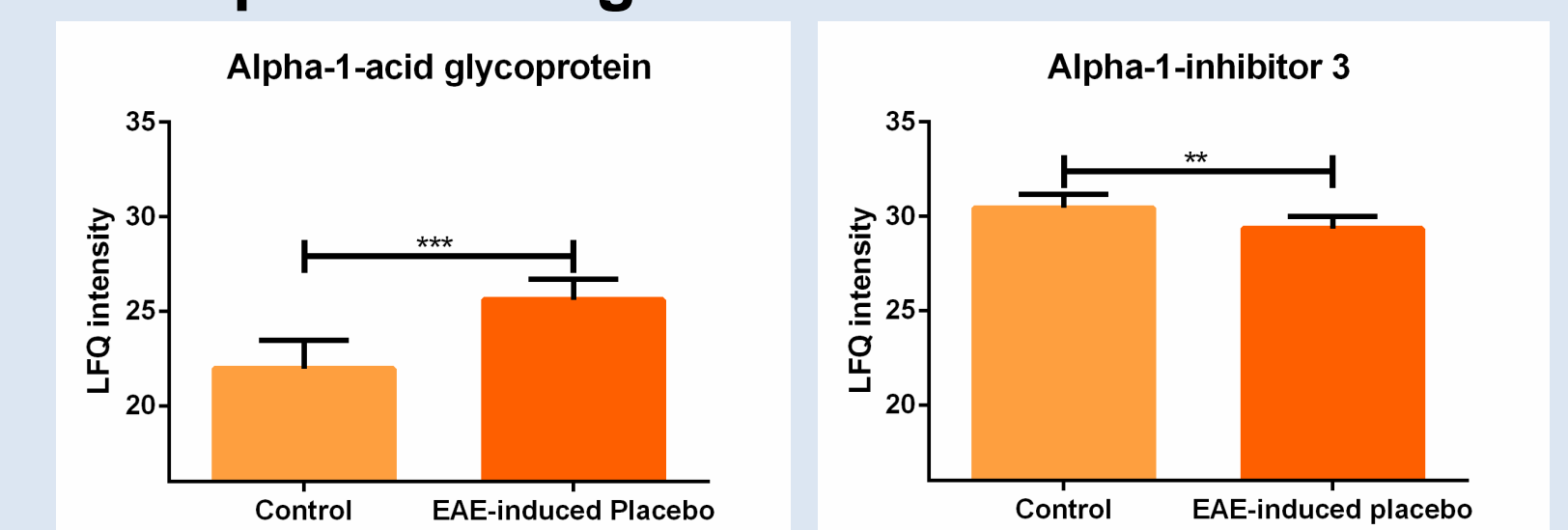


Figure 5. Serum antibody intensities to antigens involved in inflammation. Alpha-1-acid glycoprotein and alpha-1-inhibitor 3. Results are presented as mean ± SD. Number of asterisks indicates level of statistical significance (**p=0.001-0.01 and ***p=0.0001-0.001).

Examples of Antigens Involved in Multiple Sclerosis

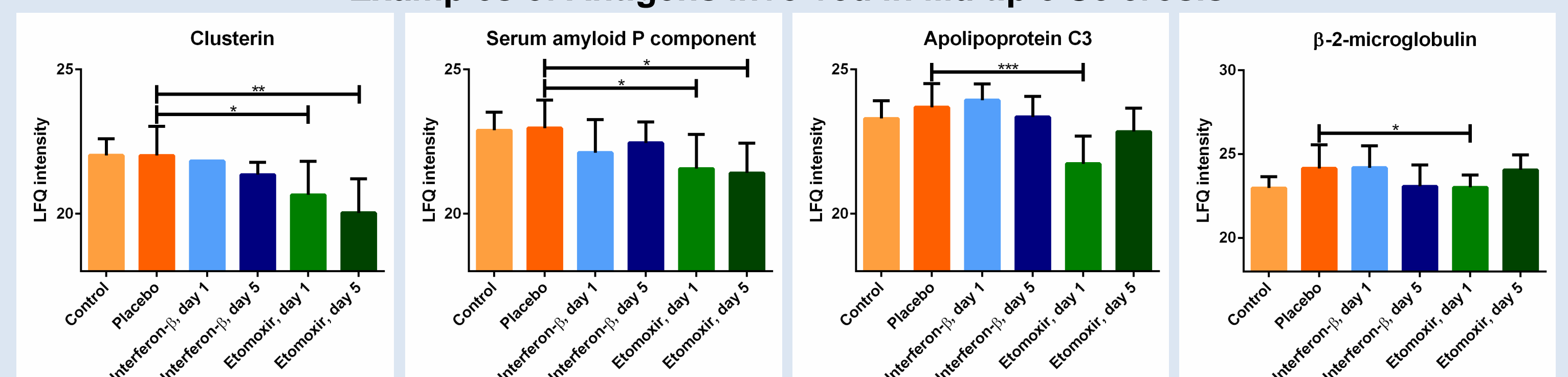


Figure 6. Serum antibody intensities of antigens involved in Multiple Sclerosis. Etomoxir treatment revealed significant decreased serum antibody intensity to clusterin, serum amyloid P component, apolipoprotein C3 and β-2-microglobulin compared to the placebo group. Results are presented as mean ± SD. Number of asterisks indicates level of statistical significance (*p=0.01-0.05, **p=0.001-0.01 and ***p=0.0001-0.001).

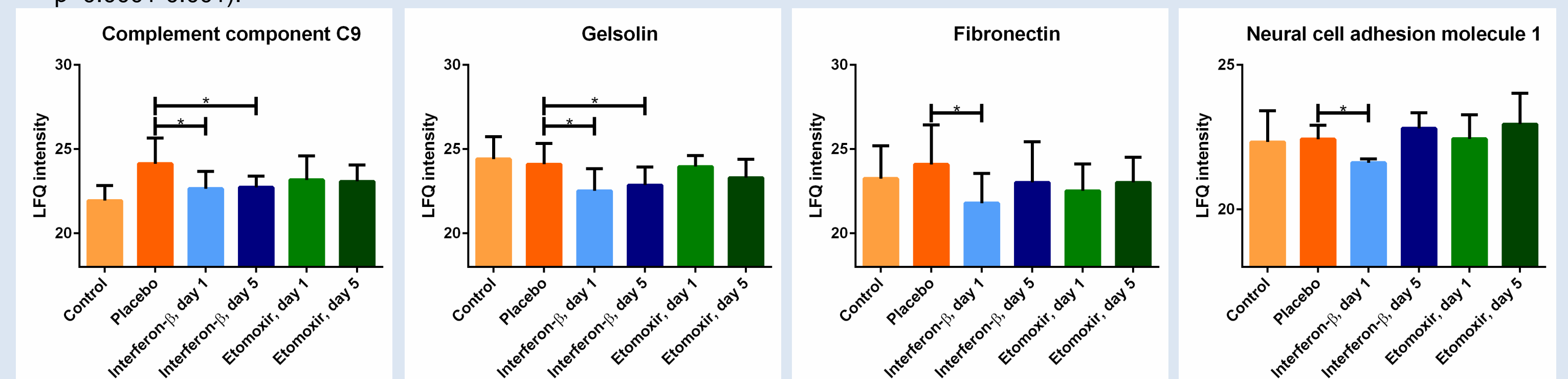


Figure 7. Serum antibody intensities of antigens involved in Multiple Sclerosis. Interferon-β treatment resulted in significant decreased serum antibody intensity to complement component C9, gelsolin, fibronectin and neural cell adhesion molecule 1 compared to the placebo group. Results are presented as mean ± SD. Number of asterisks indicates level of statistical significance (*p=0.01-0.05).