Skin autofluorescence in cardiovascular disease
Mulder, Douwe Johannes

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Summary, General Discussion, and Future Perspectives
Summary and General Discussion

Oxidative stress is a major player in the inflammatory process leading to atherosclerotic plaque formation. Several lines of evidence have pointed out that oxidative stress not only initiates the first step of oxidative modification of low density lipoprotein (LDL) particles in the vascular wall, but is also strongly involved in the development of endothelial dysfunction and ultimately plaque disruption, leading to acute ischemic events.

In Chapter 1 we have illustrated the potential usefulness of measuring oxidative stress in patients with, or at risk for, cardiovascular disease (CVD), by reviewing currently available biomarkers for oxidative stress. Most clinical research has been performed with markers for inflammation. The clinically extensively used acute phase protein, C-reactive protein (CRP), currently provides the most robust marker, when measured using a high sensitivity method. It has been shown in large epidemiological studies that apparently healthy subjects with slightly elevated levels of CRP are at increased risk for developing CVD. These subjects are thought to suffer from low grade inflammation of the vascular wall, with moderately elevated levels of CRP that would not have been detected using normal sensitivity methods. Also, CRP has been shown to be of prognostic significance in acute coronary syndromes, elevated levels predicting major cardiovascular events both in the short as well as the long term. However, little evidence is available that CRP itself is of pathophysiological significance. Elevated CRP levels produced by the liver may merely be a bystander effect of inflammation elsewhere in the body (such as the respiratory system and adipose tissue), stimulated by cytokines such as interleukin 6. Furthermore, recent reports suggest that CRP is only a moderate risk marker (odds ratio 1–2) and provides no additional prognostic information beyond traditional risk factor assessment in healthy populations. It is plausible that additional biomarkers are needed to complement the predictive power of CRP. Several novel markers for inflammation may be of potential use. These include fibrinogen, several cytokines (interleukin (IL)-6, IL-10, IL-18), CD40 ligand and neopterin. However, since inflammation is actually a response to injury caused by an excess in reactive oxygen species (ROS), it may be pathophysiologically more suitable to measure oxidative stress parameters for vascular risk assessment. Several markers have been suggested and extensively studied. These include direct measurement of lipid peroxidation products (e.g. isoprostanes, conjugated dienes, aldehydes), oxidized LDL, oxidized LDL antibodies, in vitro susceptibility of LDL to oxidation, enzymes that produce oxidative stress (myeloperoxide, A2 phospholipases), and antioxidant enzymes. However, most of these markers are volatile, require stringent sampling and storage conditions, and also require highly sophisticated assay methods that are only available in specialized laboratories. Thus, they are expensive, time consuming and not always well reproducible. Therefore, there remains a need for ongoing research for other markers, especially those that will be predictive of early stages of atherosclerosis and easily applicable on a large scale basis as well as in clinical practice.

Biomarkers and interventions with lipid lowering agents

It is still unclear whether currently available interventions with statins and other agents significantly affect levels of oxidative stress biomarkers and whether lowering levels of...
oxidative stress is beneficial with respect to clinical outcome. With currently available potent statins it is possible to lower LDL cholesterol extensively; yet, it is not known whether an intensified lipid lowering regimen may also result in more reduction in inflammatory and oxidative stress. In Chapter 2 we addressed this question by randomizing patients with known CVD that were already being treated with simvastatin 40 mg, to continue this regimen or to receive atorvastatin 80 mg, a more potent statin. We show that switching to atorvastatin significantly improves total cholesterol, LDL cholesterol, and triglycerides, but does not influence any of the biomarkers for inflammatory and oxidative stress. This lack of effect is in agreement with other studies investigating the effect of statins compared with placebo that report varying effects on biomarkers9. These results stress the importance of a continuing search for new and possibly more reliable markers of oxidative stress or inflammation in atherosclerosis.

Advanced Glycation Endproducts (AGEs) and skin autofluorescence

AGEs are a heterogeneous group of compounds, classically associated with diabetes and renal disease, which are the end products of the slowly occurring, carbohydrate-driven glycation of proteins, known as the Maillard reaction10. In Chapter 1, however, evidence is provided that much of the AGE burden is produced under influence of oxidative stress, making them potential new markers for oxidative stress. The principal reason that AGEs may be uniquely suitable markers for measuring oxidative stress is that these are highly stable compounds. Since AGEs link to long-lived proteins (e.g. collagen), they are thought to reflect "metabolic memory".

In Chapter 2 we outline that available laboratory techniques to measure AGEs in serum or plasma present several challenges. Available assays are, as is the case with measuring most oxidative stress markers, costly, time consuming and not very reproducible. Another important drawback of measuring circulating AGEs, particularly low molecular weight AGEs, is that their levels are highly influenced by kidney function. Also, circulating AGEs may not reflect the levels in the diseased tissue (e.g. vasculature). It has been shown that measuring AGEs in skin can be a more appropriate approach. However, this requires taking skin biopsies, which is not suitable for large scale studies and clinical practice, especially when sequential measurements are desired to monitor disease or therapy.

In Chapter 2 we introduce an alternative approach for measuring AGEs which is more feasible for clinical practice, i.e. skin autofluorescence (skin AF). Skin AF is measured by the AGE-Reader, which uses the characteristic fluorescence properties that some AGEs encompass. By measuring skin AF, AGEs in the skin can be non-invasively quantified. We provide evidence from previous clinical studies that skin AF is strongly related to AGEs measured in skin biopsies. Furthermore, skin AF is elevated in the diseases classically associated with accelerated AGE accumulation (e.g. diabetes and renal disease) and is also of prognostic relevance, since it is an independent predictor of long term cardiovascular morbidity and mortality in these patients.

In Chapter 7, we provide data of healthy young subjects. From these subjects, we obtained skin samples taken from the forearm and also performed a skin AF measurement at this same site. Specific AGEs were measured from homogenates using collagen linked fluorescence (CLF), carboxy-methyl-lysine (CML) and carboxy-ethyl-lysine (CEL) assays.
As expected, skin AF correlated strongly with CLF, but also with CML. The latter compound may be considered a marker for oxidative stress, since its production is mainly derived from lipid peroxidation and reactive carbonyl species (RCC), both highly influenced by oxidative stress\textsuperscript{11}. These associations, therefore, provide evidence that skin AF is a non-invasive index for glycation as well as for oxidative stress. Furthermore, from a physical point of view, it is not surprising that skin AF is related to oxidative stress products. In vitro studies as well as rat skin studies have shown that besides several AGEs, several lipid peroxidation products encompass characteristic fluorescence properties. For example, both 4-hydroxynonenal (HNE) and malondialdehyde (MDA), two lipid peroxidation products formed at high rates in oxidized LDL (oxLDL) are known to exhibit fluorescent properties at specific wavelength combinations (e.g. 360/430 nm and 387/455 nm) as adducts to amino groups of protein (Advanced Lipoxidation Endproducts), which probably contribute to the spectrum that is measured with the AGE-Reader\textsuperscript{12,13}. Accordingly, oxLDL also produces fluorescence light and changes the fluorescence pattern of atherosclerotic plaques\textsuperscript{14}. Furthermore, in rat studies it has been shown that these lipid peroxidation products cross-link collagen in the skin and accumulate linearly with age\textsuperscript{15}. In conclusion, the small validation data set presented in this thesis confirms previous validation studies in diabetes, renal failure, and older healthy controls, that skin AF is a reliable, non-invasive marker for glycation as well as oxidative stress derived AGEs\textsuperscript{16,17}.

In Chapter 1 we provide evidence that AGEs are involved in the pathophysiology of atherosclerotic disease. AGEs may contribute to atherosclerosis in several ways by cross-linking cellular matrix and basement membrane proteins, modification of lipoproteins, quenching of nitric oxide, and by promoting receptor mediated inflammation and oxidative stress in the vascular wall. In the latter mechanism, the cellular receptor for AGE (RAGE) is thought to play an important role; by engaging this receptor, AGEs promote upregulation of several pro-inflammatory pathways that ultimately lead to enhancement of inflammatory and oxidative stress. These data suggest that AGEs may be applied as markers of atherosclerosis in diabetes and renal disease, but also in other diseases associated with oxidative stress as well as in predicting disease in healthy subjects. In Chapters 4–6 we test the hypothesis

![Figure 1. Bar chart with mean and standard error of the mean of skin autofluorescence (AF) in healthy young controls (YC), matched controls (MC), high risk subjects with >3 vascular risk factors (HR), patients with stable coronary artery disease (sCAD), and patients with an acute ST-elevation myocardial infarction (STEMI). MC are matched on age with HR, sCAD, and STEMI. Arrow indicates that increasing skin AF reflects the progression of atherosclerosis.](image-url)
that skin AF, as a validated non-invasive marker for AGEs, may be a novel risk marker in atherosclerosis.

Skin AF in CVD
As mentioned before, a suitable marker for CVD should be elevated in several stages of the disease. In Chapters 4–6 the results of three studies in subjects with sub-clinical atherosclerosis, stable coronary artery disease, and acute myocardial infarction respectively, are presented. These three disease entities represent the different stages of atherosclerosis development. This comprises initiation of the atherosclerotic plaque in subjects with multiple risk factors for atherosclerosis, potentially manifesting as increased Intima Media Thickness (Chapter 4), propagation of the plaque, potentially leading to luminal narrowing, which is typical in patients with stable coronary artery disease (Chapter 5), and finally, complication as a result of plaque erosion and rupture, leading to acute coronary syndromes, including acute myocardial infarction (Chapter 6). In these chapters we demonstrate that skin AF is indeed elevated in each of these conditions and that there is a strong relation between disease stage and the level of skin AF (see Figure 1).

Skin AF and its relation to other markers
As our hypothesis was that skin AF is a marker for inflammation and oxidative stress in atherosclerosis, it should be associated with other markers of this process. In Chapter 4 we demonstrate that in subjects with sub-clinical atherosclerosis, skin AF already correlates with two well validated markers for inflammation, white blood cell count (WBC) and CRP, independent of age. In these subjects, skin AF was also associated with other vascular risk factors, such as obesity, glucose, and smoking.

In Chapter 5 we demonstrate that skin AF correlates with two experimental markers for inflammatory stress, neopterin and the soluble receptor for AGEs (sRAGE). As outlined in Chapter 1, neopterin reflects monocyte and macrophage activation and is a robust prognostic biomarker in stable CAD and ACS. We have also observed that neopterin correlates with skin AF in the population studied in Chapter 4 (e.g. patients with multiple risk factors), these results have not been included in this thesis (abstract: Mulder, Cambridge, 2006). Additionally, sRAGE is of considerable interest since it is thought to reflect the expression of cellular RAGE, which is the major receptor for AGEs. Engagement of this receptor results in activation of pro-inflammatory cellular systems (such as nuclear factor kappa B), leading to inflammatory and oxidative stress. Of note, some previous studies have shown that sRAGE is decreased in patients with hypertension and stable CAD; however, we found elevated levels in patients with stable CAD compared with controls and significant positive correlations with skin AF, but also with CRP. Others have reported elevated levels in patients with renal disease. This reflects the complicated nature of atherosclerosis, and a prognostic study is warranted to determine whether elevated levels of sRAGE are protective or harmful.

Finally, in Chapter 6 we demonstrate that skin AF is also independently associated with CRP, when measured in the first 72 hours following acute myocardial infarction. From these data we conclude that skin AF is strongly associated with inflammatory stress in different stages of atherosclerotic disease, independent of age and traditional risk factors. These data confirm previous studies that have shown that skin AF is related to CRP in
patients on hemodialysis and in renal transplant recipients. Importantly, in the latter population, skin AF correlated inversely with plasma levels of vitamin C, which is a strong antioxidant and hence an indirect marker of oxidative stress.

**Skin AF and sub-clinical atherosclerosis**

In Chapters 4 and 7 we present data on the relation of skin AF to a widely accepted, surrogate marker for atherosclerosis, the Intima Media Thickness (IMT) of the carotid arteries. This measurement is a strong predictor of future CVD, also in apparently healthy subjects and can be measured using ultrasound techniques. In Chapter 4, we demonstrate that skin AF correlates with IMT, independently of other vascular risk factors or risk scores. In Chapter 7 we confirm this association in healthy young controls. Currently, there is only limited data available on the association of AGEs with surrogate markers for the extent of atherosclerosis. It was shown that in non-diabetic patients with CAD, levels of AGEs measured in serum correlate significantly with the number of stenotic coronary arteries. Moreover, it has been shown that AGEs measured in skin samples are associated with coronary calcium score, an emerging surrogate marker for coronary atherosclerosis. Therefore, our data are in line with these studies.

**The use of skin AF in the detection of high risk patients**

Previous studies from our center demonstrate that skin AF is an independent predictor of future cardiovascular morbidity and mortality in patients with diabetes and renal disease. In this thesis (Chapter 4) we present data that indicates that skin AF also has the potential to detect asymptomatic subjects with thickened IMT, reflecting sub-clinical atherosclerosis. These subjects are at increased risk of developing CVD. When added to two widely used risk scores, FRS and SCORE, skin AF remained independently associated with IMT. Although of a cross-sectional nature, these data provide evidence that the independent prognostic power of skin AF found in previous studies with diabetic and renal failure patients may also be true for a more general population at high risk for CVD. In Chapter 6, we confirm this cross-sectional study by presenting data indicating that skin AF is associated with the one year occurrence of major adverse cardiac events in patients admitted for an acute myocardial infarction.

**Does decreased AGE formation protect against atherosclerosis?**

In Chapter 7 we present unique data concerning patients with the very rare congenital disease glycogen storage disease 1a (GSD 1a). Although these patients have been exposed to adverse vascular risk factors (dyslipidemia and microalbuminuria) all their lives and often lose renal function later in life, they do not develop premature atherosclerosis. It has previously been shown that GSD 1a patients may even be protected from atherosclerosis, since they have significantly lower IMT values compared with healthy age matched controls. This is in contrast, for example, to patients with familial hypercholesterolemia and type I diabetes patients, who already present with increased IMT at a young age. Thus, GSD 1a patients may be protected from premature atherosclerosis. Others have already shown that these patients have lower levels of oxidative stress markers in plasma. We addressed the question whether these patients accumulate AGE to a lesser extent, protecting them...
from premature atherosclerosis. However, although our present study confirmed the lower IMT in GSD 1a, these patients did not have lower levels of AGE accumulation in the skin measured from skin samples and measured non-invasively with the AGE-Reader. Apparently other mechanisms protect these patients from atherosclerosis. A plausible explanation may be that these patients start with antiatherogenic medication (angiotensin converting enzyme inhibitors and allopurinol) at a very early age. Moreover, these patients usually have elevated levels of uric acid, which may have anti-oxidant properties, and therefore, be atheroprotective. Apparently, more research is warranted to find an explanation for this puzzling phenomenon.

Methodological considerations for the AGE-Reader

In Chapter 2, several potential limitations of measuring skin AF are discussed. Skin AF may result from other sources than AGEs alone, including the reduced form of Nicotinamide Adenine Dinucleotide(P) (fluorescence excitation/emission characteristics ~350/460 nm). However this influence seems only limited. Additionally, several lipid peroxides may affect skin AF values of the AGE reader, because their excitation/emission characteristics fall within the fields of interest of the AGE-Reader. However, this may only make the AGE-Reader more robust in measuring composite sources of oxidative stress. Furthermore we provide data on the influence of several skin types on the measurement. It appears that the current set-up cannot be used on subjects with a skin of colour, skin photo type (SPT) V or VI, and in subjects with skin reflectance of <12%. The high melanin content of these skin types results in the too high absorption of incoming (excitation) light and outgoing fluorescence light hampering reliable skin AF measurement. The studies presented in this thesis have not been confounded by this phenomenon, because subjects with these skin types were excluded from each study. However, this brings about serious limitations to the application of the AGE-Reader in clinical practice. Firstly, Figure 2b of Chapter 2 demonstrates that subjects with SPT V/VI have significantly lower skin AF levels compared with subjects with lighter skin types. Secondly, persons with a pale skin (e.g. SPT I) appear to have higher levels of skin AF (Figure 2b of Chapter 2). However, the study included only 9 such subjects and, therefore, no definite conclusion may be drawn concerning this issue. It is however of crucial importance to obtain reliable measurements of skin AF in dark skinned people, because this is the most prevalent skin type of the world. Furthermore, since the incidence of diabetes is
higher among people of Hindustanian and Negroid origin, these subjects are an important potential target group for the AGE-Reader.

Among subjects with SPT I-IV, intra individual variation is very low. In 20 healthy volunteers, skin AF was measured three times with the instrumentation used in the studies presented in this thesis, and the median coefficient of variation was 4.0% (interquartile range, 2.5–7.1%). In a previous study, seasonal variance was also low (e.g. <6%)\textsuperscript{16}.

Another limitation may be local skin abnormalities that influence skin AF measurement. For example birthmarks are highly pigmented and produce unacceptable interference with the measurement. Also lichenification, scarring, and other skin lesions may produce unwanted errors. However, since these aberrations are easily recognized they seldom cause problems in daily practice. Additionally, we have noticed that use of skin creams or sun blockers and skin tanners may also cause unwanted fluorescence and absorb the UV light from the AGE-Reader. Therefore, skin AF should be measured at a healthy skin site and without skin care products.

From the data presented in this thesis, it can also be learned that temporal changes in interstitial fluid or capillaries in the upper layer of the dermis may influence skin AF. In Chapter 2 we mentioned that an intravenous infusion of sodium fluorescein, produces temporary elevation in skin AF. Hence it may be plausible that changes in blood composition by oxidative stress may contribute to the measured fluorescence light. Chapter 6 provides preliminary evidence that the latter may be the case, because the elevated skin AF measured in the 72 hours following acute myocardial infarction declined 200 days after admission. It may be that this temporarily enhanced skin AF has been caused by acute elevations in oxidative stress-derived fluorophores, such as reactive carbonyl species or AGEs linked to proteins (for example hemoglobin or albumin). An alternative explanation is that during acute diseases increased capillary leakage of plasma constituents, including fluorescent compounds, may take place. These issues warrant further study.

Skin AF may also be affected by exogenously derived AGEs. These include food-derived AGEs and smoking\textsuperscript{36}. AGEs form in heat treated foods rich in fats, and are also abundantly found in industrially prepared foods. Around 10% of these so called glycotoxins are absorbed from the intestine and may circulate in plasma and be deposited in tissue, especially in subjects with restricted kidney function\textsuperscript{37}. We have tested if ingestion of food-derived AGEs could influence skin AF significantly (data not provided in this thesis). In 9 subjects a single meal high in AGEs, consisting of 220 ml caramel custard combined with 45

\begin{figure}[h]
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\includegraphics[width=\textwidth]{scatter_plot.png}
\caption{Scatter plot of the relation between the non-invasively measured skin autofluorescence (AF) and carboxy-methyl-lysine (CML) in young healthy controls. AU indicates arbitrary units.}
\end{figure}
grams of toasted cheese sandwich was administered. 9 other subjects drank two cups (300 ml) of black coffee only. Fasting and three hour post load skin AF was measured. Three hours following ingestion of the high AGE meal, there was a median increase in skin AF of 10.3% (IQR: 1.0–17.6; p=0.021), whereas subjects that only drank coffee showed no increase (median -3.4%; IQR: -5.8 - 3.2; p=0.37) [abstract Cambridge 2006]. These preliminary data demonstrate that food-derived AGEs may also contribute to skin AF. This needs to be confirmed in a larger and better controlled trial.

**Future Perspectives**

In this thesis, we have introduced the measurement of skin AF as an index for the cumulative oxidative stress that eventually leads to clinically relevant diseases, in particular CVD. Chronic oxidative stress may be regarded as a residue of lifetime exposure to harmful stressors in the human body. It has been suggested that, in order to prevent and restore the acute harmful effects of stress and thereby maintain homeostasis, the body uses adaptive mechanisms. These stressors include risk factors such as obesity, hypertension and hypercholesterolemia,

![Figure 4. Model of the role of oxidative stress in age-related diseases. During life, people are subjected to several stressors which result in enhanced oxidative stress. In order to keep homeostasis, adaptation to these factors occurs, referred to as allostasis. However, this constant adaptation to these stressors may lead to chronic diseases, accompanied by enhanced formation of oxidative stress products in tissue, but also in the skin. The latter can be measured as skin autofluorescence (AF) and is a reflection of a lifetime of exposure to oxidative stress. COPD indicates chronic obstructive pulmonary disease; SIRS, systemic inflammatory response syndrome; CVD, cardiovascular disease; SES, socio-economic status; AMD, age-related macular degeneration.](image-url)
but also exogenous sources such as diets high in unsaturated fatty acids and smoking. Additionally, diseases suffered during life, may also contribute to this accumulation of oxidative stress, such as systemic inflammatory response syndrome (SIRS) following infections and traumatic events, but also the response during low-grade inflammation in e.g. parodontitis and during exacerbations of rheumatic disease. In neurobiological science, the constant adaptation to these environmental demands is generally referred to as allostasis, i.e. to achieve stability through change (38). However, this concept may also hold for chronic, oxidative stress-related diseases discussed in this thesis39. When these stressors persist or occur frequently over lifetime and exceed the body’s ability to adapt, there is an increased so-called allostatic load, which may eventually lead to chronic diseases (Figure 4).

As put forward in this thesis, measuring skin AF may provide an integrative marker of the cumulative oxidative damage that mounts up over a lifetime following the aforementioned mechanism. It has been shown that AGEs can form very rapidly (within a day) in milieus high in oxidative stress40 and are only slowly degraded over time, especially in subjects with impaired renal function37. Measuring skin AF may allow us to assess the risk of developing chronic diseases or their complications at a time in life when these conditions have not yet reached a clinically detectable state and may allow early interventions (see Figure 5).

Although we have mainly focused on CAD, this concept also holds for atherosclerosis in general, including peripheral artery disease and cerebrovascular disease, but also for other chronic diseases that overrule normal aging, such as age-related macular degeneration, Alzheimer’s disease and microvascular complications of diabetes. To put it simply, skin AF may reflect the biological age of a person. In order to incorporate skin AF in this concept, several important issues need to be clarified.

Figure 5. Schematic view of the accumulation of Advanced Glycation Endproducts (AGEs) during life. During acute diseases such as sepsis as well as after exposure to vascular risk factors such as cigarette smoke, high fatty acid containing meals or hypercholesterolemia, rapid rises in oxidative stress and hence AGEs occur that degrade slowly over time and accumulate over time. This growing burden of AGEs is associated with the development of chronic diseases such as atherosclerosis. X-axis represents a person’s life in time and the Y-axis represents AGE levels. This accumulation of AGEs can be non-invasively measured as skin autofluorescence.
Chapters 1 and 3 outline the need for wider clinical availability of oxidative stress biomarkers. It is especially important to investigate whether, and if so which, markers are influenced by interventions. The results presented in Chapter 3 stress the need for new therapeutic options: the clinical effectiveness of intensification of conventional therapy with statins is unequivocal, but still only partial, since it does not consistently improve inflammation and oxidative stress markers. Furthermore, although patients with a history of CVD are intensively treated with multiple agents, such as aspirin, angiotensin converting enzyme inhibitors, statins, and other cardioprotective agents, these patients may still experience recurrent events at an increased rate. Therefore, there is still a need to find alternative pathophysiological pathways and their markers, and to develop therapeutic interventions directed at these new targets. These may include circulating markers of oxidative stress. However, this thesis emphasizes that AGEs may also be a target of therapeutic interest and that skin AF may provide an easily applicable index/marker of AGE accumulation in vivo. Furthermore, it has to be confirmed in future studies that skin AF can be modified by currently available drugs, such as antidiabetic and antihypertensive agents, aspirin, and statins. Interestingly, there is accumulating evidence that these drugs may indeed reduce AGE formation. The most illustrative data come from an ancillary study of the Diabetes Control and Complications Trial that demonstrated that 5 years of intensive glucose control attenuates the formation of AGEs in the skin, measured in skin samples. One of the measured markers was autofluorescence at 370/440 nm, which resembles the spectrum measured with the AGE-Reader. It provides a challenge to confirm this study in diabetes using skin AF as a marker for AGEs. Moreover, it needs to be established in the future whether AGEs may also be a therapeutic target in CVD in general.

In this thesis we have provided evidence that skin AF can be used as a risk marker in cardiovascular disease. However, there is still a large amount of research to be conducted to consolidate the evidence and to improve the clinical applicability of the AGE-Reader. First of all, skin AF needs to be further validated with well accepted circulating biomarkers of oxidative stress. This is a challenge since currently there is no golden standard for assessing the degree of oxidative stress. Therefore, it is appropriate to validate skin AF to a panel of markers for oxidative stress. Moreover, skin AF needs to be evaluated in other diseases in which oxidative stress is a fundamental feature, such as multiple organ failure and acute infectious disease. Studies in these patients have already started and are ongoing, but a pilot study in patients with sepsis admitted to the intensive care unit suggested that skin AF is indeed markedly and transiently elevated in this category. Preliminary evidence suggests that admission skin AF is also a predictor of one month survival and duration of ICU stay in patients with sepsis (personal communication). Also, it is of interest to measure skin AF in other diseases accompanied by chronic exposure to oxidative stress and AGE accumulation, such as chronic obstructive pulmonary disease (COPD), heart failure, and auto-immune diseases (SLE, Wegener Granulomatosis). Of course, the systemic versus local extent of such conditions and the question whether local disease is associated with systemic markers of inflammation and oxidative stress will take a central place. Interestingly, preliminary evidence exists that age related macular degeneration, a chronic eye disease, is also associated with, and perhaps causally related to local AGE accumulation, but also with
elevated skin AF [abstract: Mulder, IMARS, 2006]. Others from our hospital are working on measuring skin AF in heart failure and in auto-immune diseases. Moreover, attention is warranted to evaluate the effect of exogenously derived AGEs on skin AF. Additional studies might address the direct effects of high AGE containing meals, but also the effect of chronic exposure to high AGE food intake. This should preferably be studied in an intervention study, to limit confounding factors. Additionally, it is of interest to investigate the direct effects of cigarette smoking, since chronic smokers have higher levels of skin AF.

Concerning CVD, it is of importance to evaluate whether skin AF is also elevated and eventually of prognostic value in patients presenting with unstable angina or a non-ST-elevation myocardial infarction, because these patients are at very high risk and there are currently no accepted markers available that detect subjects at the highest risk, which need early intervention or aggressive treatment. Also, the prognostic value of skin AF should be tested in apparently healthy subjects, although this will require a large study population and a long follow-up time. Since the coronary calcium score measured with electrobeam tomography is an important emerging surrogate marker for atherosclerosis, skin AF should be compared to this technique. Another interesting item with respect to CVD is whether progression in skin AF does reflect a progression of the disease. In line with this, we have recently concluded an 18 months follow-up measurement of skin AF and IMT of the high risk population described in Chapter 4. The question is whether IMT progression is associated with skin AF progression. Preliminary data from this study indicate that skin AF progression is predicted by the presence of diabetes, smoking, and inflammation at baseline [abstract: Mulder, IMARS, 2006]. Ultimately, it should be ascertained whether increased progression of skin AF is associated with an increased risk for developing cardiovascular complications.

In addition to clinical research, much work needs to be done to improve the technical specifications of the AGE-Reader. A special point of interest is allowing reliable measurements in subjects with darker skin types. A potentially useful adjustment in the measurement is to measure a broader reflectance spectrum. This can be achieved by measuring the absorbance spectrum of white light, in contrast with the UV spectrum that is now used by the device. This will allow for a better adjustment for differences in skin pigmentation, redness, and conditions such as jaundice. Moreover, the recently developed EEMS (Excitation Emission Maps) device is capable of measuring specific excitation and emission combinations, because a monochromator has been incorporated in this device. Using this technique, it may be possible to find out which specific wavelength bands contribute the most to the spectrum measured with the AGE-Reader. This may allow for more accurate measurement of specific fluorophores in the skin.

**Conclusion**

The field of inflammation and oxidative stress research is interesting, challenging, and may provide future markers that may identify people at high risk for developing diseases that may have deleterious effects on their health or even cause them to die prematurely. Research directed at finding novel markers or expanding the knowledge is crucial. In this thesis we have presented a new concept of measuring advanced glycation-related oxidative stress, by non-invasively measuring skin autofluorescence of the human skin. We hope that
this thesis may provide a useful foundation to expand the research using this technique in cardiovascular disease and other potentially harmful conditions.
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