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CERTIFICATE

This is to certify that

Flóra Diána Gausz

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gave a presentation

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cholesterol or 7-dehydrocholesterol (7DHC) levels, such as hypercholesterolemia (HC) or Smith-Lemli-Opitz syndrome (SLOS), can be associated with elevated DP, which may play a role in their pathophysiology. In such cases decreasing the DP could be beneficial, however, no known physiological method to lower DP has been described. Measurement of the DP, especially in living cells, is difficult, yet we were able to develop a novel flow cytometric method to study the DP in models of such diseases.

Methods: We have measured the magnitude of DP in models of HC and SLOS with our novel emission ratiometric (Rem) approach using the environment sensitive fluorophore F66 and flow cytometry. The models were created in JY lymphocytoid, THP-1 monocytoid and HEK-293-T human embryonal kidney cell lines treated with methyl-beta-cyclodextrin/sterol complexes. The effects of omega-3 (alpha-linolenic-acid), omega-6 (gamma-linolenic-acid) fatty acids and stearic acid on DP were also determined in disease models.

Results: In models of HC and SLOS loading the cells with cholesterol and 7DHC resulted in dose-dependent increases in DP ($\Delta\text{Rem}_{\text{max}}$ -14%, -8%, respectively). Demonstrating the importance of a diet rich in polyunsaturated fatty acids, ω -3 fatty acid decreased, while ω -6 or saturated fatty acid increased the magnitude of DP ($\Delta\text{Rem}_{\text{max}}$ +10%, -5%, respectively). In combined treatments ω -3 fatty acid attenuated, while ω -6 fatty acid amplified the effects of cholesterol on DP.

Conclusions: The results of our novel flow cytometric method confirmed the positive effect of ω -3 fatty acid in reducing the adverse membrane biophysical alterations observed in HC and proposed ω -3 fatty acids as physiological DP lowering agents.

INVESTIGATION OF THE RELATIONSHIP BETWEEN CARDIOLIPIN AND PREDIABETES INDUCED CARDIAC DAMAGE

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Keywords: cardiolipin, remodeling, prediabetes, OGTT, mass-spectrometry

Aims: Prediabetes is a symptomless disease that mostly remains unrecognized. Prediabetes induces cardiac damage, which involves mitochondrial dysfunction. One of the main components of the mitochondrial inner membrane is cardiolipin. The cardiolipin level decreases in diabetes mellitus correlated to diabetic myocardial damage. Our work aimed to examine the relationship between prediabetes induced cardiac damage and cardiolipin levels.

Methods: We used 16 male Wistar rats. The rats were divided into two groups. The control group received a normal diet while the fructose-fed group received a 60% fructose-supplemented chow for 24 weeks. During the experiment, we measured fasting blood sugar levels and oral glucose tolerance (OGTT). In the 24th week, animal hearts were isolated and cardiac functions were examined by working heart perfusion. With mass-spectrometry, we determined cardiolipin levels in the myocardium.

Results: The results of fasting blood sugar monitoring and oral glucose tolerance test showed slightly but significantly elevated values due to fructose feeding, indicating the development of the prediabetic condition. The isolated working heart perfusion revealed diastolic dysfunction in the fructose-fed group. We detected decreased cardiolipin levels with mass-spectrometry due to the increased fructose intake compared to the control group ($28,1 \pm 2,1$ vs. $37,8 \pm 2,1$). Furthermore, we detected significant cardiolipin remodeling in the fructose-fed group. Cardiolipin is essential in mitochondrial and myocardial function, so the defect of this

molecule indicates myocardial dysfunction.

Conclusions: Fructose enriched diet indicates decreasing cardiolipin levels and cardiolipin remodeling, which cause mitochondrial dysfunction. Our results show that the decreased amount of cardiolipin can play an essential role in prediabetes induced cardiac damage.

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TISSUE PROTECTIVE EFFECT OF MESENCHYMAL STEM CELL SECRETOME CONDITIONED MEDIUM ON VASCULAR GRAFT IN A RODENT MODEL OF ARTERIAL REVASCULARIZATION

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Keywords: coronary artery bypass grafting, bioactive factors produced by m

Aims: Despite the rapid development of percutaneous intervention techniques, coronary artery bypass grafting (CABG) is still one of the most commonly performed major surgical procedures. The prognosis of revascularization via CABG strongly correlates with the long-term patency of grafts. The degree of ischemia-reperfusion injury (I/R) might influence the long-term patency by causing depletion of intracellular energy, high production of reactive oxygen species, and inflammatory cytokines in the graft tissue. The new concept to decrease this I/R injury is the pharmacological preconditioning of the graft. Stem cell-based therapies for various diseases have been actively studied in the last decade. A new discovery (Caplan et al. 2006) brought attention to the vast array of bioactive factors produced by mesenchymal stem cells (MSCs) including antiapoptotic factors, growth factors, chemokines and cytokines.

Methods: Therefore we aimed to characterize the effect of MSC secretome conditioned medium (CM) on vascular graft regarding tissue protection and repair as admixture to preservation solution used during clinically relevant animal model of CABG. Bone marrow-derived MSCs were prepared from tibia of Sprague Dawley rats (8-12 week-old) and propagated. CM was obtained from Passage-3 and tested by 90-antibody-array prior to preservation. To perform our CABG modeling procedure aortic arches were harvested from donor rats and stored in cold preservation solution w/o additional CM, then implanted heterotopically in the recipient's abdominal cavity followed by 2 hours of reperfusion. Vasomotor functional measurements using a myograph system in isolated organ baths and histomorphological changes using immunohistochemistry were analyzed.

Results: CM was characterized by a 90-antibody-array, revealing the presence of 31 factors involved in apoptosis, inflammation, and oxidative stress. As a result of CM endothelium-dependent maximal vasorelaxation (R_{max}) was improved (81.8±3.4% vs 40.9±5.6%, p<0.05), nitro-oxidative stress and cell apoptosis decreased.

Conclusions: CM is a potential preconditioning agent in arterial bypass grafting.