NAD+ levels are augmented in aortic tissue of ApoE-/- mice by dietary omega-3 fatty acids

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論文題目

NAD^+ levels are augmented in aortic tissue of $ApoE^{-/-}$ mice by dietary omega-3 fatty acids

（食餌性オメガ 3 脂肪酸により $ApoE^{-/-}$マウスの大動脈組織で NAD^+レベルが増強される）

論文の内容の要旨

[Introduction]

Nicotinamide adenine dinucleotide (NAD^+) is one of the most important molecules for life's existence, and it is reported that we can not survive without it in 30 seconds. NAD^+ declines in normal aging due to the accumulation of DNA damage and enhancing activities of NAD^+-consuming enzymes. NAD^+ deficiency has proved close to congenital malformations and metabolic disorders, including cardiovascular diseases (CVDs). An imbalance between nuclear and mitochondrial gene expression contributes to mitochondrial dysfunction, and this phenomenon could be reverted by NAD^+ replenishment. Numerous subsequent studies have indicated that NAD^+ repletion is coordinated with better healthspan and lifespan in animal models, restoring energic products. Strategies to boost NAD^+ availability show promise in vascular remodeling, which has driven intense interest in the effects of supplements on human health. Maintaining bioenergetic homeostasis via ensuring adequate NAD^+ contents enables the prevention of cardiovascular events for aged patients.

In a living organism, NAD and FAD (flavin adenine dinucleotides) are cofactors that act as signaling molecules, participating in electron transfers. They exist in oxidized forms and reduced forms. NAD^+ nucleotides are critical coenzymes for thousand of chemical reactions donating as the hydride acceptors. NAD^+ accepts a hydrogen ion (H^+) and two electrons ($2e^-$) to perform its roles, transforming into $NADH + H^+$. This capability is used for glycolysis, the Krebs cycle, and oxidative phosphorylation, allowing mitochondrial networks to convert sugars and fats into ATP cellular energy. NADPH is the cosubstrate of NADPH oxidases (NOXs). NOXs are one of the major reactive oxygen species in the heart and have emerged as the primary source of oxidative stress underlying varied CVDs. The main groups of NAD^+-consuming enzymes are Poly (ADP-ribose) polymerases (PARPs), Sirtuins, and CD38. n-3 polyunsaturated fatty acids (n-3 PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have multiple biological effects. At the cell level, EPA + DHA mixture activated SIRT1 signaling by enhancing mRNA level of nicotinamide phosphoribosyltransferase, enabling subsequent NAD^+ biosynthesis. In this study, we aimed to detect simultaneously three redox couples NAD, NADP, and FAD and test whether or not an enhancement of NAD^+ levels in the aortic wall.

[Materials and Methods]

Protocols and procedures for the care and use of the experimental animals were carried out following the guidelines established by the Pharmaceutical Research Center of Mochida Pharmaceutical Co., Ltd. The approval numbers: P14-4010 and PMS15-013, which are the first and second year experiments of the collaboration, respectively.

We used three groups of apolipoprotein E-deficient (*ApoE*$^{-/-}$) mice, and those mice, respectively, were fed a Western diet (WD), WD + DHA (1%, w/w), and WD + EPA (1%, w/w) for three weeks and a group of wildtype, 15 mice in total. For experiments, desorption electrospray ionization - mass spectrometry imaging (DESI-MSI) was selected. It aims to detect the dynamic molecules of NAD^+/NADH, $NADP^+$/NADPH, and FAD^+/FADH spatial distribution in the aortic tissues. 2D molecular mapping constructed by High Definition Imaging (HDI) v1.4 software visualized the distributions. NAD^+ intensities were measured using the IMAGEREVEAL^{TM} MS v1.1 (Shimadzu) for the region of interest.

[Results]

Exogenous/endogenous NAD^+ images were successfully visualized. Ion imaging NAD^+, FAD^+, and their metabolites were distributed plentifully in the aortic root . Oxidized/reduced forms of NAD and FAD were detected in the atherosclerotic and non-atherosclerotic regions. DHA-treated and EPA-treated groups had significantly higher NAD^+, NADH, $NADP^+$, NADPH, and nicotinic acid adenine dinucleotide (NAAD) than the nontreated control. Interestingly, NAM (an NAD^+ precursor) levels remained almost unchanged.

[Discussion]

DESI-MSI, a non-destructive technique, has a charged jet of solvent to inject micro-droplets onto a sample surface, where ions are collected and desorbed into the gas phase at atmospheric pressure, minimizing sample preparation. DESI is called soft ionization, whereas matrix-assisted laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS) utilize UV laser beams and ion guns (hard ionization). That's why three redox pairs were detectable in both reactants and products for all fifteen samples.

Long-chain polyunsaturated fatty acids are structural components of cellular membranes. Ingestion of DHA or EPA induced an alternation of lipids species, including cholesterols and triglycerides. The possibility of increasing NAD^+ levels triggered by n-3 PUFAs metabolism (desaturation, elongation, and β-oxidation) and associated enzymes, such as the delta-5/6 desaturases, play an impact on n-3 PUFAs conversion and glycolytic NAD^+ recycling.

In general, enzymes are adapted to work best under plentiful amounts of cofactors. There is now consensus that Sirtuins underlie aspects of calorie restriction. Calorie restriction without malnutrition is considered the gold standard in biogerontology, which is an effective way to delay aging and age-related diseases. Hence, understanding mechanistic changes of NAD^+ metabolism in response to n-3 PUFA natural supplement may allow the design of mixable precursors that keep NAD^+ available in cells or tissues.

Given special NAD^+ functions, our finding serves as an approach targeting CVDs treatment. Reducing the size of atherosclerotic lesions is required for further research of NAD^+-dependent proteins.

[Conclusion]

Oral administration of adding DHA or EPA to a high-fat diet led to higher NAD^+ levels in the aortic wall.