Identification and imaging of lipids in tissues using TOF-SIMS

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin
vid Göteborgs Universitet kommer offentligen att försvaras i hörsal Gösta Sandell,
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av

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Avhandlingen baseras på följande delarbeten:

I: Per Malmberg, Håkan Nygren, Katrin Richter, Yun Chen, Frida Dangardt, Peter Friberg and Ylva Magnusson. Imaging of Lipids in Human Adipose Tissue by Cluster Ion TOF-SIMS.
Microscopic research and technique Volume 70, Issue 9 828-835

II: Ylva Magnusson, Peter Friberg, Peter Sjövall, Frida Dangardt, Per Malmberg and Yun Chen. Lipid imaging of human skeletal muscle using TOF-SIMS with Bismuth cluster ion as a primary ion source.
Clinical Physiology and functional imaging (In press)

III: Ylva Magnusson, Peter Friberg, Per Malmberg, Håkan Nygren and Yun Chen. Application of multivariate analysis of TOF-SIMS spectra for styding the effect of high glucose intake on aortic lipid profile.
Submitted

IV: Ylva Magnusson, Peter Friberg, Peter Sjövall, Jakob Malm and Yun Chen. TOF-SIMS analysis of lipid accumulation in the skeletal muscle of ob/ob mice.
Submitted

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Abstract

Introduction: Normal lipid metabolism in the adipose tissue, skeletal muscle and aortic wall is important for the physiological function of these tissues. Dyslipidemia that is often associated with intake of high energy diet and sedentary lifestyles can lead to the development of insulin resistance and cardiovascular diseases. Existing methods for imaging the heterogeneous distribution of lipids in the skeletal muscle and adipose tissue is limited. Our aim is to, without probing and chemical fixation, identify and image the spatial distribution of lipids in the skeletal muscle, adipose tissue and aorta, and to reveal an altered lipid pattern in the skeletal muscle associated with obesity and in the aorta associated with high glucose intake. To achieve this, we used time-of-flight secondary-ion mass spectrometry (TOF-SIMS) equipped with a bismuth (Bi)-cluster gun which is a new technique for molecular imaging of biological samples. Principal component analysis (PCA) was used for studying changes between experimental and control groups.
Methods: Human adipose and skeletal muscle tissue were obtained from obese youths and aortas were taken from Wistar Rats with or without glucose drinking. The samples were prepared by high pressure freezing, freeze-fracturing. Gastrocnemius skeletal muscle was taken from obese ob/ob mice and lean wild-type mice. The tissue was cryofixed and cryosectionized. All samples were dehydrated by a freeze drying process in ultra high vaacum. The tissue was analyzed by TOF-SIMS. Semi-quantitative measurements in the rat aorta and in the mice skeletal muscle were based on principal component analysis.
Results: In the negative spectra, we identified fatty acids and triacylglycerol. In the positive spectra, we identified the phosphocholine, cholesterol and diacylglycerol. Heterogeneous distribution of these molecules was observed in the skeletal muscle and adipose tissue. By using PCA, we identified a reduced signal of cholesterol in rats with high glucose intake compared to control rats. The obese ob/ob mice showed an increased level of fatty acids and diacylglycerol. The ratio between fatty acid peaks showed changed fatty acid composition in the rat aorta associated with high glucose intake and in the mice skeletal muscle associated with obesity.
Conclusions: With the help of imaging TOF-SIMS, it is possible to depict the heterogeneous localization of fatty acids, phosphocholine, cholesterol, diacylglycerol and triacylglycerol in the adipose tissue, skeletal muscle and aortic wall. Moreover, imaging TOF-SIMS together with PCA analysis of TOF-SIMS spectra is a promising tool for studying lipid alterations in tissues.

Key words: mass spectrometry, lipids, fatty acids, diacylglycerols, triacylglycerols, TOF-SIMS, adipose tissue, aortic wall, skeletal muscle, obesity, principal component analysis

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