Effects of antibiotics, short-chain fatty acids and amino acids on Apoliprotein A-I transcription and synthesis in normal and inflamed HepG2 and Caco-2 cells

Summary

Cardiovascular diseases (CVD) are the leading cause of death in the western countries. Dyslipidemia, one of the modifiable CVD risk factors, is defined as having high plasma low-density lipoprotein (LDL) cholesterol and triacylglycerol concentrations or low levels of serum high-density lipoprotein (HDL) cholesterol. Indeed, several epidemiological studies have shown an inverse relation between HDL cholesterol levels and CVD risk. Surprisingly, recent clinical studies however showed that increasing serum HDL cholesterol concentrations by pharmacological interventions failed to reduce CVD risk. Thus, the focus has switched to improving HDL functionality instead of elevating serum HDL cholesterol concentrations. Increasing evidence suggests that HDL functionality plays a major role in inflammation and atherogenesis. For example, HDL particles promote reverse cholesterol transport (RCT), a mechanism by which accumulated cholesterol is transported from the arterial walls to the liver for excretion, which consequently reduces atherosclerosis development. Apolipoprotein A-I (ApoA-I), the major protein component of HDL particles, has been identified as an important player in explaining HDL functionality and RCT. Therefore, approaches that target to elevate ApoA-I is a promising strategy to reduce CVD risk. In this thesis we therefore focused on the effects of antibiotics, short-chain fatty acids (SCFAs) and amino acids on ApoA-I transcription. In addition, we evaluated the effects of SCFAs on ApoA-I expression in a co-culture model by culturing enterocytes and hepatocytes together in a transwell system.

In an earlier clinal trial, the plasma HDL cholesterol concentration was significantly reduced after antibiotic (amoxicillin) treatment. This finding raised the question whether this HDL cholesterol lowering effect by amoxicillin was a direct effect on lipoprotein metabolism or an indirect effect resulting from alterations in the composition of microbiota and consequent SCFAs concentrations. To investigate the potential direct effect, we examined the effects on ApoA-I transcription when different concentrations of various antibiotics were added to the HepG2 and Caco-2 cells (**chapter 3**). In line with the clinal trial, we found that amoxicillin significantly reduced both ApoA-I mRNA expression and protein secretion in HepG2 and Caco-2 cells. We also examined potential mechanisms underlying these direct effects of antibiotic on ApoA-I expression. We found that amoxicillin treatment inhibited PPARα transactivation, which was confirmed by a lowered mRNA expression of CPT1, a well-known PPARα target gene. Therefore, we concluded that antibiotic treatment can directly affects lipoprotein metabolism via inhibiting ApoA-I transcription and secretion. In **chapter 2**, we examined the potential indirect effect of the antibiotics on HDL metabolism by evaluating dose-response relationships between the different SCFAs with ApoA-I transcription. Interestingly, increasing SCFAs concentrations significantly increased ApoA-I

mRNA expression in HepG2 cells. This increase was associated with the enhanced PPARα transactivation, which indicates that the PPARα pathway is most likely involved in the positive effects of SCFAs on ApoA-I mRNA expression. These findings further suggest that the effects on serum HDL cholesterol concentrations as observed in our human intervention study with amoxicillin could be due to a combination of direct inhibitory effects on ApoA-I transcription as well as indirect effects via reduced SCFAs production. Moreover, previous studies have suggested that ApoA-I concentrations are reduced during inflammation, since ApoA-I is a negative acute phase protein. To evaluate effects of SCFAs on ApoA-I expression in the inflammatory condition, we performed our SCFAs experiments also in inflamed HepG2 cells (**chapter 4**). In line with the normal condition, SCFAs also exerted their beneficial effects on ApoA-I during inflammation. Moreover, SCFAs were able to recover the reduced level of ApoA-I expression in the inflamed HepG2 cells up the normal level, and even increased ApoA-I expression at higher concentrations. In addition, SCFAs treatment inhibited NF-κB transactivation and lowered IL-8 secretion from the inflamed HepG2 cells. Our data suggest that PPARα transactivation also underlies these anti-inflammatory effects of SCFAs in the inflamed HepG2 cells. Although SCFAs are produced mainly by microbiota in the colon, they are also produced in lower amounts in the small intestine. It is known that the intraluminally produced SCFAs are taken up by the intestinal cells and used as energy source. However, a part of the SCFAs is transported to the liver via the portal vein. In addition, their uptake by enterocytes might trigger the secretion of factors that could be transported from the enterocytes to the liver and affect hepatic processes. To study such a possible cross-talk between intestine and liver on hepatic ApoA-I expression, we evaluated the effect of C4 on hepatic ApoA-I mRNA expression in a co-culture system including Caco-2 and HepG2 cells (**chapter 5**). The positive effects of SCFAs on hepatic ApoA-I expression were also observed in these co-culture experiments after adding C4 apically to the intestinal cells. However, the effect of C4 on hepatic ApoA-I expression in this study was smaller than the effect of C4 when it was added directly to the liver cells. This latter finding indeed suggests a lower bioavailability of C4. Also, it might be the result from a cross-talk between the intestine and liver cells. In that situation the enterocytes should secrete an inhibitory factor to dampen hepatic ApoA-I expression. Finally, since some studies have suggested that amino acids influence PPARα transactivation, we explored in **chapter 6** the effects of different amino acids on ApoA-I expression in HepG2 cells. Interestingly, leucine, glutamic acid and tryptophan increased mRNA expressions of both ApoA-I and PPARα. Therefore, amino acids could be interesting targets for further studies to increase ApoA-I production.

In summary, the research in this thesis was performed to get more insights into the effects of external factors like antibiotics, SCFAs and amino acids on hepatic and intestinal ApoA-I

transcription. Besides the negative effect of antibiotic on ApoA-I transcription and secretion, our findings have shown that SCFAs have positive effects on ApoA-I expression in HepG2 cells in both normal and inflammatory conditions. Moreover, C4 treatment also increased hepatic ApoA-I mRNA expression in the intestine-liver co-culture model. Finally, different amino acids were identified as natural compounds that have the potency to transactivate PPARα and consequently increased hepatic ApoA-I expression.