Targeting the vessel wall in cardiovascular prevention
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GENERAL SUMMARY

In this thesis, I discuss three different subjects in and around the vessel wall. After a short summary, I will revisit each chapter adding a few remarks.

Part 1 focuses on the endothelial glycocalyx, the layer surfacing the vessel wall, and its possible relation with atherosclerosis. Together with Max Nieuwdorp, Hans Mooij, Lysette Broekhuizen, Erik Stroes en Hans Vink, I showed a close, inverse relation between risk factor levels (diabetes, inflammation and hypercholesterolemia) and glycocalyx volume. To a lesser extent, we were able to reverse damage by partial restoration of the glycocalyx layer. In search of the causal role of glycocalyx perturbation in atherogenesis, we stumbled on the unexpectedly large impact of glycocalyx perturbation on renal physiology.

The second part deals with myeloperoxidase (MPO). Radicals formed by this enzyme damage the vessel wall and lipoprotein particles. We investigated the association between MPO and (signs of) cardiovascular disease (CVD). However, myeloperoxidase levels in peripheral blood only marginally help us to predict who will suffer from CVD in many patient populations outside the emergency room setting.

The last chapters of part 3 dive even deeper into the vessel wall, into the foam cells. Inhibiting of acyl-coenzyme A: cholesterol acyltransferase (ACAT) in these cells does not retard, but even worsens lesion progression.

Part 1

Chapter 1 reviews the limited number of publications addressing the endothelial glycocalyx in humans and its potential role in the development in atherosclerosis. It summarizes data from experimental models leading to our hypothesis that the endothelial glycocalyx protects the vessel wall against atherosclerosis and that restoration of glycocalyx damage may prevent CVD.

In chapter 2, we present two novel tools to estimate glycocalyx dimension in humans. Our aim is to develop a clinically useful tool that will allow us to determine glycocalyx dimensions on a large scale in patients. Orthogonal polarization spectral imaging (OPS) seems to have better papers, than the systemic glycocalyx measurement. We showed in 24 volunteers that both techniques are reproducible in humans and moreover, correlate with cardiovascular risk factors. Currently, Hans Vink is developing faster, semi-automatic methods to analyze the images to curb some of the difficulties and limitations of the described analysis.
After Max Nieuwdorp had used these techniques to show that glycocalyx dimension is reduced during acute hyperglycemia and in type 1 diabetic patients, we turned to type 2 diabetes mellitus (1, 2). Disturbance in the endothelial glycocalyx could contribute to the predisposition of diabetics to vascular complications.

First, we tested the effects of sulodexide, a mixture of the glycocalyx components heparin and dermatan sulphate, in vitro on hyperglycemia induced glycocalyx dysfunction (chapter 3). We show that hyperglycemia increases the permeability of cultured endothelial cells for albumin and that sulodexide reverses this. Increased staining of glycosaminoglycans on the endothelial surface upon sulodexide supports that it restores the glycocalyx. However, it must be noted that endothelial cells were not cultured under flow, which is likely to affect glycocalyx properties (3). Mirella Gouverneur and Hans Mooij are setting up culture methods under flow.

In the following chapter 4, we measured glycocalyx volume, albumin permeability and the effect of sulodexide in 20 type 2 diabetic patients. As expected, more albumin permeates the vessel wall in diabetics and glycocalyx volume is smaller than in healthy controls. The effect of sulodexide is, unfortunately, less pronounced than in the in vitro study. This leaves room for more targeted interventions, aimed at e.g increasing endogenous substrate production by the endothelial cells rather than supplying a random choice of glycocalyx components.

As mentioned, the glycocalyx prevents the adhesion of leukocytes and thrombocytes under normal conditions. Its size exceeds that of adhesion molecules, possibly hampering exposure. In case of inflammation, but also atherogenesis, these cells attach to the vessel wall. In chapter 5, we evaluated the effect of a standardized inflammatory stimulus, i.e. a low dose of endotoxin of 1 ng/kg bodyweight on the glycocalyx, microcirculatory perfusion as well as coagulation and inflammatory parameters in 16 healthy volunteers. Endotoxin led to loss of endothelial glycocalyx and shedding of the glycocalyx constituent hyaluronan into the plasma compartment. These changes were accompanied by reduction in perfused capillary density, increased monocyte activation and thrombin generation. Blockage of TNFα using etanercept significantly attenuated these disturbances. These findings are not only relevant for atherosclerosis, but also bear importance for sepsis. Drugs targeted at the glycocalyx may improve microcirculatory perfusion and prevent profound leakage through the vessel wall.

Subsequently, we turned to another important cardiovascular risk factor, hypercholesterolemia, in chapter 6. We observed that patients with heterozygous familial hypercholesterolemia (FH), characterized by high LDL-cholesterol levels, have a significantly smaller glycocalyx volume than control subjects. After treatment with rosuvastatin for 8 weeks
LDL-cholesterol levels completely normalized, whereas glycocalyx volume only partially recovered.

Summarizing, type 2 diabetes mellitus, inflammation and hypercholesterolemia are all accompanied by disturbances of the glycocalyx. These disturbances can be partly restored. This could imply that the endothelial glycocalyx is an important barrier and that disturbance of this layer by cardiovascular risk factors mediates progression of atherosclerosis. On the other hand, the glycocalyx could also be an innocent bystander. In vitro and intravital microscopy studies, discussed in chapter 1, do suggest a causal link between glycocalyx damage and several pro-atherogenic changes. In chapter 7, we tried to directly causally link glycocalyx damage to complete process of atherogenesis. However, the method to chronically degrade the glycocalyx, continuous infusion of hyaluronidase, was complicated by an immune response against the enzyme. Although, conclusion on atherosclerosis development cannot be drawn for this study, due to these methodological flaws, it does call for further research on the role of the endothelial glycocalyx in the kidney and vascular permeability and a new study design to answer our question. As always, research raises more questions (as well as tons of work), than it answers.

Part 2

In part 2 we investigate whether MPO levels can predict the presence of CVD. In chapter 8, we show that serum MPO levels are associated with the risk of future development of coronary artery disease (CAD) in apparently healthy individuals with an odds ratio of 1.49 in the top versus the bottom quartile. For this purpose, we used samples of a large (n = 3,375) case-control study nested in the prospective EPIC-Norfolk population study. This study shows that high MPO levels can precede the onset of overt CAD by many years. However, addition of this measurement to the Framingham risk score did not substantially improve it.

Next, we decided to look for indications that MPO is associated with the risk of CVD in patients already at risk, i.e. subjects with heterozygous familial hypercholesterolemia (FH) (chapter 9). Intima-media thickness (IMT) was used as a surrogate marker of CVD. In 122 FH patients there was no relation between MPO levels and IMT or IMT progression. Moreover, MPO levels unexpectedly increased during statin therapy.

In cooperation with the Department of Cardiology, we determined MPO levels in 267 type 2 diabetes patients with anginal complaints in chapter 10 and 11. MPO levels were marginally higher in diabetic patients than in normoglycemic patients. However, MPO levels did not predict perfusion defects on myocardial perfusion scintigraphy, indicative of myocardial ischemia. This study, as the study in chapter 9, is relatively small and lack the power of the EPIC-Norfolk cohort. Moreover, they relate MPO to markers of CVD, i.e. intima-media thickness
and myocardial perfusion scintigraphy, and not the disease itself. Therefore, subtle relations are not likely to be detected.

Finally, chapter 12 shows in 126 patients undergoing myocardial perfusion scintigraphy that exercise increases plasma MPO levels. Again, there was no difference in MPO levels between subjects with and without myocardial ischemia.

In conclusion, our research suggests that MPO, although useful in selected populations, has limited value in risk assessment in primary prevention and in a non-acute setting. However, the use of MPO as a biomarker in patients with acute chest pain seems promising (4). Moreover, MPO inhibitors are in development. They will further clarify the role of MPO in the pathogenesis of CVD and may prove to be a new treatment modality.

Part 3
New treatment modalities to prevent cardiovascular disease are tested extensively in cells, animals and humans. Still, failure of new approaches to prevent cardiovascular disease can occur until very late in their development. This is illustrated by the history of ACAT inhibitors. Chapter 13 describes the rise and fall of ACAT inhibitors as a new drug to prevent CVD. It summarizes the function of ACAT, initial animal research and the data from clinical trials. The consistent negative findings in recent clinical trials have virtually eliminated the chances for this class of drugs to be introduced for cardiovascular prevention.

We reviewed the method and value of IMT imaging as surrogate marker for CVD in chapter 14. It occupies a unique position in atherosclerosis research as it enables sensitive, reproducible and noninvasive assessment of carotid artery wall. Epidemiological and clinical trial evidence support its use as a marker for generalized atherosclerosis burden and vascular disease risk.

Finally, the CAPTIVATE study in chapter 15 was the last clinical trial testing the ACAT-inhibitor pactimibe. Unfortunately, pactimibe was associated with enhanced progression of carotid atherosclerosis, as determined by IMT, as well as increased incidence of major cardiovascular events in 881 FH patients. In stead of inhibiting atherosclerosis progression by reducing foam cell formation in the vessel wall, pactimibe may even promote cardiovascular events.

This leads us to conclude that ACAT inhibition is not a viable approach to prevent CVD. Besides that, these results emphasize the potential value of performing small and relatively short imaging trials to get a quick peak at safety and efficacy before exposing large numbers of patients to new drugs in large and prolonged morbidity and mortality trials.
FUTURE PERSPECTIVES

The studies described in this thesis raise lots of additional questions. With respect to part 1 major challenges are to find a new approach to determine whether glycocalyx perturbation causes atherosclerosis progression and to improve our glycocalyx toolkit. On the one hand, basal research using animal models is essential to understand the dynamics of the endothelial glycocalyx and its role in (patho)physiology. On the other hand, easy-to-use and minimally invasive tools to estimate glycocalyx function and dimension in humans will allow large-scale population/patient studies. These will serve to firmly establish the results of our initial observational studies in small groups and will enable us to expand our knowledge of the role the glycocalyx in atherosclerosis, but also in other diseases, such as sepsis, kidney disease and cancer growth and metastasis. The final goal is to establish for the clinic whether the endothelial glycocalyx can serve as biomarker and potential target for therapy.

It will be essential to determine for which individuals under what conditions the measurement of myeloperoxidase described in part 2 has additional value in clinical practice. So far, this seems to be limited to patients with acute chest pain in the emergency room. The next challenge is to actually implement it. Besides that, MPO inhibitors are under development. Safety, efficacy and correct selection of patients that may benefit from this drug remain to be determined.

The ACAT-inhibitors discussed in part 3 do not seem to have a future as anti-atherosclerotic therapy. Results of trials investigating the anti-atherosclerotic potential of novel drugs raising HDL-cholesterol or aimed at improving reverse cholesterol transport are eagerly awaited. Keep an eye on PubMed and the publications of our department!

REFERENCES