Novel insights in cholesterol excretion
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SUMMARY

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SUMMARY

This thesis primarily deals with the re-discovery of a “forgotten” cholesterol excretion pathway: transintestinal cholesterol efflux (TICE). A start was made to unravel the mechanisms underlying this process. Furthermore, different ways to stimulate cholesterol efflux are described.

An introduction to the subject is given in chapter 1. In this chapter the importance of cholesterol for vertebrates is explained. This chapter further describes the role of the intestine in cholesterol absorption. Subsequently, the role of the intestine in cholesterol excretion is discussed and chapter 1 ends with the aim and outline of this thesis.

Fatty acid-bile acid conjugates (FABACs) are synthetic molecules designed to treat a range of lipid disorders. The compounds prevent cholesterol gallstone formation, diet induced fatty liver and increase reverse cholesterol transport in rodents. The aim of the study described in chapter 2 was to investigate the effect of FABACs on cholesterol efflux in human cells. Aramchol (the most potent FABAC) dose dependently increased cholesterol efflux from human skin fibroblasts in the absence of known efflux mediators such as apolipoprotein A-I (apoA-I) but had little effect on phospholipid efflux. A liver X receptor (LXR) agonist strongly increased aramchol induced cholesterol efflux but in ATP-binding cassette transporter A1 (ABCA1) deficient cells from Tangiers patients the aramchol effect was absent, indicating that activity of ABCA1 was required. Aramchol did not affect ABCA1 expression but plasma membrane levels of the transporter increased twofold. Aramchol is the first small molecule, which induces ABCA1-dependent cholesterol efflux without affecting transcriptional control. These findings may explain the beneficial effect of the compound on atherosclerosis.

Until recently, hepatobiliary cholesterol secretion was generally considered to be an obligate step in the pathway of excess cholesterol excretion from the body. In chapter 3 we have investigated the validity of this paradigm in mice. Direct secretion of cholesterol from the luminal side of enterocytes was studied by perfusion of isolated segments of the small intestine in mice. Cholesterol input and output measurements in different mouse models revealed that fecal neutral sterol excretion was higher than the sum of dietary cholesterol intake and biliary cholesterol secretion indicating the existence of an alternative pathway. We
showed that substantial amounts of cholesterol can be secreted directly by enterocytes. TICE is a specific process observed throughout the small intestine (proximal>medial>distal). TICE depended on the presence of a cholesterol acceptor and was strongly stimulated by bile salts and phospholipids. The capacity of TICE was sufficient to account for the missing cholesterol in the balance studies. The contribution of TICE to total cholesterol excretion in mice is approximately twice that of the biliary pathway. So, in mice, the intestine plays a significant role in removal of cholesterol from the body.

In chapter 4 we investigated whether the activity of TICE could be influenced by dietary factors. In addition, we studied the role of cholesterol acceptors at the luminal side of the enterocyte. Mice were fed Western-type diet {0.25 % (w/w) cholesterol; 16 % (w/w) fat}, a high fat diet {no cholesterol; 24 % (w/w) fat}, or high cholesterol diet {2 % (w/w)} and TICE was measured by isolated intestinal perfusion. Bile salt/phospholipid mixtures served as cholesterol acceptor. Western-type and high fat diet increased TICE by 50 % and 100 %, respectively. In contrast, the high cholesterol diet did not influence TICE. Intestinal scavenger receptor class B type 1 (Sr-B1) mRNA and protein levels correlated with the rate of TICE. Unexpectedly, TICE was significantly increased in Sr-B1 deficient mice. Apart from the long term effect of diets on TICE, acute effects by luminal cholesterol acceptors were also investigated. The phospholipid content of perfusate was the most important regulator of TICE, whereas bile salt concentration or hydrophobicity of bile salts had little effect. TICE can be manipulated by dietary intervention. Specific dietary modifications might provide means to stimulate TICE and, thereby enhance total cholesterol turnover.

Peroxisome proliferator-activated receptors (PPARs) are involved in the regulation of energy homeostasis and lipid metabolism. PPARδ activation leads to an increase in fecal neutral sterol secretion. This phenomenon cannot be explained by an increase in hepatobiliary cholesterol secretion, nor, sufficiently, by reduction of cholesterol absorption. Therefore we hypothesized in chapter 5 that PPARδ activation would lead to stimulation of TICE. To establish whether activation of PPARδ leads to an increased rate of TICE, intestine perfusions were performed in GW610742 (a PPARδ agonist) treated FVB mice. To counteract a possible effect of PPARδ activation on cholesterol absorption, ezetimibe (a cholesterol absorption inhibitor) and ezetimibe/GW610742 treated mice were also evaluated. PPARδ agonist treatment stimulated both fecal neutral sterol excretion, and TICE, in the
GW610742 treated mice. This effect was markedly reduced by ezetimibe treatment. Intestinal Rab9 expression was significantly increased upon GW610742 treatment. In conclusion, activation of PPARδ, by GW610742 treatment stimulates TICE. This finding provides an interesting target for development of drugs aiming at the prevention of atherosclerosis.

In chapter 6 the origin of intestinally secreted cholesterol is discussed, followed by the process underlying TICE. Subsequently, the presence of TICE in humans is evaluated and in the final part of this chapter the regulation of TICE in man versus mice is discussed.