Phytosterols and atherosclerosis: The effect of phytosterols on development of atherosclerosis in the heterozygous Watanabe Heritable Hyperlipidemic rabbit

Cardiovascular disease (CVD) is the major cause of premature deaths worldwide. Coronary heart disease is the most common CVD, caused by atherosclerosis in the coronary arteries. Atherosclerosis is a multifactorial disease influenced by both genetic and environmental factors. WHO has in 2007 listed in its “Guidelines for assessment and management of cardiovascular risk” the following risk factors to influence progressive atherosclerosis: hypertension, abnormal blood lipids, diabetes, unhealthy diet, physical inactivity and smoking. Phytosterols (plant sterols and plant stanols) are known for decades for their natural ability to reduce cholesterol levels in the blood. In the last decade numerous food products added phytosterol esters have been placed on the market, e.g. yellow fat spread, yoghurt, dressing. The products are being marketed as a natural means for people who want to lower their blood cholesterol levels. The aim of this Ph.D. project was to investigate the effects of phytosterols on the development of atherosclerosis in the aorta of heterozygous Watanabe Heritable Hyperlipidemic (WHHL) rabbits. The main advantage of animal studies to human studies in atherosclerosis research is the possibility to study, as a supplement to clinical endpoints, morphological and biochemical endpoints not easily accessible in humans. In this Ph.D. project atherosclerotic changes in aorta were evaluated quantitatively and qualitatively to measure both the extent and severity of the lesions. Morphological endpoints included macroscopic and histological examinations. Macroscopically, the extent of atherosclerosis was measured by calculating the area of aorta covered by plaque. Histologically, the severity of the lesions was determined qualitatively by evaluation of lesion type (fatty streak, fibrous plaque or advanced lesion) and quantitatively by stereological methods applied to evaluate the area of the intima and the ratio of intima:media on cross sections from three defined places on the aorta.

The biochemical endpoint was the cholesterol content in the inner layer of the entire aorta, which is considered a combined measure of extent and severity of aortic atherosclerosis. The first study investigated the effect of phytosterols derived from rapeseed oil (RSO), a source rich in phytosterols, containing up to 15 % of the species specific brassicasterol. At present the use of phytosterol blends containing higher than 3% brassicasterol are not accepted on the European market. The aim of the study was to investigate the effect of dietary supplementation of RSO derived sterol, with high content of brassicasterol, and stanol esters on the development of atherosclerosis in cholesterol-fed heterozygous WHHL rabbits. Furthermore, the distribution of phytosterols in aortic tissue was studied. After an 18-week feeding period it was concluded, that RSO sterol and stanol esters are well-tolerated and have hypocholesterolemic effects. Furthermore, the development of experimental atherosclerosis was significantly inhibited when measured both by morphological and biochemical endpoints in cholesterol-fed heterozygous WHHL rabbits. The second study investigated the effect of a commercial plant stanol ester blend on established atherosclerosis. The aim of this study was to investigate if dietary intervention with plant stanol esters can cause regression of already established experimental atherosclerosis in the cholesterol-fed heterozygous WHHL rabbit model. After a 12-week induction period with cholesterol feeding the presence of atherosclerotic lesions in the aorta was confirmed by autopsy in a group of the animals. After the following 12-week intervention period with stanol ester feeding there was a significant reduction in aortic atherosclerosis evaluated biochemically; the cholesterol content in the inner layer of the entire aorta was significantly less in the intervention group than in the group which did not receive plant stanols, yet still higher that after the induction period. The study failed however, to show significant effects on the morphological endpoints. It was concluded that intervention by plant stanol ester feeding did not cause regression but retarded progression of established aortic atherosclerosis in cholesterol-fed heterozygous WHHL rabbits. Phytosterols have been subjected to multiple human and animal studies that report significant and comparable hypocholesterolemic effects. A number of animal studies have shown that atherogenesis can be significantly reduced and in some cases even inhibited by sterol or stanol ester feeding due to their hypocholesterolemic effect. So far, only two studies in transgenic mouse models have addressed the effect of plant sterols and stanols on already existing atherosclerosis. The effects of plant sterols and stanols on regression of atherosclerosis are more ambiguous. Thus, more attention should be given to the effect of plant sterols and stanols on regression of atherosclerosis. Further animal studies might be helpful to clarify the effects of phytosterols on established atherosclerotic lesions. Both studies showed a significant increase in circulating levels of plant sterols and stanols and significant incorporation of both plant sterols and stanols into vascular tissues in response to treatment. Further studies are needed to elucidate the long-term biological and physiological effects on the vascular tissues and stability of the plaques.