Summary, discussion, and future perspectives
**General summary**
Along with ageing of the population, the prevalence of many age related diseases is rising. The troublesome complications that accompany these diseases have a high impact on patients’ daily live. To reduce this impact, it is not only important to treat the diseases well, but also to detect the diseases in an early stage. Especially, diseases that affect the eye, since the cells of the eye have little or no regenerative capacity. Clinical parameters can help to identify patients at higher risk of diseases and complications, but they are not sensitive and specific enough. This emphasizes the need for new biomarkers that could be used (in addition to clinical parameters) to identify patients at high risk of specific diseases and complications.

This thesis addresses the role of an emerging group of biomarkers, called advanced glycation endproducts (AGEs), in diabetes mellitus (DM) and in rhegmatogenous retinal detachment (RRD). Both diseases are important causes for visual disability. In diabetes mellitus, an accelerated ageing process occurs that affects multiple organ systems. Among long-term microvascular complications, diabetic retinopathy is the most prevalent one. Its characteristic retinovascular damage affects the majority of diabetes patients and can eventually lead to blindness in advanced stages of the disease. In RRD patients, the separation of the neurosensory retina from the underlying retinal pigment epithelium is a direct threat for visual perception. Although the prognosis of RRD has greatly improved due to advances in vitreo-retinal surgery, the development of proliferative vitreoretinopathy (PVR) remains an important cause for impaired visual outcome after surgical treatment.

Dicarbonyl stress and (subsequent) AGE accumulation occur during ageing in general, but at an advanced rate during oxidative and glycaemic stress. Elevated levels of AGEs have been shown in tissues of diabetes patients and in several different cell structures of the eye in distinct ocular disorders. AGEs can be assessed non-invasively in the skin due to fluorescent properties of several AGEs. This technique referred to as skin autofluorescence (SAF) has been used to assess AGE accumulation in healthy individuals and in several age-related disorders. In this thesis, we aimed to evaluate the role of (locally formed) AGEs and the potential of SAF as a biomarker for occurrence of disease and development of complications in DM and RRD.

**Overview of main findings**
In Chapter 2 the technique of skin autofluorescence, its validation as a marker of tissue AGE accumulation, and its use as a clinical tool for the prediction of long-term complications in type 1 and type 2 diabetes mellitus (T1DM and T2DM) is reviewed. It has been extensively shown that skin autofluorescence is associated with a wide variety of long-term complications in both T1DM and T2DM. Some evidence is available for the predictive value of SAF in the development of complications, but further evidence is needed.

Chapter 3 shows that skin autofluorescence improved the performance of the FINDRISC model as accepted tool for diabetes detection in a very large and recently recruited population cohort. In a subset of almost 80,000 participants of the LifeLines cohort study, discrimination, calibration
and reclassification criteria showed the significance of the addition of SAF as a new biomarker to conventional diabetes risk factors. Furthermore, a simplified model (only including age, BMI, and SAF) with re-estimated score points was shown to have a similar performance in diabetes detection as the FINDRISC model in combination with SAF. However, some overall model estimates identified the FINDRISC with SAF as the best performing model.

RRD patients with a large surface area of detachment and PVR grade B or C are identified as patients with a severe retinal detachment. Although these patients have elevated risk of retinal redetachment after primary surgery, these characteristics are not specific in identifying patients at high risk who may benefit from specific treatment. In a pilot study (n=33 RRD patients), the potential value of AGEs in RRD was addressed by assessing the concentration of AGEs in various tissues (plasma, vitreous, and skin). Chapter 4 shows the results and the interpretation of this pilot study. Firstly, it was concluded that SAF did not contribute in the identification of patients at increased risk for developing RRD, since SAF values from RRD patients did not differ from SAF values that were previously assessed in a healthy population. Secondly, it was shown that the concentration of AGEs in plasma, vitreous, and skin were not related. This might be partly due to the assessment of SAF and plasma AGEs 3 to 4 years after vitreous sampling. Furthermore, major differences in AGE turnover rate between plasma and long-lived tissues, like skin and vitreous, might explain this. Thirdly, SAF and vitreous pentosidine were both elevated in patients with a severe retinal detachment as compared to patients with a less severe retinal detachment. Since severe retinal detachments are associated with the development of PVR and ensuing retinal redetachment, some support was shown for the potential of SAF as a non-invasive and practically applicable detector of RRD patients who have higher risk of developing retinal redetachment.

In a subsequent prospective cohort study (n=410), the predictive value of SAF in identifying patients at high risk of retinal redetachment was investigated. Only RRD patients who were scheduled for trans pars plana vitrectomy surgery were included in the study. Patients with conditions that are known to affect AGE levels (i.e. severe renal disease, active infection, and inflammatory disease) or SAF measurements (i.e. skin abnormalities and skin reflectance <8%) were excluded from participation. Several AGEs and their α-dicarbonyl precursors were measured in vitreous samples of 45 patients with a retinal redetachment within 3 months and compared with samples of 45 patients without a redetachment, matched on characteristics that are known to influence AGE accumulation (i.e. age, eGFR, DM, smoking, intraocular lens implantation, and PVR). Chapter 5 shows that skin autofluorescence was not suitable to identify patients at high risk for redetachment after vitrectomy surgery. Furthermore, all measured vitreous AGEs and α-dicarbonyls were comparable in the redetachment patients and the matched controls. Thus, our study did not support an independent role for AGEs in the development of redetachment, or for SAF in predicting retinal redetachment. Logistic regression analysis showed that only pulse pressure was additionally and independently related to retinal redetachment besides the well-known risk factors surface area of detachment and PVR grade C.
The final research question addressed in this thesis concerned AGE levels in the vitreous of diabetic patients before the onset of (proliferative) diabetic retinopathy (DR). Chapter 6 is a cross-sectional analysis of 31 T2DM and 62 non-diabetic RRD patients (matched on age, eGFR, smoking, intraocular lens implantation, and PVR) from the prospective cohort study described in the previous paragraph. This study provides an overview of several specific AGEs and their α-dicarbonyl precursors. The analysis revealed that only pentosidine and 3-deoxyglucosone concentrations were increased in the vitreous of RRD patients with diabetes compared to the matched non-diabetic RRD patients.

**Limitations**

A limitation of the above mentioned studies is that SAF is not a direct measure of AGEs in the skin. Since substances other than AGEs have fluorescent properties, these can contribute to the level of SAF. However, the used wavelength band of the AGE Reader was selected such that the major contribution in fluorescence comes from fluorescent AGEs. Moreover, it was shown that specific wavelengths were not preferable over the currently used broad wavelength band of the AGE Reader in differentiation between diabetic and non-diabetic subjects or between diabetic subjects with and without diabetes-related chronic complications.\(^1\)

Furthermore, it should be noted that SAF measurements may be influenced by the use of skin products\(^2\) and lifestyle factors.\(^3,4\) To be able to take these factors into account, patients were asked about the use of skin products and their current and previous smoking habits before SAF measurements were performed.

Selection bias is a common type of error that can result from applied selection criteria and from factors affecting the study participation, while it leads to a study sample that is not representative of the population intended to be analysed. For example, previous studies show that individuals who are old, single, immigrant, or have a low socioeconomic status are less inclined to participate in large population based cohort studies.\(^5,6\) Furthermore, participants to cohort studies often have less chronic diseases and have a better level of functioning than those who do not participate.\(^7,8\) These trends were also seen in LifeLines. However, in a study addressing the representativeness of LifeLines, it was concluded that LifeLines is broadly representative of the population in the north of the Netherlands on socioeconomic characteristics, lifestyle, diseases, and general health, that the risk of selection bias is low, and that risk estimates in LifeLines can be generalized to the general population.\(^9\) This may also apply for our study with LifeLines data, but care should be taken in generalizing conclusions since we used a sub-set of the LifeLines population in which both SAF measurements and biochemical measurements were available.
Discussion and future perspectives

FINDRISC & SAF in diabetes detection

The FINDRISC is commonly used as a first assessment tool to identify those who may need further blood glucose testing to confirm the presence of diabetes. SAF was previously shown to be useful as a cost-effective, simple, and reproducible test for diabetes screening in intermediate risk groups. In the LifeLines cohort study, as shown in chapter 3, the performance of the FINDRISC and SAF combined was significantly better than both methods separately. Furthermore, the combination of a simplified model (including age, BMI, and SAF) may represent an accurate alternative in a setting where measurement of waist circumference and answers about medication use and history of high blood glucose seems unreliable or inappropriate. One can think of screening settings in supermarkets and in groups that avoid or have never been exposed to healthcare. In addition, because of its easy and quick performance, the simplified model may improve the generally low participation of persons in screening programmes.\(^1\)

The demonstration that SAF improves the FINDRISC in identifying individuals with increased risk of diabetes opens prospects for, partly already ongoing, studies on related applications. An important opportunity to address is the possible value of SAF alone or in combination with conventional models in the prediction of diabetes. Furthermore, it would be interesting to investigate whether the addition of SAF to the FINDRISC model would be able to predict cardiovascular complications in diabetes and cardiovascular disease (CVD) more accurately. The FINDRISC has already been shown to be valuable in the prediction of cardiovascular events in the same population as the FINDRISC was developed\(^1\) and in a randomly selected Finnish population of men aged 45 to 74 years.\(^1\) SAF has already been shown to be valuable in the prediction of cardiovascular events in diabetic populations,\(^2,3,4\) in patients with coronary disease,\(^5\) and in patients with peripheral artery disease.\(^6\) Finally, it will be important to confirm validity of the current results in other populations, especially non-Caucasians.

SAF as predictor of diabetes complications

In chapter 2, we showed that several cross-sectional studies (performed by several research groups in different racial groups) and few prospective studies show consistent evidence of an association between SAF and end-organ complications in diabetes. Among these complications, it has been shown that SAF is associated with retinopathy, even after correction for age and presence of nephropathy.

Chapter 6 describes the vitreous content of several advanced glycation endproducts (AGEs) and its precursors (α-dicarbonyls) in RRD patients with type 2 diabetes mellitus and matched controls. This work shows that some of the compounds are elevated in the diabetes subgroup, consisting of patients with a relatively short duration of diabetes and without extensive microvascular damage to the retina. This is a unique cohort since vitreous samples are not usually available in diabetes patients without proliferative diabetic retinopathy (PDR). Furthermore, AGEs were expressed per mmol of lysine to adjust for differences in protein amount in the vitreous biopsy in order to lower the impact of sampling errors. Previous studies have addressed the AGE content.
of the vitreous in PDR patients. However, most of these studies investigated an overall measure of AGEs with enzyme-linked immunosorbent assay (ELISA) or investigated only the concentration of 1 or 2 different AGE(s).

The overview of several important AGEs and their precursors measured with the current golden standard for AGE measurements (UPLC MS/MS) can be used as a reference for future studies concerning AGEs and their role in diabetic retinopathy. A direct comparison of our results with most previous studies concerning vitreous AGEs is not correct, because of differences in methodologies or because AGEs were not expressed per protein content of the samples. Therefore, prospective studies with standardized AGE measurements in diabetes patients are needed to further elucidate the exact role of different AGEs and α-dicarbonyls in the development of diabetic retinopathy.

Prospective studies with a long period of follow-up are also needed to establish the role and potential benefit of SAF in disease management of diabetes patients. If these prospective studies, with adequate group size, would confirm that SAF is predictive of diabetic complications (preferably in patients with different ethnicities), SAF may be able to identify diabetes patients that would benefit most from AGE focused therapy. Despite promising results of AGE breakers and AGE inhibitors in animal experiments, safety and/or efficacy in human clinical studies seems to be a concern. Although pharmacological treatment is, thus, not yet available, lifestyle adjustments are also of interest in lowering AGE accumulation. Since smoking increases circulating AGE levels, smoking cessation would probably be beneficial in reducing AGE accumulation. Furthermore, several studies have focussed on the effect of high- and low-AGE diets. Overall, the evidence indicates that consumption of a high AGE diet increases circulating levels of AGEs and pro-inflammatory biomarkers, specifically TNFα, in healthy and overweight individuals and patients with T1DM, T2DM, or chronic kidney disease (CKD). Furthermore, it has been suggested that consumption of a high AGE diet may increase biomarkers of oxidative stress in healthy individuals, increase cardiovascular risk factors in patients with diabetes, and promote CKD in overweight males. Therefore, dietary AGEs may play a role in the promotion of chronic conditions such as T2DM, CVD, and CKD through increasing oxidative stress and inflammation. However, due to a lack of high quality randomised trials, more research is required.

In order to decrease the impact of dietary AGEs, ongoing research is addressing possibilities of lowering dietary AGE intake of individuals in daily life. Besides advising consumers to mainly eat food naturally low in AGEs such as fish, legumes, vegetables, fruits, and whole grains, consumers can be educated about low-AGE generating cooking methods such as poaching, steaming, and boiling. Another possible strategy to decrease the impact of dietary AGEs is lowering the uptake of dietary AGEs by the intestines. Recently, two trials have addressed the effect of sevelamer carbonate ingestion in binding AGEs and their effect on circulating and cellular AGE concentration.
Role of AGEs and pulse pressure in retinal redetachment

Previous studies have suggested that AGEs may play a role in proliferative vitreoretinopathy, which is considered to be an important cause of retinal redetachment. An explanation that SAF and vitreous AGEs did not come forward as independent risk factors for redetachment in our prospective cohort study may be the current way of treatment. Since the presence of PVR is important in choosing surgery methods and proceedings, this may have led to a smaller proportion of redetachments due to PVR than one may expect based on previous research. Therefore, the results of our prospective cohort study, showing that AGEs were not related to redetachments within three months due to any cause, was not sufficient to exclude a role of AGEs in PVR.

Concerning the risk factors for redetachment that were observed in the cohort study, the results were largely in agreement with previous studies. The extent of detached retina and PVR grade C are characteristics that usually emerge as important risk factors. For other risk factors, such as age and previous intraocular lens implantation (IOL), mixed results have been shown. Therefore, it was not surprising that age and IOL were no longer significant in our multivariable analysis.

Although arterial stiffness has not been considered as a risk factor for retinal redetachment previously, our results indicate that pulse pressure independently contributes to the development of redetachment after vitrectomy. A mechanism by which elevated pulse pressure might contribute to redetachments in RRD patients would be comparable to proposed mechanisms in which untreated hypertension may lead to serous retinal detachments and central serous chorioretinopathy: elevated blood pressure leads to increased intravascular pressure, which may cause changes in the retinal pigment epithelium (RPE) that impairs the RPE-mediated drainage of subretinal fluid. Furthermore, the damaging effects of an acute rise in blood pressure or chronic hypertension are demonstrated in hypertensive retinopathy, which typically follows sequential phases of histologic damage: vasoconstrictive, sclerotic, and exudative. Usually, the initial response to elevated vascular pressure is vasospasm and vasoconstriction to optimize blood flow (vasoconstrictive phase). Over time, endothelial damage with additional intimal thickening will develop (sclerotic phase). Finally, disruption of the blood-retinal barrier and leakage of plasma material into the retina will occur (exudative phase).

Previous studies concerning risk factors for redetachment did not report blood pressure or pulse pressure. Therefore, the value of this potential new risk factor should be established in other cohorts of RRD patients before any recommendations can be given. If the relation between pulse pressure and redetachment would be confirmed, it might be recommended to screen RRD patients for untreated hypertension and increased pulse pressure, as both are expressions of vascular compliance loss. Though it seems obvious to regulate the blood pressure properly, it should be further investigated whether this would also lead to risk reduction of redetachments and whether specific classes of antihypertensives should be preferred.
Concluding remarks

The overarching aim of this thesis was to provide more insight in SAF as a biomarker for occurrence of disease and development of complications in diabetes and retinal detachment. First, it can be concluded that SAF is not useful as a biomarker for occurrence and disease management of RRD. Similar vitreous AGE levels in patients with and without a redetachment support an insignificant role of AGEs in the identification of patients at high risk of retinal redetachment. However, a role of AGEs in the development of PVR was not excluded. Second, the combination of the simple to perform FINDRISC score, plus the easy, non-invasive SAF measurement provides a clinically useful tool for diabetes detection. Furthermore, a simplified model including age, BMI, and SAF may represent an accurate alternative in a setting where measurement of waist circumference and answers about medication use and history of high blood glucose seems unreliable or undesirable. The resulting early identification of patients with diabetes may be able to prevent or delay micro- and macrovascular complications by interfering early in the disease process. Finally, SAF is promising to be useful in disease management of diabetes patients. If prospective studies would confirm the value of SAF in predicting complications, SAF may be able to identify patients that may benefit most from AGE focused therapy.
References


