Type 2 diabetes (T2D) is a chronic inflammatory-based disease, which can lead to cardiovascular damage. Metformin is a first-line hypoglycaemic drug for T2D that acts mainly by lowering glucose levels. It has also been shown to have mitochondrial effects, but the underlying mechanism is yet to be elucidated. In the present study, we describe how T2D can affect mitochondrial function in peripheral blood leukocytes from T2D patients, and how metformin can modulate this effect.
Peripheral blood was extracted from 80 T2D patients and 80 healthy volunteers. Among the T2D patients, 40 had been treated with 1700 mg/day metformin for at least 1 year. Peripheral blood mononuclear cells (PBMC) were isolated following a ficoll density protocol and an erythrocyte lysis and were subsequently employed in our experiments. Mitochondrial mass, mitochondrial membrane potential and reactive oxygen species (ROS) content were analysed by flow cytometry with Mitotracker green, tetramethylrhodamine and MitoSox fluorescence, respectively. Protein analysis was performed by SDS-PAGE western blot.
We observed that mitochondrial mass and mitochondrial membrane potential were lower in leukocytes from T2D patients than in those of healthy controls, and that this effect was reversed in the presence of metformin. In parallel, mitochondrial ROS levels were higher in T2D leukocytes, while metformin reduced ROS levels. In relation to protein levels in mitochondrial complexes, leukocytes from the T2D patients displayed lower complex I, II, III and V protein content, while no changes were observed with respect to complex IV. Metformin treatment returned all the affected complexes to control levels.
In conclusion, we demonstrate that metformin modulates mitochondrial function and structure in T2D patients.
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NC61

Type 1 diabetic patients exhibit enhanced leukocyte-endothelium interaction and mitochondrial alterations

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Cardiovascular complications are the leading cause of death among patients with type 1 diabetes (TD1). Hyperglycaemia is one of the principal risk factors for developing cardiovascular diseases (CVD), mainly through the induction of oxidative stress. The overproduction of reactive oxygen species (ROS) promotes atherosclerosis by enhancing inflammation. We evaluated whether T1D patients present alterations in leukocyte-endothelium interaction, oxidative stress, and inflammatory parameters.
We recruited forty controls and forty-five patients with T1D. Anthropometric measurements were performed and blood samples obtained for biochemical determination and molecular analysis in all the subjects. Levels of leukocyte adhesion molecules (Selectin P, VCAM1 and ICAM1), proinflammatory cytokines (TNF-α and IL-6) and myeloperoxidase were analysed in the patients' serum. Interactions between patients' leukocytes and endothelial cells were evaluated using an ex vivo model. In leukocytes, we assessed mitochondrial function and determined several oxidative stress parameters with fluorescent probes: DCFH-DA for total ROS production, MitoSOX for mitochondrial ROS production and TMRM for mitochondrial membrane potential.
As expected, T1D patients exhibited higher levels of glucose and Hba1c-DCCT than controls (both $p<0.001$), and enhanced leukocyte-endothelium interactions, with reduced PMN rolling velocity ($p<0.001$) and PMN rolling flux ($p<0.01$) and greater PMN adhesion ($p<0.001$). In parallel with these results, serum from T1D patients presented higher levels of leukocyte adhesion molecules Selectin P ($p<0.05$), VCAM1 ($p<0.01$), and ICAM1 ($p<0.001$). Moreover, serum levels of TNF-α ($p<0.01$) and myeloperoxidase ($p<0.05$), but not IL-6, were higher in T1D patients. Finally, T1D leukocytes presented mitochondrial alterations, with enhanced total and mitochondrial ROS production (both, $p<0.05$) and increased mitochondrial membrane potential ($p<0.05$).
In conclusion, mitochondrial alterations, increased leukocyte-endothelium interactions, and oxidative stress may be related to the development of CVD in T1D.
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Resveratrol effects on SIRT1-SIRT3-SOD2 axis in high-glucose-challenged HUVECs

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Uncontrolled accumulation of methylglyoxal and reactive oxygen species occurs in hyperglycemia-induced endothelial dysfunction associated with diabetes. In our previous publication, we have demonstrated the ability of resveratrol (RSV) to protect high-gluocse (HG)-challenged endothelial cells mainly by activating the sirtuin 1 (SIRT1, a NAD^+-dependent deacetylase) - glyoxalase 1 pathway, enhancing antiglycative and antioxidant defences and abolishing the HG-dependent peroxidative and glycative damages (1).
Since mitochondria are a hub of oxidative stress, the aim of the present study is to investigate the role of SIRT1 on mitochondrial response to HG in endothelial cells. Our experimental model consists of primary human umbilical vein endothelial cells (HUVECs) undergoing a 24-h treatment with HG, with or without RSV and EX527 (a SIRT1 inhibitor). We evaluated the sirtuin 3 (SIRT3) mRNA levels, through RT-PCR, and SIRT3 protein levels as well as acetyl-superoxide dismutase 2 (ac-SOD2) over total SOD2, through western immunoblotting.
Our data indicate that HG treatment induce a decrease in SIRT3 protein and an increase of ac-SOD2. RSV is able to restore SIRT3 levels and reduce the acetylation levels of SOD2, suggesting that RSV may improve the mitochondrial antioxidant milieu through the activation of SIRT3/SOD2 axis. Surprisingly, when SIRT1 is inhibited both the acetylation levels of SOD2 and SIRT3 levels do not change. These findings suggest that the effect of RSV on SOD2 acetylation seems not to be SIRT1-dependent.
Our future work will focus on the investigation of mitochondrial respiratory function and morphology in order to better clarify how RSV and SIRT1 may protect the mitochondrial environment of HG-challenged HUVECs.
Reference:
Santini SJ, Cordone V, Mijit M, et al. SIRT1-Dependent Upregulation of Antiglycative Defense in HUVECs Is Essential for Resveratrol Protection against High Glucose Stress. Antioxidants (Basel). 2019;8(9):346. Published 2019 Sep 1. doi:10.3390/antiox8090346

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Involvement of Akt-p38-MAPK/Nrf2 pathway in prevention of endothelial damage by white-wine pomace product

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Hyperglycemia constitutes one of the main characteristics of diabetes, obesity and other cardiovascular diseases. This risk factor increases the oxidative stress and causes tissue damage through several mechanisms, including alterations in several signaling pathways. The main aim of this study was to investigate the mechanism involved in the preventive effect against endothelial oxidative damage of bioavailable fractions obtained from white wine pomace product (wWPP). The Nrf2/ARE pathway is involved in the increase of oxidative stress in