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Abstract Book

3rd ANKA/KNMF Joint Users Meeting

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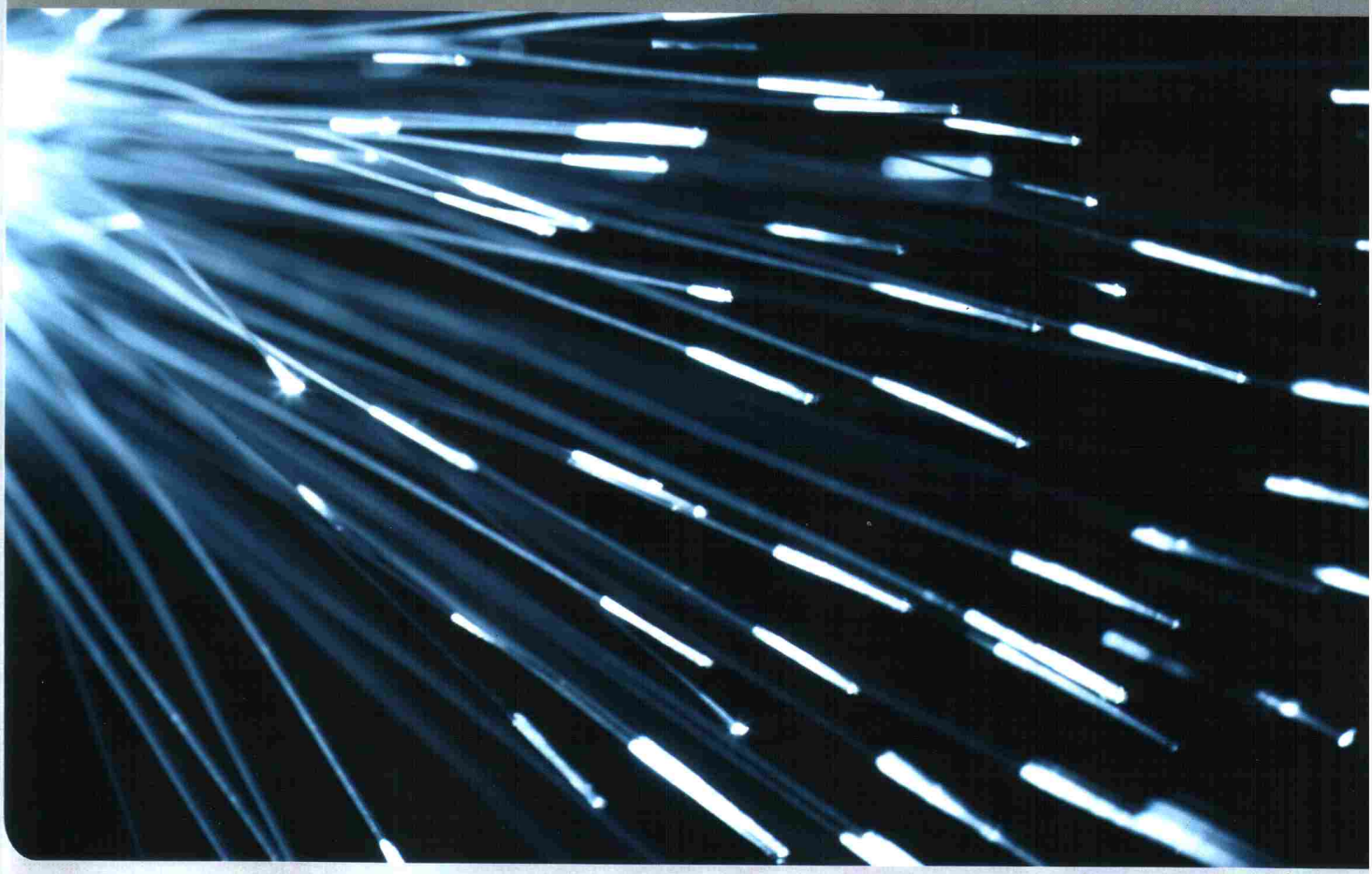

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3rd ANKA/KNMF Joint Users' Meeting

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KNMF
Karlsruhe MICRO NANO Facility

BIO-3: Effect of linoleic acid isomers from vegetable oils on elemental distribution of elements in atherosclerotic plaques of apoE/LDLR-double knockout mice

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Experiments utilizing genetically-engineered animals are of high value in studies on pathomechanism and treatment of atherosclerosis. Targeted deletion of genes for apolipoprotein E and LDL-receptor (apoE/LDLR-double knockout /-DKO) in mice leads to severe hiperlipidemia and spontaneous atherosclerosis. Synchrotron radiation microprobes were applied to characterize elemental distribution in distinct histologically defined areas of atherosclerotic lesion of apoE/LDLR-DKO mice and in atheromas after dietary and pharmacological treatments [1,2]. Isomers of conjugated linolenic acid (CLnA) present in different vegetable oils are believed to have also antiatherosclerotic abilities. The aim of the study was to combine the synchrotron radiation micro-XRF with histological stainings to assess changes in the distribution and concentration of selected elements in atherosclerotic lesions of apoE/LDLR-DKO mice fed diets supplemented with pomegranate fruit and linseed oils.

Fifteen female apoE/LDLR-DKO mice were used for this study. Up to the age of 2 months the animals were fed a commercial, cholesterol-free diet and then divided into three experimental groups fed for the following 4 months: a) AIN 93G diet (normal diet, control group, AI; n=5) b) AIN 93G diet supplemented with pomegranate fruit oil (GR; n=5) c) AIN 93G diet supplemented with linseed oil (LN; n=5). At the age of 6 months mice were sacrificed and the heart with ascending aorta were dissected out and snap-frozen. Serial 10 µm-thick cryosections of ascending aorta were cut and placed on 1,5 µm Mylar foil for µ-XRF analysis and on poly-L-lysine coated slides for histological stainings. Oil red-O staining (ORO) was used to visualize lipids and sirius red (SR) to demonstrate collagen fibers in atherosclerotic lesions.

Micro-XRF measurements were carried out at beamline FLUO of the ANKA storage ring. The photon energy was set up to 17 keV and polycapillary half-lens was used to get the final beam size on the sample of approx. 12 µm. The emitted X-ray quanta were recorded with a Vortex SMD detector. Two-dimensional maps acquired from atherosclerotic plaques (acquisition time 1,5 s per point) as well as precise point spectra (acquisition time 100 s per point) were obtained from histologically defined areas of atherosclerotic lesions.

In our study we found that the distribution of elements such as: P, S, Cl, Ca, K, Fe, Cu, Zn corresponds with histological structure of atherosclerotic lesion. Some spots of high calcium and phosphorus concentration were visible in areas related to high lipid deposition as shown in ORO stained sections. Elevated iron concentration appeared in various regions of atheroma as compared to other tissue structures. In contrast to iron, high zinc concentration occurred mainly at the periphery of the lesion. In atherosclerotic plaques of animals fed diet supplemented with pomegranate fruit oil, significantly higher concentrations of Cu and lower concentrations of P and Sr were observed in comparison to normal diet-fed animals. After treatment with linseed oil higher content of K, and lower of Ca, P, and Sr in atheromas were found. Observed differences in concentrations of some pro- and anti-atherogenic elements may confirm effectiveness of applied diets.

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