Differences in concentrations of selected elements in atherosclerotic plaques of apoE/LDLR-double knockout mice treated with low-carbohydrate high-protein and western diets

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Atherosclerosis is a most common inflammatory and degenerative vascular disease and it is strongly related to the growing epidemic of obesity in westernized populations. In obese individuals all types of diets inducing energy deficit were shown to be effective in weight loss. However, high-protein diets may produce higher weight loss in short term (3-6 months) than conventional diets. Gene-targeted apoliporotein E and LDL receptor-double knockout (apoE/LDLR-DKO) mice have been created, representing a new murine model that displays severe hyperlipidemia and atherosclerosis. We used apoE/LDLR-DKO mice to study effects of antiatherosclerotic drugs and diets [1,2] and we applied synchrotron radiation microprobes to characterize elemental composition of atheromas in this animal model [3,4]. The aim of the present study was to show changes in concentrations of selected elements in atherosclerotic plaques of apoE/LDLR-DKO mice fed low-carbohydrate high-protein (LCHP) diet and western diet (WD) comparing to normal diet. We have combined synchrotron radiation micro-XRF with histological stainings to determine distribution and concentrations of the elements in histologically defined areas of atherosclerotic lesions.

Eighteen female apoE/LDLR-DKO mice were used for the study. Up to the age of 4 months the mice were fed a commercial, cholesterol-free pelleted diet and then they were randomly assigned to one of three groups fed for the following 2 months: *i*. AIN-93G (normal) diet (n=6; AIN), *ii*. LCHP diet (carbohydrates - 17%, proteins - 52%, fat - 21%; n=6), *iii*. western diet (carbohydrates - 50%, proteins - 20%, fat - 21%; n=6). Six-month-old animals were sacrificed; hearts with ascending aorta were dissected out and snap-frozen. Serial 10 μm-thick crossections of ascending aorta were cut on cryostat and mounted either on poly-L-lysine coated slides (histology) or on 1.5 μm-thick Mylar foil (microprobes). Consecutive slides were stained with oil red O (ORO; red) for the demonstration of lipids and double immunostained: CD68 for macrophages (green) and smooth muscle actin (SMA) for smooth muscle cells (red).
All micro-XRF measurements were carried out at beamline L of the storage ring DORIS III. The primary photon energy was set to 17.5 keV by a multilayer double monochromator. A polycapillary half-lens was used for beam focusing, hence the final beam size on the sample was approximately 15 μm in diameter. Emitted elemental spectra were recorded with Vortex SDD detector. Two-dimensional maps were acquired from lesional areas of the aortic root with surrounding cardiac muscle (resolution 15 μm, time of acquisition 5 s from each point). From morphologically defined areas, precise point spectra were recorded (resolution 15 um, time of acquisition 300 s). Results were normalized to beam current, thickness of sample and time, and expressed in arbitrary units.

Histological stainings revealed more advanced atherosclerosis as expressed by total area occupied by lipids and number of macrophages in atheromas of animals fed western diet and even more severe lesions in mice subjected to LCHP diet (Fig. 1). In animals fed LCHP diet, significantly ($p<0.05$, U-test) higher concentrations of Cu and lower concentrations of S, Ca, Zn in atheromas

were seen in comparison to normal diet-fed animals (Fig. 2). In mice fed western diet, significantly higher concentrations of P, Cl, Fe, Cu and lower concentrations of Ca, K, Zn in atheromas were observed (Fig. 2).

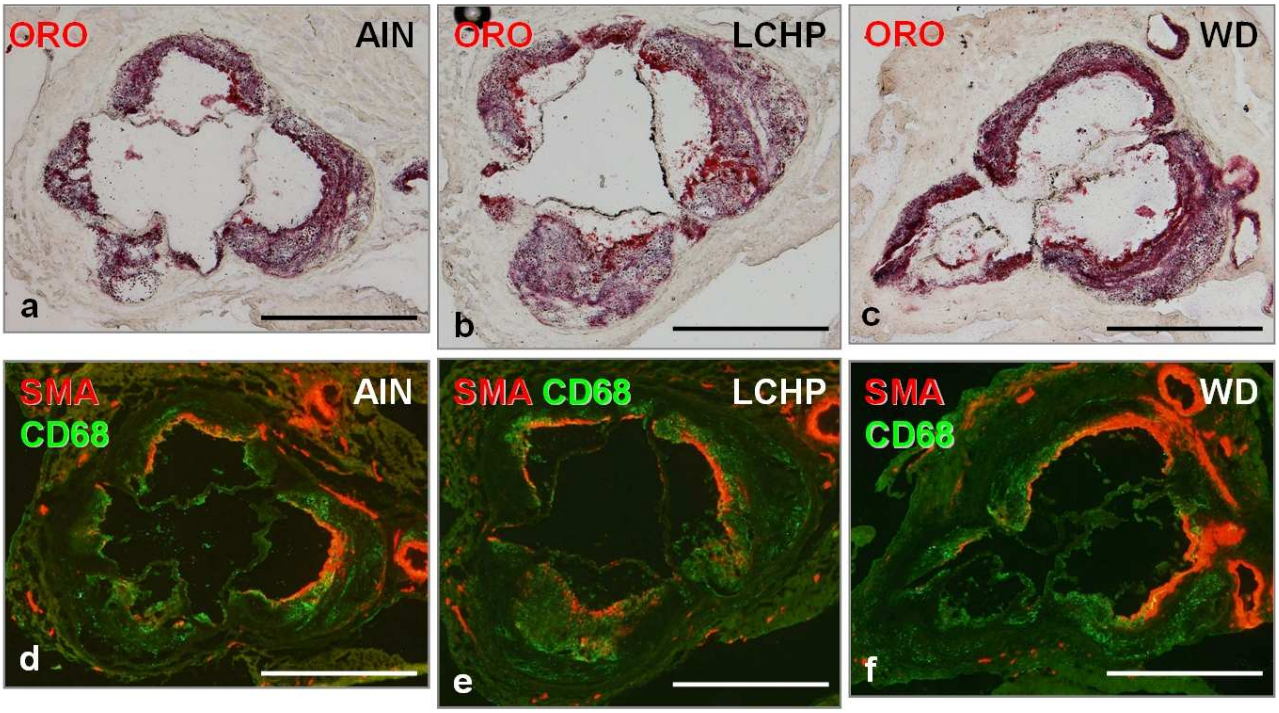

Figure 1: Histological stainings (ORO: a, b, c; CD68/SMA: d, e, f) of aortic roots of mice from control group (a, d), LCHP (b, e) and WD (c, f). Bar = 1 mm

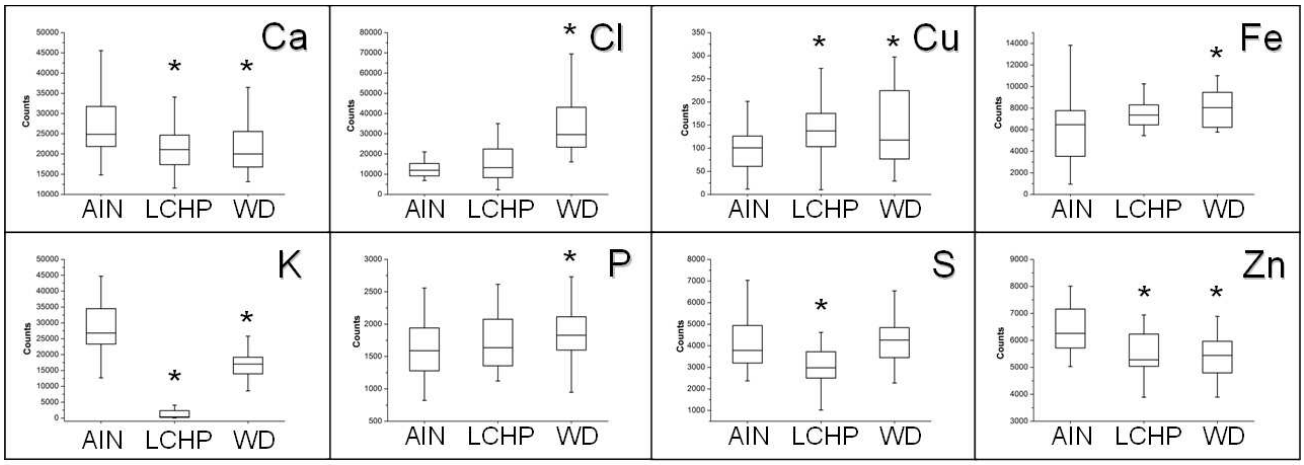

Figure 2: Relative concentrations of selected elements in atheromas of control group (AIN), animals fed low-carbohydrate high-protein diet (LCHP) and western diet (WD). Asterisks indicate significance ($p<0.05$)

References

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