5 Summary and Conclusions

It is well established that dietary proteins influence lipid metabolism. But the number of proteins examined in this connection, is so far very limited. Most of the studies are based on casein as a representative of animal proteins and soy protein as representative of plant proteins. Soy proteins are considered to be hypocholesterolemic in comparison to animal proteins mainly casein.

We performed three experiments using different animal and plant proteins. Growing male Sprague Dawley rats were used as model animal in all the experiments. In each experiment rats were fed for about 3 weeks. The diets varied only in protein source in first two experiments, but in third experiment all the plant protein containing diets (pea, lupin and soy protein) were supplemented with DL-methionine, and lupin protein containing diet was also supplemented with lysine to meet the AIN recommended amount. To mimic western diets, which are commonly rich in saturated fats and cholesterol, we chose lard as type of dietary fat and supplemented the diet with cholesterol (0.5 g/kg diet). As parameters we determined cholesterol and triglyceride concentrations in the liver, plasma and lipoproteins. To investigate the effects of dietary amino acid composition, we analyzed amino acid compositions of dietary proteins and plasma. To find out the mechanism at the genetic level we performed semiquantiative PCR for the selected genes using RNA isolated from liver.

The main aim of our first study was to investigate the effects of soy protein and its amino acids respectively, on lipid metabolism and the mechanism of action at the genetic level. To minimize the interference of other components, we decided to use ethanol washed soy protein. Soy protein isolate compared to casein led to a marked decrease of liver and VLDL cholesterol, liver and plasma triglyceride concentrations and increased bile acid excretion via faeces. Soy protein isolate lowered relative mRNA concentrations of SREBP-2, along with HMG-CoA reductase and LDL receptor, which are SREBP-2 down stream genes and play important role in the cholesterol metabolism. Soy protein also reduced the expression of SREBP-1c along with its target genes FAS and G6PDH compared to casein. The activities of FAS and G6PDH were also lowered by soy protein. This suggests that soy protein lowers fatty acid synthesis in the liver. Gene expression of insig 1 and 2, and were not different between the two groups.

Soy protein reduced MTP activity and mRNA concentration, and lowered Apo-B 100 protein and mRNA concentration thus resulting reduced assembly and secretion of VLDL particles and therefore reduced lipid concentration in plasma. But soy protein had no effect on VLDL particle size as indicated by core surface ratio.

In conclusion, this study suggests that soy protein affects cellular lipid homeostasis by down regulation of SREBP-2 and SREBP-1c and associated sterol-regulatory element regulated genes.

Besides casein, animal proteins such as those from beef, pork, poultry, or fish protein play an important role in human nutrition worldwide. Therefore, we planned a second experiment to investigate the effects of meat proteins isolated from pork, beef, and turkey, and fish protein isolated from Alaska pollack fillets and compared their effects on the lipid metabolism with casein and soy protein isolate. This study shows that proteins from beef, pork and turkey meat have similar effects as casein on the cholesterol metabolism of rats. In this study, most remarkable differences by the animal proteins were observed in their effects on triglycerides. An interesting result of this study was the triglyceride-lowering effect of pork protein compared to casein. Although we did not measure the mature fraction of SREBP-1c in nucleus and mRNA expression, the reduced activities of FAS and G6PDH in the liver suggest diminished lipogenesis via SREBP-1c. Proteins isolated from beef and turkey meats also slightly reduced concentrations of triacylglycerols in liver and plasma compared to casein, although the differences were not significant.

Proteins isolated from fish fillet had effects on cholesterol metabolism. Rats fed fish protein had higher concentrations of cholesteryl esters in liver, higher bile acid excretion via faeces, a lower concentration of cholesterol in the high-density lipoprotein fraction. Moreover fish protein feeding lowered triglyceride concentration in plasma compared to casein. As there is less knowledge available regarding the effects of fish protein on lipid metabolism, and to confirm these observations we performed a third experiment. Besides fish protein, soy protein and casein as a control, we used pea and lupin protein as additional plant proteins. In this experiment we performed additional gene expression analysis with cDNA arrays. Main effects of the fish protein on cholesterol metabolism compared to casein were an increase of liver cholesterol concentration, a reduction of the high-density lipoproteins, along with a stimulated gene expression of SR-B1, SREBP-2 and a slight increase of HMG-CoA synthase and LDL receptor. Both are target genes of SREBP-2 and indicate an activation of this transcription

factor by fish protein. However, despite the observed cholesterol accumulation in livers of rats fed fish protein the concentration of cholesterol in plasma was not increased compared to rats fed casein. The higher gene expression of LDL receptor in livers of rats fed fish protein could have possibly contributed to an increased removal of LDL from the circulation, thereby preventing a distinct LDL accumulation in plasma.

HDL is the principle vehicle for removal of surplus cholesterol from the peripheral tissues for disposal in the liver. Several genes are involved in HDL metabolism Apo-AI, a structural component of HDL, and SR-BI responsible for the selective uptake and unloading of HDL cholesterol in the liver whereas the esterification of cholesterol by LCAT is critical for optimal cholesterol uptake and maturation of HDL. Gene expressions of Apo-AI and LCAT were not different between rats fed casein and fish protein. Although the reduced concentration of HDL cholesterol observed in the fish protein fed rats could possibly be related to an increased expression of SR-BI.

In conclusion, the present findings suggest that the fish protein from Alaska Pollack exerts distinct effects on plasma and liver lipids which were at least in part caused by an altered expression of hepatic genes involved in lipid homeostasis.

Pea and lupin protein lowered cholesterol concentration in the liver and VLDL, and triglyceride in the liver, plasma, VLDL and LDL. But these proteins like soy protein could not affect plasma cholesterol concentrations. We suggest that like soy protein pea and lupin protein also lower cholesterol and triglyceride synthesis in the liver although we did not determine mRNA concentrations or enzyme activities in the liver of rats fed pea or lupin protein. Plasma taurine concentration was significantly higher in the plant protein fed rats compared to casein. Previous findings have shown that taurine supplementation reduced the concentration of triacylglycerols in liver and plasma of rats, but the mechanism is still to be investgated.

Amino acid compositions of the dietary proteins might be in part responsible for lipid lowering effects observed by these proteins. The marked differences in the amino acid composition of the dietary proteins were in cysteine, glycine, arginine, lysine aspartic acid, proline and valine.

Although the results cannot be directly interpolated to human nutrition, some effects of these proteins on the lipid metabolism could also occur in humans.