Skin Intrinsic Fluorescence in the Epidemiology of Diabetes and Complications Study: Predictors and Association with all-cause Mortality

by

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Submitted to the Graduate Faculty of the

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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Aims: The published literature suggests that higher skin intrinsic fluorescence (SIF), as a marker of advanced glycation endproducts (AGE), is associated with worse health status in type 1 diabetes (T1D). The first two aims of this dissertation test the relationship between traditional vascular disease risk factors as well as T1D-related complications, and SIF change among individuals with long-term T1D. The third aim assesses the association between SIF and all-cause mortality in T1D.

Methods: Data were from the 30-year longitudinal Epidemiology of Diabetes and Complications (EDC) study of individuals diagnosed with childhood-onset T1D at the Children's Hospital of Pittsburgh, PA, USA, between 1950-80. EDC participants were followed for 30 years biennially providing medical history, lifestyle, demographic, and diabetes self-care survey information. SIF was collected from a convenience sample of EDC participants (n=245) between 2007-14. Mortality status was evaluated as of May 2020. Regression models were run to evaluate predictors of SIF scores; Cox regression was used evaluate SIF and all-cause mortality.

Results: We observed that modifiable T1D-related complication risk factors and markers at analytic baseline, such as worse blood glucose control and lower kidney function, were associated with increased SIF scores over a mean of 5.2 years follow up. Further, increased albuminuria at analytic baseline was associated with decreased SIF scores during follow-up. Body mass index change was marginally and inversely associated with SIF score change. SIF was

univariately associated with all-cause mortality; this association remained significant after adjustment for multiple daily insulin shots/pump use, but not after adjustment for diabetes duration, A1c months, or estimated glomerular filtration rate.

Conclusion: The predictors of SIF change identified are aligned with known biological processes that occur during AGE formation, accumulation, and deposition on long lived proteins. Regarding all-cause mortality, this work builds on existing evidence that AGEs may play a role in ageing. Taken together, this work supports the existing evidence that AGEs may be a marker of complication status in T1D, and that AGEs may predict risk of accelerated ageing. Further work is needed to establish the meaningfulness of SIF scores.

Table of Contents

Preface ..... **xviii**

1.0 Background and literature review **1**

1.1 General introduction to diabetes **2**

1.2 Pathophysiology **3**

1.3 Epidemiology **4**

1.3.1 Prevalence **4**

1.3.2 Incidence **4**

1.4 Risk factors **5**

1.4.1 Genetic **5**

1.4.2 Enterovirus infection **6**

1.4.3 Breastmilk feeding **7**

1.4.4 Premature food introduction **7**

1.4.5 Seasonality of birth **8**

1.4.6 Gut microbiota **9**

1.5 Complications **9**

1.5.1 Diabetic ketoacidosis **10**

1.5.2 Hypoglycemia **12**

1.5.3 Microvascular **13**

1.5.3.1 Retinopathy **13**

1.5.3.2 Neuropathy **14**

1.5.3.3 Diabetic kidney disease **15**

1.5.4 Macrovascular 17

1.5.4.1 Cardiovascular disease 17

1.5.4.2 Coronary artery calcification 20

1.5.4.3 Stroke 20

1.5.4.4 Peripheral arterial disease 21

1.6 Mortality 23

1.7 Advanced glycation endproducts 24

1.7.1 AGE accumulation in T1D 24

1.7.2 AGEs and long term T1D 25

1.7.3 AGEs and mortality 26

1.8 Technology in T1D 30

1.8.1 The SCOUT DS® device 31

1.9 Medical device regulation in the United States 38

1.9.1 SCOUT DS® global approval status 40

1.9.1.1 SCOUT DS® US approval status 40

1.10 Summary 42

1.11 Study aims 42

1.12 Study population 43

2.0 Predictors of change in skin intrinsic fluorescence in type 1 diabetes: The Epidemiology of Diabetes Complications study 45

2.1 Synopsis 45

2.2 Introduction 46

2.3 Methods 47

2.3.1 Study participants 47

2.3.2 Predictors 48

2.3.3 Outcome 49

2.3.4 Statistical analysis 50

2.4 Results 52

2.5 Discussion 53

2.5.1 Conclusion 56

2.6 Tables and Figures 57

2.7 Supplemental information 68

3.0 Correlates of change in Skin Intrinsic Fluorescence (SIF) among individuals with type 1 diabetes in the Epidemiology of Diabetes Complications study 74

3.1 Synopsis 74

3.2 Introduction 75

3.3 Methods 76

3.3.1 Study participants 76

3.3.1.1 Participant characteristics 77

3.3.2 Predictors 77

3.3.3 Outcome 79

3.3.4 Statistical analysis 80

3.4 Results 81

3.5 Discussion 82

3.5.1 Conclusion 84

3.6 Tables and Figures 85

4.0 Skin intrinsic fluorescence scores are a predictor of all-cause mortality risk in type 1 diabetes: The Epidemiology of Diabetes Complications study 91

4.1 Synopsis 91

4.2 Introduction 92

4.2.1 Subjects 93

4.3 Materials and methods 94

4.3.1 Skin intrinsic fluorescence score 94

4.3.2 Participant characteristics 95

4.3.3 Mortality 96

4.3.4 Statistical analysis 96

4.4 Results 98

4.5 Discussion 99

4.6 Tables and Figures 104

5.0 Discussion 110

5.1 Summary 110

5.2 Literature gaps 111

5.3 Research findings 112

5.4 Strengths and limitations 115

5.5 Regulatory considerations 118

5.6 Public health impact 119

5.7 Future directions 120

5.8 Conclusion 121

Bibliography 122

List of Tables

Table 1-1 Key studies reporting on AGEs and mortality in T1D 28

Table 1-2 Studies reporting on the relationship between SIF and T1D 34

Table 1-3 Example medical device classifications according to the FDA application process 40

Table 2-1 Characteristics of EDC study participants with repeated SIF assessments at 1986-88 exam, the first SIF assessment at the 2004-06 exam, and means (or exposure years) over time (N=83) [a] 57

Table 2-2 Participant characteristics at the 2004-06 exam by SIF score change status (between 2007-08 and 2010-14) in the EDC cohort [a] 61

Table 2-3 Correlations between T1D participant characteristics at the 2004-06 exam as well as means, or exposure years, over time from 1986-88 exam to the 2004-06 exam and change in SIF score in the EDC cohort (N=83) [a] 64

Table 2-4 Multivariable analysis of participant characteristics at the 2004-06 exam and change in SIF score in the EDC cohort (n=70) [a] 66

Table 2-5 Characteristics of study participants with SIF score (n=78 unless otherwise noted) and those without SIF score (n=229 unless otherwise noted) at analytical baseline (2004-06 exam) 69

Table 3-1 EDC T1D SIF sub study participant characteristics at analytic baseline, follow-up and change over time for those with repeat SIF scores (N=60) [a] 85

Table 3-2 EDC T1D SIF sub study associations between patient characteristic change and SIF score change (n=60) [a] 88

Table 4-1 T1D participant characteristics overall and by subsequent mortality status in the EDC SIF sub-study 104

Table 4-2 Unadjusted correlations between T1D participant characteristics and SIF score in the EDC SIF sub-study, N=245 unless otherwise noted 107

Table 4-3 Cox regression models for the association between SIF score and mortality in the EDC SIF sub-study 108

List of Figures

Figure 1-1 EDC cohort SIF sub-study participant flow for each research aim 44

Figure 2-1 EDC cohort SIF sub-study participant flow for T1D predictors of SIF score change..... 67

Figure 2-2 EDC cohort SIF sub-study detailed participant flow for predictors of SIF score change..... 73

Figure 3-1 EDC SIF sub-study participant flow for those with repeat exam data and repeat SIF scores..... 89

Figure 3-2 BMI distribution at analytic baseline, follow up, and change over time 90

Figure 4-1 EDC cohort SIF sub-study participant flow for SIF and all-cause mortality .. 109

List of Abbreviations and Acronyms

A1c months	A1c months is a measure of cumulative glycemic exposure calculated in the EDC study as [(number of HbA1c units above normal at each exam)*(number of months between the midpoints of the preceding and succeeding exam intervals)]
ACEi	Angiotensin-converting enzyme inhibitors
ACR	Albumin to creatinine ratio
AER	Albumin excretion rate
AGEs	Advanced glycation endproducts
AIC	Akaike's information criterion
ARB	Angiotensin II receptor blockers
AU	Arbitrary unit
BABYDIAB	A prospective cohort study in Germany of infants followed from birth who have parents with T1D
BMI	Body mass index
CAC	Coronary artery calcification
CACTI	Coronary Artery Calcification in Type 1 Diabetes study; a cohort of T1D patients aged 20-55 years old diagnosed before age 30 and non-diabetic controls all with asymptomatic coronary artery disease
CAD	Coronary artery disease
CDSP	Confirmed distal symmetrical polyneuropathy
CGM	Continuous glucose monitor
CHD	Coronary heart disease
CI	Confidence interval
CKD-Epi	Chronic Kidney Disease–Epidemiology Collaboration equation

CVD	Cardiovascular disease
DAISY	Diabetes AutoImmunity Study in the Young; a cohort study of infants with increased risk of T1D defined as either having the high-risk HLA gene markers or a 1st degree relative with T1D
DBP	Diastolic blood pressure
DCCT/EDIC	Diabetes Control and Complications Trial / Epidemiology of Diabetes Interventions and Complications study; consists of an interventional and observational study where participants were randomly assigned to intensive or conventional treatment and followed observationally
DIaMonD	Multiple Daily Injections And continuous glucose MONitoring in Diabetes; a World Health Organization study in childhood diabetes designed to collect information on incidence, risk factors, and mortality associated with T1D in 90 centers and 50 countries worldwide
DKA	Diabetic ketoacidosis
DKD	Diabetic kidney disease
DN	Diabetic neuropathy
DPN	Diabetic peripheral neuropathy
DQA & DQB	Genes associated with T1D
DR	Diabetic retinopathy
DSPN	Distal symmetrical polyneuropathy
EDC	Epidemiology of Diabetes Complications Study, a 30-year longitudinal study of childhood-onset T1D comprised of individuals diagnosed at the Children's Hospital of Pittsburgh, PA, USA, between 1950-80
eGDR	Estimated glucose disposal rate
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
ETDRS	Early Treatment Diabetic Retinopathy Study
EURODIAB	EUROpe and DIAbetes Study; an observational study that included 1,172 subjects with T1D from 31 centers across Europe

FDA	Food and Drug Administration
FinnDiane	Finnish Diabetic Nephropathy study; a study of adults with T1D diagnosed before age 40 years
GAD	Glutamic acid decarboxylase autoantibodies
GPRD	General Practice Research Database; a large primary care database from a network of 603 UK practices in which prescription and diagnosis data are recorded
GWAS	Genome-wide association study
HbA1c	Hemoglobin A1c
HDL	High density lipoprotein
HG	Hypoglycemia
HGI	Hemoglobin glycation index
HLA	Human leukocyte antigen
HTN	Hypertension
IAA	Insulin autoantibody
IDE	Investigational device exemption
INS	Insulin gene on chromosome 11
IQR	Interquartile range (25%, 75%)
LDL	Low-density lipoprotein
LED	Light emitting diode
MDC	MedStar Health Research Institute Diabetes Complications study; cohort of patients with T1D followed for clinical care for at least 4 years and a mean of 10 years
MDI	Multiple daily insulin shots/pump use
NHIS	National Health Interview Study; an annual cross-sectional household interview conducted by the Center for Disease Control in the US

NHS	Nurses Heath Study; an ongoing US cohort established in 1976 of 121,701 female registered nurses aged 30-55 who completed a mailed questionnaire and are followed biennially
Non-HDL	Non- high-density lipoprotein
ON	Overt nephropathy
OR	Odds ratio
PAD	Peripheral arterial disease
PDC	Pediatric Diabetes Consortium study; 7 pediatric diabetes centers that established a cohort of children with new-onset T1D under the age of 19 years with the intent to evaluate the first 12-24 months of therapy for T1D in US youth
PDR	Proliferative diabetic retinopathy
PMA	Premarket approval
PY	Person years
RAAS	Angiotensin aldosterone system inhibitors/antihypertensive treatment
RMSE	Square root of the variance of residuals
SAF	Skin auto-fluorescence
SBP	Systolic blood pressure
SD	Standard deviation
SEARCH	SEARCH for diabetes in youth study; a 6-center observational study of US youth with physician diagnosed diabetes
SIF	Skin intrinsic fluorescence
SMR	Standardized mortality ratio
SNP	Single nucleotide polymorphism
sRAGE	soluble Receptor for AGEs
T1D	Type 1 diabetes

T1D Exchange Clinic Registry	T1D Registry study of adults and children in the US; a registry of adults and children with diagnosed T1D, including patient self-reported incidence rates of DKA events
T2D	Type 2 diabetes
TEDDY	The Environmental Determinants of Diabetes in the Young study; an international cohort of children with higher risk genes for T1D
US or USA	United States of America
UV-A	Ultraviolet-A light
WBC	White blood cell
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy study of patients diagnosed with T1D before the age of 30 who were taking insulin and also received primary care in Wisconsin, US, between 1979-80
WHR	Waist to hip ratio

Preface

To my committee: I am grateful for your expert guidance during my dissertation. You have each mentored me in unique ways that I will carry forward in my scientific endeavors. To my committee co-chair Tom: thank you for helping me grow new skills to become a better scientist over these past 7+ years. It has been a long and winding road, and I especially thank you for your endurance and commitment. To my committee co-chair Tina: thank you for your investment in me, I learned so much from the opportunity to work closely with you. I will carry your mentoring with me in every aspect of my scientific work and career. Trevor, your expertise has been priceless and it has been an honor to work with you. Jeanine, thank you for your insights into statistical methods and assisting me to become a better analyst. Marquis, thank you for all your editing and teaching me how to write. To John: thank you for your insights on all things SCOUT DS ®. To co-author Rebecca Baqiyyah: thank you for collecting the SIF data (personally collecting SIF at patients' homes!) and for your expert manuscript writing.

I thank everyone who has worked on the EDC study in the past and who continue to do so. To those I had the privilege of working with directly, thank you for being great colleagues and for collecting such great data in the past 30+ years!

Thank you to all my friends for being alongside me in this journey, I could not have done this alone. Fouzia, your friendship has been a source of joy and support and I am grateful for traveling together on this path. Thank you, Julie, Abby, Beth, my 2013 Epi cohort, and the BR crew.

A big thanks to my family for supporting my graduate work that was a distant dream 10 years ago. Thank you to Mom, Dad, and Granddad. Thank you to my sisters and their spouses:

Jenn & Wes, Valerie & Ryan, Katy & Eric, and Rachel & Chad. Thank you to Rosie & Larry and Carol & Henry. Thank you, Jenn, for being my reader. To my many nieces and nephews, you each keep me grounded with endless joy. And the biggest thank you goes to my best friend and fiancé, Luke: thank you for all you've done to support and care for me in every way. You are a blessing and a true gift in my life.

1.0 Background and literature review

The primary goal of this dissertation research is to evaluate late-stage clinical health factors affecting persons with type 1 diabetes (T1D). Specifically, the research aims to address the relationship between advanced glycation end products (AGEs) and both T1D clinical factors (e.g., blood glucose control, kidney functioning, etc.) and all-cause mortality.

The existing evidence, albeit primarily from cross-sectional studies, demonstrates an association between collagen-linked AGEs and T1D, including kidney function and blood glucose [1-3]. Further, evidence suggests that higher skin intrinsic fluorescense (SIF) scores, as a measure of collagen-linked AGEs, are associated with worse health status. The first two aims of this dissertation seek to address predictors of change in SIF score in T1D.

AGEs are also associated with ageing [4], senescence, development of age-related morbidities [5], and mortality [6-8]. Existing evidence indicates AGEs are associated with incident all-cause and cardiovascular mortality in T1D [6, 8]. Evidence of causal effects between AGEs and ageing are limited, however, and no prior studies have evaluated SIF scores specifically. Thus, the third aim in this work is to research the connection between collagen-linked AGEs assessed with SIF scores and all-cause mortality in T1D patients.

The three Aims of this dissertation evaluate SIF scores, as a measure of collagen-linked AGEs, assessed with the SCOUT DS® device [9]. The process to produce a SIF score is non-invasive, quick, and has no known side effects [10]. The benefit of the SCOUT DS® is that it does not require overnight fasting, a blood draw, or waiting time for laboratory results [11].

An additional component of this project is to describe the United States of America (US) Food and Drug Administration (FDA) medical device approval process as it relates to the SCOUT DS® data in this project.

The following sections provide a targeted background literature review, the research paper for each Aim, and, finally, a discussion regarding the research in entirety.

1.1 General introduction to diabetes

Diabetes is a metabolic disorder of carbohydrate, fat, and protein metabolism resulting in high blood glucose concentrations; it is typically due to insulin deficiency. Since 1980, the age-standardized diabetes prevalence has increased in every country leading to a quadrupling of the number of adults with the disease [12]. Globally, the prevalence of all types of diabetes is estimated at 387 [13] to 422 million cases [12]. Classification of diabetes falls into four major categories [14], as below:

1. T1D: due to autoimmune destruction of pancreatic beta cells resulting in absolute insulin deficiency;
2. Type 2 diabetes (T2D): due to progressive loss of pancreatic beta cell insulin secretion and background insulin resistance;
3. Gestational diabetes: diabetes diagnosed in the second or third trimester of pregnancy; and
4. Diabetes due to other causes: for example, drug or chemical induced diabetes.

T1D, the focus of this work, accounts for 5-10% of all diabetes diagnoses [14]. The development of T1D has three distinct clinical stages [14]. The biological processes in stages 1 and

2 are often undetectable as there are typically no outwardly noticeable signs or symptoms at this point. During these early stages, multiple autoantibodies are developing and changes in fasting plasma glucose and hemoglobin A1c (HbA1c) begin. Stage 3, clinical onset, occurs with outward manifestation of symptoms as a consequence of poor blood glucose regulation [14]. T1D typically presents in children with polyuria, polydipsia, and weight loss [15, 16]. Unfortunately, in people with T1D, glucose and HbA1c levels rise long before awareness of having T1D. This means that, in general, short-term complications (due to exposure to poorly-regulated blood glucose) are underway by the time a diagnosis is made.

Lacking the biological ability to produce adequate insulin, people with T1D rely on external insulin for proper digestion of food and regulation of blood glucose. Due in large part to the destructive nature of short- and long-term exposure to irregular and high blood glucose, T1D is a considerable cause of morbidity, mortality, and health related costs worldwide accounting for an estimated $10.5 billion direct and $4.4 billion indirect medical costs annually [17].

1.2 Pathophysiology

T1D is an autoimmune disease characterized by the destruction of beta cells. These pancreatic beta cells, located in the islet of Langerhans, produce the insulin required for the metabolism of glucose. Properly functioning beta cells release insulin to initiate glucose transport out of the blood and into the body's cells. In T1D, healthy beta cells are mistakenly marked as foreign, which initiates immune-mediated destruction of the healthy cells [18, 19]. This destruction eventually results in inability to produce adequate insulin for proper digestion and, ultimately, a reliance on external sources of insulin.

1.3 Epidemiology

1.3.1 Prevalence

Current T1D prevalence estimates in the US are between 2.6/1000 persons to 3.4/1000 persons [20]. National data on T1D prevalence is sparse due to difficulty in classifying diabetes by type [21]. In self-reported data collected in 2016 from the National Health Interview Study (NHIS, an annual cross-sectional household interview conducted by the Center for Disease Control), the crude prevalence of T1D was 0.55% (1.3 million) [21]. The SEARCH study, a 6-center observational study of US youth with physician diagnosed diabetes, estimated that there are 1.93 T1D cases per 1000 persons under the age of 20 [22].

1.3.2 Incidence

The current incidence of T1D in the US is estimated at 22.9/100,000 person years for those aged 0-64 years [23]. The multiple Daily Injections And continuous glucose MONitoring in Diabetes (DIaMonD) study, a World Health Organization multinational project for childhood diabetes program designed to collect information on incidence, risk factors, and mortality associated with T1D in 90 centers and 50 countries worldwide, estimates that the annual incidence increase of T1D in those aged <=14 years is ~2.8% worldwide [24]. Temporal and geographic trends indicate increases in the incidence of T1D between 2002-03 in Asia, Europe, and North America, but not in Central America or the West Indies [15]. A plateau in incidence was seen in Australia between 2003-16, following a peak in 2003 [25]. Decreased incidence was observed between 2002-03 in Central America and the West Indies [15]. In Norway, incidence of T1D among children aged 0-14

is suggested to have leveled off between 1989-2012 with no significant increases observed in that time frame [26]. However, significant regional variations in incidence persist in Nordic countries [27].

Incidence of T1D is increasing in the US. Between 2001-15, incidence in US youth aged 10-14 increased by 1.9% annually [23]. Typically, US T1D incidence rates tend to increase between ages 0-14 with peaks in presentation at ages 5-7 and 10-14 years [15, 18]; the most rapid increases are seen in those under the age of 5 years [28]. There are also observed variations of incidence by region in the US: 3.8% increase per year in east south central, 3.1%/year in the mountain region, 2.7%/year in east north central, and 2.4%/year in south Atlantic, and 2.4%/year in west north central [23]. There are also variations in incidence of T1D by race in the US [15]; T1D disproportionately affects Hispanics, with annual rate of increase greater among Hispanics than non-Hispanic whites (4.2% vs. 1.2%, $p<0.001$) [29].

1.4 Risk factors

1.4.1 Genetic

The etiology of T1D includes several factors, most of which are not fully understood. Twin and family studies have suggested that up to half of T1D susceptibility is due to familial aggregation and genetic mutations [30]. While over 50 genetic regions are associated with T1D susceptibility, the human leukocyte antigen (HLA) region on chromosome 6 and the insulin gene (INS) on chromosome 11 have thus far demonstrated the largest influence. In fact, the HLA region on chromosome 6p21, accounts for 50% of familial aggregation [31].

The HLA region is responsible for identification of self vs. non-self-immune responses, which clearly has a role in autoimmune disease including T1D. The Environmental Determinants of Diabetes in the Young study (TEDDY), an international cohort of children with higher risk genes for T1D, found HLA-DR4 associated with insulin autoantibodies (IAA) and HLA-DR3 associated with glutamic acid decarboxylase autoantibodies (GAD) in children up to age 6 years [32].

Specifically, the most common T1D HLA associations are with the DQA and DQB genes. The highest risk haplotypes are HLA class II DR4-DQA1*03:01-DQB1*03:02 (also called "DR4-DQ8") and HLA class II DRB1*03:01-DQA1*05:01-DQB1*02:01 (also called "DR3-DRQ2") [31]. Ninety percent of people with T1D carry DR4-DQ8 or DR3-DQ2 [33, 34] and about 30% of T1D patients carry both, which is quite high when compared to the general population where only 2% carry both [31].

Second to the HLA region, the INS on chromosome 11p15.5 is also associated with T1D [31, 35]. INS is responsible for regulating insulin amount and insulin immune tolerance [31]. It contains three major insulin variable number tandem repeats of which the shortest repeats are associated with highest T1D risk [31, 35].

1.4.2 Enterovirus infection

Genetic predisposition to T1D is not enough to guarantee a diagnosis [36], rather, both genetic and environmental factors are needed to initiate T1D [37]. Several environmental triggers are thought to increase the likelihood of developing T1D. One such environmental trigger is enterovirus infections. In some cases, patients experience cold and gastrointestinal symptoms prior to diagnosis, which might be a signal of viral activity [38]. This type of infection has been

connected to IAA [39] and has been found in the pancreas of newly diagnosed T1D patients (specifically Coxsackie virus B1) [38].

1.4.3 Breastmilk feeding

Duration and timing of breastmilk feeding in infancy is hypothesized to impact IAA risk in infants in both detrimental and protective ways. Several prospective studies suggest a protective effect of breastfeeding on risk of T1D [40]. However, duration of breastfeeding greatly impacts risk; short duration of breastfeeding and a short duration of exclusive breastfeeding has been associated with increased risk of IAA in children [41].

In the Diabetes Autoimmunity Study in the Young (DAISY study, a cohort of infants with increased risk of T1D, defined as either having the high-risk HLA gene markers or a 1st degree relative with T1D), longer duration of breastmilk feeding months only slightly decreased T1D risk (although this was not statistically significant) [28]. In contrast, the BABYDIAB study, a prospective cohort in Germany of infants followed from birth who have parents with T1D, reported no difference in IAA risk for duration of total breastfeeding and duration of exclusive breastfeeding [42]. Interestingly, findings further indicated that infants without any breastfeeding had the lowest IAA risk [42].

1.4.4 Premature food introduction

Premature introduction of foods in infancy has been connected to increased T1D risk. Introduction to gluten and non-gluten cereal between 0-3 months of age [40, 42, 43] and introduction to fruit before 4 months of age [28] produces increased IAA risk in infants. Similar foods, when

introduced at >=6 months of age, pose lower risk of IAA development in infants [44]. This suggests that there may be a window of exposure for specific foods outside of which exposure increases IAA risk in infants.

Cow's milk introduction may also have a role in IAA and T1D risk, although the research remains largely inconclusive [45]. It has been associated with increased risk of IAA [42, 46] and T1D [47, 48], however, most prospective birth cohort studies have not shown any link to exposure to cows' milk and IAA or T1D [40]. These inconsistent reports suggest that genetic and environmental factors may modify these effects of cow milk on IAA and T1D. For example, the DAISY study found cow milk protein intake was associated with IAA development in children with low/moderate genetic T1D risk but was not associated with IAA development in children with high genetic risk for T1D [46].

1.4.5 Seasonality of birth

In some regions, seasonality patterns for month of birth in relationship to T1D diagnosis show that higher rates of T1D incidence are seen among those born in the spring season and lower rates among those born in the fall season in Europe, New Zealand, and Israel [15]. In the US, spring births are also associated with increased likelihood of T1D, but not in all US regions [49]. Excess of observed to expected births in the US for incidence of T1D incidence later in life has been observed for April-July births, but only in the more northern latitudes and the same trends have not seen in southern locations [15, 49].

Appropriate vitamin D level and type enhance the immune system; insufficient vitamin D is associated with increased incidence of autoimmune disease [50]. Vitamin D is hypothesized to decrease the risk of T1D due to how it reduces the immune response in T-cells [40]. In Belgium,

monthly average of daily hours of sunlight were inversely related to diagnosis of new patients with T1D [51]. In studies evaluating vitamin D intake by the mother while pregnant and risk of T1D in offspring, conflicting findings have indicated decreased risk, no effect, and lower risk [40]. Individual studies evaluating the effect of vitamin D consumption in infants have found no association with IAA [52]. However, a meta-analysis of observational retrospective studies showed a protective effect of supplemental vitamin D in infants, however, this research was limited by the observational and retrospective nature of the data [53].

1.4.6 Gut microbiota

A conflict between immune cells and gut microbiota during childhood activates immunoregulatory mechanisms, which control autoimmune reactions. Known as the 'hygiene hypothesis', one theory suggests that lacking microbiota variety suppresses the immune system, ultimately leading to autoimmunity [54]. It follows that probiotic intake in children with the intent to increase microbiota has been related to reduced risk of IA. In the TEDDY study, early exposure (between the age of 0-27 days) to probiotics was protective against IAA when compared to later (after age 27 days) or no exposure [32, 55].

1.5 Complications

Those with T1D are at increased risk of short- and long-term complications. Acute complications comprise those related to extreme changes in blood glucose; i.e., low blood glucose can lead to hypoglycemia, and high blood glucose can lead to hyperglycemia. These types of

complications can be transient and recurrent. Chronic complications can be described in two major categories: microvascular and macrovascular. Most of these complications can be delayed or prevented with good blood glucose control. A large proportion of T1D patients experience both short- and long-term complications over the course of their lives. Acute complications, such as ketoacidosis [56] and hypoglycemia [57], can develop quickly from the destruction of beta cells and poor blood glucose control. Longer term complications arise from chronically elevated blood glucose and can include visual impairment, nervous system deficits and loss of function, kidney disease, cardiovascular disease including coronary artery disease, stroke, and excess mortality.

1.5.1 Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is a result of increased ketone levels in the blood from insulin deficiency and excess insulin counter-regulatory hormones that leads to alteration in the metabolism of carbohydrates, proteins, and lipids [56]. Signs and symptoms of DKA include hyperglycemia [56], thirst, frequent urination, nausea, vomiting, pain, fatigue, fruity breath smell, and in the most extreme cases confusion, coma, and death.

One of the challenges with DKA data is that standardized definitions are not typically applied which makes comparing data across studies difficult. Estimates of DKA in T1D are primarily drawn from self-report and hospital discharge-reported events via medical records. In the T1D Exchange Clinic Registry study (a US registry of adults and children with diagnosed T1D), patient self-reported incidence rates of DKA events (recall between 3-12 months) ranged from 50-100 events per 1000 people in adults [57-59]. Similar incidence rates are estimated in Canadian hospital admissions data (103 to 128 per 1000 people) [60].

DKA is an acute transient and reversable consequence of hyperglycemia that can be resolved with insulin and proper monitoring of blood glucose. All people with T1D are at risk of DKA in the presence of massively elevated blood glucose. Over time, patients with T1D may become more aware of the signs of DKA and able to prevent it. The Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications study, or DCCT/EDIC studies, consist of an interventional study where T1D participants were randomly assigned to intensive or conventional treatment and its observational follow-up. When it was shown that patients benefited from intensive diabetes treatment, the trial was stopped and participants were followed up observationally in the EDIC study. The DCCT/EDIC study found that over the course of 12-18 years follow up, DKA events decreased, with some participants no longer experiencing DKA (defined as venous pH <7.3 or HCO_3 <15 mEq/l) [61].

Younger people with T1D typically have the highest prevalence of DKA, and prevalence decreases with increasing age [60, 62]. The Pediatric Diabetes Consortium (PDC) study (comprised of 7 pediatric diabetes centers that established a cohort of children with new-onset T1D under the age of 19 years with the intent to evaluate the first 12-24 months of therapy for T1D in US youth [63]) showed that 34% of children with new-onset T1D present with DKA [62]. The risk of DKA at T1D onset was higher in children aged <3 years (54%) versus those aged >=3 years (33%) [62].

Lower risk of DKA has been associated with private health insurance, higher family income, and family history of T1D [62]. In the PDC study, DKA was non-significantly associated with being African American, lower parental education level, and children living with someone other than both parents [62]. Additionally, a higher prevalence of DKA has been associated with female gender, non-white race [60], lower income [58], no private insurance, and higher HbA1c [57]. A study of youth continuous glucose monitor (CGM) users evaluated CGM data and patient self-

reported information on DKA resulting in an overnight hospital stay in the prior 3 months. A non-significant trend was identified towards decreased DKA frequency in children (age <13 years) who used CGM compared to not using CGM [64], suggesting that better glucose management may assist in reduction of DKA.

1.5.2 Hypoglycemia

Hypoglycemia (HG) is an acute, transient, and reversable state of severely low blood glucose [65]. In the Diabetes Care Standards of Medical Care in Diabetes, HG is classified into 3 levels based on glucose concentrations:

- Level 1 (mild hypoglycemia) is glucose between 54 mg/dL to 70 mg/dL;
- Level 2 (moderate hypoglycemia) is glucose <54 mg/dL; and
- Level 3 (severe hypoglycemia) is an event where altered mental and/or physical status requires assistance [66].

Mild and moderate HG events are characterized with self-reported symptoms [65]. Estimates differ by age group with younger children at higher risk of HG [65]. The T1D Exchange Clinic Registry asked for self-reported and parent-observer-reported HG events and found that 9.6% reported an event for ages 2<6 years; 5.2% reported an event in ages 6<13 years; 6.3% in ages 13 <18 years; and 6.9% in ages 18<26 years [57]. Among adolescents in the DCCT, incidence rate of severe HG events was 86 per 100 person years (PY) in intensively treated participants and 28 per 100 PY in conventionally treated participants [67]. Post DCCT incidence rates of severe HG are much lower and estimated at 24 per 100 PY in ages <7 years in girls and 19 per 100 PY in ages 7-12 years [68].

A study using the T1D Clinic Exchange Network data linked the occurrence HG events with more HG unawareness, greater glucose variability, and worse cognitive test scores [69, 70]. After adjusting for age in years, severe HG was more common in those who were non-Hispanic black, from low household incomes, without private insurance, had longer T1D duration, higher HbA1c, and used multiple daily insulin doses instead of CGM to manage T1D [57].

1.5.3 Microvascular

1.5.3.1 Retinopathy

The prevalence of diabetic retinopathy (DR) has been decreasing over time, although this complication continues to affect a large proportion of the T1D patient population. Thus, in 1984, the prevalence of DR was estimated at 97.5% for those with 15 or more years of T1D duration [71], whereas recent prevalence estimates suggest that 71.5% of T1D patients with 30 years duration of T1D develop this disease [72].

The 25-year cumulative rate of progression to DR was 83% in the Wisconsin Epidemiologic Study of Diabetic Retinopathy study (WESDR, is a study of patients diagnosed with T1D before the age of 30 who were taking insulin and also received primary care in Wisconsin between 1979-80) [73]. Compared to WESDR, the Epidemiology of Diabetes Complications Study (EDC) cohort, a 30-year longitudinal study of childhood-onset T1D comprised of individuals diagnosed at the Children's Hospital of Pittsburgh, PA, USA between 1950 and 1980, had lower overall cumulative incidence of proliferative retinopathy (47%) over 30 years diabetes duration [61]. Similarly, the DCCT/EDIC conventional treatment group had a 30-year cumulative incidence of proliferative retinopathy of 50%, whereas incidence was lower in the DCCT/EDIC intensive therapy group (21%) [61].

Proliferative diabetic retinopathy (PDR) is a severe progressive form of visual impairment leading to decreased visual acuity and vision loss [74]. It is characterized by alterations in the retina of the eye including growth of abnormal blood vessels and fibrous tissue [74]. Sometimes this also includes macular edema, which is swelling of the retina [74].

The WESDR found that the prevalence of proliferative retinopathy by 35 years duration declined among those diagnosed in 1975-80 compared to those diagnosed between 1922-74. In the EDC study similar trends were identified: those diagnosed between 1975-80 had borderline improved complication free survival compared to those with an earlier T1D diagnosis (1965-69) [75].

Progression of DR in T1D is more likely in those who are male, have higher HbA1c and increases in HbA1c over time, have increases in diastolic blood pressure (DBP) [73], and have hypertension (HTN) [76]. Smoking, systolic blood pressure (SBP), and high density lipoprotein (HDL) are also associated with higher risk of DR [77]. Cumulative incidence has been found to be highest in those with later in life diabetes onset [77].

1.5.3.2 Neuropathy

Diabetic peripheral neuropathy (DPN) pathology is complex and involves many factors [78]. Typically, the factors leading to neuropathy include increased mitochondrial production of free radicals, increased oxidative stress, formation of AGEs, downregulation of the soluble receptor for AGEs (sRAGE), activation of genes involved in neural damage, endoplasmic reticulum stress with resulting unfolded or misfolding of proteins, and activation of apoptotic processes [79].

Prevalence of DPN increases with T1D duration. In the SEARCH study, the age-adjusted prevalence of peripheral neuropathy was reported at 8.5% in those with a mean age of 21 years

and with at least 5 years of T1D duration [80]. In the EDC cohort (mean T1D duration of 25 years), prevalence of neuropathy was lowest in participants aged 18-29 years (18%), and increased with age, being 58% in patients >=30 years of age [81]. DPN has been associated with T1D duration, HbA1c, HDL-and low-density lipoprotein (LDL)- cholesterol [82], and smoking [81].

Other than glycemic control, there are no preventative interventions for diabetic neuropathy (DN) [83] and thus duration and level of hyperglycemia are important determinants of DN. In the DCCT/EDIC study, the prevalence of DPN increased in both the intensive insulin therapy group (9% to 25%) and the conventional therapy group (17% to 35%) over 13-14 years follow up, although it remained substantially higher in the latter throughout the follow-up period [84].

Cumulative incidence of DN in the EURODIAB Study (an observational study that included 1,172 T1D patients from 31 centers across Europe) was 23.5% over a mean follow up time of 7.3 +/-0.6 years (mean age 33.6 +/-10 years, mean diabetes duration of 14.9 +/- 8.9 years) [83]. In this study, participants who developed incident DN had higher baseline total and LDL cholesterol, higher fasting triglycerides, higher body mass index (BMI), higher albumin excretion rate (AER), and lower estimated glomerular filtration rate (eGFR) [83].

1.5.3.3 Diabetic kidney disease

Diabetic kidney disease (DKD) is characterized by metabolic changes that alter the kidney and promote inflammation and fibrosis. In early diabetes these include hyper-aminoacidemia, which is a promoter of glomerular hyperfiltration and hyper-perfusion, and hyperglycemia [85]. DKD is a progressive condition, starting with glomerular hyperfiltration, progressing to albuminuria, declining eGFR, and finally, end stage renal disease (ESRD) [85]. Identification of

declining kidney function has historically been detected via low eGFR and albuminuria. However, the natural history of DKD is changing and lack of albuminuria and low eGFR do not always precede DKD [85].

DKD is a risk factor for cardiovascular disease (CVD), ESRD, and mortality in T1D [86]. Early identification of those with increased risk of kidney complications is therefore crucial. In the Finnish Diabetic Nephropathy Study (the FinnDiane study is a nationwide Finnish study of adults with T1D diagnosed before age 40 years where T1D patients with prior incident stroke were followed for CVD events or coronary artery disease (CAD)-related death for up to 14 years after the incident stroke event), progression of diabetic nephropathy was related to increased risk of CVD and premature death [87]. The presence of microalbuminuria (urinary albumin excretion 20-200 ug/min) and macroalbuminuria (urinary albumin excretion >200ug/min) have been associated with 2.8 to 9.2 times higher standard mortality ratios in those with T1D [88]. Conversely, those with regressed albuminuria have reduced risk of CVD and premature death [87-89].

Some degree of kidney disease is universal after long duration T1D. For example, in the EDC study, by 50 years T1D duration, 88% of participants had microalbuminuria (AER 20-199), 72% macroalbuminuria (AER>=200), and 60% had end stage renal disease (dialysis or kidney transplantation) [90]. In the T1D Exchange Clinic Registry, the frequency of renal disease (defined as microalbuminuria, macroalbuminuria, glomerular filtration rate <60 ml/min, renal failure, receiving dialysis, or post-kidney transplant) in the total sample was 8% at enrollment (median T1D duration of 7 years) and increased with T1D duration to 21% at 30 years duration, and 29% at 50 years T1D duration [59].

After diabetes duration of 30 years, cumulative incidence of nephropathy (defined as AER>=300 mg/24h, serum creatinine>=2mg/dL, on dialysis, or renal transplant) in the

DCCT/EDIC was 25% in the conventional treatment group and 9% in the intensive treatment group [61]. In another study of the T1D Exchange Clinic Registry, those without evidence of kidney damage (albumin to creatinine ratio (ACR) <30mg/g creatinine and eGFR>=60ml/min) at baseline were evaluated at 5 years follow up for incident decreased eGFR or albuminuria defined as two consecutive albumin/creatinine ratios or two of the past 3 measurements >30ug/mg. Among the 3,940 participants (mean age 41 years, mean T1D duration 21 years), 17% progressed to eGFR <60 ml/min or albuminuria or both; 7% experienced albuminuria; 8% progressed to eGFR <60 ml/min; and 2% progressed to eGFR <60 ml/min and albuminuria [86]. Factors that influence progression to poor kidney function include longer diabetes duration, lower annual household income [86], smoking, blood pressure, lipids, obesity, and glycemia [91].

1.5.4 Macrovascular

1.5.4.1 Cardiovascular disease

CVD and CVD-related events are some of the most frequent long-term complications of T1D [92-94]. High occurrence of specific CVD in T1D including CAD and coronary heart disease (CHD), have been documented since the late 1970s. Unfortunately, despite intensive management of risk factors, it remains clear that T1D is associated with increased risk of CVD [95].

There is some evidence of an association between HbA1c and CVD risk. Results from a meta-analysis involving 9,123 T1D patients from 13 studies confirmed that the risk of CVD associated with each 1% increase in HbA1c was moderate and, in this case, did not reach statistical significance (RR=1.15, 95% CI 0.92-1.43) [96]. Similarly, in a 10-year follow up study from the EDC, glycemia was not found to be independently predictive of CAD in T1D, but rather, insulin resistance-related factors were [97].

Further evidence for the role of blood glucose in CVD is evidenced by decreased CVD incidence in those on intensive treatment [61, 98]. After diabetes duration of 30 years, cumulative incidence of CVD in the DCCT/EDIC study was 14% in the conventional treatment group and 9% in the intensive treatment group [61]. After 11 years of follow up in the EDIC study, despite HbA1c levels being identical by previous intervention assignment, those formerly on intensive treatment had an event rate of 0.38 per 100 PY versus 0.80 per 100 PY in conventional treatment (p=0.007) [98].

In the EDC cohort, a study evaluating CAD morbidity found that HbA1c was predictive of CAD morbidity during a mean follow up of 15 years (mean age 33.1 years, mean T1D duration 24.8 years); per each 1% increase in baseline HbA1c, unadjusted relative risk of non-fatal CAD was 1.15 (95% CI: 1.01-1.30) [99]. Similar findings persisted in the EDC cohort with increasing follow up time: during 25 years of follow-up, each 1% increase from baseline HbA1c was associated with 1.26-fold risk of CVD (95% CI 1.07-1.45), and after adjustment for baseline factors this moderate association persisted at 1.13-fold increased risk (95% CI 0.99-1.32) [100].

Not surprisingly, as age in years and T1D duration increase, risk of CVD increases in T1D [101]. Absolute risk of major CHD events in the General Practice Research Database (GPRD, which is a large primary care database from a network of 603 UK practices in which prescription and diagnosis data are recorded) was 7.3 in men and 5.5 in women per 1000 person years after 4.7 years follow up [94]. Incidence of CVD in T1D for those without any prior CVD is as high as 46% after 25 years follow up [100]. The EURODIAB study reported 7 year follow up incidence of 9% for total coronary events (baseline mean age of 38.1 years and mean T1D duration of 19 years) [102]. At 10 years follow up, the EDC reported incidence of total coronary events of 16% (mean age at

baseline of 33 years and T1D duration of 24.9 years) [97]. After another 8 years of follow up (18 years total follow up) CAD incidence increased to 29.5% in men and 25.5% in women [103].

Risk of occurrence of a second CVD event increases with longer T1D duration. By 14 years follow-up in the FinnDiane study, 72% of participants, who had experienced a prior event, had a second CVD-related event [104]. In shorter term follow up, the EURODIAB reported that of those with a prior CVD event, 176 participants (~8% of the 2,181 total) had a second CVD event during a median of 7.3 years of follow up [105].

CVD occurs earlier and more often in those with T1D than those without T1D [92-94]. Those with T1D have an overall 10-fold greater risk of CHD mortality compared with the general US population [106]. By 55 years of age, those with T1D have 6-fold greater (35%) cumulative CAD mortality rate compared to the expected rate from Framingham study (4% for women, 8% for men) [107]. Mortality in WESDR from all causes was 7.5 times more than expected from the general Wisconsin population (baseline mean age 28.4 years, baseline mean T1D duration not reported, study recruited 25% of sample with 0-14 years duration and remaining with +15 years T1D duration) [108]. WESDR also reported a standardized mortality ratio (SMR) from ischemic heart disease of 9.1 (for men) and 13.5 (for women) for those with a diabetes diagnosis before 30 years of age (mean age of 28.4 years in those with T1D diagnosis before age 30, and 66.7 years in those diagnosed with T1D after age 30) [108]. Recent mortality estimates from the EDC study indicate that CVD mortality SMR ranged from 19 to 33 suggesting an increase in CVD events in this cohort of long-standing T1D [109].

1.5.4.2 Coronary artery calcification

Coronary artery calcification (CAC) is a marker of atherosclerotic plaque that is likely to result in impaired cardiac function [110] and excess mortality [111]. In adults with T1D, the Coronary Artery Calcification in Type 1 Diabetes study (CACTI study, a cohort of T1D patients aged 20-55 years old diagnosed before age 30 and non-diabetic controls all with asymptomatic coronary artery disease) found that in men the prevalence of CAC for those with T1D was 48% and 39% in controls; for women, in those with T1D the prevalence was much lower (27%), and controls even lower (12%) [112]. This study also found that glycemia is a risk factor for CAC progression. During the 4-year follow-up of CACTI study participants, those with HbA1c values >7.5% had a 7-fold greater progression of CAC compared with those with lower HbA1c levels [113]. Other risk factors for CAC include diabetes duration [113], BMI [113], low plasma adiponectin levels [111], cholesterol [112], and gender [112].

Early vascular ageing may predict incident CAD in those with T1D. in the EDC study, the risk of CAD increased with increasing systolic blood pressure (SBP), DBP, pulse pressure, and arterial pressure [114]. Significant findings were observed for cognitive impairment and CAC in the EDC cohort: participants with greater CAC burden at EDC baseline (1986-88) was associated with cognitive impairment 14 years later [115].

1.5.4.3 Stroke

Stroke is another vascular issue heightened in persons with T1D. The traditional clinical definition of stroke is an acute-onset focal neurological deficit persisting for more than 24-hours [116]. There are two major types of stroke: ischemic (insufficient blood supply to a portion of the

brain) and hemorrhagic (blood vessel rupture [116]). Ischemic stroke accounts for about 85% of all T1D strokes, and the other 15% are hemorrhagic [116].

People with T1D have 3 to 4 times the risk of ischemic and hemorrhagic stroke compared to the general population [117-119]. In the Nurses Heath Study (NHS, an ongoing cohort established in 1976 of 121,701 female registered nurses aged 30-55 who completed a mailed questionnaire and are followed biennially) the risk of hemorrhagic stroke was almost four times higher in those with T1D than those without T1D (relative risk 3.8, 95% CI 1.2-11.8) [120]. In the course of 15 years of follow up in the EDC study, 31 strokes (4.7%) occurred in participants who had no prior stroke history [118]. In WESDR, adjusted cumulative incidence of stroke was 5.9% over 20 years follow up time [121] and total CHD/stroke incidence was 8.6% over 5.7 years follow up time [122]. In the FinnDiane study, 32% of participants had a recurrent stroke during the 13 year follow up [104]. In a study of African Americans (mean age 27.5 +/-10.8, mean diabetes duration of 10.4 +/- 8.6 years) with T1D in New Jersey who were hospitalized and discharged with T1D diagnosis, 3.1% experienced stroke over 6 years follow up as reported via self-report and confirmed with medical records [123].

Factors that relate to incident stroke include older age and longer diabetes duration [118]. Survival after ischemic stroke is predicted by SBP, non-HDL, white blood cells (WBC), and pulse [118]. In the DCCT study, it was found that intensive diabetes treatment reduced the risk of stroke, nonfatal myocardial infarction, or death from CVD by 57% [98].

1.5.4.4 Peripheral arterial disease

Peripheral arterial disease (PAD) refers to partial or complete obstruction of the peripheral arteries and occurs when blood vessels narrow and reduce blood supply to the tissues in the lower

extremities [124]. In T1D, arterial calcification predicts renal and cardiovascular mortality, and lower extremity arterial calcification is associated with CAD [125]. Non-severe PAD is typically asymptomatic and estimates of prevalence and incidence are difficult to establish. In its most severe form, PAD results in lower extremity amputation; these events are simpler to estimate.

Glycemia in individuals with T1D increases the risk of lower extremity amputation [124, 126]. An incremental increase of 1% in HbA1c in the WESDR study was associated with 1.39 times the odds of incident amputation in those diagnosed with T1D before the age of 30 years (95% CI: 1.22-1.59, mean age of 27.9 years at baseline, mean diabetes duration of 14.4 years at baseline) and 1.25 times the odds in those diagnosed after the age of 30 years (OR: 1.25, 95% CI: 1.09-1.43, mean age of 64.4 years at baseline, mean diabetes duration of 10.9 years at baseline) [127]. In a Swedish cohort the cumulative probability of lower extremity amputation by age 65 was 11% for women and 20.7% for men (mean age of 19.7 years at baseline and mean study follow up of 12.5 years) [128]. In the same cohort, standardized incidence ratios (ratio of observed events to the expected number of events) of lower extremity amputations were as high as 85.8 (95% CI 72.9-100.3) [128].

Predictors of lower extremity amputation include age [82, 129], T1D duration [82, 129], waist to hip ratio [82], total cholesterol [82], LDL [82, 129], non-HDL, white blood cells, blood pressure, AER, heart rate, microvascular complications, smoking, and eGDR [129]. Being male, T1D duration, autonomic neuropathy all independently predicted odds of lower extremity arterial calcification in the EDC study [125].

1.6 Mortality

Prior to the 1940s mortality in T1D was common and occurred 1-2 years after diagnosis, often due to acute complications [130]. In the past 40 years, survival for those with T1D has improved dramatically, increasing life expectancy overall. In the EDC study, life expectancy from birth increased by about 15 years for those diagnosed between 1965-80 (life expectancy of 68.9 years) vs those diagnosed 1950-64 (life expectancy of 53.4 years) [131]. Reductions in all-cause, CVD and renal mortality were further observed in those diagnosed after 1970 compared with those diagnosed between 1965-69 [75]. This increased life expectancy is likely partly attributable to improved glucose monitoring and control and a resulting decline in complications [131]. The exact causal process and mechanism are unclear beyond the known development of kidney and cardiovascular complications. Despite these encouraging data and advances in treatment, individuals with T1D have an excess risk of mortality compared to the non-diabetic population [132]. Thus, findings from the EDC study suggest that people with T1D continue to have an average of 4.6 life years lost compared to the general population [131]. Further, compared to those without T1D, it is estimated that there is a 3 to 18-fold excess risk of death in T1D [132].

Today, both acute and chronic complications account for nearly all the excess premature mortality in T1D, particularly CVD [99, 121, 130, 133]. The FinnDiane study found that 53% had died of a CVD or diabetes related cause [104]. Although CVD is one of the major drivers of mortality in T1D, higher mortality risk has also been associated with history of hypoglycemia, albuminuria, renal insufficiency [134], and hyperglycemia [135].

While studies have documented that there is increased mortality in T1D, there is evidence that this is driven by renal disease. In the FinnDiane Study, individuals with normo-albuminuria

have an equivalent mortality to that of the general age and sex matched Finnish population over a 7 year follow up [88]. The EDC study confirmed these findings indicating that in the absence of renal disease the 20-year mortality risk in T1D is similar to the general population [89]. Interestingly, those with normo-albuminuria died to non-diabetes related causes compared to those with renal disease who primarily died from diabetes related complications [89].

1.7 Advanced glycation endproducts

AGEs are a heterogeneous group of compounds which accumulate in plasma and tissues [136]. They are formed as a result of non-enzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids [137]. Although the chemical nature of AGEs is not yet well defined, they are known to be products of glycol-oxidation, and the formation of AGEs occurs in everyone as a natural part of ageing and normal metabolic processes.

1.7.1 AGE accumulation in T1D

Despite being an essential part of normal physiology, an increased production and accumulation of AGEs may promote cellular destruction. There is emerging evidence that AGE (especially those that deposit in long-lived proteins) cause damage and may contribute to chronic complications in diabetes. Thus, AGE accumulation is particularly harmful; it is estimated that those with T1D have three times greater accumulation of AGE than those without T1D [138, 139]. This harmful accumulation be partly due to declining kidney functioning and inability to properly clear AGEs [140, 141]. Since the kidneys play a role in processing and clearing AGEs [142, 143], it has

been speculated that declining kidney functioning with increased T1D duration may be involved in a deficit in AGE detoxification [144] which may contribute to AGE accumulation [138, 139]. Further, AGEs also contribute to the development of chronic kidney disease [145].

1.7.2 AGEs and long term T1D

AGEs have been shown to have an important role in the oxidative damage of proteins [146], contributing to T1D complications [144, 147-149]. In patients with T1D, several studies have concluded that collagen-linked fluorescent AGEs are cross-sectionally associated with factors such as HbA1c [1-3], AER >30 mg/24hr [1], CAC [1, 150] and distal symmetrical polyneuropathy (DSPN) [151].

AGEs have also been associated with increased likelihood of adverse neurological outcomes [151, 152]. AGE accumulation within myelin is thought to corrupt nerve conduction and ultimately interfere with nerve functioning [151]. The deposit of AGEs in the peripheral nerves can also lead to neural lesions [152]. In the DCCT study, the AGE concentrations were higher in the skin biopsies of participants who had neuropathy [148]. Further, high AGEs were associated with a greater likelihood of autonomic neuropathy and confirmed distal symmetrical polyneuropathy (CDSP) [151].

Studies have also identified associations between AGEs and retinopathy. In one study of collagen-linked AGE in T1D patients, associations were seen between AGE and retinopathy (OR=6.77, 95% CI=1.33-34.56) as well as between AGE and early nephropathy (OR=13.44, 95% CI=2.00-93.3) [153]. In another study with Japanese patients with T1D, AGE levels assessed with skin auto-fluorescence (in arbitrary units, AU) were significantly higher in patients with diabetic retinopathy compared to those without (2.21AU +/-.0.8AU vs. 1.97AU+/-0.06AU) [139]. In WESDR, AGEs were significantly associated with PDR [154].

Several deleterious mechanisms of AGE are linked to CVD events in T1D, including progression of CVD [155]. Significant correlations have been found between AGE and arterial stiffness (r=0.41) and SBP (r=0.42) and DBP (r=0.36) [138]. Having high AGE levels over time have been associated with a three-fold increased risk of incident CVD events (HR 4.13, 95% CI 1.30-13.07) [156]. AGE contribute to the chronic tissue damage seen in T1D through intracellular glycation in abnormal and stable cross links on collagen with glucose. It is hypothesized that glycated LDL binds with collagen and contributes to vessel occlusion. Glycated HDL can also cause reduction in cellular antioxidant capacity. The glycation of lipids in people with T1D is hypothesized to lead to abnormal formation of foam cells and promotion of atherosclerosis [157]. These chemical and physical modifications can explain some of the chronic tissue damage seen in diabetes such as abnormal vascular rigidity and arterial stiffness [136].

AGE levels have also been moderately associated with CAC. In the DCCT/EDIC study, each unit change in SIF score predicted an expected change of +1.07 in CAC score (β=1.07 +/- 0.33) [1]. The EDC study identified a 2.5 greater likelihood of CAC for each unit change in AGE as assessed with a SIF score (OR 2.51, 95% CI 1.37-4.59) [150]. Also, in the EDC, AGE and CAD were significantly associated (OR 3.5, 95% CI 2.1-6.1) [158].

1.7.3 AGEs and mortality

AGEs are associated with ageing [4], senescence, development of age-related morbidities [5], and mortality [6-8]. The production of altered proteins from glycation is one of the most common hallmarks of ageing and increased risk of death [5, 159]. Even though scientific understanding is incomplete, production of detectable AGEs has been implicated in ageing in general [159, 160] through promotion of protein modification, cellular stiffness, organ dysfunction, vessel rigidity, bone

fragility, tissue damage, and muscle stiffness, and oxidative stress [161]. AGEs are also linked to several signs of declining cognitive health including the pathogenesis of Alzheimer's disease and cognitive impairment [161].

Two research centers have evaluated AGEs in relation to mortality in T1D (see Table 1-1) [6, 8, 162]. In Nin et. al 2010 & 2011, 169 T1D patients in Denmark were followed over a median of 12.3 years during which 82 of the patients died [6, 162]. Both the receptors for AGE [162] and plasma AGEs [6] were significantly associated with all-cause mortality. In a study by Meerwaldt et al 2007, 48 T1D patients, 69 T2D patients, and 43 control participants were recruited from a diabetes outpatient center in The Netherlands and assessed with the AGE Reader (Diagnoptics Technologies B.V., Groningen, The Netherlands) [8]. The AGE Reader assesses collagen-linked AGEs with a skin autofluorescent (SAF) score [163, 164] and has been previously associated with end-organ complications in T1D [139, 165-167]. During a 5 year follow up, there were 11 CHD mortality events in the T1D group [8]. Univariate analysis in the T1D group indicated that age, mean A1c, creatinine, HTN, hemodialysis treatment, triglycerides, smoking, CHD at baseline, and SAF were significantly associated with CHD-mortality [8]. These covariates were further tested for effect on CHD-mortality in Cox regression models that included SAF. SAF scores were independently significantly associated with CHD-mortality; SAF was stronger than age, mean A1c, triglycerides, and smoking as an independent predictor of CHD-mortality but not hemodialysis treatment or CHD at baseline [8].

Table 1-1 Key studies reporting on AGEs and mortality in T1D

Reference	Study population	Study design	Covariates	AGE assessment	Key findings
Meerwaldt 2007 [8]	48 T1D patients from diabetes outpatient center in The Netherlands Mean age 45±15 years and mean diabetes duration of 20±11 years at baseline	Case control longitudinal observational study over 5 years follow up to observe incident CHD mortality	Age, mean A1c, T1D duration, creatinine, HTN, microalbuminuria, hemodialysis treatment, triglycerides, LDL, BMI, smoking, CHD at baseline	AGE Reader SAF score	11 mortality events over 5 years in T1D SAF was independently associated with CHD mortality (OR= 6.0, 95% CI 2.5-14.2, p<0.001) Association persisted in multivariate model (OR=2.0, 95% CI 1.3-2.7, p<0.01) adjusted for covariates
Nin 2010 [162]	169 T1D patients with diabetic nephropathy (DN)[a] and 170 T1D patients with normo-albuminuria[b] from the outpatient Steno Diabetes Center in Denmark Mean age of deceased 45.5±9.9 years, mean diabetes duration of 30.3±10 years	Case-control longitudinal observational study over 13 years (1993-2006) to observe incident fatal and nonfatal CVD and all-cause mortality	Model 1. Age, sex, A1c, case control status, T1D duration Model 2. Model 1+ mean arterial pressure, smoking, total cholesterol, renin-angiotensin aldosterone system inhibitors/antihypertensive treatment (RAAS), baseline medication, eGFR	Plasma sRAGE	82 mortality events over 12.3 years median follow up sRAGE was associated with all-cause mortality per unit increase in baseline level of log-sRAGE: 1. HR: 2.4, 95% CI 1.5-4.07, p=0.001 2. HR: 1.9, 95% CI 1.1-3.31, p=0.02

Reference	Study population	Study design	Covariates	AGE assessment	Key findings
Nin 2011 [6]	169 T1D patients with DN[a] and 1740 T1D patients with normo-albuminuria[b] from the outpatient Steno Diabetes Center in Denmark Mean age of deceased 45.5±9.9 years, mean diabetes duration of 30.3±10 years	Case-control longitudinal observational study over 13 years (1993-2006) to observe incident fatal and nonfatal CVD and all-cause mortality	Model 1: age, sex, A1c, case control status, T1D duration Model 2: model 1 + BMI, mean arterial pressure, smoking, total cholesterol Model 3: model 2+ RAAS inhibitors	Plasma AGE	82 mortality events over 12.3 years median follow up AGEs were associated with incident all-cause mortality, per each 1 SD unit increase in AGE: 1. HR: 1.3 (95% CI 1.1-1.7, p=0.013) 2. HR: 1.3 (95% CI 1.1-1.7, p=0.013) 3. HR: 1.4 (95% CI 1.1-1.7, p=0.011)

[a]DN defined as persistent macroalbuminuria [>300 mg/24 h] in at least two out of three previous consecutive 24-h urine collections, in the presence of diabetic retinopathy, and in the absence of another kidney or urinary tract disease

[b]normo-albuminuria defined as urinary excretion rate <30 mg/24 h

1.8 Technology in T1D

Ongoing patient self-management, diabetes education, and clinical support are key to reducing the risk of long-term complications of T1D. Without adequate and appropriate oversight, the maintenance of blood glucose is challenging, if not impossible. Daily therapy for people with T1D includes self-monitoring of blood glucose levels, identification of the correct type of insulin and dose needed, and self-administration of the appropriate insulin type and dose [14]. Significant technological advancements in T1D devices benefit patients in these daily therapy-related decisions and actions.

For several years in the 20th century, T1D patients were bound to the clinic for both glucose testing and insulin administration. By the mid-1960s, patients could monitor and treat themselves without the need to travel to a medical clinic. This was possible with the development of finger stick technology and insulin and syringes they could take and administer at home outside the clinic. Self-monitoring of blood glucose began in the 1980s when insulin pumps worn by the user were available to deliver accurate insulin doses with the push of a button. Few, however, used these devices at that time due to availability, clinical understanding, and cost. With increasing ability to self-monitor and treat outside the clinic setting the technology to support T1D patients has become an essential component of daily monitoring and treatment.

T1D technology today is breaking new ground with the establishment of the CGM and the artificial pancreas. The CGM automatically and constantly measures blood glucose levels and alarms the user when blood sugars are out of range. Insulin can be self-administered via injection and/or through insulin pumps worn by the user. In 2016, the first 'artificial pancreas' was approved

by the US FDA. This device continuously monitors glucose via a closed loop system that automatically adjusts the delivery of long-acting or basal insulin based on blood sugar levels [168].

Technology used by medical practitioners in the clinical setting is important in T1D care. This technology is designed to capture clinical characteristics of the patient with the intent to identify complications and optimize patient quality of life [14]. In the past decade, considerable attention has been given to making clinical knowledge available 'just in time' to the physician. However, just in time clinical tests are not yet regularly applied in T1D; clinicians rely on blood samples for HbA1c, fasting plasma glucose, two-hour plasma glucose oral glucose tolerance tests, and symptoms of hyperglycemia to diagnose a patient [169]. Most of these tests require laboratory assays to be performed and results are not immediately available.

1.8.1 The SCOUT DS® device

Historically, accurate and reliable ways to measure type and amount of AGE included blood and urine samples, and skin biopsies. Some types of AGEs (i.e., collagen-linked fluorescent AGE) can be assessed with non-invasive light technology [9]. The process takes a few minutes, is non-invasive, and has no known side effects [10].

Two devices are designed for this purpose: the SCOUT DS ® by RISE Life Science, Corp., and the AGE Reader by DiagnOptics Technologies BV. Participants place their forearm on the device and light is shone on the volar side of the forearm approximately three inches from the elbow. After the reading, a score is produced that represents fluorescence, of which some is emitted by presence of collagen-linked AGEs. These two devices employ different type and wavelength of light. Briefly, the AGE Reader employs SAF and emits ultraviolet-A (UV-A) light with a peak wavelength of 360-370 nm. Reflected light is measured with photodiodes. SAF is

calculated as the ratio of excitation light (300-420 nm) to emitted light (420-600 nm) and is reported as a score in AU [170]. In contrast, the SCOUT DS® employs intrinsic fluorescence and emits light emitting diode (LED) light. Skin fluorescence is excited with a LED centered at 375nm (LED1) and detected at 435-655nm using the 0.5 mm source/detector spacing of the channel 2 optical probe. Skin reflectance is measured with excitation LED and broadband LED. These values are used in an intrinsic correction equation (to compensate for distortion of raw fluorescence by skin absorption and scattering) where F is fluorescence and ג is the emission wavelength:

$$f(ג) = \frac{\mathrm{F}(ג)}{R_x^{kx} R_m\,(ג)^{km}}$$

Fλ is divided by reflectance values at the excitation and emission wavelengths, R_x and R_m (λ). The reflectance values are then adjusted by the dimensionless exponents, k_x and k_m where k_x =0.6 and k_m =0.2. The resulting intrinsic fluorescence, (fλ), is integrated over the 441 to 496nm spectral region to produce an overall SIF score in AU. The measured fluorescence is quantified as a SIF score.

The focus of this dissertation work is SIF scores collected with the SCOUT DS ®. SIF scores produced by the SCOUT DS® device have been found to be correlated with abnormal glucose tolerance, fasting plasma glucose, and A1c tests. The benefits of the SCOUT DS® over these tests are that it does not require overnight fasting, blood draw, or waiting time for laboratory results [11].

The key body of literature regarding the SCOUT DS® device SIF scores and T1D clinical factors includes analysis from 11 articles (Table 1-2). The EDC and DCCT/EDIC studies were the first to evaluate SIF in T1D. These studies identified SIF score associations with CAC [1, 150], autonomic neuropathy and CDSP [151], CAD [158], glycemic exposure over a mean of 16.6 years [2, 3] age, eGFR, smoking status [2], AER [1], and caffeine intake [171]. In addition to this, a locus on

chromosome 1 [172] and the NAT2 acetylator status tag single nucleotide polymorphism (SNP) have been associated with SIF scores in participants in the DCCT/EDIC study [173]. Further, at Children's Hospital New Orleans, SIF scores were associated with age, diabetes duration, and sex (SIF was higher in boys) [174]. And in the WESDR study, SIF was associated with proliferative diabetic retinopathy [154].

Table 1-2 Studies reporting on the relationship between SIF and T1D

Reference	Study population	Study design	Covariates	Associations with SIF
Conway 2010[150]	105 participants from the EDC study of childhood onset T1D, mean age 49, mean diabetes duration 40 years.	Cross sectional	CAC calculated with isotropic interpolation from electrocardiogram signals.	Each SD change in SIF associated with 2.5 greater likelihood for prevalence of CAC.
Conway 2011[151]	111 participants from the EDC study of childhood onset T1D, mean age 49, mean diabetes duration 40 years.	Cross sectional (SIF assessed 2 years after clinical factors)	Autonomic neuropathy defined as electrocardiogram abnormal heart rate response to deep breathing expiration to inspiration ratio of <1.1. CDSP defined as presence of two or more: symptoms, sensory/motor signs, tendon reflexes.	Each SD change in SIF associated with 2.6 greater likelihood of AN. SIF was higher in those with CDSP and AN.
Felipe 2011[174]	110 children aged 5-20 years with T1D of at least 1-year duration attending diabetes clinics at Children's Hospital New Orleans. Mean age of 13.2, mean diabetes duration of 5.9 years.	Cross sectional	T1D duration, age, and sex were collected at the visit. HbA1c, blood glucose levels were extracted from medical records. Hemoglobin glycation index (HGI) was calculated as difference between observed HbA1c minus HbA1c level predicted from the patients observed blood glucose based on population regression of HbA1c on blood glucose.	SIF scores were significantly associated with mean HbA1c. Significant correlations between SIF and mean blood glucose, mean HGI, mean HbA1c, age, diabetes duration. SIF levels increased with age and were higher in girls than boys.
Conway 2012[158]	172 participants with mean age 48, mean diabetes duration 36 years. 112 participants from the EDC study of childhood onset T1D with mean follow up of 18 years. 60 participants from MDC participants with a mean of 10.3 years T1D care.	Cross sectional	CAD defined as history of myocardial infarction, revascularization, or stenosis >50%.	Significant association with CAD in men but not women. Each SD change in natural log SIF was univariately associated with 3.5 greater likelihood of CAD, 1.7 greater likelihood after adjustment.

Reference	Study population	Study design	Covariates	Associations with SIF
Aroda 2013[3]	172 participants with T1D from the EDC study and MDC study. Mean age of 48.9 years, mean diabetes duration of 36.1 years.	Cross sectional	Blood samples were assayed for HbA1c. Mean HbA1c was calculated by taking the sum of an individual's HbA1c values over the duration of follow-up and dividing by the number of HbA1c measurements taken for that individual. Average HbA1c years was 16.6.	SIF correlated with most recent HbA1c, long-term mean HbA1c. SIF was significantly associated with mean HbA1c (0.065, 95% CI 0.033-0.097) after adjustment for age, duration, recent creatinine, site. SIF was significantly associated with most recent HbA1c (0.038, 95% CI 0.014-0.062) after adjustment for age, duration, recent creatinine, site.
Orchard 2013[175]	1,185 participants from DCCT/EDIC follow up visit in 2010-11. Mean age 51.5 years, mean diabetes duration of 29.8 years.	Cross sectional	AER was measured using a timed 4-hr. urine collection and expressed per 24 hr. Total glycemic exposure (mean HbA1c) was calculated as: (pre-DCCT: DCCT eligibility HbA1c * duration of diabetes at study baseline) + (DCCT mean HbA1c * years of follow-up in DCCT) + (EDIC mean HbA1c * years of follow-up in EDIC). CAC was detected with multi-slice or electron beam computed tomography, with of present/absent thresholds of CAC>0 and CAC>200 Agatston units from testing done in EDIC year 12. CAN was assessed via sinus arrhythmia.	Statistically significant associations between SIF and AER >30mg/24 hr. and CAC after adjustment for mean HbA1c. Associations were weaker in DCCT intensive group, and insignificant after adjustment. Associations in the conventional group remained for CAN, AER >30mg/24 hr., and CAC after adjustment.

Reference	Study population	Study design	Covariates	Associations with SIF
Cleary 2013[176]	1,185 participants from DCCT/EDIC follow up visit 16/17. Mean age 51.5 years, mean diabetes duration of 29.8 years.	Cross sectional	Demographics and smoking were self-reported. Mean HbA1c was calculated by taking the mean of the HbA1c values of the given time period. Total mean HbA1c was calculated by summing (DCCT/EDIC eligibility HbA1c*duration of diabetes at study baseline), (DCCT mean HbA1c*years of follow-up in DCCT), and (EDIC mean HbA1c*years of follow up in EDIC) and dividing by total duration of diabetes. eGFR was calculated from serum creatinine using the CKD-Epi equation. Categorical variable for eGFR was defined as <60mL/min/m^2. Skin tone was measured by the SCOUT device and was calculated by summing light reflectance.	Log SIF correlation increased with mean HbA1c as the time period increased. In multivariate analyses log SIF was significantly associated with mean HbA1c, age, estimated glomerular filtration rate <60mL/min/m^2, smoking status, skin tone, and clinic latitude <37° N.
Eny 2014[173]	1,082 participants from the DCCT/EDIC study at the 16/17 follow up exam. Participants were between 35-67 years of age.	Cross sectional	Genome-wide association study (GWAS) data	SIF is significantly associated with NAT2 acetylator phenotype.
Eny 2015[171]	1,441 DCCT/EDIC participants participating in the year 16/17 follow up visit.	Cross sectional (SIF assessed 4 years after food diary)	Diet in the DCCT was assessed from years 1983-93 with a modified Burke-type diet history at baseline, year 2, 5, and end of study. Diet in EDIC was assessed biennially from years 1-12 (1994-2006) with the Harvard food frequency questionnaire.	Mean caffeine intake during EDIC was positively associated with SIF, and accounted for 11% of variance unadjusted, and 3.8% after adjustment. Caffeine intake during DCCT was consistently positively associated with SIF.

Reference	Study population	Study design	Covariates	Associations with SIF
Roshandel 2016[172]	Analysis of GWAS studies of SIF on directly genotyped SNPs in T1D (DCCT/EDIC, WESDR).	Meta-analysis of genome-wide association studies	Ungenotyped autosomal single nucleotide polymorphisms (SNP) were imputed using 1000 Genomes data.	Locus rs7533564 on chromosome 1 is associated with SIF after adjustment for time-weighted HbA1c.
Klein 2017[154]	414 WESDR participants in the 32-year follow up visit (2012-14). Mean diabetes duration of 42.2 years and mean age of 55.5 years.	Cross sectional	For each eye, the maximum grade of diabetic retinopathy in any of the 7 standard photographic fields was determined for each of the lesions using the Early Treatment Diabetic Retinopathy Study (ETDRS) classification scheme.	SIF was statistically significantly associated with PDR after adjusting for diabetes duration, A1c, and fibrogen.

Acronyms: AER: albumin excretion rate; CAC: coronary artery calcification; CAD: coronary artery disease; CAN: cardiac autonomic neuropathy; CDSP: Confirmed distal symmetrical polyneuropathy; CI: confidence interval; CKD-Epi: Chronic Kidney Disease–Epidemiology Collaboration equation; DCCT/EDIC: Diabetes Control and Complications Trial /Epidemiology of Diabetes Interventions and Complications; EDC: Epidemiology of Diabetes Complications Study; eGFR: estimated glomerular filtration rate; ETDRS: Early Treatment Diabetic Retinopathy Study; GWAS: genome-wide association study; HbA1c: hemoglobin A1c; HGI: hemoglobin glycation index; MDC: MedStar Health Research Institute Diabetes Complications study; PDR: proliferative diabetic retinopathy; SIF: skin intrinsic fluorescense; SNPs: single nucleotide polymorphisms; T1D: type 1 diabetes; WESDR: Wisconsin Epidemiologic Study of Diabetic Retinopathy

1.9 Medical device regulation in the United States

All technology or medical devices approved for marketing in the US are required to establish evidence of safety and efficacy according to US FDA standards. The burden of establishing this evidence falls on the manufacturer of the new medical device. Medical devices are defined by the FDA as any equipment "*intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action… and which is not dependent upon being metabolized for the achievement of its primary intended purpose*" [177].

Each manufacturer begins the approval process by establishing a classification for the medical device. This classification is based primarily on three factors [178, 179]:

1) the perceived potential risk of harm to the patient and/or user based on the intended use of the device (i.e., the device indication);

2) availability of existing evidence; and

3) existence of a predicate device to compare the new device to.

There are also three device classifications [180]:

1) Class I is a low risk device;
2) Class II is a moderate risk device; and
3) Class III is a high-risk device.

Device classification determines the type of evidence required to evaluate safety and effectiveness of the device. Class I & II devices are evaluated by comparing the new device to an existing device and establishing substantial equivalence between the new and existing devices [178,

[179]. The existing device is typically one already marketed and manufactured for use. The FDA calls these existing devices predicate devices. Class III medical devices do not have any existing devices to compare with. Evidence to demonstrate safety and effectiveness of a Class III device is typically based on new clinical trial data.

The FDA application to request approval for a new medical device is determined by the device classification [180, 181]. Application types are listed below:

1. 510(k): a submission for Class I & II devices to demonstrate the new medical device is substantially equivalent to an existing device.
2. Premarket approval (PMA): a submission for Class III devices that includes new clinical trial data of safety and effectiveness.
3. De Novo classification request: a submission for Class III devices that demonstrates the device should be re-classified to Class I or II.
4. Investigational device exemption (IDE): a submission to support any of the other three application types.

After a medical device is approved by the US FDA, the manufacturer must adhere to regulatory reporting requirements for production and distribution of the medical device, the details of which are beyond the scope of this document [182]. Table 1-3 displays a summary of FDA device classification, level of risk, typical evidence required to support an FDA application, corresponding application type, and an example device for the scenario.

Table 1-3 Example medical device classifications according to the FDA application process

FDA classification	Perceived device risk based on class	Evidence to support FDA application	FDA request/ application type	Example device
I & II	Low & Moderate	Existing device to compare with	510(k)	I: Pacemaker charger with predicate device II: Blood pressure alarm with predicate device
III	High	Existing device to compare with and also need to collect new clinical data	PMA	Replacement heart valve
III re-classified to I or II	Undetermined	Request for re-classification	De Novo	SCOUT DS®
An investigational device	Undetermined	Investigational device is used in a clinical study to collect safety and effectiveness data	IDE	Use of device in a clinical trial to evaluate safety and effectiveness of device

1.9.1 SCOUT DS® global approval status

Globally, the SCOUT DS® device has been tested in 20 clinical studies and is approved in 33 countries as a screening tool for diabetes [183]. However, in the US, the SCOUT DS® is not approved for marketing and is currently considered a non-significant risk device; it has been historically used in clinical trials after review and approval by local institutional review board.

1.9.1.1 SCOUT DS® US approval status

In this section, a hypothetical example is provided for SCOUT DS® approval in the US. Following the methods outlined above in Section 1.9, the SCOUT DS® device classification would be based on the perceived risk of harm to the patient and/or user. First, the intended

indication would be defined. For example, the SCOUT DS® could be indicated as "a tool for identification of a patient's long-term historical glucose control and kidney function and as a way to identify mortality risk in T1D which could be particularly useful to identify patients who need more aggressive management of long-term complications".

Next, a literature review would be performed to identify any existing evidence. The SCOUT DS® has been applied in the observational setting for clinical research purposes in diabetes, however, there is little known about its performance in a prospective setting. After an indication is identified and the literature reviewed, the next step is to identify a predicate device; there is no predicate for the SCOUT DS ®. Given the lack of predicate the SCOUT DS® is automatically classified in Class III. However, the device poses little risk to the user and administrator and thus it can be re-classified to a lower risk category via a De Novo application. The De Novo is suitable when the manufacturer believes that the device is not truly a high-risk Class III device and should be re-classified from Class III to Class I or II.

The evidence demands for a De Novo request are high. For this approach, prospective research performed in the US would be necessary to support the De Novo request. The existing body of literature and evidence regarding SCOUT DS® and T1D demonstrates its ability to correlate with some health factors (such as those mentioned above, e.g. A1c, eGFR) in the research setting. This type of data supports the external validity real-world application of the device but is clearly not adequate for demonstrating the accuracy and predictive ability of SIF scores. Prospective clinical trial data, beyond what is currently available, would be necessary to demonstrate the safety and effectiveness of the SCOUT DS®.

1.10 Summary

The existing cross sectional research provides an overall snapshot of the relationship between SIF scores with T1D clinical factors and AGEs with T1D clinical factors. However, because data are primarily evaluated at one time point, it is difficult to infer temporal associations between an exposure and outcome. The gaps in the T1D literature are primarily based on the lack of prospective data regarding predictors of SIF and the role of SIF in mortality risk. It is hypothesized that traditional renal disease risk factors, including T1D related risk factors and complications, predict SIF scores. It is further hypothesized that SIF scores may indicate mortality risk in individuals with T1D. The specific objectives of this dissertation are designed to address the literature gaps as noted.

1.11 Study aims

This research includes the following three aims:

Aim 1: Evaluate predictors of SIF score change in adults with T1D. It is hypothesized that a worse risk factor profile will be associated with increased SIF scores.

Aim 2: Evaluate associations between change in T1D complications risk markers and SIF score change in adults with T1D. In general, it is hypothesized that worsening T1D complications risk markers will be associated with increased SIF scores.

Aim 3: Evaluate the relationship of SIF scores and all-cause mortality risk. It is hypothesized that a higher SIF score will be associated with higher risk of mortality.

1.12 Study population

For all three aims, data from the 30-year longitudinal EDC study of childhood-onset T1D was analyzed. The EDC is comprised of individuals diagnosed with childhood-onset T1D at the Children's Hospital of Pittsburgh, PA, USA, between 1950-80 (Orchard, Dorman et al. 1990, Orchard, Dorman et al. 1990). Since the EDC baseline examination (1986-88), participants have been followed prospectively for 30 years (as of 2019) biennially providing medical history, lifestyle, demographic, and diabetes self-care survey information.

The SCOUT DS ® device (RISE Life Science Corp.) was administered to a convenience sample of EDC participants (n=245) between 2007 and 2014 to assess presence of collagen-linked AGE. Within the 245 SIF observations, there were 444 SIF assessments performed; it is these assessments that are used for each of the 3 aims (see Figure 1-1). Each SIF value was adjusted for age and gender based on normative values reported in people without diabetes [184].

All procedures for the EDC study were approved by the Institutional Review Board at the University of Pittsburgh. All participants provided written informed consent prior to any study procedures.

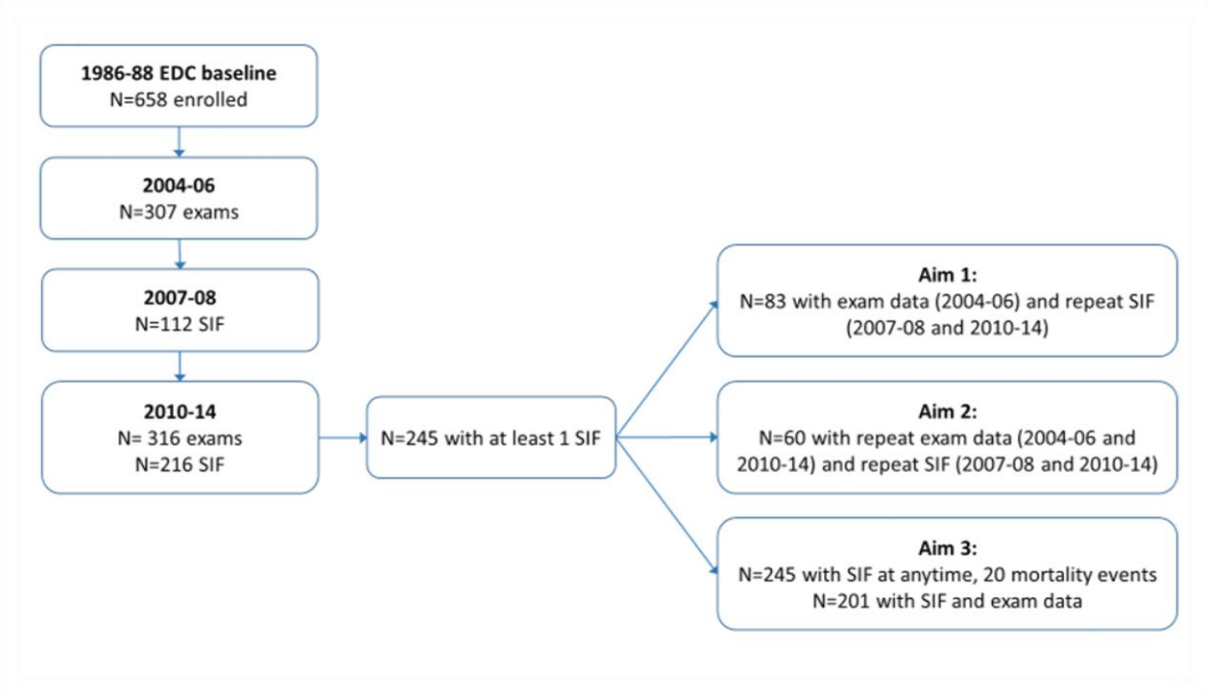

Figure 1-1 EDC cohort SIF sub-study participant flow for each research aim

2.0 Predictors of change in skin intrinsic fluorescence in type 1 diabetes: The Epidemiology of Diabetes Complications study

2.1 Synopsis

Background: SIF scores are indirect measures of AGE. SIF scores are cross-sectionally associated with T1D complications such as increased albumin excretion rate, coronary artery calcification, and neuropathy. We assessed predictors of SIF score change in those with T1D.

Methods: Data from the 30-year longitudinal Epidemiology of Diabetes Complications study of childhood-onset T1D were used to assess AGEs measured with a SIF score produced by the SCOUT DS® device. SIF scores were assessed twice in 83 participants: between 2007-08 and again between 2010-14. Regression analyses were used to assess independent predictors of SIF score change.

Results: At baseline, mean age was 47.9+6.9 years, diabetes duration was 36.7+6.4 years, and median HbA1c was 7.1 (interquartile range: 6.5, 8.5). During a mean follow-up of 5.2+0.9 years, mean change in SIF score was 2.9+2.8 arbitrary units. In multivariable linear regression models, log HbA1c ($p<0.001$), log eGFR ($p<0.001$), overt nephropathy (defined as AER >=200 µg/min, $p=0.06$), and MDI exposure years ($p=0.02$) were independent predictors of SIF score change.

Conclusion: Increases in SIF score over 5 years were related to increased glycemic levels and decreased kidney function. MDI and glomerular damage were related to a decreased SIF score. This is one of the first studies with repeated SIF assessments in T1D and provides unique, albeit preliminary, insight about these associations.

2.2 Introduction

Type 1 diabetes (T1D) prevalence is increasing in the US, affecting more children and adults each year [13, 15, 23]. Today, patients are thought to be living longer due to improved treatment and management [131]. With this increased life expectancy, however, comes the added burden of addressing chronic complications.

Emerging evidence suggests that advanced glycation end-products (AGEs) may contribute to some of these chronic complications in diabetes [150, 151, 154, 158]. AGEs are a heterogeneous group of compounds which accumulate in plasma and tissues [136]. They are formed as a result of non-enzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids [137]. Although the chemical nature of AGEs has not been fully defined, they are known to be products of glycol-oxidation in ageing and normal metabolic processes [136]. Despite being an essential part of normal physiology, an increased production and accumulation of AGEs may promote cellular damage [136].

There is preliminary evidence from cross-sectional studies of an association between HbA1c and collagen-linked fluorescent AGEs in T1D [2, 3, 175]. This evidence suggests that increased skin intrinsic fluorescence (SIF) scores are associated with increased albumin excretion rate (AER) (>30 mg/24hr) [175], coronary artery calcification (CAC) [150, 175], distal symmetrical polyneuropathy, and autonomic neuropathy [151]. However, because these data were only evaluated at one time point, it is difficult to infer temporal associations.

Historically, accurate and reliable ways to measure type and amount of AGEs included blood and urine samples, and skin biopsies. Collagen-linked fluorescent AGEs can be assessed with non-invasive light technology [185] using the SCOUT DS® device that produces a SIF score [163, 186]. The Epidemiology of Diabetes Complications (EDC) study collected SIF scores in a sub-

set of participants at two follow up time points. Using these data, our work seeks to identify independent predictors of longitudinal SIF score change. We hypothesize that risk characteristics related to disordered glucose metabolism (e.g., blood glucose control), AGE clearance, and overall cellular damage and inflammation (e.g., AER and white blood cell count) would predict SIF score increases.

2.3 Methods

2.3.1 Study participants

Participants were recruited from the EDC cohort study, a longitudinal study comprised of individuals diagnosed with childhood-onset (aged <17 years old) T1D at the Children's Hospital of Pittsburgh, Pittsburgh PA, USA between 1950-80 [187, 188]. The purpose of the EDC study is to identify characteristics associated with T1D complications. Since the EDC baseline examination (1986-88), participants have been followed prospectively for 30 years, biennially providing medical history, lifestyle, demographic and diabetes self-care survey information. Study participants also attended clinical examinations biennially up to 10 years and again at 18 years and 25 years. All participants provided written informed consent prior to any study procedures. The SCOUT DS ® device (RISE Life Science, Corp.) was administered to a convenience sample of EDC participants between 2007-08 and again as part of the 2010-14 follow-up exam to identify presence of collagen-linked AGE. Risk characteristics were selected from the 2004-06 EDC exam and the 2010-14 EDC exam. We will refer to the 2004-08 time-frame as 'analytic baseline' and

the 2010-14 exam as 'follow up'. All procedures for the EDC and the SIF sub-study were approved by the Institutional Review Board at the University of Pittsburgh.

2.3.2 Predictors

Race/ethnicity, gender, smoking status, daily number of alcoholic drinks consumed, medication use, insulin units/day, and pump use were self-reported. Height was measured using a stadiometer and weight using a balance beam scale. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist to hip ratio was calculated by dividing waist by hip circumference. Multiple daily insulin shots/pump use (MDI) was defined as either use of an insulin pump or at least three insulin shots a day. The insulin units per kg of weight were calculated.

Systolic (SBP) and diastolic (DBP) blood pressure were measured three times and the average of the second and third measures were used in analyses. Hypertension (HTN) was defined as blood pressure $\geq$140/90 mmHg or use of anti-hypertensive medications. Blood samples were assayed for lipids, lipoproteins, glycosylated hemoglobin (HbA1c), and creatinine. Stable glycosylated hemoglobin (HbA1) was measured with ion exchange chromatography (Isolab, Akron, OH) for the first 18 months [189] and with automated high-performance liquid chromatography (Diamat, BioRad, Hercules, CA) for the next 10 years. Extensive duplicate samples were run using both techniques, and no systematic differences were seen (r^2=0.95; Diamat [HbA1] = -0.018+1.00 Isolab [HbA1) [190]. After 10 years, HbA1c was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY). All HbA1 and HbA1c values were converted to Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) aligned HbA1c values by using a regression formula derived from duplicate

analyses [190]. Total cholesterol was measured enzymatically [191, 192] and low-density lipoprotein (LDL) was calculated using the Friedewald equation [193]. High density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure [194] evaluated from fasting blood samples. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL. White blood cell count was obtained using a Coulter counter.

Albumin and creatinine from three timed urine samples (24 h, overnight, and 4-h clinic) were used to calculate AER and albumin to creatinine ratio (ACR). Overt nephropathy (ON) was defined as AER>=200 μg/min. In the 10% of urine collections deemed inadequate based on creatinine excretion, and during the 2010-14 follow-up exam where AER was not assessed, an albumin to creatinine ratio (DCA Vantage System) >0.3 mg/mg was used to define ON [195]. The Chronic Kidney Disease Epidemiology Collaboration equation was used to calculate estimated glomerular filtration rate (eGFR) [196]. Impaired eGFR was defined as eGFR less than or equal to 60 mL/min/1.73m^2. Glucose disposal rate was estimated (eGDR) via an equation previously derived from hyperinsulinemic-euglycemic clamp studies of 24 study participants chosen to represent the full spectrum of insulin resistance [197].

2.3.3 Outcome

To assess SIF, participants placed their forearm on the SCOUT DS ® device and light was shone on the volar side of the forearm. Skin fluorescence was excited with a light emitting diode (LED) centered at 375nm (LED1) and detected at 435-655nm using the 0.5 mm source/detector spacing of the channel 2 optical probe (see supplemental information). Skin reflectance was measured with excitation LED and broadband LED. These values were used in an intrinsic

correction equation (to compensate for distortion of raw fluorescence by skin absorption and scattering) where F is fluorescence and λ is the emission wavelength:

$$f(\lambda) = \frac{\mathrm{F}(\lambda)}{R_x^{kx} R_m\,(\lambda)^{km}}$$

Fλ was divided by reflectance values at the excitation and emission wavelengths, R_x and R_m (λ). The reflectance values were then adjusted by the dimensionless exponents, k_x and k_m where k_x =0.6 and k_m =0.2. The resulting intrinsic fluorescence, (fλ), was integrated over the 441 to 496nm spectral region to produce an overall SIF score in arbitrary units (AU). Participants were excluded for arm tattoos, wounds, injuries, or rashes on the underside of the forearm. To remove skin care products, the forearm was cleaned prior to the scan.

A modified SCOUT DS ® device was used at the follow up time point. The modified SCOUT DS® device had a redesigned spectrometer to eliminate a ghost reflection and improve accuracy of the measurement for diabetes screening. The SIF scores from the modified device were converted for compatibility with the analytic baseline SIF scores (see Supplemental information) [198]. Collagen-linked AGE differ by gender [184] and age [199], therefore, each SIF value was adjusted for these attributes [184].

2.3.4 Statistical analysis

To assess variables related to SIF score change, analyses first focused on covariates measured at the analytic baseline. Subsequent analyses evaluated continuous variables as updated mean values from EDC study baseline (1986-88) to the analytic baseline. Non-normally distributed continuous variables were log transformed. For categorical exposure variables the years of exposure from EDC study baseline to analytic baseline were used.

The outcome variable, change in SIF score, was calculated by subtracting analytic baseline SIF score from the individual follow up SIF score.

Descriptive characteristics of participants were assessed at EDC study entry (1986-88), analytic baseline, and as updated means (continuous variables) or years of exposure (categorical variables). T-tests and the Wilcoxon rank sum test, as appropriate, were used to assess differences in continuous variables between increased and decreased SIF scores over time; for categorical variables, the Chi-Square or the Fisher's exact test was used. Pearson or Spearman correlation coefficients were used to evaluate the association between participant characteristics and change in the SIF scores as a continuous variable. Linear regression models (using `proc GLM` in SAS) were applied to assess independent predictors of change in SIF scores.

Models were obtained in a forward fashion by sequentially considering blocks of variables that represented similar characteristics. For variables that represent the same clinical factor only one of the variables was included in analysis at a time. Blocks included variables representing demographic characteristics, diabetes control, blood pressure and lipids, and kidney disease and inflammation. Subsequently, significant variables from each block were combined into one model, also allowing for the number of visits a participant attended, the time period between SIF assessments, angiotensin-converting enzyme inhibitors (ACEi) medication use, and analytic baseline SIF score. Models were limited to observations that had no missing exam data at analytical baseline. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

2.4 Results

Eighty-three participants had a SIF score assessed at both the analytic baseline (n=112) and follow up (n=189) time points (Figure 2-1).

Characteristics at analytic baseline of the individuals who had SIF scores versus those who did not were compared (see Supplemental information). Compared to those without SIF scores, those with SIF scores were older ($p<0.01$), older at age of T1D onset ($p<0.01$), had higher eGDR ($p<0.0$), lower AER ($p=0.01$), lower non-HDL cholesterol ($p=0.05$), lower SBP ($p<0.01$), fewer had ON ($p=0.04$), and fewer ever smoked ($p=0.02$); but were of similar diabetes duration ($p=0.9$); HbA1c ($p=0.06$); eGFR ($p=0.6$); and MDI exposure years ($p=0.4$).

Table 2-1 shows the characteristics of the 83 SIF study participants from EDC study entry to the analytic baseline. At analytic baseline, participants were on average 47.9 ($\pm$ 6.9) years of age, 56.6% (n=47) were female, and the average diabetes duration was 36.7 ($\pm$ 6.4) years. Most participants were Caucasian (n=81, 97.6%). The median HbA1c was 7.1% (6.5, 8.5). The majority of participants had normal or mildly decreased kidney function (defined as eGFR<60mL/min/m^2, 85.5%), and most used MDI (76.3%).

The mean SIF score for the entire sample (n=83) at analytic baseline was 4.6 ($\pm$ 4.3 AU); mean change in SIF scores was 2.9 AU ($\pm$ 2.8). Table 2-2 compares participant characteristics at analytic baseline by type of SIF score change (increase or decrease). Most characteristics at analytic baseline were similar between participants with the exception of non-HDL cholesterol and triglycerides which were both significantly lower in those with decreased SIF score ($p=0.02$, $p=0.03$, respectively).

Correlations between risk factors at analytic baseline and change in SIF scores were also evaluated. Significant correlations were observed for eGFR (r^2= -0.3, p=0.03) and updated mean SBP (r^2=0.2, p=0.06) (Table 2-3).

In multivariable models, higher HbA1c, lower eGFR, and fewer years of MDI exposure were independently associated with increased SIF scores. Conversely, ON was independently associated with decreased SIF scores. These associations are presented in detail in Table 2-4. The r-square of the final model was 26.9%. Models were limited to 70 observations with complete data on all clinical factor variables of interest at analytic baseline.

2.5 Discussion

This study aimed to identify possible predictors of change in SIF scores based on data from a longitudinal cohort of adults diagnosed with T1D in childhood. We observed that modifiable risk factors, such as worse glucose control (i.e., HbA1c) and lower kidney function were associated with increased SIF scores. Increased albuminuria in the range of overt nephropathy (AER >=200 µg/min) was associated with a decrease in SIF scores during follow-up.

The results of our work indicate that higher HbA1c is related to increased SIF scores. A connection between HbA1c and AGEs is well-known: AGEs are formed by normal physiologic metabolic processes of glycol-oxidative chemistry in reducing sugars. The higher the blood glucose levels, the more AGEs form as a result of the glycation processes. Our research findings are consistent with existing cross-sectional research from the DCCT/EDIC study where historical HbA1c was strongly correlated with AGEs [2, 3, 200].

Our findings indicate an association between longer exposure to MDI and a reduction in SIF scores. This finding was independent of HbA1c concentrations. It is possible that MDI reflects additional protective behaviors and/or factors beyond average or mean glucose exposure, for example less extreme glucose excursions, that have beneficial health effects and are reflected in reduced SIF scores.

We identified a link between worse kidney function (lower eGFR) and increased SIF scores. As the kidneys play a role in processing and clearing AGEs [142, 143], it has been speculated that declining kidney functioning with increased T1D duration may be involved in a deficit in AGE detoxification [144], which may contribute to up to three times the amount of normal AGE accumulation [138, 139]. It is also known that AGEs contribute to the development of chronic kidney disease [145].

Our study results revealed that the presence of overt nephropathy (urinary AER >=200 µg/min) was associated with decreased SIF scores (although borderline statistically significant at p=0.0552) (see Table 2-4) in multivariable analyses accounting for renal function. It should be noted that in this multi-variable finding, only 6 observations in our sample had overt nephropathy and thus these findings warrant exploration in a larger sample. It is known that the kidney filters some AGEs and that high AER reflects glomerular membrane damage which can result in greater protein leakage and ultimately bigger molecules, including AGEs, leaking into the urine. Indeed, other research has found that in patients with T1D, urinary excretion of AGEs increases with worsening albuminuria suggesting that AGEs are excreted in the context of reduced renal functioning [201]. AER is inversely associated with and is modified by eGFR. This may explain our inverse association between high AER and decreased SIF scores after controlling for the effect of reduced renal function. Thus, if eGFR is accounted for, that is if the effect of decreased AGE

clearance is removed, then those with severe glomerular damage and leakage will have greater renal loss of AGEs (high AER) and possibly decreased SIF. We were not able to find any prior work demonstrating that AER >=200 µg/min is related to decreased SIF scores after adjusting for eGFR. While these novel observations need confirmation in other studies, they raise intriguing insights into AGE physiology.

A recent study has evaluated the relationship between T1D and progression of SAF [140]. In Rajaobelina et al, the AGE Reader device assessed collagen-linked AGEs via a SAF score. While both the SCOUT DS® and AGE Reader scores reflect glycol-metabolic memory, the devices employ different type and wavelength of light. Rajaobelina et al found that among baseline variables, eGFR was associated with progression of SAF; those with mildly impaired eGFR (<90 mL/min/1.73m^2) demonstrated the highest progression [140]. In stratified analysis (eGFR <90 mL/min/1.73m^2 and/or AER $\geq$30 mg/24h vs. those without kidney impairment at baseline) in those without kidney impairment, continuous subcutaneous insulin infusion (CSII) was significantly associated with reduced SAF progression [140]. Interestingly, those treated with CSII also had a mean decrease in AGE score of -17.1% [140].

In our analysis (Table 2-4), 26.9% of the variance in SIF score change was explained by analytic baseline levels of HbA1c, eGFR, MDI, and overt nephropathy. There are likely several other factors that affect changes in SIF scores that were not identified in our research. The literature indicates that AGEs reflect broad components of a person's overall health and 'biological age' and we know that factors such as diet, smoking, environmental exposures [5], skin fluorescence/pigment, and hemoglobin levels [175] impact AGEs. Smoking exposure is related to increased collagen-linked AGE [202, 203]. However, smoking was not significantly associated with SIF score change in our work, this may be due small numbers of smokers in our sample.

This study has limitations. First, not all participants in the EDC study cohort were originally asked to participate in the SIF sub-study. Given this, there could be confounding factors in the sample of which we are unaware. However, after performing a comparison of characteristics of participants and non-participants, we found that generally there are no differences in major risk factors. The analytic sample was also small. A larger sample size may have provided us greater statistical power to clarify the relationships.

Another limitation is the technology producing the SIF score. While it is clear the SCOUT DS® assesses cross-linked fluorescent AGEs, it remains unknown which exact AGE compounds are being captured with a SIF score. In addition, the SIF score is calculated based on the amount of cross-linked AGEs per unit of collagen. Although SIF scores are intrinsically corrected to attempt to account for this, the only truly accurate way to assess collagen unit is by skin punch. Given this lack of clarity, there could be minor measurement error in the SIF dataset.

2.5.1 Conclusion

Our research reported herein is the first study assessing SIF score changes over time. Our analyses provide the first insights into possible predictors of SIF score changes in people with T1D. This work demonstrated that worse glucose control and lower kidney function were associated with increased SIF scores. Conversely, overt nephropathy, was associated with decreased SIF scores over time. Thus, our findings suggest that the mechanisms leading to AGE production and accumulation are partially driven by aspects of glucose control and kidney function. Future work in this area should focus on confirming our novel findings in people with childhood-diagnosed T1D to better understand SIFs potential in the natural history of complications.

2.6 Tables and Figures

Table 2-1 Characteristics of EDC study participants with repeated SIF assessments at 1986-88 exam, the first SIF assessment at the 2004-06 exam, and means (or exposure years) over time (N=83) [a]

Participant Characteristic	EDC Study Entry (1986-88 exam)	First SIF assessment (2004-06 exam)	Updated means or exposure years between 1986-88 to 2004-06 exams
Difference in SIF score (from 2007-08 to 2010-14)	X	2.9 (2.8)	X
SIF score (2007-08)	X	4.6 (4.3)	X
SIF score increased	X	71 (85.5%)	X
Age	27.5 (6.8)	47.9 (6.9)	X
Female	47 (56.6%)	47 (56.6%)	X
Caucasian	81 (97.6%)	81 (97.6%)	X
Age of onset	10.2 (6.1-12.4)	X	X
Diabetes duration	18.3 (6.4)	36.7 (6.4)	X
Body mass index	23.0 (2.7)	26.1 (3.9)	28.9 (3.2)
HbA1c	8.2 (7.6-9.1)	7.1 (6.5-8.5)	8.1 (7.7-8.9)

Participant Characteristic	EDC Study Entry (1986-88 exam)	First SIF assessment (2004-06 exam)	Updated means or exposure years between 1986-88 to 2004-06 exams
HbA1c months	437.3 (269.5-744.4)	981.8 (665.7-1369.9)	X
Total insulin units/weight (kg)	0.7 (0.6-0.9)	0.6 (0.5-0.8)	0.7 (0.6-0.8)
eGFR	114.1 (88.0-123.0)	80.2 (72.1-96.9)	100.1 (86.2-110.9)
Impaired eGFR (<60mL/min/m^2)	80 (96.4%)	65 (85.5%)	X
eGDR (mg/kg/min)	8.6 (7.6-9.5)	8.2 (6.4-9.8)	8.4 (7.0-9.2)
AER (µg/min)	10.2 (6.5-39.2)	5.6 (3.8-32.7)	11.8 (5.9-47.3)
Overt nephropathy (AER >=200 µg/min)	3 (4%)	6 (7.9%)	X
Total cholesterol (mg/dl)	174 (159-197)	167.5 (151-185)	179.8 (170.5-197.8)
Non-HDL cholesterol (mg/dl)	114.7 (105.4-142.3)	106.5 (91-127)	126.9 (108.4-139.5)
HDL cholesterol (mg/dl)	55.8 (13.3)	60.5 (17.6)	56.9 (13.7)
LDL cholesterol (mg/dl)	100.5 (91.5-116.2)	94 (78-112)	109.5 (97.5-121.3)
Triglycerides (mg/dl)	76 (56-95)	72.5 (46.5-89.5)	78.3 (61-105)
MDI	6 (7.5%)	61 (76.3%)	X
MDI exposure years	6 (7.5%)	X	6 (2-8)
Diastolic blood pressure (mmHg)	70 (66-77)	64 (59-71)	70.6 (64.8-74.9)
Systolic blood pressure (mmHg)	108 (101-114)	113 (102-119)	112 (105.6-119.8)

Participant Characteristic	EDC Study Entry (1986-88 exam)	First SIF assessment (2004-06 exam)	Updated means or exposure years between 1986-88 to 2004-06 exams
Pulse (beats/min)	77.1 (10.4)	73.5 (11.7)	73.5 (6.9)
White blood cell *1000/m^3	5.8 (5.1-6.9)	6.1 (4.7-6.9)	6.3 (5.6-7.3)
ACEi inhibitor medication use	3 (3.7%)	43 (53.8%)	X
ACEi inhibitor medication exposure years	X	X	4 (0-8)
Triglyceride lowering medication	X	35 (43.8%)	X
Triglyceride lowering medication exposure years	X	X	0 (0-4)
LDL lowering medication use	X	33 (41.3%)	X
LDL lowering medication exposure years	X	X	0 (0-6)
Blood pressure lowering medication	X	16 (20%)	X
Blood pressure lowering medication exposure years	X	X	0 (0-4)
Hypertension[b]	11 (13.3%)	19 (24.1%)	X
Hypertension exposure years	X	X	0 (0-6)
Total alcohol intake per day[c]	1 (0-4)	0 (0-2)	2.4 (0.4-5.5)
Smoking exposure years	X	X	0 (0-11)
Current smoker	12 (14.5%)	6 (7.6%)	X

Participant Characteristic	EDC Study Entry (1986-88 exam)	First SIF assessment (2004-06 exam)	Updated means or exposure years between 1986-88 to 2004-06 exams
Never smoked	61 (73.5%)	58 (73.4%)	X
Past smoker	10 (12.1%)	15 (18.9%)	X

[a]mean and (SD), median (25%, 75%) if at least one time-frame is nonparametric, or n (%) where appropriate

[b] defined as ≥140/90 mmHg or use of anti-hypertensive medications

[c] one serving of alcohol defined as one 12-ounce bottle of beer, 4-ounce glass of wine, or 1.5 ounce of liquor

Table 2-2 Participant characteristics at the 2004-06 exam by SIF score change status (between 2007-08 and 2010-14) in the EDC cohort [a]

Participant Characteristics	SIF score decreased (n=11)	SIF score increased (n=72)	p-value
SIF at analytic baseline (2007-08)	6.1 (4.3)	4.4 (4.3)	0.20
SIF at follow up (2010-14)	5.4 (4.5)	8.4 (5.0)	0.08
Mean change in SIF	-1.1 (0.6)	+3.6 (2.4)	<0.01
Age (2004-06)	47.1 (6.4)	48.0 (7.1)	0.77
Age (2010-14)	52.1 (6.2)	53.0 (7.1)	0.7
Female	6 (12.8%)	41 (87.2%)	--
Caucasian	11 (13.6%)	70 (86.4%)	--
Age of onset	8.6 (5.1-10.7)	10.3 (6.2-12.4)	0.39
Diabetes duration	36.9 (4.9)	36.7 (6.6)	0.63
BMI	24.6 (3.0)	26.4 (4.1)	0.18
Waist to hip ratio	0.9 (0.1)	0.9 (0.1)	0.98
HbA1c	7.1 (6.1-7.9)	7.3 (6.6-8.7)	0.27
HbA1c months	1080.5 (397.8)	1010.7 (474.6)	0.64
Total insulin units/weight (kg)	0.6 (0.1)	0.7 (0.2)	0.15
eGFR	93.3 (85.0-100.7)	78.3 (70.9-96.3)	0.09
Impaired eGFR (<60mL/min/m^2)	10 (13.9%)	55 (86.1%)	--

Participant Characteristics	SIF score decreased (n=11)	SIF score increased (n=72)	p-value
eGDR (mg/kg/min)	8.8 (1.4)	7.9 (2.3)	0.20
AER (µg/min)	7.5 (3.8-128.2)	5.1 (3.8-30.4)	0.36
Overt nephropathy (AER >=200 µg/min)	2 (33.3%)	4 (66.7%)	0.21
Total cholesterol (mg/dl)	158.5 (18.5)	171.2 (32.7)	0.08
Non-HDL cholesterol (mg/dl)	97.2 (14.1)	110.9 (29.5)	0.02
HDL cholesterol (mg/dl)	61.3 (14.7)	60.4 (18.1)	0.88
LDL cholesterol (mg/dl)	86 (16.7)	95.8 (26.5)	0.24
Triglycerides (mg/dl)	50 (45-70)	77 (52-91)	0.03
MDI	8(13.1%)	53 (86.9%)	0.72
MDI exposure years	6 (2-6)	6 (3-8.5)	0.46
Diastolic blood pressure (mmHg)	61.2 (6.7)	65.5 (10.6)	0.19
Systolic blood pressure (mmHg)	108 (102-116)	113 (102-122)	0.42
Pulse (beats/min)	73.5 (10.6)	73.5 (11.9)	0.99
White blood cell *1000/m^3	5.7 (4.4-6.3)	6.2 (5-7.1)	0.08
ACEi inhibitor medication use	6 (13.9%)	37 (86.1%)	--
ACEi inhibitor medication exposure years	4 (0-6)	4 (0-8)	0.89
Triglyceride lowering medication	7 (20%)	28 (80%)	0.20

Participant Characteristics	SIF score decreased (n=11)	SIF score increased (n=72)	p-value
Triglyceride lowering medication exposure years	0 (0-4)	0 (0-4)	0.34
LDL lowering medication use	6 (18.2%)	27 (81.8%)	0.34
LDL lowering medication exposure years	4 (0-6)	0 (0-5)	0.60
Blood pressure lowering medication	1 (6.3%)	15 (93.8%)	0.45
Blood pressure lowering medication exposure years	0 (0-0)	0 (0-4)	0.37
Hypertension[b]	1 (5.3%)	18 (94.7%)	0.28
Hypertension exposure years	0 (0-0)	0 (0-6)	0.16
Total alcohol intake per day[c]	0 (0)	0 (0-2)	0.43
Current smoker	2 (33.3%)	4 (66.7%)	0.31
Never smoked	8 (13.8%)	50 (86.2%)	ref
Past smoker	1 (6.7%)	14 (93.3%)	0.31

[a] mean and (SD), median (25%, 75%) if at least one time-frame is nonparametric, or n (%) where appropriate. T-test run for normally distributed variables, Wilcoxon rank sum for non-normally distributed variables

[b] defined as $\geq$140/90 mmHg or use of anti-hypertensive medications

[c] one serving of alcohol defined as one 12-ounce bottle of beer, 4-ounce glass of wine, or 1.5 ounce of liquor

Table 2-3 Correlations between T1D participant characteristics at the 2004-06 exam as well as means, or exposure years, over time from 1986-88 exam to the 2004-06 exam and change in SIF score in the EDC cohort (N=83) [a]

Participant Characteristic	r (p-value) at 2004-06 follow-up exam	r (p-value) for updated means from 1986-88 exam to 2004-06 follow-up exam
Age	0.16 (0.16)	0.15 (0.16)
Age of onset	0.16 (0.16)	X
Diabetes duration	0.08 (0.50)	0.06 (0.59)
BMI	0.08 (0.48)	-0.004 (0.97)
Waist to hip ratio	0.02 (0.88)	0.005 (0.96)
HbA1c	0.07 (0.53)	0.007 (0.95)
HbA1c months	0.10 (0.37)	X
Total insulin units/weight (kg)	0.05 (0.73)	-0.03 (0.79)
eGFR	-0.25 (0.03)	-0.14 (0.21)
eGDR (mg/kg/min)	-0.08 (0.50)	-0.11 (0.34)
Total cholesterol (mg/dl)	0.008 (0.94)	0.13 (0.25)
Non-HDL cholesterol (mg/dl)	0.02 (0.84)	0.08 (0.46)
Albumin excretion rate (µg/min)	-0.04 (0.72)	0.16 (0.14)
Total alcohol intake per day[b]	0.09 (0.45)	-0.03 (0.84)
HDL cholesterol (mg/dl)	-0.03 (0.79)	0.07 (0.50)

LDL cholesterol (mg/dl)	0.03 (0.79)	0.05 (0.64)
Triglycerides (mg/dl)	0.06 (0.61)	0.04 (0.72)
MDI exposure years	X	-0.19 (0.08)
Diastolic blood pressure (mmHg)	-0.01 (0.93)	0.03 (0.76)
Systolic blood pressure (mmHg)	0.17 (0.13)	0.22 (0.05)
Pulse (beats/min)	-0.04 (0.73)	0.16 (0.15)
White blood cell *1000/m^3	0.21 (0.07)	0.15 (0.18)
ACEi inhibitor medication exposure years	X	0.04 (0.72)
Triglyceride lowering medication exposure years	X	-0.16 (0.14)
LDL lowering medication exposure years	X	-0.13 (0.25)
Blood pressure lowering medication exposure years	X	0.18 (0.11)
Hypertension exposure years	X	0.16 (0.15)
[a]Pearson correlations calculated for normally distributed variables; Spearman correlations calculated for non-normal variables [b]one serving of alcohol defined as one 12-ounce bottle of beer, 4-ounce glass of wine, or 1.5 ounce of liquor		

Table 2-4 Multivariable analysis of participant characteristics at the 2004-06 exam and change in SIF score in the EDC cohort (n=70) [a]

Clinical factor [b]	Estimate	95% confidence interval limits	p-value
log HbA1c	4.99	1.44 to 8.55	<0.01
log eGFR	-3.49	-5.40 to -1.58	<0.01
Overt nephropathy (AER >=200 µg/min)	-2.44	-4.94 to 0.06	0.06
MDI exposure years	-0.18	-0.33 to -0.03	0.01

[a] multiple linear regression models were run for blocks of clinical factors in a stepwise fashion, removing one factor at a time. A p-value of p<=0.05 was the model inclusion criteria

[b] the resulting statistically significant clinical factors after all models were run adjusting for ACEi medication use, the time between SIF score assessments, SIF score at 18-year, and number of study visits attended

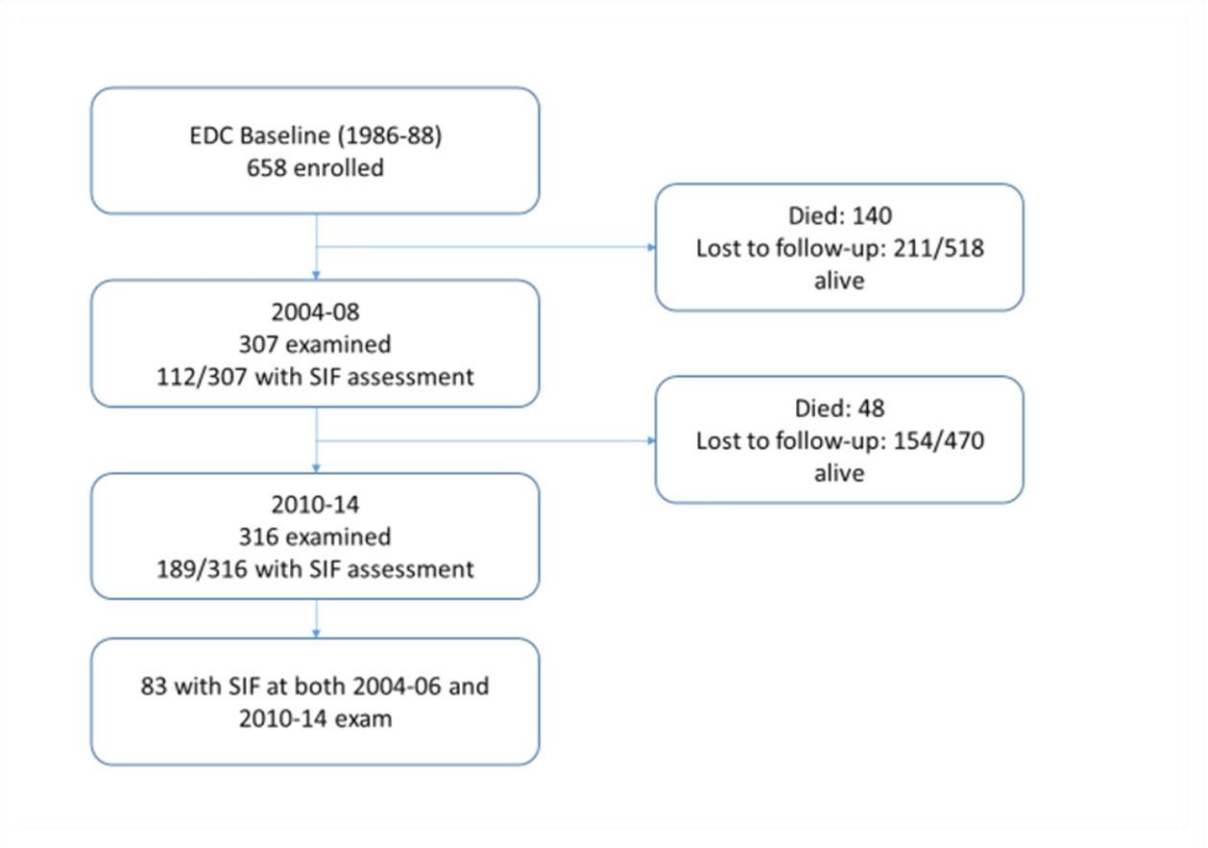

Figure 2-1 EDC cohort SIF sub-study participant flow for T1D predictors of SIF score change

2.7 Supplemental information

A conversion of SIF scores between the old and new SCOUT DS ® device was performed (as per Maynard, J, *Conversion of EDC fluor spectra SCOUT DS (R)*, 2018 [198]). SIF scores collected at 2004-06 exam were collected from two different SCOUT DS® devices. To ensure they were equivalent, we utilized the identical spectral range and measured a diffusely reflecting calibration standard on each generation of SCOUT DS to develop a linear correction to normalize the SIF measurements.

The selection of channel 2 optical probe (CH2) of the SCOUT DS ® device is further described here. The SCOUT has two optical channels that interrogate different depths of the skin. Channel 2 has a center to center spacing of the source and receiver fiber optics of 500 microns, whereas channel 1 has a spacing of 375 microns. Over the years we found that the Channel 2 spacing gave us the best results because it penetrated deeper into the dermal layer of the skin and was less susceptible to surface reflections (specular light) from the skin that did not contain useful information related to AGEs.

Table 2-5 displays the characteristics of the study participants not included in the SIF-sub study. Figure 2-2 displays the detailed study participant flow.

Table 2-5 Characteristics of study participants with SIF score (n=78 unless otherwise noted) and those without SIF score (n=229 unless otherwise noted) at analytical baseline (2004-06 exam)

Participant Characteristic	With SIF score	No SIF score	p-value
Age	47.5 (6.7)	44.9 (7.8)	<0.01
Female	44 (56.4%)	114 (49.8%)	0.3
Caucasian	76 (97.4%)	226 (98.7%)	0.6
Age of onset	10.2 (6.2-12.4)	7.7 (4.5-11.0)	<0.01
Diabetes duration	36.3 (32.2-40.1)	35.4 (30.7-43.1)	0.9
Body mass index	25.5 (22.9-28.7)	26.8 (24.2-30.6)	0.01
Updated mean body mass index	24.3 (22.5-26.9)	25.1 (23.4-27.5)	0.1
HbA1c	7.1 (6.5-8.5)	7.7 (6.8-8.9)	0.06
Updated mean HbA1c	8.1 (7.7-8.8)	8.5 (7.9-9.5)	<0.01
HbA1c months	962.9 (654.9-1359.8)	1053.9 (776.5-1401.7)	0.07
Total insulin units/weight (kg) n 45; 120	0.6 (0.5-0.8)	0.6 (0.5-0.8)	0.5
Updated mean total insulin units/weight (kg)	0.6 (0.6-0.8)	0.7 (0.6-0.8)	0.5
eGFR n 76; 221	80.2 (72.1-96.9)	80.6 (60.8-97.3)	0.6
Updated mean eGFR	99.8 (86.4-110.9)	100.2 (87.8-111.6)	0.5
Impaired eGFR (<60mL/min/m^2) n 76; 221	11 (14.5%)	52 (23.5%)	0.1
eGDR (mg/kg/min) n 76; 228	8.2 (6.4-9.8)	7.4 (5.6-8.9)	<0.01

Participant Characteristic	With SIF score	No SIF score	p-value
Updated mean eGDR	8.4 (7.3-9.2)	7.6 (6.6-8.8)	<0.01
Albumin excretion rate (µg/min) n 76; 228	5.6 (3.8-32.7)	9.2 (4.9-63.0)	0.01
Updated mean albumin excretion rate	11.6 (5.9-47.3)	17.4 (7.6-161.1)	0.04
Overt nephropathy (AER >=200 µg/min) n 76; 228	6 (7.9%)	41 (17.9%)	0.04
Total cholesterol (mg/dl) n 78; 228	167.5 (151-185)	173 (151.5-197.5)	0.2
Updated mean total cholesterol	182.8 (23.9)	185.5 (29.1)	0.7
Non-HDL cholesterol (mg/dl) n 78; 228	106.5 (91-127)	114 (92-139)	0.05
Updated mean non-HDL cholesterol	126.8 (108.4-139.2)	128.8 (109.2-148.4)	0.3
HDL cholesterol (mg/dl) n 78; 228	58 (46-73)	55 (47-68)	0.3
Updated mean HDL cholesterol	54.0 (46.3-65.9)	52.8 (46.3-62.3)	0.3
LDL cholesterol (mg/dl) n 62; 195	94 (78-112)	98 (79-124)	0.2
Updated mean LDL cholesterol	108.7 (19.3)	111.8 (26.8)	0.3
Triglycerides (mg/dl) n 68; 200	72.5 (46.5-89.5)	76 (51-107)	0.2
Updated mean triglycerides	78.1 (61-100.7)	89 (64.5-113.7)	0.09
MDI	59 (75.6%)	177 (77.3%)	0.8
MDI exposure years	6 (4-8)	6 (4-10)	0.4
Diastolic blood pressure (mmHg) n 78; 227	64.9 (10.2)	66.8 (10.9)	0.2
Updated mean diastolic blood pressure	69.8 (7.0)	70.6 (7.8)	0.4

Participant Characteristic	With SIF score	No SIF score	p-value
Systolic blood pressure (mmHg) n 78; 227	113 (102-119)	117 (107-129)	<0.01
Updated mean systolic blood pressure	111 (105.5-118.9)	113.4 (107-122.1)	0.1
Pulse (beats/min)	72 (64-84)	72 (66-84)	0.4
Updated mean pulse	73.4 (7.1)	74.1 (6.5)	0.4
White blood cell *1000/m^3 n 74; 217	6.1 (4.7-6.9)	6.2 (5.1-7.6)	0.2
Updated mean white blood cell *1000/m^3	6.3 (5.6-7.3)	6.4 (5.7-7.3)	0.6
ACEi inhibitor medication use	42 (53.9%)	124 (54.1%)	0.9
ACEi inhibitor medication exposure years	4 (0-8)	4 (0-8)	0.3
Triglyceride lowering medication	34 (43.6%)	97 (42.4%)	0.8
Triglyceride lowering medication exposure years	0 (0-4)	0 (0-4)	0.7
LDL lowering medication use	32 (41%)	98 (42.8%)	0.8
LDL lowering medication exposure years	0 (0-6)	0 (0-4)	0.9
Blood pressure lowering medication	15 (19.2%)	63 (27.5%)	0.1
Blood pressure lowering medication exposure years	0 (0-4)	0 (0-6)	0.2
Hypertension [b] n 78; 228	18 (23.1%)	77 (33.8%)	0.08
Hypertension exposure years	0 (0-6)	0 (0-6)	0.3
Total alcohol intake per day [c] n 76; 226	0 (0-2)	0	0.2
Updated mean total alcohol intake per day [c]	2.4 (0.5-5.5)	2.8 (0.8-5.6)	0.6

Participant Characteristic	With SIF score	No SIF score	p-value
Smoking exposure years n 28; 118	0 (0-10)	1.5 (0-11)	0.6
Current smoker	6 (7.8%)	27 (11.9%)	Ref
Never smoked	58 (75.3%)	138 (60.8%)	0.07
Past smoker	13 (16.9%)	62 (27.3%)	0.07
Ever smoked (vs. never) n 77; 227	19 (24.7%)	89 (39.2%)	0.02

[a] mean and (SD), median (25%, 75%) if at least one time-frame is nonparametric, or n (%) where appropriate

[b] defined as ≥140/90 mmHg or use of anti-hypertensive medications

[c] one serving of alcohol defined as one 12-ounce bottle of beer, 4-ounce glass of wine, or 1.5 ounce of liquor

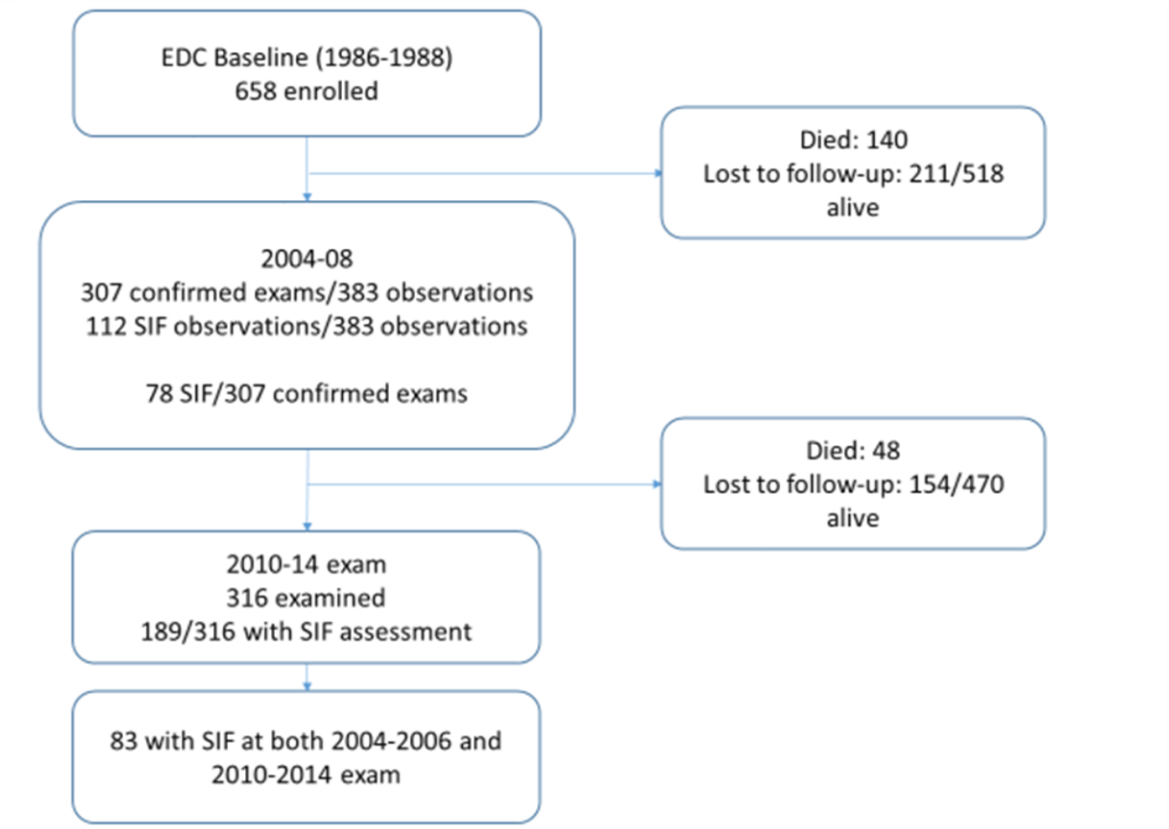

Figure 2-2 EDC cohort SIF sub-study detailed participant flow for predictors of SIF score change

3.0 Correlates of change in Skin Intrinsic Fluorescence (SIF) among individuals with type 1 diabetes in the Epidemiology of Diabetes Complications study

3.1 Synopsis

Background: Simple screening techniques can aid in identification of T1D complication risk and subsequent complication management. The deposition of AGEs may have a role in T1D complication development and their presence can be detected with non-invasive SIF. This report examines how change in diabetes characteristics correlate with change in SIF in adults with T1D.

Methods: Data from the longitudinal Epidemiology of Diabetes Complications study of childhood-onset T1D were analyzed. Sixty EDC participants had repeat SIF assessment in 2007-08 and again between 2010-14. Regression analyses were used to determine if change in T1D risk characteristics predicted change in SIF.

Results: At time of first SIF, mean age was 45.8 (+/- 6.7) years, diabetes duration was 36.6 (IQR: 33.1, 40.2) years, and median HbA1c was 7.1% (IQR: 6.4, 8.3). SIF increased in the majority of our sample (mean 2.9 arbitrary units +/- 2.9). A moderate association was identified for BMI change (β=-0.3, 95% CI: -0.6 to 0.04, p=0.09) with SIF score change.

Conclusion: Our research is the first to report on change of T1D risk characteristics and concurrent SIF change. BMI may be an important correlate with SIF change. Further work is needed to fully characterize SIF as an approach for monitoring the role of AGEs in T1D.

3.2 Introduction

Non-invasive screening techniques can assist in type 1 diabetes (T1D) risk identification and management. Skin intrinsic fluorescent (SIF) scores, a non-invasive indirect measure of advanced glycation end-products (AGEs), are one such potential tool to aid in clinical screening for T1D risk characteristics. SIF scores have been associated with T1D complications such as increased albumin excretion rate (AER), neuropathy, and coronary artery calcification.

AGEs are a heterogeneous group of compounds which accumulate in plasma and tissues [136]. They are formed as a result of non-enzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids [137]. Although the chemical nature of AGEs is not fully defined, they are known to be products of glycol-oxidation. Despite being an essential part of normal physiology, increased production and accumulation of AGEs may promote cellular damage [136, 160].

Historically, accurate and reliable ways to measure the type and amount of AGEs include blood and urine samples, and skin biopsies. Collagen-linked fluorescent AGEs, however, can also be assessed with non-invasive light technology [9] using the SCOUT DS® device that produces a SIF score [163, 186]. One benefit of using a SIF score is that it is non-invasive and requires no biological sampling, lab assays, and storage. The current body of literature regarding SIF and T1D primarily includes cross-sectional research on associations between SIF and risk characteristics and complications [2, 3, 150, 151, 158, 175]. This evidence suggests that SIF scores are associated with increased AER [175], coronary artery calcification (CAC) [150, 175], distal symmetrical polyneuropathy, and autonomic neuropathy [151].

To further characterize and identify the utility of SIF for monitoring T1D complications, this report examines if changes in T1D risk characteristics are related to changes in SIF in adults with childhood diagnosed T1D. The research reported herein is, to the best of our knowledge, one

of the first assessing change in T1D risk characteristics in relationship to SIF score change. In unpublished work, our research team has observed that baseline factors of worse historic glucose control (i.e., HbA1c) and lower kidney function were associated with increased SIF scores [204]. Thus, we hypothesize that that worsening of risk markers related to disordered glucose metabolism (e.g., blood glucose control), AGE clearance, lipids, and overall cellular damage and inflammation maybe associated with increased SIF score over time

3.3 Methods

3.3.1 Study participants

Participants for this evaluation were from the Epidemiology of Diabetes Complications Study (EDC) cohort which is comprised of individuals diagnosed with childhood-onset T1D at the Children's Hospital of Pittsburgh, PA, USA, between 1950-80 [187, 188]. A major goal of the EDC study is the identification of characteristics associated with T1D complication development. Since the EDC baseline examination (1986-88), participants have been followed prospectively for 30 years, biennially providing medical history, lifestyle, demographic, and diabetes self-care survey information. Study participants also attended clinical examinations biennially for the first 10 years and again at 18 (2004-06), 25 (2010-14), and 30 years (2016-19).

The SCOUT DS ® device (RISE Life Science Corp.) was administered to a convenience sample of EDC participants in 2007-08 (n=112) and again between 2010-14 (n=189) to assess presence of collagen-linked AGEs. This SIF convenience sample comprises those who attended an EDC study celebratory function in 2007, those who consented to having a researcher come to

their home to collect SIF score, and those attending the 2010-14 EDC clinical examination. Regularly scheduled clinical exams in the EDC study measured participant characteristics during 2004-06 and again between 2010-14. The 2004-06 clinical exam and SIF assessment (2007-08) will be referred to as 'analytic baseline' and the 2010-14 time-frame as 'follow-up'. All procedures for the EDC and the SIF sub-study were approved by the Institutional Review Board at the University of Pittsburgh and participants provided written informed consent prior to study procedures.

3.3.1.1 Participant characteristics

Participants self-reported race/ethnicity and gender (collected once at EDC baseline). A1c months, or cumulative glycemic exposure, was calculated as [(number of HbA1c units above normal at each exam)*(number of months between the midpoints of the preceding and succeeding exam intervals)] [190]. Height was measured using a stadiometer and weight using a balance beam scale. White blood cell count was obtained using a Coulter counter.

3.3.2 Predictors

Self-reported questionnaires were used to capture smoking status, daily number of alcoholic drinks consumed (one serving defined as one 12-ounce bottle of beer, 4-ounce glass of wine, or 1.5 ounce of liquor), medication use (including angiotensin-converting enzyme inhibitors, ACEi, and angiotensin II receptor blockers, ARB), insulin units/day, and pump use. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist to hip ratio was calculated by dividing waist by hip circumference. Multiple daily insulin

shots/pump use (MDI) was defined as either use of an insulin pump or at least three insulin shots a day. Insulin dose is expressed as units per kg of body weight.

Systolic (SBP) and diastolic (DBP) blood pressure were measured three times and the average of the second and third measures were used in analyses. Hypertension (HTN) was defined as blood pressure $\geq$140/90 mmHg or use of anti-hypertensive medication. Blood samples were assayed for lipids, lipoproteins, hemoglobin A1c (HbA1c), and creatinine. HbA1c was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY). All HbA1 and HbA1c values were converted to Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) aligned HbA1c values by using a regression formula derived from duplicate analyses [190]. Total cholesterol was measured enzymatically [191, 192] and low-density lipoprotein (LDL) was calculated using the Friedewald equation [193]. High density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure [194] evaluated from fasting blood samples. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL.

Albumin and creatinine from three timed urine samples (24 h, overnight, and 4-h clinic) were used to calculate AER and albumin-creatinine ratios (ACR). During the 2010-14 follow-up exam where AER was not assessed, an albumin to creatinine ratio (DCA Vantage System) was used [195]. The Chronic Kidney Disease Epidemiology Collaboration equation was used to calculate estimated glomerular filtration rate (eGFR) [196]. Glucose disposal rate was estimated (eGDR) via an equation previously derived from hyperinsulinemic-euglycemic clamp studies of 24 study participants chosen to represent the full spectrum of insulin resistance [197].

3.3.3 Outcome

The SCOUT DS ® device assessed SIF as an estimate of collagen-linked AGEs. Participants placed their forearm on the device and light was shone on the volar side of the forearm. Skin fluorescence was excited with a light emitting diode (LED) centered at 375 nm (LED1) and detected at 435-655 nm using the 0.5 mm source/detector spacing of the channel 2 optical probe. Skin reflectance was measured with excitation LED and broadband LED. These values were used in an intrinsic correction equation (to compensate for distortion of raw fluorescence by skin absorption and scattering) where F is fluorescence and λ is the emission wavelength:

$$f(\lambda) = \frac{\mathrm{F}(\lambda)}{R_x^{kx} R_m\,(\lambda)^{km}}$$

Fλ was divided by reflectance values at the excitation and emission wavelengths, R_x and R_m (λ). The reflectance values were then adjusted by the dimensionless exponents, k_x and k_m where k_x =0.6 and k_m =0.2. The resulting intrinsic fluorescence, (fλ), was integrated over the 441 to 496 nm spectral region to produce an overall SIF score in arbitrary units (AU). Participants were excluded for arm tattoos, wounds, injuries, or rashes on the underside of the forearm. To remove skin care products, the forearm was cleaned prior to the scan.

A modified SCOUT DS ® device was used for the second SIF scan at follow-up. The modified device had a redesigned spectrometer to eliminate a ghost reflection and improve accuracy of the measurement for diabetes screening. SIF scores from the modified device were converted for compatibility with the analytic baseline SIF scores [198]. As collagen-linked AGE also differ by gender [184] and age [199], each SIF value was adjusted for these attributes [184].

3.3.4 Statistical analysis

Risk characteristics were selected from the clinical examinations closest to the two SIF assessments: the 2004-06 clinical examination for the first SIF assessment in 2007-08 and the 2010-14 exams for the second SIF assessment, which took place concurrently with this clinical examination.

Risk characteristic change was calculated by subtracting the analytic baseline value from the follow-up exam value. Similarly, the outcome variable, SIF score change, was calculated by subtracting the individual 2007-08 SIF score from the 2010-14 SIF score. Categorical medication use and comorbidity status variables were characterized as change in status between 2004-06 and 2010-14. Variables with missing data at analytic baseline were estimated by carrying forward the most recently known value in the prior 10 years. Non-normally distributed continuous variables were log transformed prior to inclusion in regression models.

Linear regression models were used to identify independent predictors of SIF score change. They were obtained in a forward fashion by sequentially considering blocks of risk factors that represented similar characteristics. Blocks included demographic characteristics, diabetes control, blood pressure and lipids, and kidney disease and inflammation. Subsequently, variables with $p<=0.20$ from each block were combined into one full model also allowing for the analytic baseline clinical factor variable, analytic baseline SIF scores, and the time period between SIF assessments. Given the small sample size, we selected a p-value of $<=0.1$ to retain variables in the model.

All models were limited to observations that had no missing data at analytical baseline. To evaluate model fit, the square root of the variance of residuals (RMSE) was used. RMSE indicates the absolute fit of a model and is used to determine how close the observed data points are to the model's predicted values. It is the standard deviation of the unexplained variance and lower values

indicate better fit. R^2 was also produced, which is a relative measure of model fit. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

3.4 Results

Sixty participants had covariate data available at two time points and repeat SIF scores (Figure 3-1). Characteristics of these participants at analytic baseline, follow-up, and change over time are displayed in Table 3-1. Mean follow up time was 5.2 years (range of 3.5 to 7 years) between SIF assessments and 6.9 years between EDC exams. At analytic baseline, participants had a mean age of 45.8 (+/- 6.7) years and a median diabetes duration of 36.6 (IQR: 33.1-40.2) years. The median HbA1c was 7.1 (IQR: 6.4-8.3). The majority of participants were women (57%), Caucasian (97%), and used multiple daily insulin shots or an insulin pump (78%). The mean SIF score at analytic baseline was 4.3 (+/- 4.2) arbitrary units (AU) and increased to 7.2 (+/- 4.9) AU at follow-up, with a mean change over time of 2.9 (+/- 2.9) AU. SIF score increased between analytic baseline and follow-up in 83% of study participants, with an average increase of 0.60 AU per year.

BMI change was the only variable considered in the final full combined model (i.e., $p<=0.2$ in each block). Change in BMI ranged from -8.57 to 6.26 (Figure 3-2). Results indicate a marginally significant association for BMI change with SIF change (β=-0.3, 95% CI: -0.6 to 0.04, p=0.09) (Table 3-2). The RMSE of the model was 2.9 and the R^2=0.06.

3.5 Discussion

This report examines the relationship between T1D risk characteristics change and change in SIF in adults with childhood diagnosed T1D. We observed a moderate inverse association between BMI change and SIF score change. Increased BMI was moderately associated with decreased SIF.

We saw a small mean change in BMI over follow up, with most participants remaining in the overweight category (overweight category is defined as a BMI between 25 to 29.9). The biological processes involved in the BMI—AGE relationship might be explained by work done outside T1D. In healthy participants, BMI is inversely associated with serum AGEs; higher BMI is related to suppressed AGEs [205]. Excess accumulation of visceral and peripheral adipose tissue may play a role in trapping AGEs and possibly have the capacity to drive lower serum AGEs [206-208]. Although this other work is performed in the general population [206], healthy participants [205, 207], and type 2 diabetes [208], it may or may not be directly comparable to our T1D research. However, the work does shed light on some of the biological processes involved in how AGEs accumulate. If having more fat, and thus higher BMI, truly does 'trap' circulating AGEs, this might result in reduced availability of AGEs for deposit in long-lived proteins such as collagen. Given SIF is a measure of collagen-linked AGEs, we might be seeing some of these biologic processes taking place in our negative, albeit non-significant, BMI—SIF association.

Another possible explanation for the negative BMI – SIF association may relate to glucose control. Although the vast majority (78.3%) of study participants already practiced MDI at analytic baseline, this proportion increased to 88.3% by the follow-up assessment and was accompanied by a small increase in mean BMI (25.6 vs. 26.2). As the EDC study and others have previously shown that intensive therapy is associated with weight gain in T1D [209 210 211], it is

possible that the negative association between change in BMI and change in SIF may be due to weight gain with MDI exposure. In our other work [204] , we found an association between longer exposure to MDI and a reduction in SIF scores, independent of HbA1c concentrations. It is possible that, although MDI increases BMI, MDI reflects additional protective behaviors and/or factors beyond average or mean glucose exposure, for example less extreme glucose excursions, that have beneficial health effects and are reflected in reduced SIF scores.

This research study is characterized by the following limitations. First, only a convenience sample of participants in the EDC study cohort were originally asked to participate in the SIF sub-study which could affect the generalizability of the findings. The analytic sample was also small which may have exacerbated the effect of missing data. We tried to account for this by last observation carried forward for some risk characteristic data, but our sample remained small. Further, due to the small sample size, we also chose an alpha of 0.10 instead of the more common value of 0.05. A larger sample size may have also provided greater statistical power to clarify the relationships.

Since T1D and SIF are time-dependent processes, the findings could be biased by age; to attempt to reduce such bias we adjusted SIF scores by age. These results were adjusted for time between SIF assessments, but were not adjusted for time between BMI assessment; however, if we had adjusted for both we most likely would have over-adjusted. Another limitation is the SIF score technology. While it is clear the SCOUT DS® assesses cross-linked fluorescent AGEs, it remains unknown which exact AGE compounds are being reported with SIF scores. In addition, SIF scores are calculated based on the measure of cross-linked AGEs per unit of collagen. Although SIF scores are intrinsically corrected to attempt to account for this, the only truly accurate

way to assess collagen unit is via skin punch biopsy. Given this, there could be minor measurement error in the SIF data.

Considering the pathways involved in AGE formation [147], many other compounds need to be characterized to fully understand the observed association. Skin fluorescence also has multiple determinants beyond AGE. The DCCT/EDIC study found that SIF is not simply a function of AGE formation and glycemic exposure, but that it may reflect may other factors, such as skin pigment and hemoglobin levels [1]. Studies have also reported that a low-AGE diet leads to reduction in serum AGEs, and is ultimately accompanied by reduction in markers of inflammation, oxidative stress, and endothelial dysfunction [212]. In addition, physical exercise has been shown to reduce circulating levels of AGEs [212]. The analysis we conducted did not include any diet or physical exercise exposure data, all of which influence life expectancy [161] and AGE levels. Given this is the first reporting of such a finding, more work in this area needs to be performed to understand these associations in more depth.

3.5.1 Conclusion

In conclusion, our study adds to the existing evidence regarding the potential correlates of AGE changes in T1D. Taken together, SIF has demonstrated potential as a non-invasive tool to aid in T1D complication risk identification and possibly inform subsequent management. While whether SIF scores have a causal association with complications in T1D has to be decided by experimental and intervention studies, the current results identify a potential and relevant correlate of SIF change to consider.

3.6 Tables and Figures

Table 3-1 EDC T1D SIF sub study participant characteristics at analytic baseline, follow-up and change over time for those with repeat SIF scores (N=60) [a]

Participant Characteristic	Analytic baseline	Follow up	Change between timepoints [b]
SIF score	4.3 (4.2)	7.2 (4.9)	2.9 (2.9)
SIF score increased	-	50 (83.3%)	-
Age	45.8 (6.7)	52.6 (6.8)	6.8 (0.7)
Female	34 (56.7%)	-	-
Caucasian	58 (96.7%)	-	-
Age of onset	10.2 (6.1-12.6)	-	-
Diabetes duration	36.6 (33.1-40.2)	43.6 (40-46.9)	6.8 (6.4-7.3)
Body mass index	25.6 (3.9)	26.2 (4.6)	0.6 (2.6)
Waist to hip ratio	0.9 (0.8-0.9)	0.9 (0.8-0.9)	0.003 (-0.02-0.03)
Ever smoker / started smoking	15 (25%)	14 (23.3%)	0 [c]
HbA1c	7.1 (6.4-8.3)	7.7 (7.2-8.8)	0.7 (0.1-1.1)

Participant Characteristic	Analytic baseline	Follow up	Change between timepoints [b]
A1c months	902.9 (660.3-1416.4)	1029.8 (713.1-1579.7)	76.1 (24-153.7)
Total insulin units/weight	0.6 (0.4-0.7)	0.5 (0.4-0.7)	-0.03 (-0.2-0.02)
MDI /started	47 (78.3%)	53 (88.3%)	6 (10%) [d]
ACR	6.6 (3.9-42.2)	11.4 (5.9-41.7)	2.1 (-0.9-7.2)
Total cholesterol	169.4 (27.5)	178.7 (31.9)	9.3 (36.4)
Non-HDL cholesterol	108.5 (26.4)	112.8 (32.8)	4.3 (34.1)
HDL cholesterol	60.9 (17.8)	65.9 (19.6)	4.9 (12.7)
LDL cholesterol	95.5 (82-113.5)	93.5 (77-120)	0 (-13.5-14)
Triglycerides	71 (45-89.5)	67.5 (44-83)	-1 (-20.5-19)
Diastolic blood pressure	64.4 (11.1)	64.4 (9.5)	-0.03 (9.9)
Systolic blood pressure	113.6 (16.5)	113.8 (18.1)	0.2 (18.1)
Pulse	72.7 (11.9)	74.4 (9.7)	1.7 (11.8)
ACEi/ARB inhibitor medication use /started	33 (55%)	29 (48.3%)	3 (5%) [e]
LDL lowering medication use/started	23 (38.3%)	32 (53.3%)	11 (18.3%) [f]
Blood pressure lowering medication /started	11 (18.3%)	10 (16.7%)	3 (5%) [g]
Hypertension / incidence	14 (23.3%)	13 (22.8%)	5 (8.3%) [h]
eGFR	80.9 (21.6)	80.7 (23.2)	-0.2 (14.6)

Participant Characteristic	Analytic baseline	Follow up	Change between timepoints [b]
eGDR	8.4 (6.9-9.9)	8.2 (7.2-9.8)	-0.3 (-0.9- 0.3)
White blood cell count	6.2 (1.8)	6.0 (1.9)	-0.1 (1.7)
Total alcohol intake per day	0 (0-2)	0 (0-2)	0

[a] mean and standard deviation for normally distributed variables, median (IQR) for non-normally distributed variables, and n (%) for categorical variables

[b] change in all categorical variables is defined as started medication or worsened/became diagnosed, change for non-normal variables is reported as median change

[c] n=14 observations that remained smokers between time points

[d] n=47 observations that maintained MDI between time points

[e] n=23 observations that maintained ACEi/ARB treatment between time points

[f] n=21 observations that maintained lipid treatment between time points

[g] n=7 observations that maintained blood pressure treatment between time points

[h] n=8 observations that remained hypertensive between time points

Table 3-2 EDC T1D SIF sub study associations between patient characteristic change and SIF score change (n=60) [a]

	β (95% CI), p-value
BMI change	-0.3 (-0.6 to 0.04), p=0.09
RMSE/R^2	2.9/0.06
[a] model also adjusted for analytic baseline BMI, analytic baseline SIF, and time between SIF assessment in years	

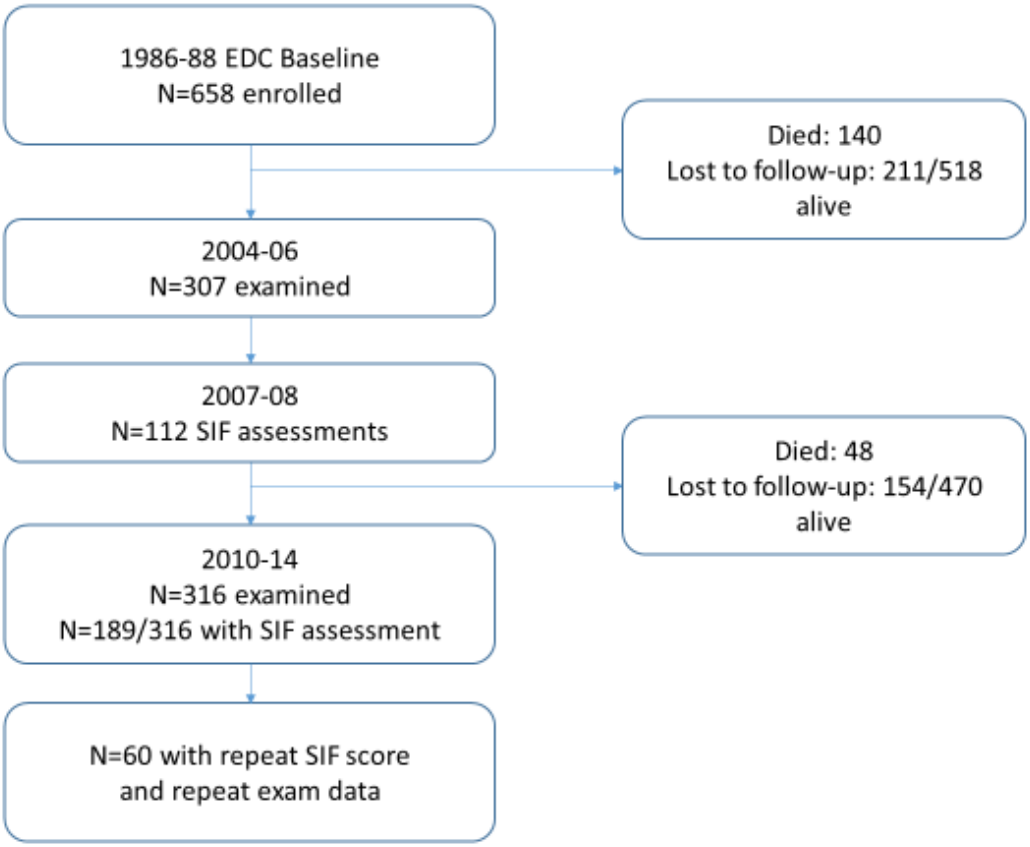

Figure 3-1 EDC SIF sub-study participant flow for those with repeat exam data and repeat SIF scores

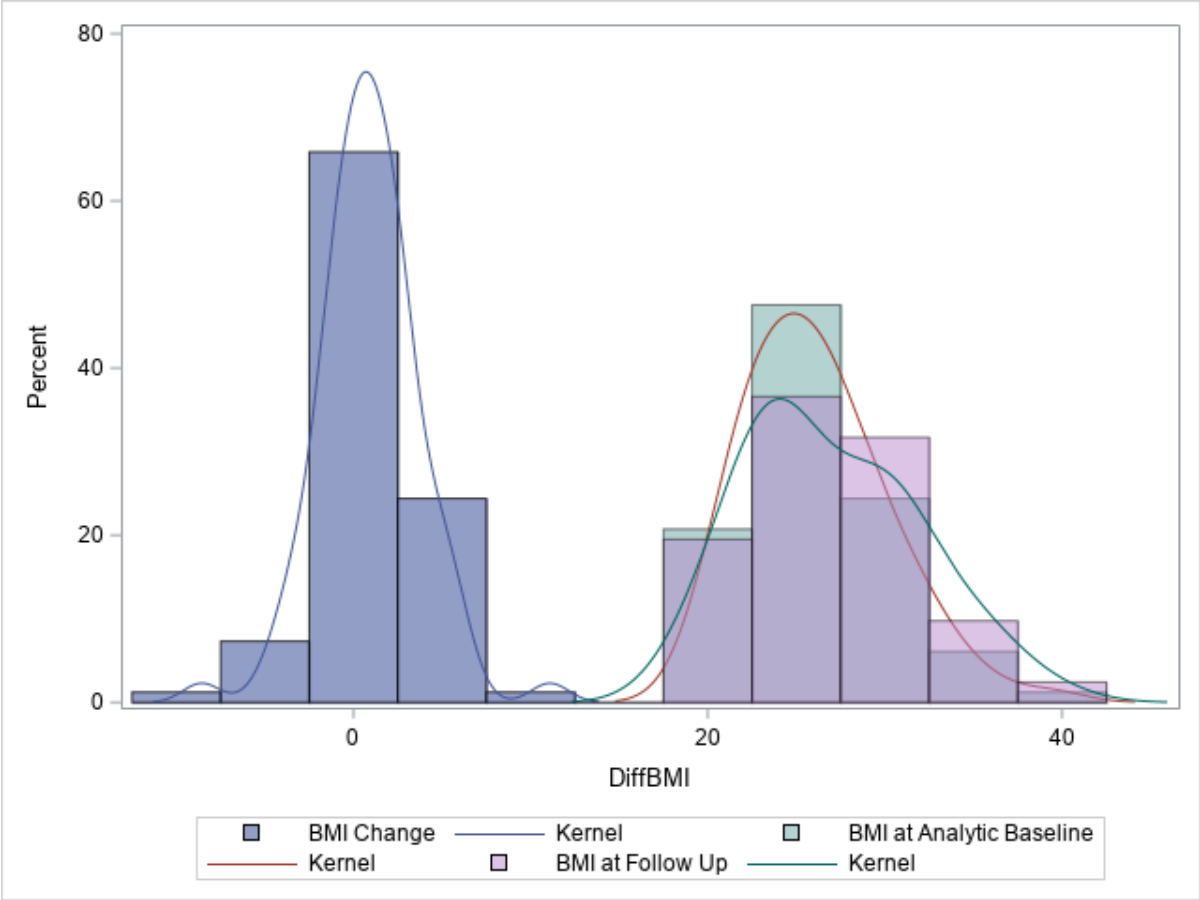

Figure 3-2 BMI distribution at analytic baseline, follow up, and change over time

4.0 Skin intrinsic fluorescence scores are a predictor of all-cause mortality risk in type 1 diabetes: The Epidemiology of Diabetes Complications study

4.1 Synopsis

Aims: We assessed the association of skin intrinsic fluorescence (SIF) scores, as a measure of advanced glycation end-products (AGE), with all-cause mortality in type 1 diabetes (T1D). Methods: This is an observational retrospective study of a convenience sample from the Epidemiology of Diabetes Complications (EDC) study. AGEs were measured with a SIF score between 2007 and 2014; vital status was assessed in 2020. Results: Among 245 participants, mean age was 48.6 ± 7.4 years, median diabetes duration was 39.5 years (IQR: 34.2, 44.9), and 53.5% were female. Compared to survivors, the deceased (n = 20) were older, with higher SIF scores, longer diabetes duration, lower body mass index (BMI), and an adverse risk factor profile (all $p \leq 0.05$). Univariate Cox regression showed a marginal association between SIF score and mortality (HR: 1.1, 95% CI 0.9–1.2, p = 0.06), which persisted after adjustment for multiple daily insulin shots/pump (MDI) use (HR: 1.1, 95% CI 1.0–1.2, p = 0.04). This association was attenuated after adjustment for T1D duration, A1c months, or estimated glomerular filtration rate (eGFR). Conclusion: In individuals with long duration T1D, SIF scores adjusted for MDI predicted all-cause mortality, although this association was attenuated after adjustments. Given the nature of sampling and small number of events, our findings require replication.

4.2 Introduction

In the past 40 years, survival for those with type 1 diabetes (T1D) has improved dramatically [75]. In the Epidemiology of Diabetes Complications (EDC) study, life expectancy from birth increased by about 15 years for those diagnosed between 1965-1980 (life expectancy of 68.9 years) compared to those diagnosed between 1950 and 1964 [131]. This increased life expectancy is likely partly attributable to improved glucose monitoring and control and a resulting decline in complications [131]. The exact causal process and mechanism are unclear beyond the known development of kidney and cardiovascular complications. Despite these encouraging data and advances in treatment, individuals with T1D have an excess risk of mortality compared to the non-diabetic population [132]. Thus, findings from the EDC study suggest that people with T1D continue to have an average of 4.6 life years lost compared to the general population [131].

AGEs are associated with ageing [4], senescence, development of age-related morbidities [5], and mortality [6-8]. Plasma AGEs have been associated with incident all-cause and cardiovascular mortality in T1D [6]; skin autofluorescent AGEs have been associated with all-cause and cardiovascular mortality in T1D and type 2 diabetes (T2D) [8] as well as in hemodialysis patients [213].

AGEs have further been shown to have an important role in the oxidative damage of proteins [146], contributing to T1D complications [144, 147-149]. In patients with T1D, several studies have concluded that collagen-linked fluorescent AGEs are cross-sectionally associated with factors such as glycosylated hemoglobin (HbA1c) [1-3], albumin excretion rate (AER) >30 mg/24hr [1], coronary artery calcification (CAC) [1, 150] and distal symmetrical polyneuropathy [151]. Thus, AGE accumulation is particularly harmful; it is estimated that those with T1D have three times greater

accumulation of AGE than those without T1D [138, 139]. This may be partly due to declining kidney functioning and inability to properly clear AGEs [140, 141].

Collagen-linked fluorescent AGEs can be assessed with non-invasive light technology [9] using the SCOUT DS® device (RISE Life Science Corp., Toronto, Canada) [186] that produces a skin intrinsic fluorescence (SIF) score. There are no known studies that have assessed the relationship between SIF scores and all-cause mortality in T1D. In a sub-set of patients with T1D in the EDC study, we assessed the association of collagen-linked AGE, assessed with a SIF score, and all-cause mortality.

4.2.1 Subjects

Participants for this evaluation were from the EDC cohort study, which is comprised of individuals diagnosed with childhood-onset T1D at the Children's Hospital of Pittsburgh, PA, USA, between 1950 and 1980 [187, 188]. Following a baseline examination (1986-88), participants were followed prospectively for 30 years (as of 2019), biennially providing medical history, lifestyle, demographic, and diabetes self-care survey information. Study participants also attended clinical examinations biennially up to 10 years and again at 18 (2004-07), 25 (2010-14), and 30 years (2016-19).

The SCOUT DS ® device was administered to a convenience sample of EDC participants (n=245) between 2007 and 2014 to assess presence of collagen-linked AGE. This convenience sample comprised those who attended a 'thank you' luncheon after the EDC 20 Year Anniversary in 2007, anyone living within a 50-mile radius of the University of Pittsburgh who consented to having a researcher come to their home to collect SIF scores, and those attending the 2010-14

follow up examination. All-cause mortality was assessed up to May 6, 2020. All procedures for the EDC and the SIF sub-study were approved by the Institutional Review Board at the University of Pittsburgh. All participants provided written informed consent prior to all study procedures.

4.3 Materials and methods

4.3.1 Skin intrinsic fluorescence score

To assess SIF, participants placed their forearm on the SCOUT DS ® device and light was shone on the volar side of the forearm approximately three inches from the elbow. Skin fluorescence was excited with a light emitting diode (LED) centered at 375 nm and detected at 435-655 nm using the 0.5 mm source/detector spacing of the channel 2 optical probe. Skin reflectance was measured with excitation LED and broadband LED. These values were used in an intrinsic correction equation (to compensate for distortion of raw fluorescence by skin absorption and scattering) where F is fluorescence and λ is the emission wavelength:

$$f(\lambda) = \frac{\mathrm{F}(\lambda)}{R_x^{kx} R_m\,(\lambda)^{km}}$$

Fλ was divided by reflectance values at the excitation and emission wavelengths, R_x and R_m (λ). The reflectance values were then adjusted by the dimensionless exponents, k_x and k_m where k_x =0.6 and k_m =0.2. The resulting intrinsic fluorescence, (fλ), was integrated over the 441 to 496 nm spectral region to produce an overall SIF score in arbitrary units (AU). Participants were excluded for arm tattoos, wounds, injuries, or rashes on the underside of the forearm. To remove skin care products, the forearm was cleaned prior to the scan.

4.3.2 Participant characteristics

Race/ethnicity, gender, smoking status, daily number of alcoholic drinks consumed (one drink is defined as one 12-ounce bottle of beer, 4-ounce glass of wine, or 1.5 ounce of liquor), medication use, insulin units/day, and pump use were self-reported. Height was measured using a stadiometer and weight using a balance beam scale. Multiple daily insulin shots/pump use (MDI) was defined as either use of an insulin pump or at least three insulin shots a day.

Hypertension (HTN) was defined as blood pressure $\geq$140/90 mmHg or use of anti-hypertensive medications. Blood samples were assayed for lipids, lipoproteins, HbA1c, and creatinine. Stable glycosylated hemoglobin (HbA1) was measured with ion exchange chromatography (Isolab, Akron, OH, USA) for the first 18 months [189] and with automated high-performance liquid chromatography (Diamat, BioRad, Hercules, CA, USA) for the next 10 years in the EDC study. Extensive duplicate samples were run using both techniques, and no systematic differences were seen (r^2=0.95; Diamat [HbA1] = -0.018+1.00 Isolab [HbA1). The difference between the means of the two methods (0.158 [HbA1]) was not statistically significant [190]. After 10 years, HbA1c was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY, USA). All HbA1 and HbA1c values were converted to Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) aligned HbA_{1c} values by using a regression formula derived from duplicate analyses, where DCCT/EDIC HbA1c= [(EDC HbA1) *(0.83) + 0.14]; and DCCT/EDIC HbA1c= [(EDC HbA1c – 1.13) / 0.81]. A1c months were calculated as [(number of HbA1c units above normal at each exam)*(number of months between the midpoints of the preceding and succeeding exam intervals)] [190]. Total cholesterol was measured enzymatically [191, 192] and low density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation [193]. High density lipoprotein (HDL) cholesterol was

determined by a heparin and manganese procedure evaluated from fasting blood samples [194]. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDLc. White blood cell count was obtained using a counter S-plus intravenous line.

Albumin and creatinine from three timed urine samples (24 h, overnight, and 4-hour clinic) were used to calculate AER and albumin to creatinine ration (ACR). Overt nephropathy (ON) was defined as AER>=200 µg/min. In the 10% of urine collections deemed inadequate based on creatinine excretion, and during the 2010-14 follow-up exam where AER was not assessed, an albumin to creatinine ratio (DCA Vantage System) >0.3 mg/mg was used to define ON [195]. The Chronic Kidney Disease Epidemiology Collaboration equation was used to calculate estimated glomerular filtration rate (eGFR) [196]. Impaired eGFR was defined as eGFR less than or equal to 60 mL/min/1.73m^2. Glucose disposal rate was estimated (eGDR) via an equation previously derived from hyperinsulinemic-euglycemic clamp studies of 24 study participants chosen to represent the full spectrum of insulin resistance [197].

4.3.3 Mortality

Vital status was assessed as of May 6, 2020. For all deaths, the next of kin or close relative was contacted and interviewed, a copy of the death certificate obtained and, where appropriate, the hospital record and/or autopsy record was requested.

4.3.4 Statistical analysis

T-tests or the Wilcoxon rank sum test, for parametric and non-parametric variables, respectively, were used to assess differences in continuous variables between those who survived

and the deceased; for categorical variables, the Chi-Square or Fisher's exact test was used. Pearson or Spearman correlation coefficients were used to evaluate the presence of a linear association between continuous variables and SIF score. Descriptive data are presented in Table 4-1 as mean (standard deviation, SD) for normally distributed variables and median (inter-quartile range, IQR) for non-normally distributed variables, or n (%) for categorical variables. Participant characteristics were selected from the examination cycle closest to the date of the SIF assessment. Missing values for covariates were calculated using the last observation carried forward from 2000-04 exam data.

Unadjusted and adjusted Cox regression models were constructed to evaluate the ability of SIF score to predict all-cause mortality. Akaike's information criterion (AIC) was reviewed for model fit. Covariates for adjustment were selected from the literature based on being associated with both SIF score and mortality in T1D; these included T1D duration, A1c months, eGFR, and MDI. Non-normally distributed continuous covariates were log transformed. Because of the small number of events, Cox models allowed for only one covariate at a time. The proportional hazards assumption of Cox regression models was evaluated using Schoenfeld residuals and loess smooths. Martingale residuals were used to test departures from linearity [214, 215]. There was minimal pattern of Schoenfeld residuals indicating that the data met the proportional hazards assumptions. The Martingale residuals supremum test for the proportional hazards assumption was not significant ($p=0.21$) indicating that the non-linearity proportional hazards assumption was met. Outlier observations were identified; when Cox models were run excluding outliers, the results were unaltered and thus we present results for the total cohort. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

4.4 Results

The study flow is displayed in Figure 4-1. Characteristics of the 245 study participants at the exam closest to the SIF date are presented for the entire sample and by subsequent mortality status in Table 4-1. Overall, the mean time between SIF assessment and date of mortality was 6.1 years; mean age was 48.6 years and median duration of diabetes was 39.5 years; 53.5% were female. The majority of participants were Caucasian (n=239, 97.6%).

Compared to those who survived, the deceased were more likely to be older, with longer diabetes duration and to have higher SIF scores, HbA1c, A1c months, systolic blood pressure (SBP), and median AER. They also had lower BMI, eGFR, eGDR, total insulin units per kg of weight. They were less likely to use MDI, to have hypertension (HTN), and have normal or mildly decreased kidney function (i.e., eGFR =>60mL/min/m^2).

Correlations between risk factors and SIF score were also evaluated (Table 4-2). Statistically significant (p<=0.05) positive correlations were observed for age, T1D duration, waist to hip ratio (WHR), A1c months, white blood cells, triglycerides, SBP, and AER. Statistically significant (p<=0.05) negative correlations were observed for eGFR and eGDR.

The unadjusted and multivariable adjusted relationship of SIF with mortality is presented in Table 4-3. Unadjusted SIF score (Table 4-3, Model 1) statistically significantly predicted all-cause mortality (HR: 1.1, 95% CI 1.0-1.2, p=0.02) in the full sample of N=245 with 20 mortality events. In the sample limited by no missing covariate data (N=201 with 16 mortality events), the univariate Cox regression model showed a marginal association between SIF score and mortality (HR: 1.1, 95% CI 0.9-1.2, p=0.06, Table 4-3, Model 2), which persisted after adjustment for MDI (HR: 1.1, 95% CI 1.0-1.2, p=0.04, model 3). However, the effect of SIF score was no longer significant (p>0.05) after adjusting for T1D duration, log A1c months, or log eGFR, all of which

were significant covariate predictors of mortality (all p<=0.05 in Table 4-3, Models 4, 5, and 6, respectively).

4.5 Discussion

Our goal was to evaluate the relationship between collagen-linked AGE, assessed via SIF scores, and all-cause mortality in a convenience sample of T1D patients from the EDC study. Our results indicate that SIF is univariately associated with all-cause mortality. This association remained significant after adjustment for MDI, although adjustment for diabetes duration, A1c months, or eGFR rendered SIF non-significant.

The relationship between SIF scores and all-cause mortality was of interest to us because existing evidence suggests that the production of altered proteins from glycation are one of the most common hallmarks of ageing and increased risk of death [5, 159]. Ageing is somewhat driven by increased deposition of AGEs and increased expression of the receptors for AGEs [5, 161]. Even though scientific understanding is incomplete, such production of detectable AGEs has been implicated in ageing in general [159, 160] through promotion of protein modification, cellular stiffness, organ dysfunction, vessel rigidity, bone fragility, tissue damage, and muscle stiffness, and oxidative stress [161]. Thus, we hypothesized that SIF scores might be an indication of ageing processes and ultimately risk of all-cause mortality in T1D. In addition to this, it is known that the accumulation of AGEs is particularly harmful in T1D; people with T1D can have up to three times greater accumulation of AGE compared to those without T1D [138, 139]. This is primarily due to accelerated AGE formation [216] and decreased AGE clearance capabilities [142-144, 217]. Further, experimental breakage of AGE crosslinks has been shown to reduce development of experimental

diabetes-related complications [218]. Notably, AGE breakers can reverse the effects of AGE on age-related increases in myocardial stiffening [219] and the use of AGE inhibitors in experimental models have demonstrated kidney protection in diabetes [220].

Interestingly, although adjustment for diabetes duration, A1c months or eGFR reduced the significance of SIF for mortality risk, its effect size remained largely unaltered. This may suggest that power to detect an effect was limited due to the small number of events in this sub-cohort. A larger sample size may have provided greater statistical power to clarify the relationship between SIF score and mortality by allowing for multivariable adjustment. T1D duration, A1c months, eGFR, and MDI are also all potential confounders that influence both SIF scores and all-cause mortality in T1D. The kidneys are especially related to AGEs. As the kidneys play a role in processing and clearing AGEs [142, 143], it has been speculated that declining kidney functioning (i.e., eGFR) with increased T1D duration may be involved in a deficit in AGE detoxification [144], which may contribute to up to three times the amount of normal AGE accumulation [138, 139] and development of chronic kidney disease [145]. Given our work is the first reporting of such findings, additional research in this area would further understanding of these associations.

Our study builds on the growing body of evidence that AGEs may play a role in ageing. There are no other reports of evaluation of SIF scores and all-cause mortality in T1D and thus our research adds the first study assessing this association. Nevertheless, two other research centers previously evaluated AGEs in relation to mortality in T1D (see Table 1-1) [6, 8, 162]. In Nin et. al 2010 & 2011, 169 T1D patients in Denmark were followed over a median of 12.3 years during which 82 of the patients died [6, 162]. Both the receptors for AGE [162] and plasma AGEs [6] were significantly associated with all-cause mortality. However, as plasma AGEs differ from collagen-linked AGEs and may not represent the same types of AGEs, thus comparability to our results is

difficult. In a study by Meerwaldt et al 2007, 48 T1D patients, 69 T2D patients, and 43 control participants were recruited from a diabetes outpatient center in The Netherlands and assessed with the AGE Reader (Diagnoptics Technologies B.V., Groningen, The Netherlands) [8]. The AGE Reader assesses collagen-linked AGEs with a skin autofluorescent (SAF) score [163, 164] and has been previously associated with end-organ complications in T1D [139, 165-167]. During a 5 year follow up, there were 11 coronary heart disease (CHD) mortality events in the T1D group [8]. Univariate analysis in the T1D group indicated that age, mean A1c, creatinine, HTN, hemodialysis treatment, triglycerides, smoking, CHD at baseline, and SAF were significantly associated with CHD-mortality [8]. These covariates were further tested for effect on CHD-mortality in Cox regression models that included SAF. SAF scores were independently significantly associated with CHD-mortality; SAF stronger than age, mean A1c, triglycerides, and smoking as an independent predictor of CHD-mortality but not hemodialysis treatment or CHD at baseline [8]. Our results demonstrated a less robust association in terms of an independent role for SIF in predicting mortality. A potential explanation may relate to our assessment of all-cause rather than cardiovascular disease (CVD)-related mortality. AGEs are thought to play a major role in progression of CVD [155], vascular rigidity, and arterial stiffness [136]. AGEs on long-lived proteins, such as skin collagen, may serve as a measure of cumulative metabolic stress and metabolic burden by hyperglycemia and hyperlipidemia [8] and thus we hypothesize that collagen-linked AGE may be a stronger predictor of CHD-mortality than overall mortality. There could also be potential measurement differences between the AGE Reader and the SCOUT DS® devices. The AGE Reader emits ultraviolet-A light and reflected light is measured with photodiodes [170]. In contrast, the SCOUT DS® employs intrinsic fluorescence and emits LED light. SIF scores are thought to be a more reliable measure of fluorescense across varying skin types and pigmentation because

the device compensates for optical absorption by hemoglobin and melanin, whereas autofluorescence does not [150].

Strengths of our study include the relatively large number of study participants with SIF assessments. Another strength is that the EDC is a well-characterized cohort. Moreover, this is the first study to report on SIF scores and all-cause mortality in T1D, and adds to the growing body of AGE-related evidence in T1D.

Nonetheless, our research study has limitations. First, not all participants in the EDC study cohort were originally asked to participate in the SIF sub-study which could affect the generalizability of the findings. Since T1D and SIF are time-dependent processes, our findings could be biased by age; to attempt to reduce such bias we adjusted SIF scores by age. Another limitation is the SIF score technology. While it is clear the SCOUT DS® assesses cross-linked fluorescent AGEs, it remains unknown which exact AGE compounds are being reported with a SIF score. In addition, the SIF score is calculated based on the amount of cross-linked AGEs per unit of collagen. Although SIF scores are intrinsically corrected to attempt to account for this, the only truly accurate way to assess collagen unit is via skin punch biopsy. Given this, there could be minor measurement error in the SIF data. Further work is needed to fully validate the SCOUT DS ® device for reliability in measuring physiologic outcomes. Finally, considering the pathways involved in AGE formation [147], many other compounds need characterized to fully understand the observed association.

Our analysis did not include any diet or physical exercise exposure data, all of which influence life expectancy [161] and AGE levels. Studies have reported that a low-AGE diet leads to reduction in serum AGEs, and is ultimately accompanied by reduction in markers of inflammation, oxidative stress, and endothelial dysfunction [212]. In addition, physical exercise has been shown to

reduce circulating levels of AGEs [212]. Skin fluorescence also has multiple determinants beyond AGE. The DCCT/EDIC study found that SIF is not simply a function of AGE formation and glycemic exposure, but that it may reflect may other factors, such as skin pigment and hemoglobin levels [1].

In conclusion, our study provides the first insight into the relationship between SIF scores and all-cause mortality in people with T1D. Our results support the existing evidence that AGEs may play a role in accelerated ageing in T1D and suggest that SIF scores may detect biomarkers indicating risk of all-cause mortality. Whether SIF scores have a causal association with all-cause mortality in T1D has to be decided by experimental and intervention studies. Future work in this area should also focus on identifying SIF score thresholds for mortality risk in T1D. Our work also demonstrates that non-invasive SIF may provide a novel approach for monitoring the role of AGEs in T1D.

4.6 Tables and Figures

Table 4-1 T1D participant characteristics overall and by subsequent mortality status in the EDC SIF sub-study

Participant Characteristics	Overall cohort (n=245)	Deceased (n=20)	Survived (n=225)	p-value
SIF score (AU)	6.2 (3.3-9.8)	8.2 (5.1-13.7)	5.9 (3.2-9.5)	0.03
Age (years)	48.6 (7.4)	52.8 (6.8)	48.2 (7.4)	<0.01
Female	131 (53.5%)	11 (55%)	120 (53.3%)	0.88
Caucasian	239 (97.6%)	18 (90%)	221 (98.2%)	0.08
Age of onset (years)	8.8 (5-11.8)	8.0 (4.4-9.3)	8.9 (5.1-11.9)	0.25
Diabetes duration (years)	39.5 (34.2-44.9)	46.2 (42.9-49.7)	38.8 (34.1-44.2)	<0.01
BMI (kg/m^2) (n=243, 20, 223)	26.6 (23.6-29.9)	26.3 (21.3-26.9)	26.6 (23.9-30.6)	0.05
WHR (n=227, 17, 210)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.28
Ever smoked (vs. never smoked n=241, 20, 221)	86 (35.7%)	9 (45%)	77 (34.8%)	0.36
HbA1c (mmol/mol) (n=233, 17, 216)	60.7 (50.8-73.8)	69.4 (55.2-91.3)	60.7 (50.8-71.6)	0.05
HbA1c (%) (n=233, 17, 216)	7.7 (6.8-8.9)	8.5 (7.2-10.5)	7.7 (6.8-8.7)	0.05
A1c months (n=241, 20, 221)	1113.3 (751.3-1512.9)	1420.9 (1078.7-1753.7)	1070.7 (720.8-1495.5)	0.01
Total insulin units/kg weight (n=215, 16, 199)	0.6 (0.4-0.7)	0.5 (0.4-0.5)	0.6 (0.4-0.7)	0.02

Participant Characteristics	Overall cohort (n=245)	Deceased (n=20)	Survived (n=225)	p-value
MDI (n=214, 19, 195)	185 (86.5%)	12 (63.2%)	173 (88.7%)	<0.01
Total cholesterol (mg/dl) (n=233, 17, 216)	172 (151-195)	179 (157-195)	172 (149-194.5)	0.49
Non-HDL cholesterol (mg/dl) (n=233, 17, 216)	108 (93-133)	122 (89-132)	108 (93-133.5)	0.85
HDL (mg/dl) (n=233, 17, 216)	57 (46-72)	64 (49-73)	56 (46-71.5)	0.32
LDL (mg/dl) (n=193, 12, 181)	96 (79-118)	97.5 (69-120)	96 (80-118)	0.97
Triglycerides (mg/dl) (n=222, 17, 205)	72 (50-102)	81 (68-103)	71 (49-98)	0.19
Diastolic blood pressure (mmHg) (n=231, 17, 214)	65.4 (9.6)	62.7 (10.7)	65.6 (9.4)	0.22
Systolic blood pressure (mmHg) (n=233, 17, 216)	116 (106-127)	122 (118-129)	115 (105-125)	0.02
Pulse (beats/minute) (n=233, 17, 216)	72 (64-80)	72 (64-80)	73 (64-82)	0.61
ACEi medication use (n=233, 18, 215)	101 (43.4%)	12 (66.7%)	89 (41.4%)	0.04
LDL lowering medication use (n=238, 20, 218)	104 (43.7%)	10 (50%)	94 (43.1%)	0.55
Triglyceride lowering medication use (n=112, 18, 94)	51 (45.5%)	8 (44.4%)	43 (45.7%)	0.92
Blood pressure lowering medication (n=238, 20, 218)	69 (28.9%)	9 (45%)	60 (27.5%)	0.09
Hypertension (n=233, 19, 214)	76 (32.6%)	10 (52.6%)	66 (30.8%)	0.05
eGFR (mL/min/m^2) (n=226, 17, 209)	80.7 (67.1-96.5)	49.5 (42.2-84.9)	81.3 (70.2-97.2)	<0.01
eGFR =>60mL/min/m^2 (n=226, 17, 209)	181 (80.1%)	7 (41.2%)	174 (83.3%)	<0.01
eGDR (mg/kg/min) (n=223, 17, 206)	7.5 (2.2)	6.3 (2.3)	7.6 (2.2)	0.02
AER (µg/min) (n=106, 15, 91)	7.5 (4.3-59.1)	101.9 (22.3-252.4)	5.9 (3.9-29.5)	<0.01

Participant Characteristics	Overall cohort (n=245)	Deceased (n=20)	Survived (n=225)	p-value
White blood cell count ($10^3/m^3$) (n=211, 14, 197)	6.1 (5-7.2)	6.5 (5.1-7.9)	6.1 (5-7.2)	0.68
Alcoholic beverages consumed per day (n=229, 16, 213)	0 (0-3)	0	0 (0-3)	0.08

Table 4-2 Unadjusted correlations between T1D participant characteristics and SIF score in the EDC SIF sub-study, N=245 unless otherwise noted

Participant Characteristic	r (p-value)	Participant Characteristic	r (p-value)
Age (years)	0.3 (<0.01)	Total cholesterol (mg/dl) (n=233)	-0.06 (0.35)
Age of onset (years)	-0.03 (0.68)	Non-HDL cholesterol (mg/dl) (n=233)	-0.05 (0.46)
Diabetes duration (years)	0.2 (<0.01)	HDL cholesterol (mg/dl) (n=233)	-0.02 (0.79)
BMI (kg/m^2) (n=243)	0.01 (0.87)	LDL cholesterol (mg/dl) (n=193)	-0.1 (0.08)
WHR male (n=105)	0.2 (0.04)	Triglycerides (mg/dl) (n=222)	0.2 (0.02)
WHR female (n=122)	0.2 (0.02)	Diastolic blood pressure (mmHg) (n=231)	-0.08 (0.23)
HbA1c (%) (n=233)	0.08 (0.19)	Systolic blood pressure (mmHg) (n=233)	0.2 (0.02)
A1c months (n=241)	0.2 (<0.01)	Pulse (beats/minute) (n=233)	-0.03 (0.67)
Total insulin units/kg weight (n=215)	-0.1 (0.12)	eGFR ($mL/min/m^2$) (n=226)	-0.3 (<0.01)
White blood cell count ($10^3/m^3$) (n=211)	0.1 (0.05)	eGDR (mg/kg/min) (n= 223)	-0.2 (<0.01)
Alcoholic beverages consumed per day (n=229)	-0.05 (0.44)	AER (µg/min) (n=106)	0.5 (<0.01)

Table 4-3 Cox regression models for the association between SIF score and mortality in the EDC SIF sub-study

	Model 1 **HR (95% CI),** **p-value [a]**	**Model 2** **HR (95% CI),** **p-value [b]**	**Model 3** **HR (95% CI),** **p-value [b]**	**Model 4** **HR (95% CI),** **p-value [b]**	**Model 5** **HR (95% CI),** **p-value [b]**	**Model 6** **HR (95% CI),** **p-value [b]**
SIF (AU)	1.1 (1.0 to 1.2), p=0.02	1.1 (0.9 to 1.2), p=0.06	1.1 (1.0 to 1.2), p=0.04	1.1 (0.9 to 1.2), p=0.34	1.1 (0.9 to 1.2), p=0.22	1.1 (0.9 to 1.2), p=0.35
MDI	N/A	N/A	0.3 (0.1 to 0.8), p=0.02	N/A	N/A	N/A
Diabetes duration (years)	N/A	N/A	N/A	1.1 (1.0 to 1.2), p<0.01	N/A	N/A
A1c months	N/A	N/A	N/A	N/A	6.8 (1.7 to 27.6), p<0.01	N/A
eGFR ($mL/min/m^2$)	N/A	N/A	N/A	N/A	N/A	0.4 (0.2 to 1.0), p=0.05
AIC	201.9	162.5	159.7	156.9	155.9	161.4

[a] total sample of 245 observations with 20 mortality events

[b] missing covariate data for 44 observations; analysis included 201 observations with 16 mortality events

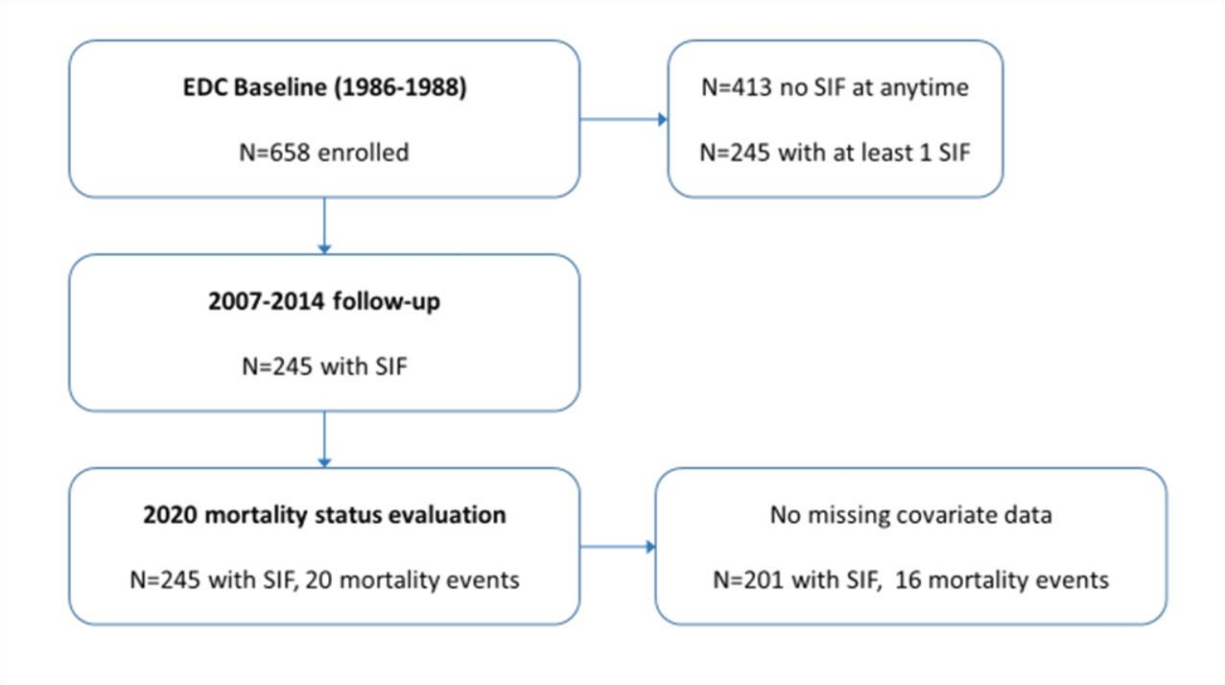

Figure 4-1 EDC cohort SIF sub-study participant flow for SIF and all-cause mortality

5.0 Discussion

5.1 Summary

Through this work, we observed that modifiable T1D-related complication risk factors and markers at analytic baseline, such as worse blood glucose control and lower kidney function, were associated with increased SIF scores over follow up. Further, increased albuminuria at analytic baseline was associated with decreased SIF scores during follow-up. Body mass index change was marginally and inversely associated with SIF score change. SIF was univariately associated with all-cause mortality; this association remained significant after adjustment for multiple daily insulin shots/pump use, but not after adjustment for diabetes duration, A1c months, or estimated glomerular filtration rate. The predictors of SIF change identified are aligned with known biological processes that occur during AGE formation, accumulation, and deposition on long lived proteins. Regarding all-cause mortality, this work builds on existing evidence that AGEs may play a role in ageing. This work demonstrates that SIF scores may be a useful tool in identification of historical glucose and kidney status, but not as a prospective screener for identifying real-time changes in status. This is in accordance with prior learnings from the DCCT/EDIC where SIF demonstrated stronger correlations with historical HbA1c over 10 years than over 5 years. Taken together, our work supports the existing evidence that AGEs may be a marker of complication status in T1D, and that AGEs may predict risk of accelerated ageing. Prospective studies are needed to fully establish the predictive ability of SIF scores to identify T1D patients who have a worse historical risk profile (i.e., worse blood glucose, worse kidney functioning).

5.2 Literature gaps

The first major gap identified in the published literature was a lack of prospective data regarding predictors of SIF in T1D. The published literature suggests that higher SIF scores, as a measure of collagen-linked AGEs, are associated with worse health status. This existing evidence, albeit primarily from cross-sectional studies, demonstrates an association between collagen-linked AGEs and kidney function, blood glucose, and some T1D complications [1-3]. Further, this emerging evidence suggests that AGEs have an important role in the oxidative damage of proteins [146], contributing to some chronic complications in T1D [144, 147-151, 154, 158]. Thus, despite being an essential part of normal physiology, AGE accumulation and deposit is particularly harmful because it promotes cellular destruction [136, 138, 139]. Even though scientific understanding is incomplete, such production of detectable AGEs has been implicated in promotion of protein modification, cellular stiffness, organ dysfunction, vessel rigidity, bone fragility, tissue damage, and muscle stiffness, and oxidative stress [161]. However, little is known about the prospective relationship of T1D morbidity as it relates to SIF. To address this literature gap, the first two aims of this dissertation test the relationship between T1D-related complication markers and changes in SIF scores among individuals with childhood-onset T1D.

Another major gap in the existing SIF literature involves mortality. In the past 40 years, survival for those with T1D has improved dramatically [75]. This increased life expectancy is likely partly attributable to improved glucose monitoring and control and a resulting decline in complications [131]. Despite these encouraging data and advances in treatment, individuals with T1D have an excess risk of mortality compared to the non-diabetic population [132]. AGEs are associated with ageing [4], senescence, development of age-related morbidities [5], and mortality [6-8].

Specifically in T1D, plasma AGEs have also been associated with incident all-cause and cardiovascular mortality [6]; and skin autofluorescent AGEs have been associated with all-cause and cardiovascular mortality [8]. Evidence for a causal association between AGEs and ageing is limited, however, and no prior studies evaluate SIF scores specifically in relationship to all-cause mortality in T1D. To fill this gap, the third aim in this work tested the association of collagen-linked AGE, assessed with a SIF score, and all-cause mortality in T1D.

5.3 Research findings

In the first aim, the objective was to identify predictors of SIF score change. We evaluated predictor variables (e.g., T1D complication markers) at one time point, analytic baseline, with the outcome of SIF score change over time (between analytic baseline and follow up, mean of 5.2 years later). We observed that modifiable T1D-related complication markers at analytic baseline, such as worse glucose control and lower kidney function were associated with increased SIF scores. Further, increased albuminuria at analytic baseline, after controlling for kidney function was associated with decreased SIF scores during follow-up. The connection between HbA1c and AGEs is well-known; the higher the blood glucose levels, the more AGEs form as a result of the glycation processes. Our research findings are thus consistent with existing cross-sectional research from the DCCT/EDIC study where historical HbA1c was strongly correlated with AGEs [2, 3, 200]. In this work we found that longer exposure to MDI was related to a reduction in SIF scores, independent of HbA1c concentrations. It is possible that MDI reflects additional protective behaviors and/or factors beyond average or mean glucose exposure, for example less extreme glucose excursions, that have beneficial health effects and are reflected in reduced SIF scores.

Regarding eGFR, lower kidney function was related to increased SIF scores. This finding is in concert with the biological mechanisms involved with kidney function and AGE clearance. The kidneys play a role in processing and clearing AGEs [142, 143], and AGEs contribute to the development of chronic kidney disease [145]. It has been speculated that declining kidney functioning with increased T1D duration may influence inadequate AGE detoxification [138, 139, 144]. Somewhat perplexing are the findings regarding the presence of overt nephropathy (urinary AER >=200 µg/min) being associated with decreased SIF scores. It should be noted that in this multi-variable finding, only 6 observations in our sample had overt nephropathy and thus these findings warrant exploration in a larger sample. Biologically, other research indicates that in patients with T1D, urinary excretion of AGEs increases with worsening albuminuria suggesting that AGEs are excreted in the context of reduced renal functioning [201]. AER is also inversely associated with and is modified by eGFR. We observed that when eGFR is accounted for, that is if the effect of decreased AGE clearance is removed, those with severe glomerular damage and leakage have greater renal loss of AGEs (high AER) and possibly decreased SIF.

The goal of aim 2 was to identify the relationship between change in T1D-related complication markers as predictors of SIF score change. The goal of this aim was to characterize changes in SIF scores and changes in T1D factors as hypothesis generating evidence to support future work on minimally important differences in SIF score. Thus, in this aim we evaluated change in both T1D complication markers and SIF scores between analytic baseline and follow up (mean 5.2 years). We observed that BMI change was marginally associated with SIF score change, although the direction of this inverse association was unexpected; the initial hypothesis was that increasing BMI is, in general, considered a marker of worse health and thus would be connected to increased SIF. The biological processes involved in the BMI—AGE relationship might be

explained by work performed outside of the T1D population. In healthy participants, BMI is inversely associated with serum AGEs; higher BMI is related to suppressed AGEs [205]. Excess accumulation of visceral and peripheral adipose tissue may play a role in trapping AGEs and possibly have the capacity to drive lower serum AGEs [206-208]. Although this other work is performed in the general population [206], healthy participants [205, 207], and T2D [208], and may or may not be directly comparable to our research, it may shed light on some of the biological processes involved in how AGEs accumulate. If having more fat, and thus higher BMI, truly does 'trap' circulating AGEs, this might result in reduced availability of AGEs for deposit in long-lived proteins such as collagen. Given SIF is a measure of collagen-linked AGEs, we might be seeing some of these biologic processes taking place in our negative, albeit non-significant, BMI—SIF association.

In the third aim, the SIF score was the predictor, rather than the outcome as in the previous two aims. Our goal was to evaluate the relationship between SIF scores at analytic baseline and risk of incident all-cause mortality over follow up (mean of 6.1 years, maximum 11.9 years). Our results indicate that SIF was univariately associated with all-cause mortality (HR: 1.1, 95% CI 0.9-1.2, p=0.06). This association remained significant after adjustment for MDI (HR: 1.1, 95% CI 1.0-1.2, p=0.04), although adjustment for diabetes duration, A1c months, or eGFR rendered SIF non-significant. Interestingly, although adjustment for diabetes duration, A1c months or eGFR reduced the significance of SIF for mortality risk, its effect size remained largely unaltered (i.e., the 95% CI was between 0.9 to 1.2 for SIF). This may suggest that power to detect an effect was limited due to the small number of events in this sub-cohort. Biologically, existing evidence suggests that the production of altered proteins from glycation are one of the most common hallmarks of ageing and increased risk of death [5, 159]. Further, both the receptors for AGE [162] and

plasma AGEs [6] have been found to be significantly associated with all-cause mortality. Although there are no other reports of evaluation of SIF scores and all-cause mortality in T1D, skin autofluorescence is independently significantly associated with CHD-mortality [8]. Our results demonstrated a less robust association compared to other research (95% CI 1.0-1.2 vs. 2.5-14.2, respectively) in terms of an independent role for AGEs in predicting mortality. However, a potential explanation may relate to our assessment of all-cause rather than CVD-related mortality. AGEs are thought to play a major role in progression of CVD [155], vascular rigidity, and arterial stiffness [136]. Overall, our results support the existing evidence that AGEs may play a role in accelerated ageing in T1D and suggest that SIF scores may detect biomarkers indicating risk of all-cause mortality.

5.4 Strengths and limitations

To our knowledge this research is the first work assessing exposure to T1D complication risk with SIF score change, and the relationship of SIF with all-cause mortality.

A major strength of our study is that participants are from the well-characterized EDC cohort comprised of individuals diagnosed between 1950-80 with childhood-onset T1D at the Children's Hospital of Pittsburgh, Pennsylvania, USA [187, 188]. Moreover, these are the first results reporting on complication markers associated with SIF score change, and SIF scores as a predictor of all-cause mortality in T1D, adding to the growing body of AGE-related evidence in T1D.

There are a few notable limitations in the analyses presented. The sample size for each aim was small and thus our findings may be limited in power to demonstrate associations. This was especially relevant in the evaluation of SIF scores and mortality where a larger sample size

may have provided greater statistical power to clarify the relationships and allow for multivariable adjustment.

An additional limitation is that the SIF sub-study sample was a non-random convenience sample, that is, not all participants in the EDC study cohort were originally asked to participate in the SIF sub-study. It is also unclear if there was self-selection bias in those who agreed to participate in the SIF sub-study. Self-selection bias occurs when participants in a particular sub-group of the population are more or less likely to participate than participants in another category. In our work, it may have been that participants who agreed to participate in the SIF sub-study were systematically different from those who chose not to participate. However, after performing a comparison of characteristics of participants and non-participants, we found there were generally no differences in participant characteristics. For example, in the 83 participants with change in SIF vs those without SIF at the 2004-06 EDC exam frame, we found there were statistically significant differences ($p<=0.05$) for age, age of T1D onset, BMI, WHR, ACR, eGDR, non-HDL, LDL, and SBP; of which only BMI was found significant in paper 2. Comparing those with change in SIF vs those who had no SIF at the 2010-14 time-frame, we found there were statistically significant differences ($p<=0.05$) for BMI, updated mean HbA1c, and eGDR.

Another important issue is survivor bias. Participants included in these analyses had to first survive to 2007-08 when the first SIF assessment occurred and then for aim 1 & 2 also further survive to the second SIF assessment (2010-14). Thus, participants included in this evaluation may be systematically different from many who were not. As noted above, a comparison of characteristics of participants and non-participants at analytic baseline found generally no major differences in participant attributes. Comparing those who survived to those who were deceased, we found there were statistically significant differences ($p<=0.05$) for SIF score, age, T1D

duration, BMI, HbA1c, A1c months, total insulin units per kg weight, MDI use, SBP, HTN, eGFR, eGDR, and AER. Further, since T1D and SIF are time-dependent processes, our findings could be biased by age; to attempt to reduce such bias we adjusted SIF scores by age[199]. In addition, collagen-linked AGE differ by gender [184] and therefore, each SIF value was also adjusted for gender.

One more limitation is that several other factors known to affect SIF scores were not identified. Skin fluorescence has multiple determinants and reflects broad components of a person's overall health and general characteristics. We also 43 know that factors such as diet and environmental exposures impact AGEs [5]. We were not able to account for these factors in our analysis. An individual's amount of skin collagen also affects the SIF score. The only way to assess the true amount of collagen is through skin punch biopsy, which we did not do. It should be noted that although SIF scores were intrinsically corrected (measured fluorescence is corrected for skin reflectance measured at the excitation and emission wavelength that are adjusted by separate excitation) to account for an individual's amount of skin collagen, we cannot be certain the correction accounted for all collagen variation in participants.

And finally, the technology producing the SIF score has limitations. While it is clear the SCOUT DS® assesses, in part, cross-linked fluorescent AGEs, it remains unknown which exact AGE compounds are being captured with a SIF score. Given this lack of clarity, there could be measurement error in the SIF dataset. Overall, due to the nature of sampling and small number of events, our findings require replication in larger cohorts. Given this is the first reporting of such findings, more work in this area needs to be performed to understand these associations in more depth.

5.5 Regulatory considerations

An additional component of this work is to describe the FDA medical device approval process and how the data from this work could support an FDA application for the SCOUT DS® device. Our work supports the use of SIF scores in T1D in the real-world setting.

The SCOUT DS® could be indicated as "a tool for identification of a patient's long-term historical glucose control and kidney function and as a way to identify mortality risk in T1D which could be particularly useful to identify patients who need more aggressive management of long-term complications". With this hypothetical indication additional prospective studies would be needed to evaluate the ability of SIF to identify those with higher risk of mortality and with a more severe historical blood glucose control and kidney function risk profile. One way to do this would be to perform a prospective trial to test the true positive and false positive rate between the SCOUT DS® and tests for detecting abnormal HbA1c and abnormal kidney functioning. Further, sensitivity, specificity, and positive and negative predictive values of the SCOUT DS® would be evaluated for these criteria. And finally, the test re-test reliability of SIF scores would be evaluated. This would be a prospective cohort trial of patients with T1D. Study duration would be 12 months with every 3-months follow up. At each visit, all participants would be screened with SIF and have a full set of exam data collected including biological samples to evaluate blood glucose, kidney functioning, and lipids.

The work presented in this dissertation establishes a foundation for the SCOUT DS® device. That is, our research identified that long-term T1D complications status and risk of all-cause mortality might be reflected in a SIF score. With this evidence, our work provides additional real-world evidence supporting the theory that tissue AGE are a measure of cumulative metabolic stress and trigger inflammatory reactions in T1D. Even with our findings and the body of cross-

sectional research, additional prospective research would be necessary to support a regulatory agency approval. Overall, these findings support further work in larger prospective studies of SIF scores in T1D.

5.6 Public health impact

Despite advances in T1D treatment, individuals with T1D have an excess risk of morbidity and mortality compared to the non-diabetic population [132]. Thus, mechanisms to identify patients' risk of morbidity and mortality remain important in the long-term management of T1D. Our work herein has focused on identification of complications and mortality in later T1D as identified with SIF. The deposit of AGEs, as identified with a SIF score, may have a role in detection of long-term complication status and mortality risk in T1D.

In the context of a broad spectrum of health management for T1D, SIF scores could be particularly helpful in providing a snapshot of a patient's health. Other technology designed to measure collagen-linked AGEs has been validated to aid in screening for CVD risk assessment in T2D [221], setting the precedence for the use of this type of technology in the health care setting. Our work suggests that the presence of long-term complications in T1D can be identified with a non-invasive SIF score. We also found that SIF scores can predict all-cause mortality. Thus, the SCOUT DS® device may be a valuable tool for identification of a patient's long-term historical glucose control and kidney function and as a way to identify mortality risk. This could be particularly useful to identify patients who need more aggressive management of long-term complications.

In addition, the SIF score could also be useful to evaluate the historical status of a new patient; that is, SIF could help a clinician quickly and easily see a snapshot of a patient's health and where they are in the disease spectrum. With this ability to identify important long-term T1D complication status, it provides clinicians with another tool in the management of these complications. The unique benefit of a SIF score is that it does not require any biological sampling or lab work to estimate risk. This potentially makes the tool more widely accessible in areas where it is not possible to perform traditional blood and urine sampling (e.g., mobile health clinics, public health screening clinics, or community led health screening events).

5.7 Future directions

This work establishes a foundation for future research of SIF scores in the clinical setting. While we identified that SIF scores can possibly detect T1D historical health status, we were not able to identify thresholds for SIF scores. Important work is still needed to fully characterize SIF scores. That is, it will be important to further define SIF score thresholds in relationship to T1D complications status. For example, we do not know what specific SIF score value corresponds to historical 10-year HbA1c, or if a change in albuminuria can be detected with a SIF score change. In establishing these types of scoring interpretations, a SIF score could possibly help monitor patient status and the effects of therapy over the long term. With regards to mortality, we found a promising signal that SIF may predict all-cause-mortality risk; whether SIF scores have a causal association with all-cause mortality in T1D has yet to be decided by experimental studies.

5.8 Conclusion

The predictors of SIF score change identified are aligned with known biological processes that occur during AGE formation, accumulation, and deposition in long lived proteins. Regarding all-cause mortality, our work builds on the growing body of evidence that AGEs may play a role in ageing. Taken together, this work supports the existing evidence that AGEs may be a marker of complication status in T1D, and that AGEs may predict risk of all-cause mortality. Further work is needed to fully validate the SCOUT DS® device and the interpretation of SIF scores before the tool could be implemented in clinical practice.

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