ummary

Advanced glycation endproducts (AGEs) are biomarkers of metabolic stress and are ıought to contribute to the increase of long-term complications in chronic age-related iseases, such as diabetes and renal failure. Until recently, all AGE-assays needed invasive ampling. However, tissue autofluorescence is related to the accumulation of AGEs. Therefore we developed a noninvasive Autofluorescence Reader (AFR), to measure skin AGE accumulation noninvasively. The aims of this thesis were to validate the AFR as a neasure of AGE accumulation, to evaluate skin autofluorescence as a clinical tool and its relationship with long-term complications in diabetes and renal failure.

The AFR is indeed able to measure skin autofluorescence of the skin noninvasively with a high reproducibility. Measurements at different body sites (calf vs arm) show comparable results and ,as discussed, the production and testing of different types of the AFR, including a version with a monochromator, have proceeded to application in various clinical studies (**chapter 5**). Skin autofluorescence measured with the AFR is related to the accumulation of AGEs, i.e. collagen-linked fluorescence, pentosidine, and carboxymethyllysine (CML). This relationship was found in healthy subjects, type 1 and type 2 diabetic patients, and hemodialysis patients (**chapter 6,10**).

Increased levels of skin autofluorescence in diabetic patients are associated with parameters of metabolic burden (HbA1c, triglycerides, low density lipoproteins (LDL)) and with renal dysfunction and ageing (**chapter 6,8,9**). In hemodialysis patients, skin autofluorescence is related to the presence of diabetes, hyperlipidaemia (triglycerides and LDL), duration of renal failure and dialysis treatment (**chapter 10**). Furthermore, a strong correlation between skin autofluorescence and inflammation (C-reactive protein) was observed in these patients. In "healthy" subjects, skin autofluorescence correlates with ageing and smoking habits (**chapter 11**). Overall, skin autofluorescence seems to be a biomarker of ageing, cumulative metabolic stress, and renal function.

In diabetic patients, skin autofluorescence is strongly associated with the presence of long-term complications, including microvascular complications and coronary heart disease (CHD). Increased skin autofluorescence is associated with nerve conduction abnormalities (velocity and amplitude) and autonomic dysfunction (heart rate variability and baroreflex sensitivity), even before clinical manifestations of diabetic neuropathy (**chapter 8**). Increased

skin autofluorescence was also observed in patients with microalbuminuria, diabetic retinopathy, hypertension and CHD. Multivariate analysis showed that skin autofluorescence was more strongly associated with long-term complications, compared to current measures of metabolic burden, such as HbA1c or LDL. We hypothesize that skin autofluorescence may represent the long-term effects of a final common pathway for various risk factors. Thus, based on the present work, skin autofluorescence may integrate these factors and be more informative than the actual levels of these risk factors per se.

Prospective mortality studies in this thesis showed that skin autofluorescence is a strong and independent predictor of mortality in diabetic patients and in hemodialysis patients. Again, skin autofluorescence replaced other measures of cumulative metabolic stress as predictors of survival. These results are the first to show the predictive value of AGE accumulation (**chapter 9,10**).

The original Maillard hypothesis on the formation of AGEs proposed that chemical modification of proteins by reducing sugars (glycation of proteins) in diabetes altered the structure and function of tissue proteins, precipitating the development of diabetic complications. Interactions between AGEs and AGE specific receptors (e.g. RAGE) may be a major mechanism by which AGEs contribute to the development of long-term complications. Interaction with RAGE results in the production of cytokines, which stimulates proliferation of mesangial cells, arterial smooth cells and increases collagen synthesis. Binding to RAGE further activates endothelial cells, resulting in higher levels of endothelial adhesion molecules like VCAM-1, and activation of transcription factor NF-kB. Endothelial adhesion molecules and NF-kB further increase monocyte adhesivity and vascular permeability, accelerating atherosclerosis. AGEs initiate inflammatory-proliferative processes and propagate inflammation in established macrovascular disease. Trough the interaction with RAGE, AGEs induce oxidative stress. AGEs modify LDL, making it less able to be cleared, promoting uptake by macrophages and inducing inflammation (**chapter 2**).

Several limitations of the studies are discussed in this thesis. Not all AGEs exhibit fluorescent properties. ELISA-based assays have shown increased accumulation of specific AGEs before increased autofluorescence in diabetes. Fluorescence represents group reactivity, which fails to provide quantitative information on concentrations of individual compounds. Importantly, the current understanding of physiological AGEs indicate that the major AGEs quantative are not fluorescent. Another limitation in our studies is that we do not yet know the degree to which the relation of autofluorescence with AGE accumulation is affected by other skin fluorophores such as the oxidation degradation product of tryptophan, N-

formylkynurenine, or elastin linked fluorescence. In particular, advanced lipoxidation endproducts (ALE) may also contribute to tissue and skin autofluorescence.

Skin autofluorescence is related to the accumulation of specific AGEs, cumulative metabolic stress, and to long-term complications in diabetes and renal failure. Our study supports the important clinical impact of AGE accumulation in the pathogenesis of cardiovascular disease and warrants for interventions aimed at reducing AGE accumulation. The non-invasive AFR may become a clinical desktop tool for follow-up in metabolic control and risk assessment, but also provides a novel approach for monitoring the role of AGEs in disease. As expected, many questions have risen as our understanding of noninvasive skin autofluorescence measurements is increasing. Many are beyond the scope of this thesis. At best our results on the presence of long-term complications are correlative, and it remains a question whether the correlation with survival is causative. Several follow-up studies have been started and are in progress. Will we discover the value of skin autofluorescence in daily clinical practice?, or will it remain an Aladdin's magical lamp?