Understanding Metabolic Memory: A Tale of Two Studies

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The results of the Diabetes Control and Complications Trial (DCCT) have given rise to much encouragement in the battle to stave off the complications of type 1 diabetes, showing dramatic declines in the development of severe retinopathy, nephropathy, and neuropathy in those treated intensively compared with conventional therapy. Particularly encouraging has been the continuing difference between the two groups despite both having similar HbA_{1c} (~8%) since the end of DCCT, when 96% of participants entered the observational Epidemiology of Diabetes Interventions and Complications (EDIC) study. This continuing relative benefit has been termed "metabolic memory," which implies altered metabolic regulation. Based on evidence from both the Epidemiology of Diabetes Complications (EDC) prospective cohort study of childhood-onset type 1 diabetes and DCCT/EDIC, we show that the metabolic memory effect can be largely explained by lower cumulative glycemic exposure in the intensive therapy group, and, on average, the development of complications increases with greater glycemic exposure, irrespective of whether this results from a high exposure for a short time or a lower exposure for a longer time. Thus, there is no need for a concept like "metabolic memory" to explain these observations. Potential mechanisms explaining the cumulative glycemic effect are also briefly discussed.

The adoption of intensive diabetes therapy over the past 20–25 years has contributed to a reduced risk of some (1,2) but not all (3) complications in type 1 diabetes. However, despite improvements in average glycemic control, the vascular complications of diabetes continue to exert an enormous burden in terms of death and disability, quality of life, and health care costs (4). The Diabetes Control and Complications Trial (DCCT) definitively established the benefit of intensive diabetes therapy for preventing or delaying microvascular complications of type 1 diabetes (5). In the subsequent observational Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study, former members of the DCCT conventional therapy arm continued for some years to develop both micro- and macrovascular complications at a greater rate than former intensive arm members, despite similar levels of glycemic control in the two groups following the end of the randomized trial (6). This continuing increased risk, despite improvements in glycemic control, has been termed "metabolic memory" (7). A similar effect has also been observed in type 2 diabetes, where it was referred to as a "legacy effect" (8,9). However, the mechanisms behind this effect have not been fully elucidated and it is unclear whether continued protection against complications associated with a period of intensive diabetes therapy indicates a long-term change in metabolism or simply a lower cumulative glycemic exposure.

MEASURES OF ESTIMATED CUMULATIVE GLYCEMIC EXPOSURE IN TYPE 1 DIABETES

Cumulative glycemic exposure has been estimated using different measures in epidemiologic research. Recent analyses using DCCT/EDIC data have used time-weighted updated mean HbA_{1c} and found it to be the strongest risk factor for progression of retinopathy (10) and cardiovascular disease (CVD) after age (11) and that it explains all of the DCCT treatment group effect on CVD risk (12). In the Epidemiology of Diabetes Complications (EDC) study, an observational, prospective cohort study of childhood-onset type 1 diabetes, cumulative glycemic exposure has previously been estimated using a metric called "A1 Months," which is calculated by multiplying the number of HbA_1 % units above the upper limit of normal (7.3%) at each study visit by the number of months elapsing between the midpoints of the previous and subsequent visits, and

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summed over the entire follow-up time (13). While both the time-weighted updated mean HbA_{1c} and the A1 Months summarize cumulative exposure to hyperglycemia, a metric like A1 Months facilitates greater ease of interpretation for patients and clinicians. In earlier EDC analyses incorporating the first 10 years of follow-up data, the mean exposure when microvascular complications were first observed was ≥1,000 A1 Months (approximately equivalent to 900 A1c Months) (13), a value above which risk rose dramatically for kidney and nerve complications. A1 Months also easily demonstrates the idea that the same level of cumulative glycemic exposure can be reached by either a greater exposure for a relatively short period of time or a lesser exposure for a longer period of time. For example, 1,000 A1 Months can be reached by having an average HbA_1 4 units above normal (i.e., 7.3%, the upper limit of normal) for 21 years or an average of 2 units above normal for 42 years. More recently, Dekker et al. (14) calculated a measure of cumulative glycemic exposure based on the area under the curve of HbA_{1c} above "normal" (7.0%) over 15 years and found that increasing exposure was associated with diabetic foot ulcers. This approach is conceptually similar to A1 Months but more difficult to utilize clinically, as the interpretation may be less clear for patients.

REVISTING CUMULATIVE GLYCEMIC EXPOSURE AND COMPLICATION RISK

To examine whether the cumulative glycemic exposure and complication risk association is consistent over time and by cohort, we now *1*) update the EDC A1 Months analysis using A1c Months (based on HbA_{1c}, rather than HbA_1), focusing exclusively on the subcohort diagnosed during 1965–1980, who experienced the majority of their natural history under contemporary treatment guidelines (e.g., HbA_{1c} testing and self-monitoring of blood glucose), and incorporating a total of 25 years of follow-up for complication incidence, *2*) explore whether cumulative glycemic exposure shows similar associations in an independent cohort (DCCT/EDIC), and *3*) examine whether cumulative glycemic exposure explains the metabolic memory effect in DCCT/EDIC.

EDC Study Cohort

The Pittsburgh EDC study is a prospective cohort study of childhood-onset (<17 years old) type 1 diabetes. All participants ($n = 658$) were diagnosed or seen within 1 year of diagnosis at Children's Hospital of Pittsburgh between 1950 and 1980. The analyses reported here are restricted to the more recently diagnosed 1965–1980 subcohort ($n = 434$), who had very low mortality prior to study baseline and the majority of their diabetes duration under contemporary management. The cohort has been described in detail elsewhere (15,16). In brief, participants have been followed since 1986–1988, initially with biennial examinations for 10 years and thereafter with biennial questionnaires and further examinations at 18 and 25 years post-baseline. Research protocols were approved by the University of Pittsburgh institutional review board, and all participants provided written informed consent.

EDC participants were followed for 25 years to ascertain incidence of complications, including proliferative diabetic retinopathy, microalbuminuria, overt nephropathy, end-stage renal disease (ESRD), coronary artery disease (CAD), and confirmed distal symmetric polyneuropathy (CDSP). A detailed description of complication ascertainment can be found in the Supplementary Data.

Assessing HbA_{1c} and A1c Months in the EDC Study

In the EDC study, HbA_1 was assessed at baseline and repeated at 2, 4, 6, 8, and 10 years, and HbA_{1c} was assessed at 14 and 18 years of follow-up. For the first 18 months of the study, HbA_1 was measured in fasting blood samples using microcolumn cation exchange (Isolab, Akron, OH). For the remainder of the first 10 years of follow-up, HbA_1 was measured using automated high-performance liquid chromatography (Diamat; Bio-Rad, Hercules, CA). The two assays had high agreement ($r = 0.95$; Diamat $HbA_1 = -0.18 + 1.00$[Isolab HbA_1]). HbA_1 values were converted to DCCT-aligned HbA_{1c} values using a regression equation derived from duplicate assays (DCCT $HbA_{1c} = 0.14 + 0.83$[EDC HbA_1]) (17). At the 14- and 18-year examinations, HbA_{1c} was measured using the DCA 2000 Analyzer (Bayer Healthcare LLC, Elkhart, IN) and converted to DCCT-aligned HbA_{1c} by the equation DCCT $HbA_{1c} = (\text{EDC } HbA_{1c} - 1.13)/0.81$.

The methodology used to calculate A1 Months has been reported in detail previously (13). We have now updated the calculation using HbA_{1c} (rather than HbA_1). Supplementary Fig. 1 depicts the calculation of A1c Months with examples from three EDC participants. Briefly, A1c Months is calculated by summing the product of the number of months from diabetes diagnosis to the midpoint between the baseline (1986–1988) and visit two × HbA_{1c} units above normal (6.1%, which corresponds to 7.3% HbA_1 units used in the original analysis) at baseline plus the product of the number of months from the midpoint between baseline and visit two × HbA_{1c} units above normal at visit two plus the product of the number of months from the midpoint between visit two and visit three × HbA_{1c} units above normal at visit three and so on to include all follow-up time and HbA_{1c} measurements up to 18 years. It is important to note that the A1c Months calculated at each interval is added to the cumulative total from the preceding interval, providing an estimate of the cumulative glycemic exposure from diabetes diagnosis at each time point.

Updating the A1c Months Analysis

In the main EDC analyses, participants with prevalence of a given complication at study baseline were excluded from the corresponding analyses. At each study visit, an A1c Months variable capturing glycemic exposure up to that visit was calculated and time-varying Cox models were fit

to assess the hazard ratio associated with each 100–A1c Month increment. Categories of A1c Months based on approximate quintiles of the overall distribution (<570 [reference group], 570–769, 770–969, 970–1,249, and ≥1,250 A1c Months) were also created, and time-varying Cox models were fit to estimate the hazard ratio associated with each category. These categories were not treated as ordinal variables, thus linearity across the categories was not assumed. The attributable risk percent (AR %), or the proportion of cases that can be attributed to excess glycemic exposure (i.e., that associated with ≥2% average excess HbA_{1c} exposure over follow-up, corresponding to approximately 900 A1c Months on average), was also calculated for each complication, with the corresponding 95% CI.

Cumulative Glycemic Exposure and Complications in EDC

Baseline characteristics of the 1965–1980 diabetes diagnosis subcohort of the EDC study are shown in Table 1. The mean (SD) A1c Months at complication development and hazard ratio (95% CI) associated with each 100-unit increase in A1c Months are shown in Table 2. The absolute mean A1c Months exposure at complication development was smallest for microalbuminuria at 844 A1c Months, followed by approximately 900 A1c Months for proliferative retinopathy and CDSP, 1,100 A1c Months for overt nephropathy and CAD, and nearly 1,300 A1c Months for ESRD. Increasing A1c Months was significantly associated with an increased risk of all of the complications, with hazard ratios per 100–A1c Month increment ranging from 1.09 (95% CI 1.04, 1.14) for CAD to 1.20 (95% CI 1.14, 1.27) for overt nephropathy. Importantly, when stratified by tertile of diabetes duration, the absolute mean exposure at complication diagnosis was approximately 900 A1c Months for proliferative retinopathy, microalbuminuria, and CDSP, regardless of diabetes duration category (Table 3). Similarly, though higher, the absolute mean A1c Months at development of overt nephropathy, ESRD, and CAD also did not differ by diabetes duration.

Table 1—Characteristics of the EDC 1965–1980 type 1 diabetes diagnosis subcohort at study baseline (1986–1988)

Characteristic	Mean (SD), unless otherwise noted
n	434
Age (years)	23.4 (5.6)
Diabetes duration (years)	14.8 (4.3)
Age at diabetes onset (years)	8.6 (4.1)
Female sex, % (*n*)	49.8% (216)
African American, % (*n*)	2.3% (10)
HbA_{1c} (%; mmol/mol)	8.91 (1.57); 74 (17.2)
A1c Months	528.6 (319.7)
Prevalent complications, % (*n*)	
Proliferative retinopathy	16.1% (70)
Microalbuminuria	39.2% (170)
Overt nephropathy	18.2% (79)
ESRD	1.2% (5)
CDSP	14.1% (61)
CAD	4.8% (21)

Figure 1 shows a forest plot of the hazard ratio and 95% CI associated with each A1c Month quintile, compared with the reference group with <570 A1c Months. Though the pattern varies a little for each complication, the hazard ratio generally increased by A1c Month quintile, reaching 10.6 (3.5, 32.5) for the top quintile for ESRD but only 2.7 (1.4, 5.2) for CAD.

The AR % associated with a ≥2% average excess HbA_{1c} exposure for each complication is shown in Table 4. Consistent with the results above, AR % was lowest for CAD at 30%, while it was somewhat higher for proliferative retinopathy, microalbuminuria, and CDSP, ranging from 44% to 48%, and highest for overt nephropathy (80%) and ESRD (77%).

Comparison With DCCT/EDIC

Data from the DCCT/EDIC study were supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Central Repositories. A1c Months was calculated using all available HbA_{1c} measures from DCCT baseline through EDIC year 8. We report A1c Months at DCCT baseline, DCCT closeout, and EDIC year 8. A subgroup of the EDC cohort with comparable characteristics to the DCCT was selected for comparison analyses. This EDC subgroup has been utilized in a previous joint analysis with the DCCT/EDIC investigators (1) and comprises a subset of the post-1965 diabetes diagnosis cohort of the EDC. The participants selected for this DCCT-comparable subgroup were 13–39 years old with diabetes duration <15 years and retinopathy grade <30 at study baseline (n = 161). To maximize comparability, the three complications (proliferative retinopathy, overt nephropathy/albuminuria, and CVD) examined in the aforementioned joint analysis (1) are also compared here. These complications were originally chosen because they were ascertained using similar methodology in both studies. Notably, the methods used to determine neuropathy in EDC and DCCT/EDIC were not comparable, so it was excluded from the analyses.

To determine whether the cumulative incidence rates in DCCT/EDIC differ from what would be expected based on EDC A1c Months exposure, the cumulative incidence of each complication across the range of A1c Months was estimated using Weibull regression for interval-censored data within the DCCT-comparable subgroup of EDC. Additional details regarding the modeling can be found in the Supplementary Data. The mean A1c Months of 534 and 729 observed in the intensive and conventional treatment arms, respectively, at EDIC year 8 (mean diabetes duration 20 years) were used as the comparison reference points. The EDC-estimated cumulative incidences of proliferative retinopathy, overt nephropathy, and CVD at 534 and 729 A1c Months were compared with the published cumulative incidences, which were also derived using

Table 2—A1c Months and complications, in ascending order of A1c Months, in the EDC 1965–1980 diabetes diagnosis subcohort (*n* = 434)

Complication	A1c Months at the time of incidence, mean (SD) and median complication (p25, p75)	Hazard ratio per 100 A1c Months (95% CI)*	*P* value
Microalbuminuria (*n* at risk = 234**, 99 events)	844 (423) 782 (573, 1,043)	1.17 (1.11, 1.23)	<0.0001
Proliferative retinopathy (*n* at risk = 347**, 192 events)	907 (383) 866 (620, 1,187)	1.14 (1.10, 1.18)	<0.0001
CDSP (*n* at risk = 325**, 127 events)	963 (444) 919 (629, 1,246)	1.12 (1.07, 1.16)	<0.0001
CAD (*n* at risk = 413**, 98 events)	1,068 (469) 986 (725, 1,398)	1.09 (1.04, 1.14)	0.0004
Overt nephropathy (*n* at risk = 314**, 61 events)	1,139 (537) 1,035 (744, 1,471)	1.20 (1.14, 1.27)	<0.0001
ESRD (*n* at risk = 429**, 53 events)	1,265 (495) 1,274 (873, 1,618)	1.18 (1.12, 1.25)	<0.0001

p25, 25th percentile; p75, 75th percentile. *Estimated using time-varying Cox models. **Prevalent cases at baseline excluded from analysis.

Weibull models, at 20 years' diabetes duration in DCCT/EDIC (1).

The mean HbA_{1c} during DCCT was 7.2% (55 mmol/mol) in the intensive group and 9.1% (76 mmol/mol) in the conventional group. At DCCT closeout, the conventional therapy group had, on average, 180 greater A1c Months compared with the intensive group (mean A1c Months 509 and 329, respectively, $P < 0.0001$). At EDIC year 8, this differential was maintained and the former conventional therapy group had an average of 195 greater A1c Months compared with the intensive group (mean A1c Months 729 and 534, respectively, $P < 0.0001$). The predicted cumulative incidence curves for proliferative retinopathy, overt nephropathy, and CVD in the DCCT-comparable subgroup of EDC and the reported cumulative incidences at 20 years of diabetes duration for the intensive and conventional therapy arms of DCCT/EDIC are shown in Fig. 2, while Table 5 compares numerically these predicted cumulative incidences at 534 and 729 A1c Months in EDC with the reported (1) cumulative incidence at 20 years of diabetes duration in DCCT/EDIC in the conventional and intensive therapy arms. The relative differential in the 20-year cumulative incidences reported for all three complications between the

Table 3—Mean A1c Months at complication development by diabetes duration in the EDC 1965–1980 diabetes diagnosis subcohort

	Type 1 diabetes duration at baseline (years)			
	<12	12–16	≥17	*P* value
Microalbuminuria	*n* at risk = 106 759 ± 339 (43)	*n* at risk = 69 879 ± 530 (29)	*n* at risk = 59 941 ± 404 (27)	0.19
Proliferative retinopathy	*n* at risk = 132 885 ± 353 (62)	*n* at risk = 118 858 ± 355 (68)	*n* at risk = 97 984 ± 434 (62)	0.15
CAD	*n* at risk = 134 897 ± 431 (19)	*n* at risk = 138 1,091 ± 517 (30)	*n* at risk = 141 1,119 ± 446 (49)	0.21
CDSP	*n* at risk = 123 993 ± 477 (38)	*n* at risk = 116 922 ± 467 (50)	*n* at risk = 86 987 ± 383 (39)	0.70
Overt nephropathy	*n* at risk = 124 1,071 ± 550 (27)	*n* at risk = 99 1,114 ± 554 (20)	n at risk = 91 1,309 ± 486 (14)	0.40
ESRD	*n* at risk = 139 1,217 ± 463 (16)	*n* at risk = 144 1,259 ± 516 (16)	*n* at risk = 146 1,264 ± 525 (21)	0.99

Values are mean ± SD (number of cases). Prevalent cases of each complication at baseline were excluded from the respective analysis.

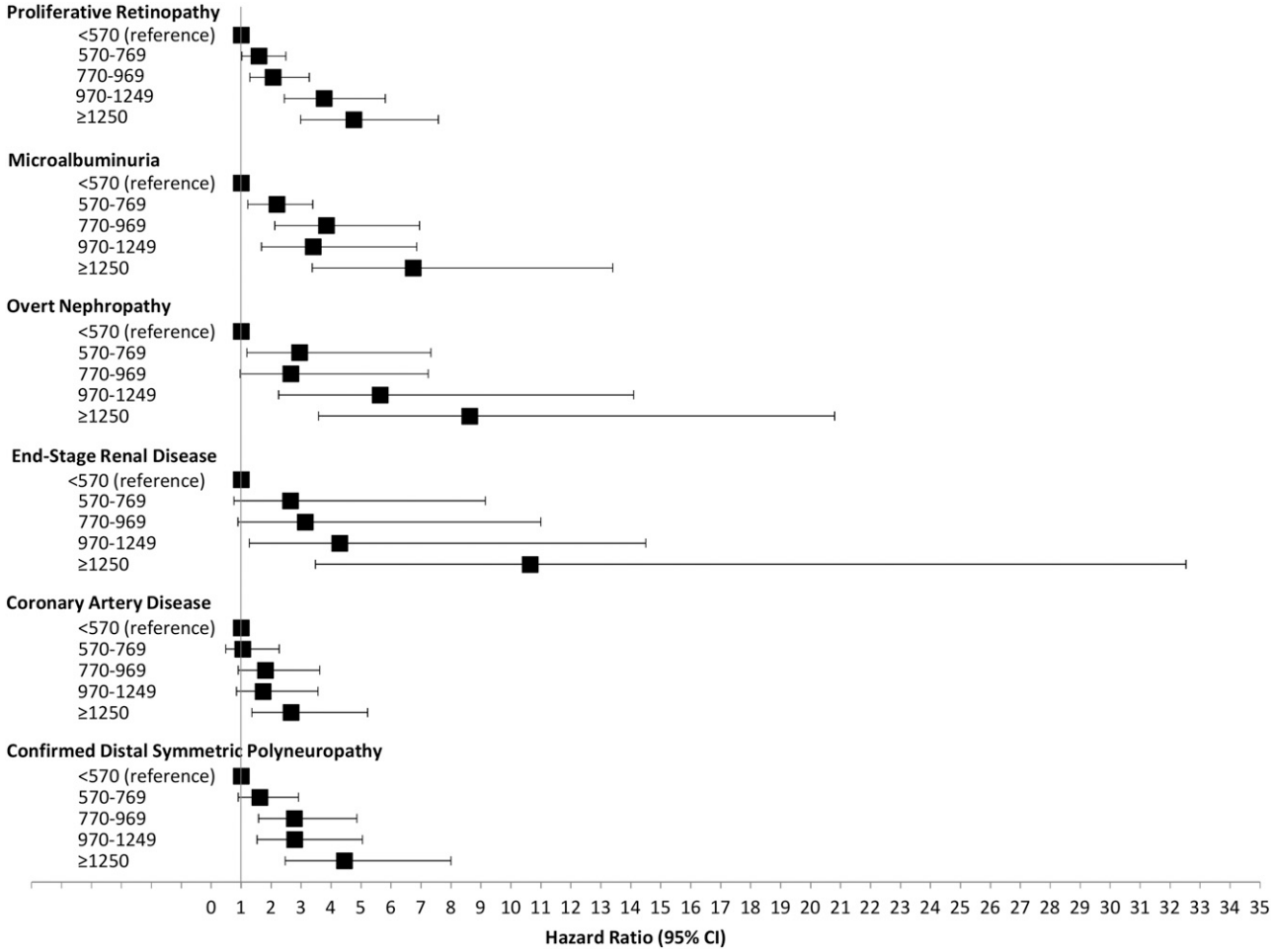

Figure 1—Hazard ratios associated with each A1c Month quintile, compared with the reference group (<570 A1c Months). Error bars are 95% CI.

DCCT/EDIC intensive and conventional therapy arms (1) is close to that predicted by the EDC cumulative incidence curves based on A1c Months. Thus, for proliferative retinopathy, there was a 49% predicted relative risk reduction (RRR) associated with 534 versus 729 A1c Months compared with a 61% (1) RRR observed with intensive therapy versus conventional, while for overt nephropathy the corresponding RRRs were 54% and 67% and for CVD 41% and 40%. The somewhat higher absolute incidences in DCCT/EDIC likely reflect the older age of onset in DCCT/EDIC (3), while the lower CVD incidences likely reflect the exclusion of high-risk participants in DCCT/EDIC.

Table 4—AR % associated with ≥2% average excess HbA_{1c} exposure in the EDC 1965–1980 diabetes diagnosis subcohort

Outcome	AR % (95% CI)
Proliferative retinopathy	47.7% (30.7, 64.8)
Microalbuminuria	43.9% (17.0, 70.9)
Overt nephropathy	80.5% (44.3, 100)
ESRD	76.6% (34.8, 100)
CDSP	47.1% (22.4, 71.9)
CAD	30.4% (0.0, 64.0)

Limitations of the EDC–DCCT/EDIC Comparison

There are some important differences in the measurement of HbA_{1c} between the EDC and DCCT/EDIC studies that should be noted. In the EDC study, HbA_{1c} was measured every 2 years during the first 10 years of follow-up and once more at 18 years. HbA_{1c} was measured more frequently in DCCT/EDIC, with quarterly assessments during DCCT and annual assessments during EDIC. This difference in frequency of measurement means that the precision of HbA_{1c} measures over time, and thus also that of A1c Months, is higher in DCCT/EDIC. Additionally, in EDC, HbA_{1c} measurements were aligned to DCCT values using a validated regression equation (described in ASSESSING HBA1C AND A1C MONTHS IN THE EDC STUDY), which also introduces additional variability into the EDC measurements.

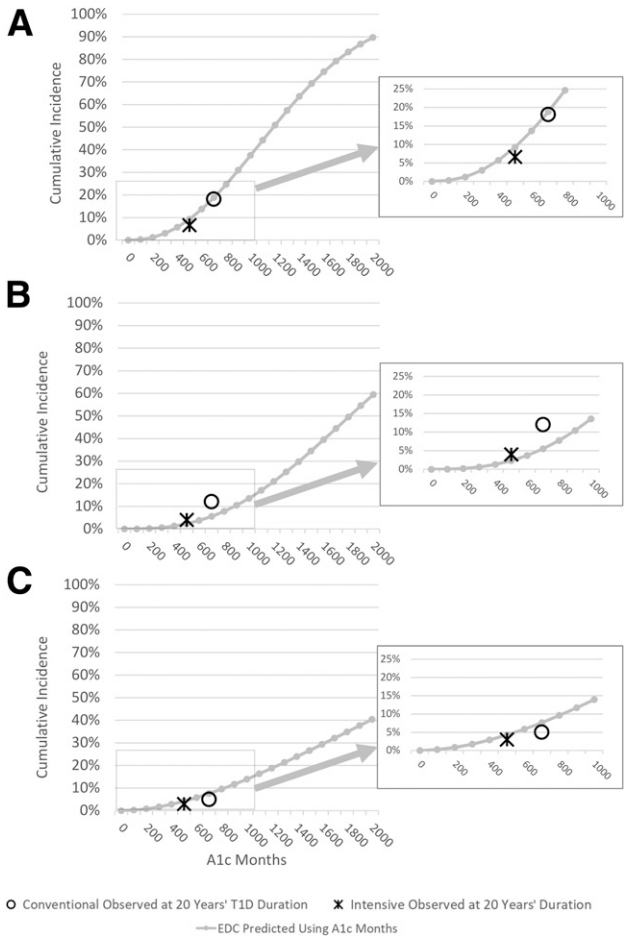

Figure 2—Predicted cumulative incidence of retinopathy (*A*), overt nephropathy (*B*), and CVD (*C*) by A1c Months in the DCCT-eligible subgroup of the EDC cohort (line) and the observed cumulative incidence in the DCCT/EDIC conventional (circle) and intensive (star) groups at 20 years' duration (corresponding observed mean A1c Months 729 and 534, respectively). The inset panels provide a closer view of cumulative incidence curve at <1,000 A1c Months. T1D, type 1 diabetes.

CUMULATIVE GLYCEMIC EXPOSURE EXPLAINS COMPLICATION RISK WITHOUT INVOKING A "METABOLIC MEMORY"

While the concept of metabolic memory is provocative, isolating such an effect from the concomitant effects of cumulative glycemic exposure itself is challenging and potentially misleading. Even within the DCCT/EDIC, cumulative glycemic exposure itself, estimated using updated mean HbA_{1c}, explains nearly all of the risk reduction associated with intensive therapy on retinopathy, overt nephropathy, and CVD. Importantly, by EDIC year 18, the annual rates of retinopathy were nearly identical in both the former intensive and conventional treatment groups, suggesting that the memory effect had faded (18). This observation supports the hypothesis that the memory effect was simply due to a difference in cumulative glycemic exposure during (and, in the short term, after) the trial period and that this differential matters less as the total accumulation of glycemic exposure increases in both groups over longer follow-up. Interestingly, the Veterans Affairs Diabetes Trial (VADT) also showed the suggestion of a memory or legacy effect for CVD in type 2 diabetes after 10 years of follow-up (19,20), but it is now reported to have been lost by 15 years (21), again suggesting that any memory effect conferred by earlier intensive therapy dissipates with greater total cumulative glycemic exposure.

The analyses presented here suggest that, on average, complication development occurs after sufficient glycemic exposure has been experienced, and the same level of cumulative exposure can result from either a high exposure for a short period of time or a lower exposure for a longer period of time. In EDC, approximately 900 A1c Months was the average exposure at which complication development began to occur. Supplementary Table 1 shows the number of years it would take at varying levels of excess HbA_{1c} exposure to reach 900 A1c Months. Evidence from both EDC and DCCT/EDIC supports the thesis that cumulative glycemic exposure explains most microvascular complication incidence without invoking any concept of metabolic memory. On the other hand, the role of hyperglycemia in the development of macrovascular complications is less straightforward. In EDC, the risk attributable to $\geq$2% average excess HbA_{1c} exposure over follow-up is only 30% for CAD, compared with 44–80% for microvascular complications.

POTENTIAL MECHANISMS UNDERLYING THE LONG-TERM EFFECTS OF CUMULATIVE GLYCEMIC EXPOSURE ON COMPLICATION RISK

There is some cell culture and animal model evidence that hyperglycemic exposure may result in long-lasting effects. In an early article, Roy et al. (22) demonstrated that in human endothelial cells, overexpression of fibronectin and collagen IV induced by high glucose exposure remained for 9 days of follow-up after normal glucose was restored. More recently, brief periods of high glucose exposure have been shown to increase inflammatory gene expression and oxidative stress (23) and are associated with persistent epigenetic changes (24) in cells. In animal models, retinal complications (25) continued for 2.5 years of follow-up and increased inflammation and oxidative stress in the kidneys (26) and retina (27) continued for several months of follow-up after reversal of hyperglycemia. These observations are, however, out of necessity, based on both short-term exposure and follow-up. Two mechanisms likely to relate to cumulative glycemic damage over the long term are advanced glycation end product (AGE) formation and epigenetic modifications.

Skin Collagen Glycation and AGEs

The strong associations between both skin collagen glycation and AGEs with complication development (28–33) supports the importance of a direct effect of cumulative glycemic exposure itself. In DCCT/EDIC, skin collagen glycation, as measured by the early Amadori product, furosine, was strongly associated with risk of microvascular

Table 5—Predicted cumulative incidence of complications associated with 534 and 729 A1c Months in the DCCT-eligible subgroup of the EDC cohort compared with the reported cumulative incidence in the DCCT/EDIC intensive and conventional treatment groups at 20 years' diabetes duration

	DCCT treatment group		
	Conventional (n = 730)	Intensive (n = 711)	RRR (95% CI)
A1c Months	729	534	
Proliferative retinopathy			
EDC predicted, based on A1c Months	20.5%	10.7%	48% (32, 59)
DCCT/EDIC reported, 20 years	18%	7%	61% (47, 71)
Overt nephropathy			
EDC predicted, based on A1c Months	6.1%	2.8%	54% (24, 73)
DCCT/EDIC reported, 20 years	12%	4%	67% (51, 78)
CVD			
EDC predicted, based on A1c Months	8.2%	4.8%	41% (13, 61)
DCCT/EDIC reported, 20 years	5%	3%	40% (0, 65)

complications (30–32) and subclinical CVD (33). Intensive treatment was associated with significantly lower levels of furosine compared with conventional treatment. This differential in furosine level between treatment groups was similar in both the primary and secondary cohorts of DCCT, despite longer diabetes duration and greater microvascular disease in the secondary cohort at baseline (31). Interestingly, participants with higher levels of furosine (>75th percentile) but low HbA_{1c} (<75th percentile) had double the risk of progression to retinopathy compared with participants with high HbA_{1c} but low furosine (30). In this and subsequent analyses, furosine was a stronger predictor of microvascular complications than mean HbA_{1c} (30,32).

AGEs, in addition to being markers of cumulative glycemic exposure, are actively involved in tissue damage through extra- and intracellular protein modification and the activation of signaling cascades resulting in proinflammatory mediators and reactive oxygen species (34,35). Skin intrinsic fluorescence (SIF) is a measure that partially reflects skin AGEs, as well as other fluorescence in the skin, that has been associated with complication prevalence (36–39). In a subgroup of the EDC study with SIF measured at the 20-year follow-up, there was a stronger cross-sectional association between SIF and CAD, CDSP, and autonomic neuropathy than that observed for updated mean HbA_{1c} (37,38). SIF was also strongly associated with presence and severity of coronary artery calcification (36). In cross-sectional analyses at EDIC years 16–17, moderate univariate associations between SIF and retinopathy, neuropathy, cardiac autonomic neuropathy, nephropathy, and coronary artery calcification were also observed (39). Interestingly, in DCCT/EDIC, it was observed that the correlation between HbA_{1c} and SIF increased the further back in time that measurements of HbA_{1c} were incorporated. This relationship is thought to reflect the long half-life of collagen and increasing related AGE formation over time. There was, however, one key exception: when the mean HbA_{1c} during the DCCT was included, the correlation between HbA_{1c} and SIF did not increase in the intensive therapy group but did increase in the conventional therapy group, as expected (40). Thus, the 6-year period of relatively normal glycemia in the intensive therapy group is identifiable >20 years later, in terms of lower cumulative glycemic damage.

Epigenetic Modifications

Another likely contributor to the damage cumulative glycemic exposure may cause is epigenetic modification (41–43). Knowledge of the contribution of epigenetics to vascular complication risk is still limited, but there is growing evidence for epigenetic regulation of dyslipidemia, inflammation, and glycemia (44,45). Epigenetic modifications, therefore, including DNA methylation and changes in microRNAs (42), may prove to be an important link between diabetes and long-term vascular complication risk. In a recent report examining genome-wide methylation assessed in two samples collected 16–17 years apart in DCCT/EDIC, a subset of former conventional group members with a history of poor glycemic control and progression of microvascular complications was compared with a subset of former intensive group members with a history of good glycemic control and no progression of complications (43). Differential methylation between the two groups persisted at several loci across the two time points, including at a CpG site in the thioredoxin-interacting protein (*TXNIP*) gene, which has been associated with hyperglycemia and microvascular disease (46–48). These results thus provide strong evidence of a direct relationship between past glycemic exposure and DNA methylation in type 1 diabetes that may drive complication development.

SUMMARY

There is no need to invoke a "metabolic memory" phenomenon to explain the persistence of a lower incidence of complications in the DCCT intensive therapy group compared with conventional therapy group, which can be fully explained by cumulative glycemic exposure. The effect of cumulative glycemic exposure itself is more likely mediated

by other mechanisms, including AGE formation and epigenetic modification. These effects may, therefore, be better termed "cumulative glycemic effects" and do not require the concept of "metabolic memory." Regardless of the semantics, it is clear that given sufficient diabetes duration and glycemic exposure, most patients with type 1 diabetes will develop most complications (3). Future research should thus focus on further understanding the pathogenic mechanisms for cumulative glycemic exposure itself.

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Duality of Interest. T.J.O. is a consultant for Boehringer Ingelheim International GmbH. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. R.G.M. wrote the manuscript, researched data, and performed statistical analyses. T.J.O. wrote the manuscript and researched data.

Data and Resource Availability. The EDC data sets analyzed during the current study are available from the corresponding author upon reasonable request. The DCCT/EDIC study data sets analyzed during the current study are available by request from the NIDDK Central Repository (https://repository.niddk.nih.gov). No applicable resources were generated or analyzed during the current study.

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