Severe Hypoglycemia, Risk of Recurrent Events and Weight Gain on Insulin Therapy in Patients with Type 1 Diabetes Mellitus

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ABSTRACT

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Severe Hypoglycemia, Risk of Recurrent Events and Weight Gain
on Insulin Therapy in Patients with Type 1 Diabetes Mellitus
(Under the direction of Til Stürmer, M.D., MPH)

BACKGROUND: Few studies provide epidemiologic evidence to demonstrate acute effects of severe hypoglycemia (SH) on subsequent SH in patients with type 1 diabetes mellitus (T1DM) under a clinically relevant time range. Although it is well accepted that hypoglycemia could associate with subsequent weight gain in T1DM, there is limited direct evidence to support this hypothesis.

METHODS: We conducted a secondary data analysis using data from the Diabetes Control and Complications Trial (DCCT) which randomized T1DM patients to either intensive (IT) or conventional therapy (CT) and followed them quarterly. We estimated relative risks (RR) for subsequent SH in three consecutive quarterly time windows following occurrence of SH (index SH). We estimated the effects of SH on subsequent weight change/weight (kg) in various observation periods. We used generalized estimating equations to account for the dependence of multiple-observations within a person and to adjust for confounding. Hazard ratios (HRs) of SH on substantial weight gain, overweight and obesity were estimated using Cox and marginal structural models.

RESULTS: in both treatment arms, the greatest absolute risks and RRs for subsequent SH after index SH were observed in the first time window in three consecutive quarterly windows following index SH. In IT, the estimated effect of SH on weight change/weight was close to null effect. In CT, a weight loss was observed during the 1st 3-month following SH

[adjusted difference of the means for weight change (MDWC) was -0.29 (-0.51, -0.08)] comparing to those without SH. The HRs of SH on substantial weight gain, becoming overweight or obese were also found around the null effect (HR=1) in both treatment arms.

CONCLUSIONS: This study provides direct evidence to support an acute effect of recent SH on subsequent SH episodes, and the results indicate that the immediate time periods after occurrence of SH are crucial in clinical management of T1DM to prevent subsequent SH. We did not find evidence to support an association between occurrence of SH and subsequent weight gain in patients with T1DM. Because the DCCT is a clinical trial design, one should be cautious in extrapolating our findings to all patients with T1DM.

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LIST OF ABBREVIATIONS

CI	Confidence Interval
DAG	Directed Acyclic Graph
DCCT	Diabetes Control and Complication Trial
EDIC	Epidemiology of Diabetes Interventions and Complications study
GEE	Generalized Estimating Equations
HAAF	Hypoglycemia-associated autonomic failure
A1C	Glycated hemoglobin
MBG	Mean blood glucose
TDID	Typical daily insulin dose
SBGM	Self-blood glucose monitoring
SH	Severe hypoglycemia

I. INTRODUCTION

Hypoglycemia and weight gain are two primary adverse events during insulin therapy among patients with type 1 diabetes [1-4]. Hypoglycemia has been documented to be the leading limiting factor of intensive diabetes management among patients with type 1 diabetes mellitus [5, 6]. Hypoglycemia also causes recurrent physical and psychological morbidity, higher risk of mortality and impairs defenses against subsequent hypoglycemia [1]. Although weight gain after initial insulin treatment is often perceived as desirable [4] in type 1 diabetes, excessive weight gain can not only increase diabetic morbidity and mortality when weight gain becomes a barrier to the intensification of insulin treatment, but also adversely affect cardiovascular risk profiles as well [4, 7].

Severe hypoglycemia (SH) can induce the defect in counterregulation and loss of awareness of hypoglycemia under the mechanism of hypoglycemia-associated autonomic failure (HAAF)[1]. Thus, prior SH can cause a vicious cycle of recurrent hypoglycemia [2, 8], and the effect of SH episodes is often regarded as an acute effect that the greatest risk may occur in weeks and months after episodes of prior SH [1].

Although prior studies have consistently reported prior SH as a risk factor in general for recurrent subsequent SH [9-16], epidemiological evidence which could provide information for clinical management of occurrence of SH in the patients with type 1 diabetes on insulin therapy has not been fully illustrated. Very few studies provide epidemiologic evidence to demonstrate the acute effects of SH on subsequent SH in a clinically relevant time range, and the magnitude of the effects from population levels in patients with type 1 diabetes is not completely clear either.

A number of possible mechanisms have been described to explain weight gain on insulin therapy including compensation for hypoglycemia [4]. The unpleasant symptoms and negative consequences of hypoglycemia may result in significant fear of hypoglycemia [17]. Because low blood glucose levels can be remedied by ingestion of glucose or food, following a hypoglycemic event, patients may over-react or be instructed by consuming more calories (e.g., frequent snacking) in response to the threat of subsequent hypoglycemia. However, there is also very limited direct evidence to support this hypothesis of compensation for hypoglycemia.

The objectives of this study are: 1) in a clinically relevant time range, to estimate and illustrate the acute effects of SH on risk of subsequent SH episodes in patients with type 1 diabetes; 2) to provide insight into the interplay of two primary adverse events (SH and weight gain) in patients with type 1 diabetes. Eventually, the results should provide physicians with useful information to help them better clinically manage patients with type 1 diabetes on insulin therapy to reduce recurrent SH and excessive weight gain.

II. REVIEW OF THE LITERATURE

A. Hypoglycemia in patients with type 1 Diabetes

Hypoglycemia in insulin-treated patients has been documented to be the most common adverse effect and leading limiting factor of intensive diabetes management among type 1 diabetes mellitus [5, 6]. Hypoglycemia associated with insulin treatment also causes recurrent physical and psychological morbidity, mortality and impairs defenses against subsequent hypoglycemia [1]. Hypoglycemia has been classified as “asymptomatic” or “biochemical”, which is particularly common, and “symptomatic”. Symptoms of hypoglycemia may be idiosyncratic. Neurogenic symptoms often include palpitations, tremor, hunger, and sweating. Neuroglycopenic symptoms often include behavioral changes, difficulty thinking, and/or frank confusion, and neuroglycopentic manifestations can lead to seizure, coma, and even death [3].

Hypoglycemia is regarded as a fact of life for most people with type 1 diabetes. It is estimated that the average patients with type 1 diabetes has untold numbers of episodes of asymptomatic hypoglycemia and suffers two episodes of symptomatic hypoglycemia per week [2]. Although severe hypoglycemia (SH), which requires the assistance of another individual, represents only a small fraction of the total hypoglycemia experience, it was estimated that the incidence of SH was around 115 episodes per 100 patient-years in a random sample of 267 people recruited from a population-based diabetes register in Scotland in 2001 [2]. Though the subjects in the DCCT trial (the Diabetes Control and Complication Trial) were highly selective and those who had multiple events of SH before enrollment were not eligible to participate [10, 18], almost half of the DCCT cohort still had

one or more episodes of hypoglycemia requiring assistance (severe hypoglycemia), and intensive therapy was associated with a threefold increase in the risk of SH compared with conventional therapy (the event rate per 100 patient-years was 61.2 in the intensive therapy versus 18.7 in the conventional therapy group). Unfortunately, there seems no evidence that this problem has abated over the decade and a half since it was highlighted by the final report of the DCCT in 1993 [2].

In people with type 1 diabetes, hypoglycemia is the result of the interplay of relative or absolute insulin excess and compromised physiological defenses against falling plasma glucose concentrations [1, 2]. The major conventional risk factors for hypoglycemia in type 1 diabetes includes: relative insulin excess; aggressive glycemic therapy with more stringent control goals; impaired renal function; and patient behavioral activities such as missed meals or snacks, unusual exercise, altered insulin doses and alcohol use. However, these known behaviors as risk factors alone are believed to explain only a minority of episodes of hypoglycemia in patients with type 1 diabetes [2].

Besides the above conventional risk factors, the concept of hypoglycemia-associated autonomic failure (HAAF) in diabetic patients posits that recent prior hypoglycemia as well as prior exercise and sleep can cause both defective glucose counterregulation (by reducing the epinephrine response and glucagon response) and hypoglycemia unawareness (by reducing sympathetic neural response) and thus those factors can cause a vicious cycle of recurrent hypoglycemia (Figure 1) [2, 8]. The risk factors for HFFA include the degree of absolute endogenous insulin deficiency; history of SH, hypoglycemia unawareness, or both as well as recent prior hypoglycemia, prior exercise, or sleep. Some studies suggest that two to three weeks of scrupulous avoidance of hypoglycemia should be a rational approach to reverse hypoglycemia unawareness and to improve glucose counterregulation [2, 19]. However, whether this approach can work in routine clinical practice is unknown because it

requires a labor-intensive approach with frequent, often daily, contact between health professionals and patients, frequent blood glucose monitoring and insulin adjustment to prevent episodes, particularly at night [19].

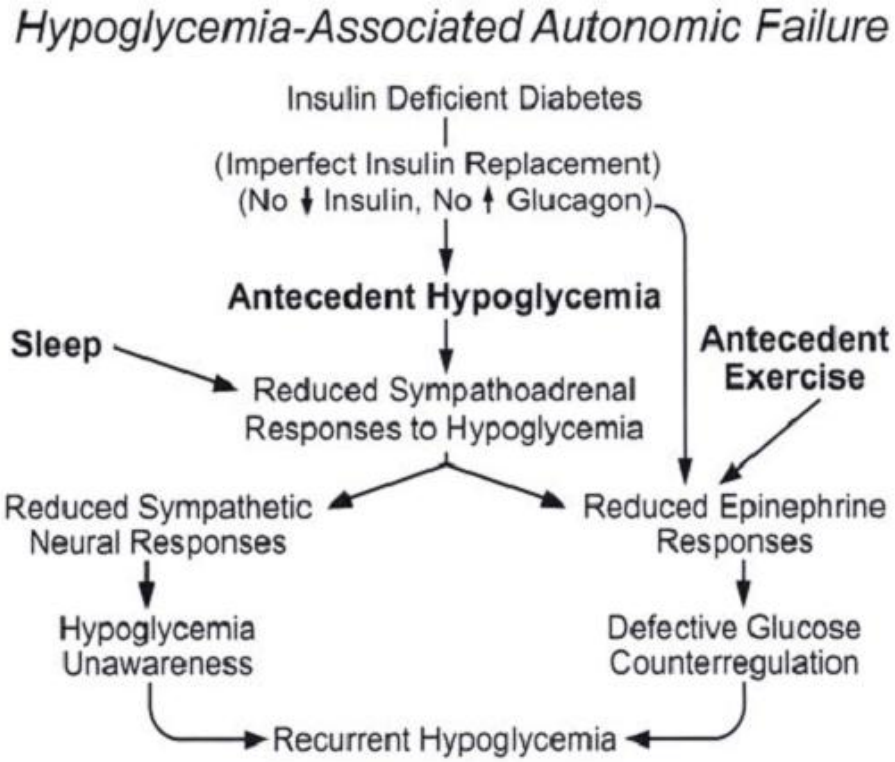

FIG. 2. Schematic diagram of HAAF in diabetes. Modified from Cryer PE: Diverse causes of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med* 350:22722–279, 2004. © 2004 Massachusetts Medical Society. All rights reserved.

Figure 1: Schematic diagram of HAAF in Diabetes

(From Cryer PPE. The barrier of hypoglycemia in diabetes. Diabetes (New York, N.Y.). 2008; 57(12):3169-3176)

The prevention of hypoglycemia involves: acknowledging and addressing the problem; applying the principles of intensive glycemic therapy (diabetes self-management

based on patient education, frequent blood glucose monitoring and appropriate and flexible insulin regiments); considering both the conventional risk factors and the indicative of HAAF [19]. American Diabetes Association Workgroup on hypoglycemia concluded that any reduction in SH (that requiring the assistance of another individual), even by as little as 10-20% would be advantageous. But the Workgroup also mentioned that a clinically important reduction in hypoglycemia should not be accompanied by any compromise in the glycemia control goals [20].

B. Risk of prior SH on subsequent SH in type 1 diabetes

SH can induce the defect in counterregulation and loss of awareness of hypoglycemia due to the HAFF, and the defect would increase the subsequent risk of recurrent SH in patients with type 1 diabetes [1]. Thus, prior SH could cause a vicious cycle of recurrent hypoglycemia [2, 8], and the effect of SH is often regarded as an acute effect that the greatest risk may occur in weeks and months after episodes of prior SH events [1].

Studies have been consistently reported SH as a risk factor in general for recurrent SH events [9-16]. An early DCCT study, which followed a cohort of 817 patients with a mean of 21 months follow-up, reported a hazard ratio 2.5 (CI: 1.7-3.9) for the effect of history of SH before the DCCT baseline on the first subsequent SH event during the follow-up [9]. The later DCCT study which used all participants (1,441 patients) further reported that per additional episode of SH was associated with a hazard ratio 1.2 (CI: 1.2-1.2) on subsequent recurrent SH episodes in the conventional therapy group and 1.1 (CI: 1.1-1.1) in the intensive therapy group respectively during a mean 6.5 follow-up years [10]. Another cohort study using the same DCCT population also reported that history of SH prior to the DCCT baseline was associated with the first, second, third, fourth and fifth SH event during the follow-up with hazard ratios 2.0, 2.3, 2.4, 2.3 and 2.7, respectively [16]. Similarly, a German study prospectively followed a cohort of 684 patients with type 1 diabetes in an average 19

follow-up months and found the occurrence of SH in the preceding year prior to the study baseline was associated SH episodes which occurred during the follow-up with a hazard ratio 2.7 (CI: 1.8-4.3) [13]. Another German cohort study which followed 636 patients with type 1 diabetes also reported to find an association between the occurrence of SH in the previous year prior to the study baseline and the subsequent occurrence of SH during six follow-up years [12]. However, these two studies have used different definition of SH compared with the DCCT study. The SH was defined as being treated by glucagon injection reported by patients themselves in these two German studies whereas in the DCCT study, SH was defined as the need for assistance from others.

Although above studies consistently have shown that the prior SH is a risk factor in general for the subsequent SH events, few studies estimated the magnitude of acute effects of SH using clinically relevant time range to define the exposure (prior SH) and outcome (subsequent SH events) to illustrate the acute effects of SH. One Scottish study which prospectively followed 94 patients with type 1 diabetes reported that the occurrence of hypoglycemia in the previous month was associated with the occurrence of hypoglycemia in next month with an odds ratio 4.6 (CI: 1.5-13.7) [15]. The effect appears greater in this study compared to estimates reported by other studies that did not restrict their definition of exposure and outcome to a short time range, and the results of this study seem to support the notion of an acute effect [1]. This study has its limitations however: first, the follow-up time was only one month and the sample is small; secondly, the definition of hypoglycemia is totally based on self-report by patients, and the investigators did not differentiate the severity of reported hypoglycemia; lastly, in this study, patients were asked to use a diary to record their hypoglycemic events after their baseline interview, and therefore those patients who reported hypoglycemic events in the baseline interview may tend to over-report the

hypoglycemic events in their one month follow-up. Thus, the greater effect reported by this study could be biased due to the study design.

The epidemiological evidence, which can illustrate the acute effects of SH and provide the magnitude of the effects, will help to improve clinical management of SH in patients with type 1 diabetes (e.g., to help physicians to consider available interventions in appropriate timing to reduce subsequent SH events when a SH event has occurred).

Table 1: Results from prior studies for the effects of severe hypoglycemia (SH) on subsequent SH events

Authors/ Publication year	Study population	Results	Comments
The DCCT study group/1991	First 817 subjects who entered the DCCT, with a mean of follow-up of 21 months	Hazard ratio for the effect of history of severe hypoglycemia (SH) at study baseline on the first occurrence of SH during the follow-up was 2.54 (CI: 1.67-3.88)	SH was defined as the hypoglycemic events requiring assistance from other people
The DCCT study group/1997	All subjects in the DCCT with a mean of follow-up 6.5 years	Hazard ratios for the effect of history of SH at study baseline on any occurrence of SH during the follow-up were 1.7 (CI: 1.36-2.13) in the conventional therapy group and 1.33 (CI: 1.17-1.52) in the intensive therapy group. Per additional episode of SH during follow-up was associated with a hazard ratio 1.16 (CI: 1.15-1.18) in the conventional therapy group and 1.1 (CI: 1.1-1.11) in the intensive therapy group	SH was defined as the hypoglycemic events requiring assistance from other people
Kilpatrick et al./2007	All subjects in the DCCT with a mean of follow-up 6.5 years	History of SH at study baseline was associated with the first SH event, second, third, fourth and fifth event during the follow-up with hazard ratios 1.98, 2.27, 2.36,2.31 and 2.64, respectively	SH was defined as the hypoglycemic events requiring assistance from other people
Mühlhauser I et al/1998	684 patients from a German community with a mean of follow-up 19 months	The occurrence of SH during the preceding 12 months at the baseline was associated recurrent SH with a hazard ratio 2.73 (CI: 1.76-4.25)	SH was defined as being treated by glucagon injection reported by patients themselves
Bott S et al/ 1997	636 patients from German hospitals with a mean of 6 years	The history of SH was associated with SH during the follow-up (P value 0.0007)	SH was defined as being treated by glucagon injection reported by patients themselves
Donnelly LA et al/2005	94 patients in Scotland with one month follow-up	The occurrence of hypoglycemia in the previous month was associated with the occurrence of hypoglycemia in next month with odd ratio **4.60** (CI: **1.54-13.68**)	The definition of hypoglycemia was totally based on self-report without differentiating severity of reported events.

C. Weight gain, and the role of hypoglycemia for weight gain during insulin treatment

Improved glycemic control on insulin is often observed to be associated with weight gain [4, 21]. At diagnosis patients with type 1 diabetes may be underweight following a period of glucosuria, osmotic diuresis and frank catabolism due to lack of insulin. Thus weight gain after initializing insulin treatment is often regarded as normalization [4, 21]. However, in the DCCT patients in the intensive therapy group continued gaining weight up to 9 years although weight gain was less rapid after the first year [22]. The prevalence of overweight defined as the body mass index (BMI) over 27.8kg/m^2 for men and 27.3kg/m^2 for women in the DCCT study population reached 33% in the intensive therapy group and 19% in the conventional therapy group at the end of the DCCT [18].

Furthermore, patients with excessive weight gain may have higher risk of cardiovascular diseases in future. Purnell et al. [7] stratified data in the intensive therapy group by quartiles of weight gain at the end of the DCCT. When they compared the first quartile (where BMI did not change appreciable) with the fourth quartile (where BMI increased by 7kg/m^2), the baseline cardiovascular diseases profiles at the beginning of the trial were similar between these two quartiles, and at the end of the DCCT both quartiles also had similar A1C levels. However, at the end of the DCCT, the first quartile showed improvements in all profiles related to cardiovascular disease risk; the fourth was associated with the negative profiles related to the increasing risk of cardiovascular diseases such as higher blood pressures, higher triglyceride (0.99 mmol/l vs 0.79 mmol/l), higher total cholesterol (4.97 mmol/l vs 4.55 mmol/l), higher low-density lipoprotein cholesterol (1.32 mmol/l vs 1.29 mmol/l) and lower high-density lipoprotein cholesterol (3.18 mmol/l 2.82 mmol/l). Thus improved glycemic control did not seem to result in improvements in the cardiovascular risk profiles among patients who gained excessive weight during the insulin treatment. Moreover, treatment adherence to prescribed insulin may be compromised by a

desire to avoid weight gain [4]. A UK study [23] reported 30% of the women admitted to intentionally reducing doses of insulin to control weight gain. Another study [24] in the United States also reported that 31% intentionally omitted insulin to avoid weight gain.

A number of possible mechanisms have been described to explain weight gain on insulin therapy including compensation for hypoglycemia [4, 21]. In many patients with diabetes, the unpleasant symptoms and negative consequence associated with hypoglycemia may result in significant fear of hypoglycemia [17][1]. Because low blood glucose levels can be remedied by ingestion of glucose or food, following a hypoglycemic event, patients may over-react or be instructed by their physicians to consume more calories than necessary (e.g., frequent snacking) in response to the threat of subsequent occurrences of hypoglycemia [4, 21].

Although the hypothesis of compensation for hypoglycemia is well accepted and assumed, there is very limited direct evidence to support it in the literature. An early DCCT study [25] with a small sample (only the subjects recruited during the first year were used for analyses) found that the 29 patients who experienced SH in the intensive therapy group gained more weight than the intensively treated subjects with no severe episodes. Another study, which re-analyzed data from a 26-week, randomized, open-label trial comparing insulin determir with NPH insulin in 476 patients with type 2 diabetes [26], reported no correlation between frequencies of hypoglycemia and weight gain for insulin detemir, while a correlation was found for NPH insulin(only *P* values for the correlations were reported in this study). However, for these two studies the temporal relation between occurrence of SH or hypoglycemia and subsequent weight gain was unclear because some of the weight gain may have occurred before the first SH or hypoglycemic episode. Moreover, in the second study, the relationship between the frequencies of hypoglycemia and weight gain in this 26-

week trial was assumed linear in their analyses. However, upon examination of their results, the linear assumption is problematic (Figure 2).

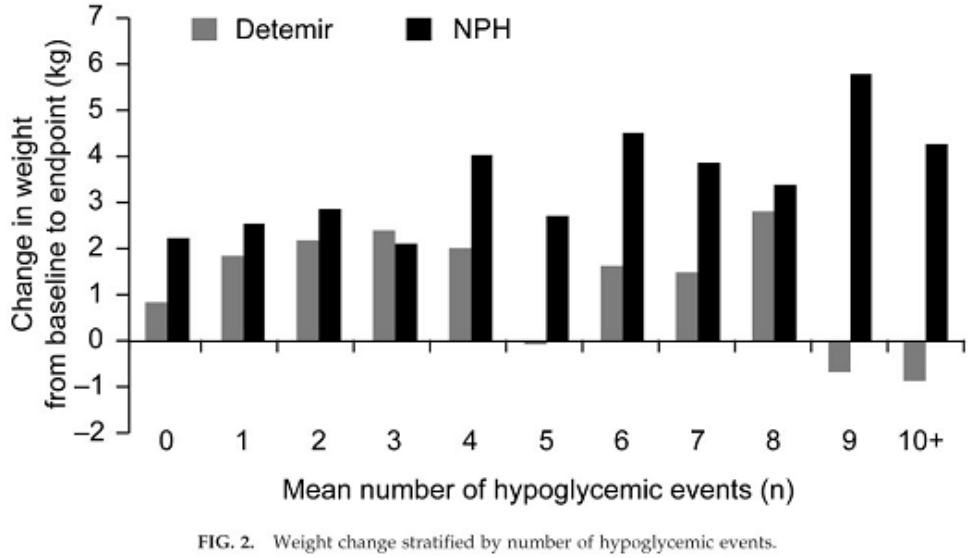

FIG. 2. Weight change stratified by number of hypoglycemic events.

Figure 2: Weight Change stratified by Number of Hypoglycemic Events

from Davies MJ, Derezinski T, Pedersen CB, Clauson P. Reduced weight gain with insulin detemir compared to NPH insulin is not explained by a reduction in hypoglycemia. Diabetes Technol Ther. 2008; 10(4):273-277.

III. STATEMENT OF SPECIFIC AIMS

We conducted a secondary data analysis using data from the DCCT trial to address the following specific aims:

A. Specific aim 1:

To estimate the effects of SH (index SH) on subsequent SH in three subsequent time windows at months 1-3, 4-6 and 7-9 after index SH in patients with type 1 diabetes.

Hypothesis 1.1:

The time window which is closest to the index SH will have the greatest risk/effect of SH among the three observation windows.

Hypothesis 1.2:

If the hypothesis 1.1 does not hold, compared to those without occurrence of index SH the greater risk of subsequent SH episodes will occur immediately after index SH in the first observation window at months 1-3.

B. Specific aim 2:

Aim 2_1: to estimate the effects of SH (index SH) on subsequent weight change/actual weight in various observation periods from 3, 6, 9, 12, 24 and 32 months after index SH, respectively;

Aim 2_2: to estimate the effects of SH (index SH) on subsequent weight change in three fixed-term time windows during the months 1-3, 4-6 and 7-9 after index SH, respectively;

Aim 2_3: to estimate the effects of occurrence of SH on the time to substantial weight gain (defined as the 5, 10, 15 and 20% weight gain from the baseline), becoming overweight or obese, respectively in patients with type 1 diabetes.

Hypothesis 2:

A short-term weight gain after index SH (e.g., 3-9 months after index SH) will be observed but not for a long-term weight gain including substantial weight gain, overweight or obesity in the DCCT population.

C. Specific aim 3:

To explore whether occurrence of SH is associated with a decrease of insulin dosing in the DCCT population. This is a supplementary analysis to provide results to assist interpretations of results in aim 1 and 2.

IV. METHODS

A. Overview of methods

We conducted a secondary data analysis using data from the DCCT trial study. The DCCT is a randomized clinical trial whose primary aim is to establish the relative effectiveness of intensive vs. conventional therapy in term of reducing microvascular and macrovascular complications among type 1 diabetes mellitus [27]. 1,441 patients from 29 clinical centers were randomized to two treatment arms and followed up for a mean of 6.5 years between 1983 and 1993.

To address study aim 1, we estimated the relative risk (RR) of SH (index SH) on subsequent SH in three subsequent time windows after the index SH based on quarterly visits in the DCCT: between the 1^{st} and 2^{nd}, between the 2^{nd} and 3^{rd}, between the 3^{rd} and 4^{th} quarterly visit after the index SH, which corresponds to approximate months 1-3, 4-6, and 7-9 after index SH, respectively (Figure 3). We also estimated transition probabilities as the risk of subsequent SH episodes in the three subsequent observation windows using the estimation models. We assigned fixed values for the covariates in the estimation models to produce predictive values as the corresponding transition probabilities. Since each patient could contribute multiple observations, we used generalized estimating equations (GEE) by specifying working correlation structure as “exchangeable” to allow for the dependence of observations within a person [28, 29].

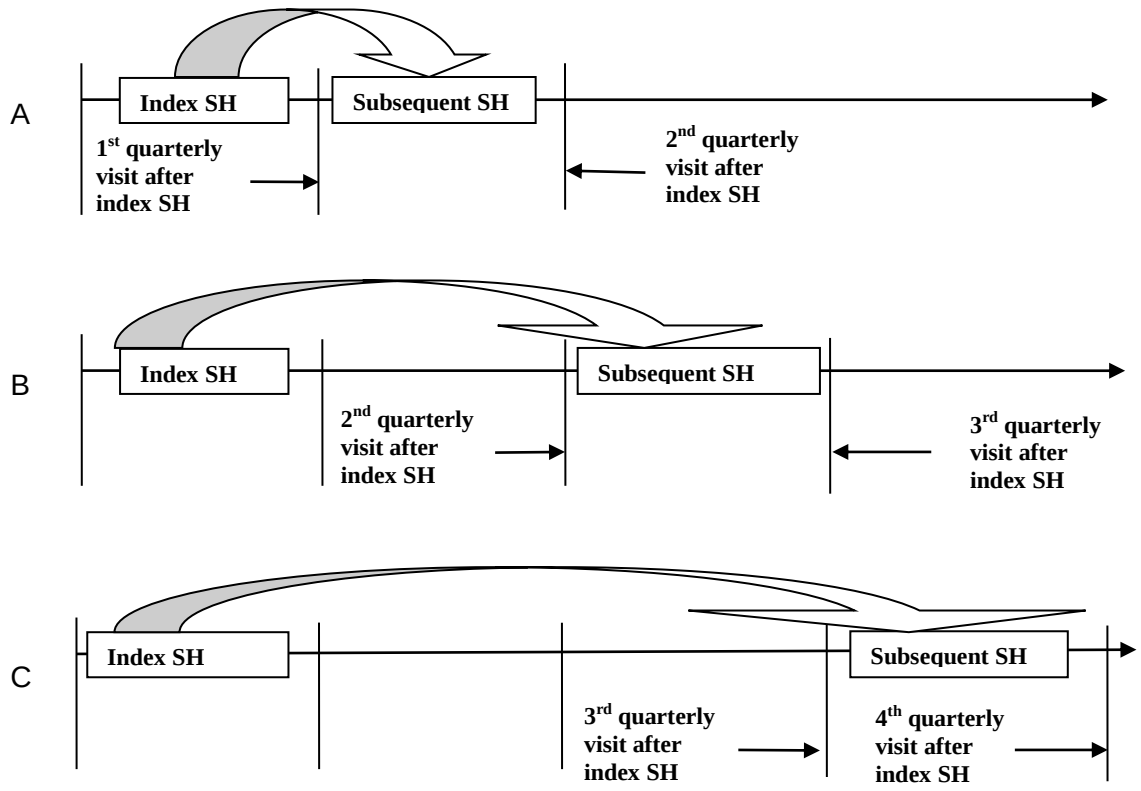

Figure 3: Study design for estimation of acute effects of severe hypoglycemia (SH) on subsequent recurrent episodes in three time windows in the DCCT population

Effects of SH (index SH) on the risk of subsequent SH were estimated in three subsequent time windows (the duration of each time is a quarterly interval) between the 1st and 2nd quarterly visit (corresponding to A in above figure), between the 2nd and 3rd quarterly visit (B in above figure), between the 3rd and 4th quarterly visit (C in above figure) after the index SH, respectively. These three time windows approximate months 1-3, 4-6, and 7-9 after the index SH, respectively. It should be noted that the above defined windows would not be the exact durations as the months 1-3, 4-6 and 7-9 after index SH because the period between the time when the index SH occurred and the time when the next quarterly visit was conducted would not be captured by this definition. However, we use months to describe the timeline after the index SH in all sections in this paper for simplicity. Both exposure (index SH) and outcome variables (subsequent SH episodes) for three observation windows were defined as dichotomized variables. Each patient would contribute multiple observations. To validly estimate the effects of index SH, the most recent values of relevant time-dependent covariates measured in the time points prior to the index SH were used to control for confounding.

To address study aim 2_1 and 2_2, we estimated the effects of SH (index SH), which occurred in a quarterly interval, on weight change/weight in the following various periods (one, two, three, four, eight, and twelve consecutive quarterly intervals), and the effects of index SH on weight change in three fixed-term time windows during the 1st, 2nd and 3rd quarterly interval. Each patient could contribute multiple observations (Figure 4). The different numbers of involved quarterly intervals from one to twelve approximated the observation periods with various durations from 3 months to 36 months after index SH, and three fixed-term time windows approximated the periods during months 1-3, 4-6 and 7-9 after index SH, respectively. Similarly, since each patient could contribute multiple observations, we used GEE by specifying working correlation structure as "exchangeable" to allow for the dependence of observations within a person.

To address study aim2_3, we used Cox proportional hazard models to estimate the hazard ratios (HRs) of SH on the substantial weight gain (defined as the 5, 10, 15 and 20% weight gain from the DCCT baseline, respectively), on becoming overweight (BMI≥25.0), or on becoming obese (BMI≥30.0) comparing patients after they had their first SH episode with all patients who did not experienced any SH episode at the same time during the follow-up (they could have a SH episode later during follow-up) [30]. Hence, the exposure in our Cox models was treated as a time-dependent variable. Furthermore, as an ancillary analysis, we further used marginal structural models (MSM) that allowed us to control for average daily insulin dose, which may be seen as a time-dependent confounder affected by prior SH.

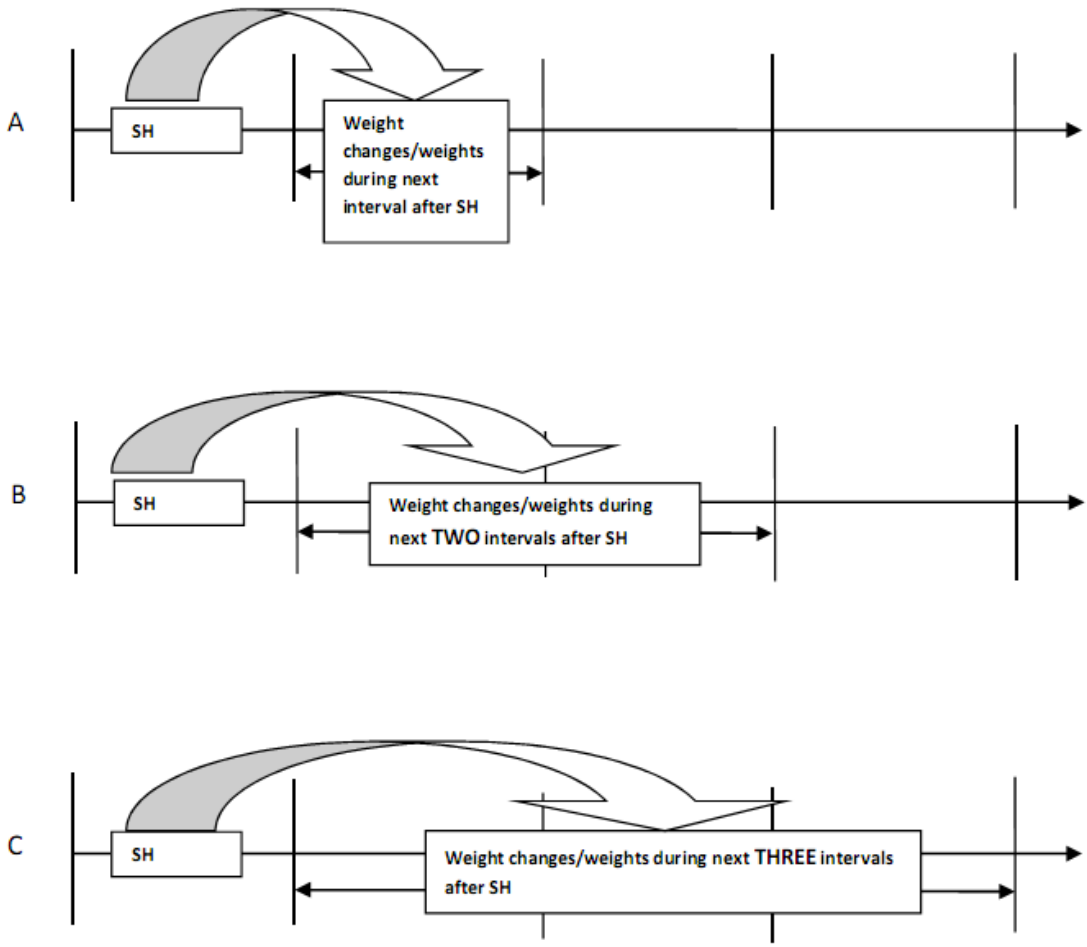

Figure 4: Diagram for study design to estimate the effects of occurrences of severe hypoglycemia (SH) on subsequent weight change/weight during various observation periods (only the first, second and third observation period are shown)

First, the effect of SH which occurred in a quarterly interval on weight change/weight in the next quarterly interval (a period approximates a duration of 3-month) was examined (the "A" in above figure). Then, we examined the effects of SH on weight during longer periods ranging from two (the "B" above included the first two consecutive quarterly intervals after SH), three (the "C" included the first three consecutive quarterly intervals after SH), four, eight and twelve consecutive quarterly intervals after SH. The different numbers of quarterly intervals approximate observation durations ranging from 3 months to 36 months after SH. We defined all time-dependent covariates using the most recent value before the occurrence of SH.

Two continuous outcome variables were defined for each observation period: 1) the first outcome variable (weight change) was the difference between the weight measured at the end of observation period and the weight at the beginning of the observation period; 2) the second was the actual weight measured at the end of observation period.

Daily changes of therapy regimens in the DCCT were not centrally documented and were not accessible for analysis, and only typical daily insulin doses (TDID) for the past quarterly interval were recorded at quarterly visits [31]. Thus, in the DCCT there is no way to tell whether the reported dose actually reflected the dose before occurrence of SH or after occurrence of SH if patients had occurred SH episodes in a quarterly interval. To account for this issue and conduct the supplementary analysis for study aim 3, new study analytic units were constructed and each analytic unit consisted of two consecutive quarterly visits to account for temporality of change of insulin dose following occurrence of SH (Figure 5). We estimated the effects of SH occurring in the first composed quarterly interval in an analytic unit on the continuous outcome (the difference of daily insulin doses between the end and beginning of the analytic unit) and the dichotomized outcome (≥ 10% dose reduction) in each analytic unit. We also used GEE to account for multiple-observations per patient and to control relevant factors. Furthermore, for continuous outcome (the difference of daily insulin doses), we also used mixed models as a sensitivity analysis.

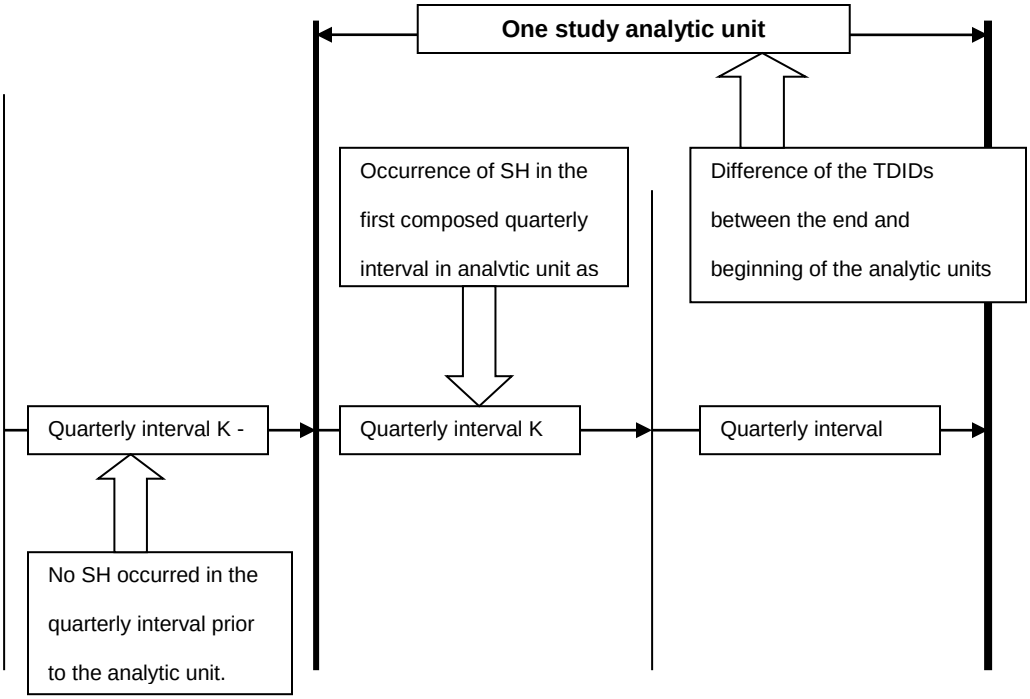

Figure 5: Diagram for Construction of an Analytic Unit for Occurrence of Severe Hypoglycemia (SH) on Changes of Typical Daily Insulin Dose (TDID) in the DCCT

The defined analytic unit consists of two consecutive quarterly intervals (quarterly intervals K and K+1 in above figure). In order to be eligible as such an analytic unit, SH must not occur in the quarterly interval (interval K-1) preceding the defined unit. Exposure status (yes/no) in each defined study unit depends on the occurrence of SH in the first composed quarterly interval (interval K). Both continuous variable (the differences of TDID between the end and beginning of unit) and dichotomized variable (≥ 10% TDID reduction) were used as the outcome for analytic unit. Only intervals after the 1st year follow-up were included in the analysis to account for probable bias due to the transition period when patients entered the trial.

B. Methods for proposed aims

1. Data source

a. The DCCT study

The DCCT is a randomized controlled clinical trial starting in 1983. 1,441 people with type 1 diabetes in the USA and Canada were randomly assigned to receive either intensive

therapy (three or more insulin injections or continuous subcutaneous insulin infusion) or conventional therapy (one or two insulin injection per day). Intensive therapy aimed to keep hemoglobin A1C as close to normal (6 %) as possible. After an average follow-up of 6.5 years, the intensive therapy group achieved a median HbA1c with 7.3% vs. 9.1% for the conventional therapy. The trial also showed that intensive treatment aimed at maintaining near-normal blood glucose values markedly reduced the risk of microvascular complications including retinopathy, nephropathy, and neuropathy [32] .

At the end of the DCCT, the occurrence of SH was three times higher in the intensive than in the conventional therapy group. Almost half the DCCT overall cohort had one or more episodes of SH. Of these, 71% had multiple events. For 10 or more episodes, the respective percentages were 14.2% of the intensive group and 2.5% of the conventional group [18]. By the end of the study, 42% of the intensively treated patients had exceeded the overweight limits at some point during follow-up as compared to 27% of the conventionally treated patients, and the pattern was uniform among men, women, adults, and adolescents [18].

During the trial, intensive therapy had specific glucose targets: 70-120 mg/dl before meals, <180 mg/dl after meals, and >65 mg/dl at 3 am. Intensive therapy group subjects could choose a regimen consisting of either multiple daily injections or continuous subcutaneous insulin infusion with an external pump, both guided by the frequent self-monitoring of blood glucose levels. All aspects of the intensive therapy regimen were subject to change as needed to reduce the risk of hypoglycemia when indicated. Subjects in the conventional therapy group used one or two insulin injections per day and monitored either urine or blood glucose without specific numeric glucose targets. The daily changes of therapy regimens were not centrally documented and were not accessible for analysis. However, during the average 6.5 years follow-up in the DCCT, every participant had

quarterly visits. At the quarterly visit, the typical daily insulin doses for the past interval were recorded and the capillary blood hemolysates for a daily blood glucose profile were collected at the day before quarterly visit at seven points throughout the day, namely pre-breakfast, post-breakfast, pre-lunch, post-lunch, pre-supper, and post-supper and bedtime. For these seven-point blood samples, capillary blood hemolysates were collected before meals, 90 min after meals and at bedtime by patients in the home the day before the quarterly visit. Blood glucose was measured in a central laboratory using a hexokinase enzymatic method. Subjects were instructed to report all episodes of suspected severe hypoglycemia (requiring assistance of others to manage hypoglycemia) immediately; all were interviewed regarding the episodes. In addition, subjects were also asked at the quarterly visits about the occurrence of any hypoglycemia [27, 31].

Patients in the intensive therapy group were seen at least monthly and telephone contact was as often daily for the first few weeks, then weekly thereafter. Their A1C results were unmasked by investigators. The patients on the intensive therapy also were instructed to perform self-blood glucose monitoring at least four times a day and also were instructed to conduct a further test a 3:00 A.M. if their glucose value was less than 65mg/dl. On the contrary, the patients on the conventional therapy were seen only quarterly and they did not have any specific glucose control targets. Their A1C values often were masked by investigators during the follow-up. The patients on the conventional therapy were not asked to take self-blood glucose monitoring early in the trial. Later with the increased use of self-blood glucose monitoring in clinical practice, this technique was made part of the conventional therapy in 1986. But they were only asked to perform at least one self-blood glucose monitoring or one urine test per day [31].

In the trial, registered dietitians tailored meal plans and educational strategies to the need and life style of each individual to achieve and maintain ideal body weight with goals of

15-20% of energy from protein, 30-35% from fat, and 50-55% from carbohydrate. Patients in the intensive therapy received more frequent and detailed instruction on individualized meal plans. Since weight gain with intensive therapy had been recognized, dietitians increased their emphasis and counseling on weight management for patients in the intensive therapy, and greater attention was given to the relation between nutrient intake and insulin to try to achieve target glycemic levels without hypoglycemia or weight gain [31].

The DCCT data is publicly accessible through the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) central repository (https://www.niddkrepository.org/niddk/home.do).

b. Study populations

For study aim 1, to allow for learning during the transition period when patients entered the trial, we only included quarterly visits after the first year of follow-up in the DCCT as analytic intervals in our analysis. We also restricted eligible patients who must be 18 years or older when they entered the DCCT trial, since younger patients were treated differently for glycemic control and would differ in term of self-management of type 1 diabetes from adult patients [31].

For study aim 2, we restricted eligible patients to be 18 years or older when they entered the DCCT to avoid weight gain as a result of adolescent growth. In addition, we also censored female patients at the time of their first pregnancy during follow-up based on their reported last menstrual dates.

For study aim 3, we also only included quarterly visits after the first year of follow-up in the DCCT to define the analytic units, and restricted eligible patients who must be 18 years or older when they entered the DCCT trial and censored female patients at the time of their first pregnancy during follow-up based on their reported last menstrual dates.

2. Measures of exposure

SH was defined as an episode in which a patient required assistance of another person and the following conditions: 1) a blood glucose level of < 50 mg/dl, or 2) prompt recovery following oral carbohydrate, intravenous glucose, or glucagons. Patients in the DCCT were instructed to report all episodes of suspected SH immediately, and all were interviewed to verify each episode [31].

For study aim 1, the exposure was a dichotomized variable to indicate the occurrence of SH in an eligible study quarterly interval.

For study aim 2_1 and 2_2, similarly, the exposure was a dichotomized variable to indicate the occurrence of SH in an eligible study quarterly interval. For study aim 2_3, although the exposure was still to indicate the occurrence of SH, it was a variable based on the occurrence of the first SH episode during the follow-up: 1) the patient was defined as unexposed prior to the 1st SH episode in their follow-up in the DCCT; 2) after the 1st SH episode, the patient was defined as always exposed.

For study aim 3, the exposure was a dichotomized variable to indicate the occurrence of SH in the first composed quarterly interval in each defined analytic unit.

3. Measures of outcome

For study aim 1, the dichotomized outcome was the subsequent occurrence of SH (the same definition as the exposure for SH above) in three observation windows after index SH (exposure).

For study aim 2_1, two continuous outcome variables were defined for each observation period with ranges from 3 to 36 months after index SH: 1) the first outcome variable (weight change) was the difference between the weight measured at the end of

observation period and the weight at the beginning of the observation period; 2) the second was the actual weight measured at the end of observation period. For study aim 2_2, the outcome was the quarterly weight change for three fixed-term time windows, respectively. In the DCCT, weight (in kilograms) was measured with patients in light clothing and stockings on the same balance-beam scale for the duration of the DCCT trial [27].

For study aim 2_3, the outcome substantial weight gain was defined as the time to 5, 10, 15 and 20% weight gain from the DCCT baseline, respectively at the first time during the follow-up. We also looked at the time to overweight (BMI values ≥25.0), and time to obesity (BMI values ≥30.0), respectively at the first time as outcomes [33].

For study aim 3, two types of outcome were defined in each analytic unit: 1) continuous variable (the differences of insulin doses between the end and beginning of analytic unit); 2) dichotomized variable (≥ 10% insulin reduction).

4. Covariate measurement

Relevant covariate information was obtained from the baseline and quarterly visit data files in the DCCT. Table 2-4 showed that the relevant covariates with their definitions and formats were used in final statistical models for each specific aim.

For continuous covariates, besides using their original values, power terms or categories, we also explored to use spline terms in estimation models for better confounding control. Spline models could combine the advantages of categorical and power models [34]. We categorized continuous covariate or created the knots for their splines based on established cut-points (e.g., BMI) or known conventions. Otherwise, we used empirical cut-points based on the actual distributions from data themselves (e.g., the different distribution percentiles). When to estimate the parameters for the main exposure, we tried several parameter specifications (original values, categories and splines) for continuous covariates

in the models and various specifications appeared to yield a similar result in term of the estimates for the main exposure. Based on the precision of the estimates for the main exposure and model fit statistics: quasilikelihood under the independence model criterion (QIC) for GEE and Akaike information criterion (AIC) for Cox models [35], the most appropriate specifications (formats) for continuous covariates were used in final models for different specific aims.

Table 2 : Covariates adjusted in the models for study aim 1

Variable	Formats	Notes
Gender	2 categories (female, male)	Measure at the DCCT baseline
Age	3 categories "18-25, 26-29, and >29 years of old "	Measure at the DCCT baseline
Duration of type 1 diabetes	3 categories "1-5, 6-10 and >10 years"	Measure at the DCCT baseline
History of SH	2 categories (Yes/No)	Based on the DCCT baseline file
Recent SH	Dichotomized variable to indicate the occurrence in the quarterly interval prior to index SH (exposure)	Updated quarterly
Rates of SH episodes from baseline to prior to the index SH	3 categories "no occurrence prior to the index SH, less or equal to the 90^{th} percentiles of the rates in corresponding treatment groups, and greater than 90^{th} percentiles"	Updated quarterly
Expected A1C	4 categories "<6%, 6-6.9%, 7-7.9% and ≥8%"	Calculated from mean blood glucose with quarterly blood glucose profiles based on previous studies [36, 37], updated quarterly
Actual A1C	4 categories "<6%, 6-6.9%, 7-7.9% and ≥8%"	Updated quarterly
Alcohol use	3 categories "no use, 1-75 grams per week, >75 grams per week"	Updated annually
Physical activities	3 categories "little, moderate and strenuous"	Updated annually
Meal plan adherence	Dichotomized variable for whether subjects claimed to have followed the meal plan almost all of the time in quarterly questionnaire	Updated quarterly
Insulin use adherence	Dichotomized variable for whether subjects claimed to have followed the prescribed insulin dose in quarterly questionnaire	Updated quarterly
Use of SMBG	3 categories "no use, often done and at least one per day"	Updated quarterly
Blood glucose variability	Standard deviation calculated from quarterly blood glucose profiles for individual subjects	Updated quarterly
Follow-up time	A restricted cubic spline with 5 knots at 5, 10, 25, 75 an 95^{th} percentiles of the months since entered the DCCT	Updated quarterly

Table 3: Covariates adjusted in the models for study aim 2_1 and 2_2

Variable	Formats	Notes
Gender	2 categories (female, male)	Measure at the DCCT baseline
Age	3 categories "18-25, 26-29, and >29 years of old "	Measure at the DCCT baseline
Duration of type 1 diabetes	3 categories "1-5, 6-10 and >10 years"	Measure at the DCCT baseline
History of SH	2 categories (Yes/No)	Based on the DCCT baseline file
Recent SH	Dichotomized variable to indicate the occurrence in the quarterly interval prior to index SH (exposure)	Updated quarterly
Rates of SH episodes from baseline to prior to the index SH	3 categories "no occurrence prior to the index SH, less or equal to the 90^{th} percentiles of the rates in corresponding treatment groups, and greater than 90^{th} percentiles"	Updated quarterly
Physical activity	3 categories "little, moderate and strenuous"	Updated annually
Meal plan adherence	Dichotomized variable for whether subjects claimed to have followed the meal plan almost all of the time in quarterly questionnaire	Updated quarterly
Insulin adherence	Dichotomized variable for whether subjects claimed to have followed the prescribed insulin dose in quarterly questionnaire	Updated quarterly
BMI prior to SH	a restricted quadratic spline with 3 knots at BMI 18.5, 24.9 and 29.9	Updated quarterly
Quarterly daily insulin dose	a restricted quadratic spline with 3 knots at 5^{th} , 50^{th} and 95^{th} percentiles	Updated quarterly
Follow-up time	A restricted cubic spline with 5 knots at 5, 10, 25, 75 an 95^{th} percentiles of the months since entered the DCCT	Updated quarterly

Table 4: Covariates adjusted in the models for study aim 2_3

Variable	Formats	Notes
Gender	2 categories (female, male)	Measure at the DCCT baseline
Age	a restricted quadratic spline with 3 knots at 5^{th}, 50^{th} and 95^{th} percentiles	Measure at the DCCT baseline
Duration of type 1 diabetes	a restricted quadratic spline with 3 knots at 5^{th} , 50^{th} and 95^{th} percentiles	Measure at the DCCT baseline
History of SH	2 categories (Yes/No)	Based on the DCCT baseline file
BMI	Continuous variable	Measured at the DCCT baseline
Average physical activities	Dichotomized variable on whether a subject had strenuous activities in 80% of follow-up time or more.	Updated annually
Average meal plan adherence	Dichotomized variable on whether a subject had claimed to follow meal plan in 80% of follow-up time or more.	Updated quarterly
Average daily insulin dose	A restricted quadratic spline with 3 knots at 5^{th} , 50^{th} and 95^{th} percentiles	Updated quarterly

5. Statistical analyses

a. Estimation of the acute effects of SH on subsequent SH

We estimated the RRs of SH (index SH) on subsequent SH in three subsequent time windows after the index SH corresponding to approximate months 1-3, 4-6, and 7-9 after index SH, respectively. It should be noted that the above defined windows would not be the exact durations as the months 1-3, 4-6 and 7-9 after the index SH because the period between the time when the index SH occurred and the time when next quarterly visit was conducted would not be captured by this definition (Figure 3). However, we used months to describe the timeline after index SH in the following sections for simplicity.

We used log-linear multivariable models to estimate RRs and their 95% confidence intervals (CI). We controlled for confounding by *a set of priori* specified covariates (Table 2) based on directed acyclic graph (DAG) [38] (Figure 6). We defined all time-dependent covariates using the most recent value before the occurrence of the index SH to avoid the

value to be in the intermediate pathway between the exposure and outcome. Since each patient could contribute multiple observations, we used GEE by specifying working correlation structure as “exchangeable” to allow for the dependence of observations within a person [28, 29]. When the log-linear models did not converge, we used Poisson models to approximate RRs as previously described [39].

We also estimated transition probabilities as the risk of subsequent SH episodes in the three subsequent observation windows using the estimation models. We assigned the following values for the covariates in the estimation models including the intercept term and to produce predictive values as the corresponding transition probabilities (except for the index SH, we fixed all covariates with the following referent values in corresponding models): occurrence of index SH (yes or no), gender (female), baseline age (>29 years), duration of disease (>10 years) and history of SH (no), most recent SH (no) and incidence rates of SH episodes (less or equal to the 90^{th} percentiles of the rates in corresponding treatment groups up to the index SH), expected A1C (6-6.9%), actual A1C (6-6.9%), alcohol use (no use), exercise activities (moderate), meal plan (adherence) and insulin adherence (adherence), use of SMBG (often done), blood glucose variability (population mean 3.89) and the follow-up time (24 months). I did not include any occurrences of SH in three subsequent time windows following index SH as the covariates in the models to obtain transition probabilities. For instance, we did not include occurrences of SH in the first 3-month observation window as the covariates in the models when estimating the transition probabilities for subsequent SH episodes in the 4-6 and 7-9 month observation windows after the index SH because the occurrences of SH in the first 3-month were treated as the intermediated effects of the index SH on the 4-6 and 7-9 month observation windows. If log-linear models could not converge, we used logistic regression to estimate transition probabilities.

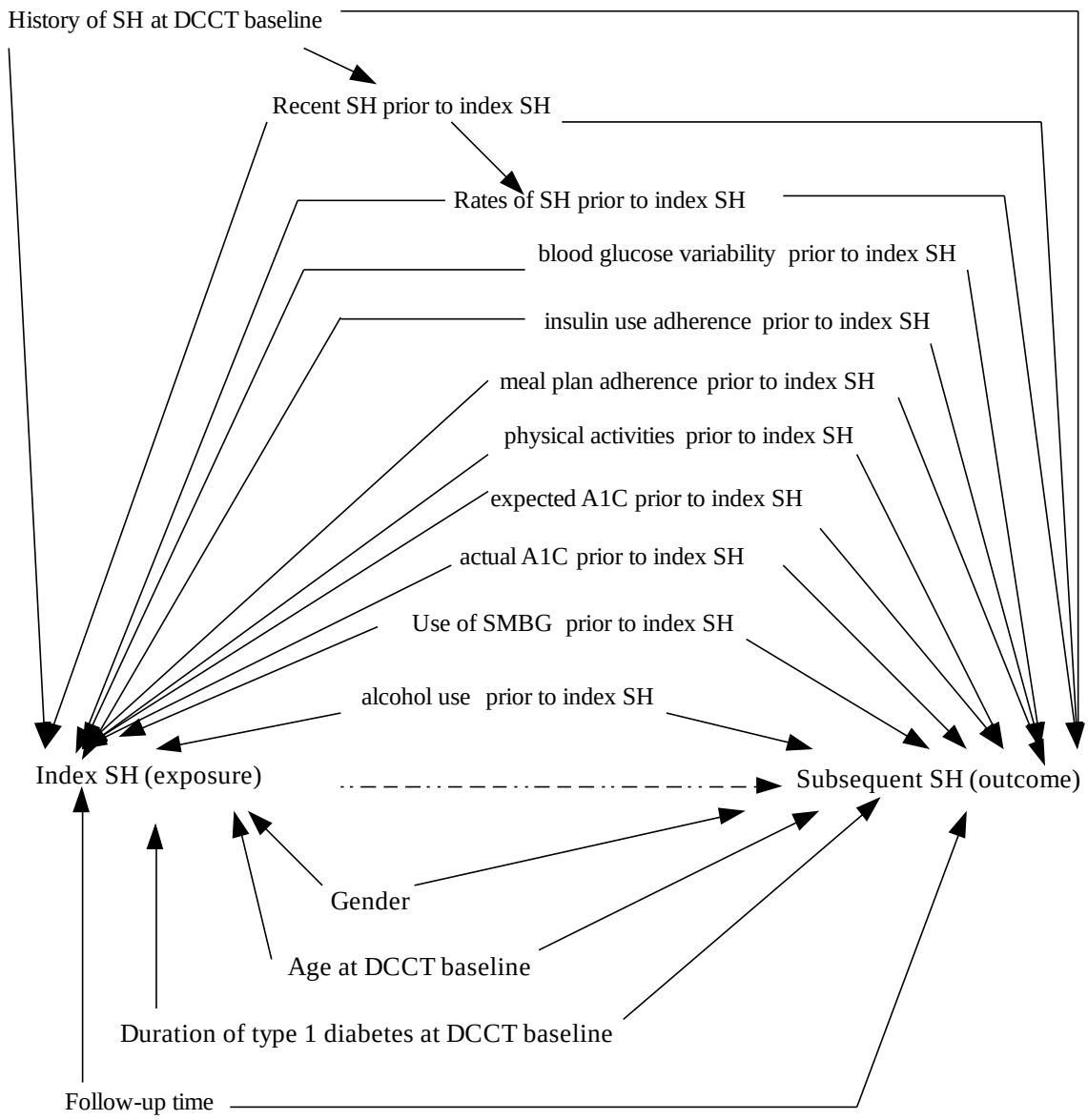

Figure 6: Directed acyclic graph for the effect of occurrence of SH (index SH) on subsequent SH episodes in observation windows following index SH.

b. Estimation of the effects of SH on subsequent weight gain over various periods

We used linear multivariable models to estimate the effects of SH (index SH), which occurred in a quarterly interval, on weight change/actual weight in various periods with

ranges approximately from 3 months to 36 months after index SH. We also looked at the effects of index SH on weight change in three subsequent fixed-term time windows at the 1st, 2nd and 3rd quarterly interval following index SH (the observation period for each time window approximated a 3-month period in this analysis), and the outcome as the weight change in this analysis was the quarterly weight change for three fixed-term time windows, respectively. The corresponding comparison intervals were the intervals without occurrence of index SH.

We controlled for confounding by including a set of priori specified covariates in the models based on DAG (Figure 7). We tried several parameter specifications (original values, categories and splines) for continuous covariates in the models. Based on the precision of the estimates for the main exposure and model fit statistics, the most appropriate specifications (formats) for continuous covariates were used in final models (Table 3). Since each patient could contribute multiple observations, similarly we used GEE by specifying working correlation structure as “exchangeable” to allow for the dependence of observations within a person [28, 29].

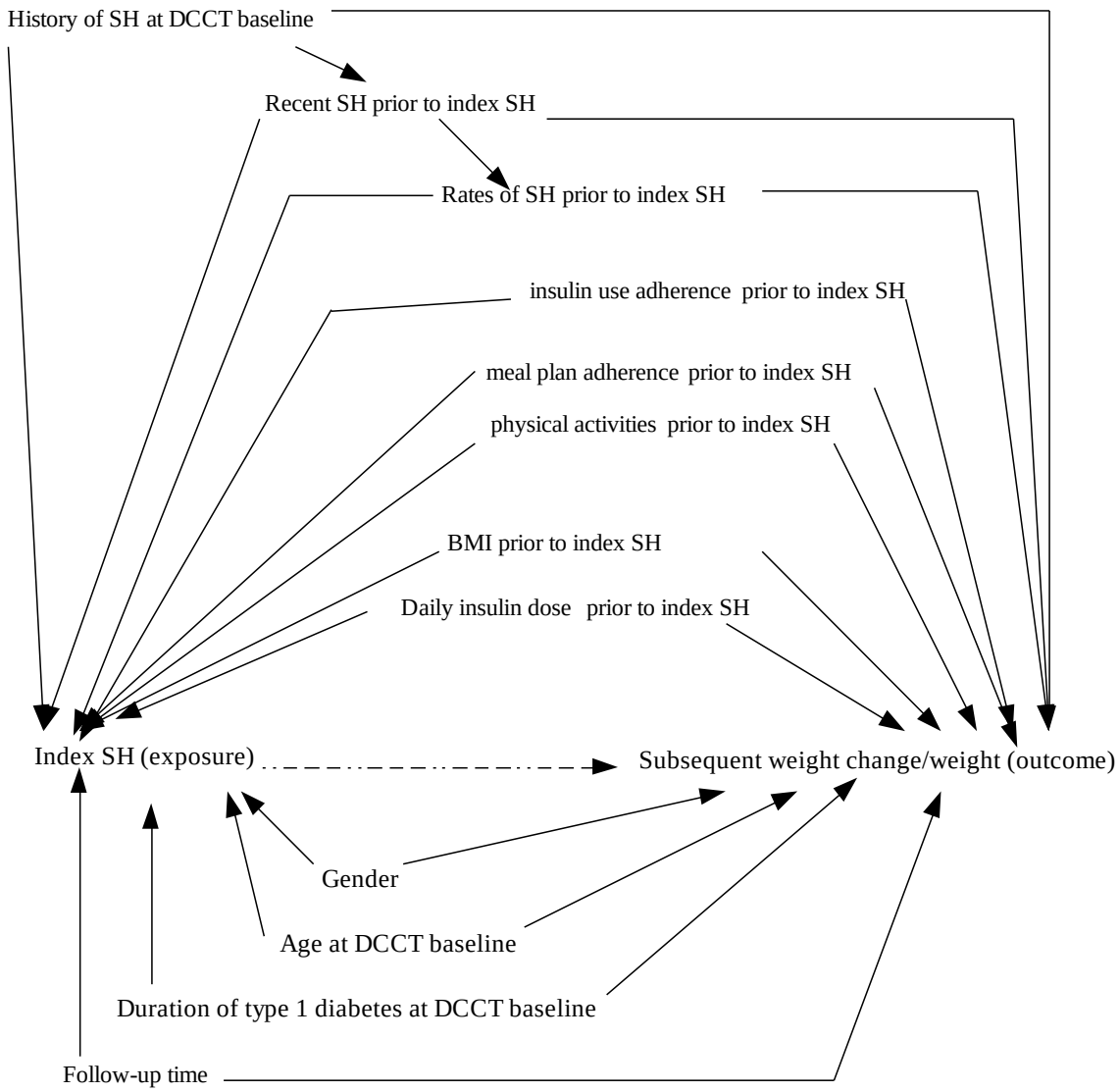

Figure 7: Directed acyclic graph for the effect of occurrence of SH (index SH) on subsequent weight change/weight in various periods following index SH.

c. Estimation of the effects of SH on substantial weight gain, overweight, and obesity

We used Cox proportional hazard models to estimate the HRs of SH on the substantial weight gain (defined as the 5, 10, 15 and 20% weight gain from the DCCT baseline, respectively), on becoming overweight (BMI≥25.0), or on becoming obese (BMI≥30.0) comparing patients after they had their first SH episode with all patients who did

not experienced any SH episode at the same time during follow-up (they could have a SH episode later during follow-up) [30]. Hence, the exposure in the Cox models was a time-dependent variable: 1) the patient was defined as unexposed prior to the 1st SH episode; 2) after the 1st SH episode, the patient was defined as always exposed. We checked the proportional hazards assumption for main exposure by adding an interaction term between the exposure and the follow-up time. We controlled for confounding by including a set of priori specified covariates (Table 4) in the models based on the DAG (Figure 8).

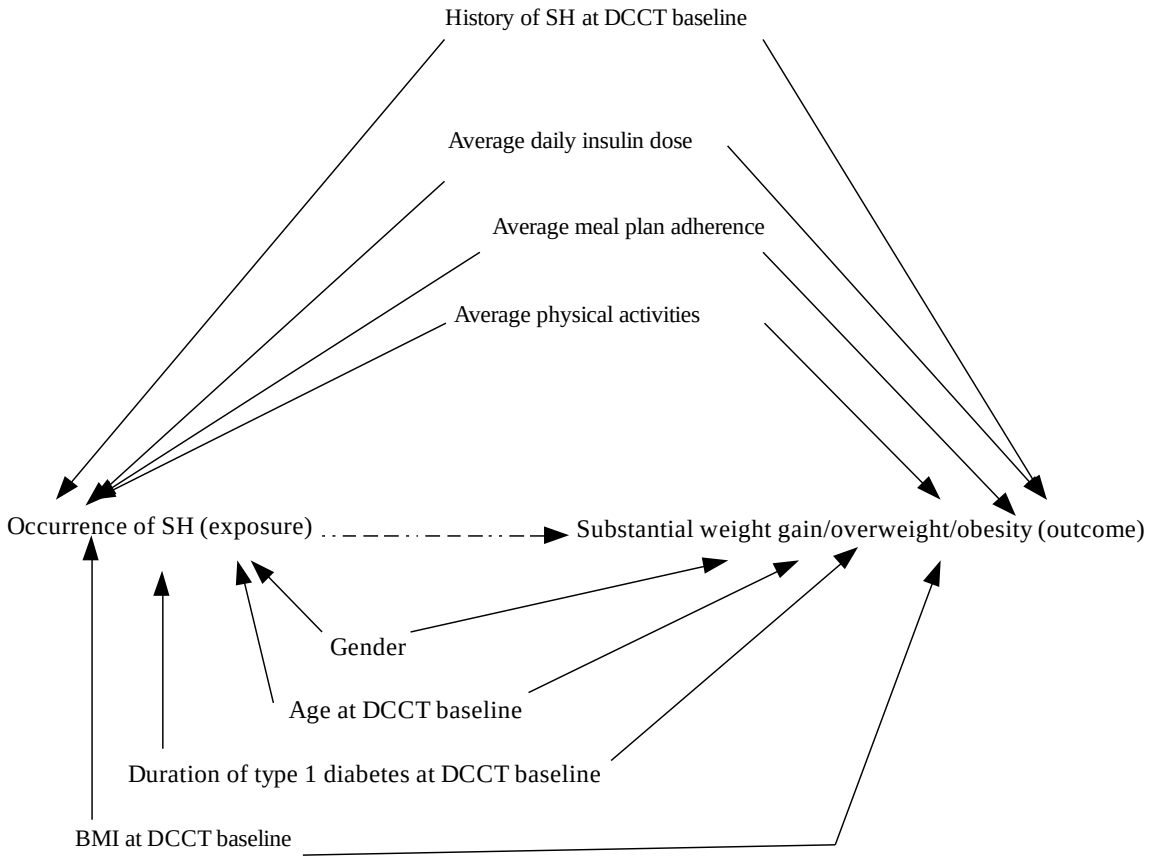

Figure 8: Directed acyclic graph for the effect of occurrence of SH on developing substantial weight gain, overweight or obesity during the follow-up.

Average daily insulin dose may be seen as a time-dependent confounder affected by prior SH (Figure 9). Under this assumption the Cox model controlling for average daily insulin dose as well as a model not controlling for it would be biased [40]. Furthermore, as an ancillary analysis, we therefore used marginal structural models (MSM) that allow us to control for average daily insulin dose that may be affected by exposure. I used weighted pooled logistic regression models to approximate the parameters of the MSM [13]. Fitting the weighted estimation of the parameters involved three sets of models: 1) a pooled logistic model estimating the time-varying exposure propensity (the probabilities to have the first SH episode during the follow-up), 2) a pooled logistic model estimating the probability of censoring, and 3) the final model weighting exposed and unexposed by the weights obtained in steps 1) and 2) to obtain the estimates for the main exposure with robust standard errors for the confidence intervals. We used a restricted spline with 3 knots for all continuous variables in all sets of models as recommended [41]. To estimate the time-dependent intercept for all three models in the MSM, we produced a restricted cubic spline for the follow-up time (the numbers of months since start of the trial) with SAS macro RCSPLNE [42]. We also examined the distribution of the estimated weights to check whether the weights were well constructed (the well-constructed weights should have a mean around 1 and narrow ranges for the estimated weights) [41].

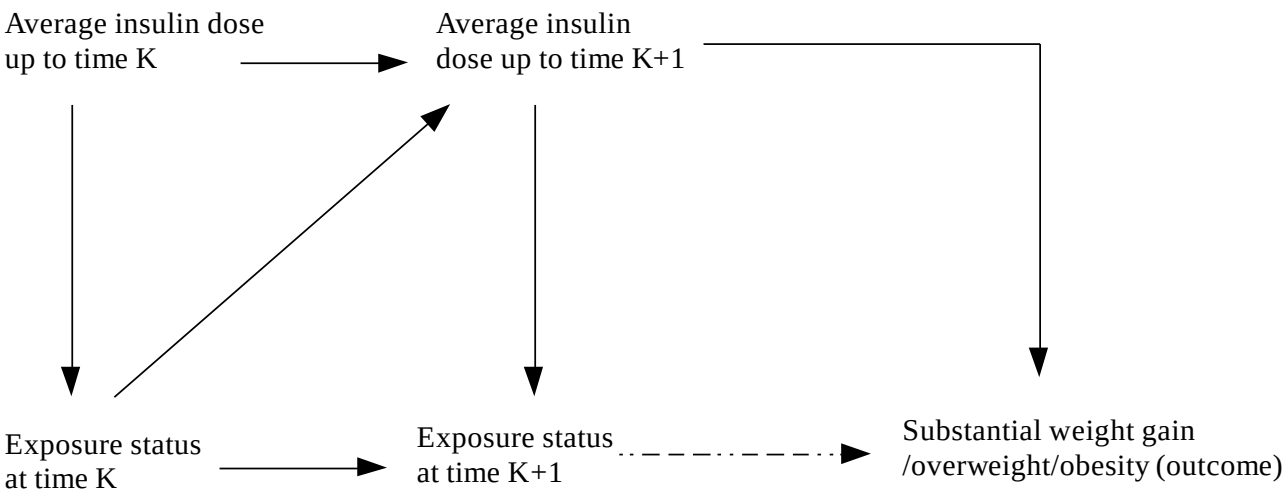

Figure 9: Conceptual directed acyclic graph to show that covariate average daily insulin dose may be an intermediate and time-dependent confounder for the association between the occurrence of severe hypoglycemia (exposure) and time to substantial weight gain, time to becoming overweight or time to becoming obese during follow-up.

d. Estimation of the effects of SH on insulin dosing changes

As a supplementary analysis, we used linear multivariable models to estimate the effects of SH occurring in the first composed quarterly interval in an analytic unit on the continuous outcome (the difference of daily insulin doses between the end and beginning of the analytic unit) and used log-linear multivariable models on the dichotomized outcome (≥ 10% dose reduction) in each analytic unit. Each analytic unit consisted of two consecutive quarterly visits to account for temporality of change of insulin dose following occurrence of SH (Figure 5). We used generalized GEE to account for multiple-observations per patient and to control relevant factors with working correlation structure “exchangeable”. Furthermore, for continuous outcome (the difference of daily insulin doses), we also used mixed models as a sensitivity analysis. However, negative variance components were

reported when we used a random effect for the intercept for each subject by covariance structure as either variance component or unstructured in the mixed models. After serial diagnostic tests, we omitted the random effect for the intercept, but correlation within the random effect was modeled by including covariance parameter in the residual variance matrix R with a toeplitz structure and toep (5) appeared a best fit.

V. RESULTS

A. Estimation of acute effects of severe hypoglycemia on subsequent episodes in type 1 diabetes

1. Introduction

Hypoglycemia is the predominant limiting factor in the glycemic management of type 1 diabetes [2]. Severe hypoglycemia (SH), which requires the assistance of another person and cannot be treated by patients themselves, can further induce hypoglycemia-associated autonomic failure, a defect in counterregulation and loss of awareness of hypoglycemia [8]. Thus, SH can cause a vicious cycle of recurrent hypoglycemia, and the effect of SH on subsequent episodes is often inferred as an acute effect with the greatest risk occurring in weeks and months immediately following SH [1, 8].

Although prior studies have consistently reported SH as a general risk factor for recurrent SH episodes [9, 10, 16], very few studies provide epidemiologic evidence to demonstrate the acute effects of SH on subsequent SH in a clinically relevant time range, and the magnitude of such effects from population levels in patients with type 1 diabetes is not completely clear. One Scottish study with 94 subjects with type 1 diabetes reported that the occurrence of hypoglycemia in the previous month was associated with the occurrence of hypoglycemia in next month, with an odds ratio of 4.6 (CI: 1.5-13.7) [15]. The effect appears greater in this study compared to estimates reported by other studies that did not restrict their definition of exposure and outcome to a short time range [9, 10, 16], and the results of this study seem to support the notion of an acute effect [1]. However, this study followed participants for only one month and had a very small sample size.

The objectives of this study are to estimate and illustrate the acute effects of SH on risk of subsequent SH episodes in patients with type 1 diabetes.

2. Methods

Study population

We used data from the Diabetes Control and Complications Trial (DCCT), which randomized 1,441 patients between ages of 13 to 39 with duration of type 1 diabetes 1-15 years to either intensive therapy or conventional therapy between 1983 and 1993. Patients were followed through quarterly clinic visits [31]. The trial was designed to compare intensive and conventional therapies and their relative effects on the development and progression of diabetic complications. Patients on intensive therapy had specific glucose targets (to keep A1C as close to 6% as possible with daily targets at 70-120 mg/dl before meals, <180 mg/dl after meals, and >65 mg/dl at 3 am) and could choose a regimen consisting of either multiple daily injections or continuous subcutaneous insulin infusion with an external pump. They also were required to conduct frequent self-monitoring of blood glucose (SMBG), were seen by their health care team monthly, and were contacted by telephone weekly. Patients randomized to the conventional therapy only saw the team quarterly, used one or two insulin injections per day, and monitored either urine or SMBG without specific glucose targets [31].

To allow for learning during the transition period when patients entered the trial, we only included quarterly visits after the first year of follow-up as analytic intervals in our analysis. We also restricted eligible patients who must be 18 years or older when they entered the trial, since younger patients were treated differently for glycemic control and would differ in term of self-management of type 1 diabetes from adult patients [31].

Definition of SH

SH was defined as an episode in which a patient required assistance of another person and the following conditions: 1) a blood glucose level of < 50 mg/dl (if available), or 2) prompt recovery following oral carbohydrate, intravenous glucose, or glucagons. Patients in the DCCT were instructed to report all episodes of suspected SH immediately, and all were interviewed to verify each episode [31].

Estimation of the acute effects of SH on subsequent SH

We estimated the relative risk (RR) of SH (index SH) on subsequent SH in three subsequent time windows after the index SH based on quarterly visits in the DCCT: between the 1st and 2nd, between the 2nd and 3rd, between the 3rd and 4th quarterly visit after the index SH, which corresponds to approximate months 1-3, 4-6, and 7-9 after index SH, respectively (Figure 3). It should be noted that the above defined windows would not be the exact durations as the months 1-3, 4-6 and 7-9 after the index SH because the period between the time when the index SH occurred and the time when next quarterly visit was conducted would not be captured by this definition. However, we use months to describe the timeline after index SH in the following sections for simplicity.

We used log-linear multivariable models to estimate relative risks and their 95% confidence intervals (CI). We controlled for confounding by the following *a set of priori* specified covariates based on directed acyclic graph [38](Figure 6): 1) time-independent variables: gender, baseline age (3 categories “18-25, 26-29, and >29 years of old ”), duration of disease (3 categories “1-5, 6-10 and >10 years”) and history of SH (dichotomized variable based on the interview as the same definition above) at the DCCT baseline; 2) time-dependent variables: recent SH (dichotomized variable to indicate the occurrence in the quarterly interval prior to index SH) and rates of SH episodes prior to the index SH (3 categories “no occurrence prior to the index SH, less or equal to the 90th percentile of the rates in corresponding treatment groups, and greater than 90th percentile”), expected A1C (4

categories “<6%, 6-6.9%, 7-7.9% and ≥8%”) calculated from mean blood glucose with quarterly blood glucose profiles as previously described [36, 37]), actual A1C (4 categories “<6%, 6-6.9%, 7-7.9% and ≥8%”), alcohol use (3 categories “no use, 1-75 grams per week, >75 grams per week”), physical activities (3 categories “little, moderate and strenuous” based on annual questionnaire), meal plan adherence (dichotomized variable for whether subjects claimed to have followed the meal plan almost all of the time in quarterly questionnaire) and insulin use adherence (dichotomized variable for whether subjects claimed to have followed the prescribed insulin dose in quarterly questionnaire), use of SMBG (3 categories “no use, often done and at least one per day”), blood glucose variability (standard deviation calculated from quarterly blood glucose profiles for individual subjects) and the follow-up time since entry of the DCCT (a restricted cubic spline with 5 knots at 5, 10, 25, 75 and 95th percentiles of the months since entered the DCCT). We defined all time-dependent covariates using the most recent value before the occurrence of the index SH. Since each patient could contribute multiple observations, we used generalized estimating equations (GEE) by specifying working correlation structure as “exchangeable” to allow for the dependence of observations within a person [28, 29]. When the log-linear models did not converge, we used Poisson models to approximate RRs as previously described [39].

We also produced transition probabilities as the risk of subsequent SH episodes in the same three subsequent observation windows using the above estimation models. We assigned the following values for the covariates in the estimation models including the intercept term to produce predictive values as the corresponding transition probabilities (except for the index SH, we fixed all covariates with the following referent values in corresponding models): occurrence of index SH (yes or no), gender (female), baseline age (>29 years), duration of disease (>10 years) and history of SH (no), most recent SH (no) and incidence rates of SH episodes (less or equal to the 90th percentile of the rates in

corresponding treatment groups up to the index SH), expected A1C (6-6.9%), actual A1C (6-6.9%), alcohol use (no use), physical activities (moderate), meal plan (adherence) and insulin adherence (adherence), use of SMBG (often done), blood glucose variability (population mean 3.89) and the follow-up time (24 months). We did not include any occurrence of SH in three subsequent time windows following index SH as the covariates in the models to obtain transition probabilities. For instance, we did not include occurrence of SH in the first 3-month observation window as the covariates in the models when estimating the transition probabilities for subsequent SH episodes in the 4-6 and 7-9 month observation windows after the index SH because the occurrence of SH in the first 3-month were treated as the intermediated effects of the index SH on the 4-6 and 7-9 month observation windows. If log-linear models could not converge, we used logistic regression to estimate transition probabilities. All analyses were performed with SAS, version 9.2 (SAS Inc., Cary, NC).

3. Results

We identified 1,220 eligible patients who contributed 22,207quarterly visits (intervals) from both treatment groups (605 patients in the intensive therapy and 615 in the conventional therapy) in the DCCT for our final analysis. The intervals that had prior SH events appeared to have lower expected and observed A1C levels in both treatment groups, and more intervals in the intensive therapy group were found to have lower expected and observed A1C levels than those in the conventional therapy group (Table V-A 1).

The highest absolute risks (transition probabilities) for subsequent SH episodes after index SH were found in the first 3-month observation window in both treatment groups (Table V-A 2): in the conventional therapy, the risks to develop subsequent SH episodes in the first 3 months, months 4-6 and months 7-9 after index SH were 19.8%, 15.4% and 15.9%, respectively; the corresponding risks were 21.5%, 21.4% and 17.3% in the intensive therapy group.

In both treatment groups, the greatest adjusted RRs on the risk of subsequent SH episodes were also observed in the first 3 months after the index SH compared to those without occurrence of the index SH (Table V-A 3): in the conventional therapy group, the RRs (95% CI) in the first 3 months, months 4-6 and 7-9 were 3.38 (2.21, 5.18), 1.37 (0.76, 2.47) and 1.95 (1.33, 2.87) respectively, and the corresponding RRs were 1.7(1.48, 1.97), 1.45 (1.2, 1.74) and 1.36 (1.16, 1.61) in the intensive therapy group.

Similar to RRs, the greatest risk differences (RDs) for the effects of index SH on subsequent episodes (calculated by transition probabilities) were also observed in the 1st observation window after the index SH in both treatment groups (Table V-A 2).

4. Discussion

We observed that the greatest risk of recurrent SH occurred in the follow-up time windows closest to recent SH in a population with type 1 diabetes. The results in our study support the notion of acute effects of SH on subsequent episodes in patients with type 1 diabetes on insulin therapy. In addition, our results based on a relatively large population are also compatible with a hypothesized underlying biologic mechanism: the recent antecedent hypoglycemia may cause defective glucose counterregulation and hypoglycemic unawareness, and, therefore, recent SH episodes can lead to more recurrent events, a vicious continuing cycle [8]. Hence, besides previous evidence to treat history of SH as a general risk factor [43], our study adds further detail for the evidence to indicate that the immediate periods after occurrence of SH are crucial to prevent subsequent SH in clinical management of type 1 diabetes.

Our study is not without its limitations with regard to available information to better address our research questions and generalization of our findings. First, the daily changes of therapy regimens in the DCCT were not centrally documented and were not accessible

for analysis. We only had quarterly data available for patients in our study and assumed that it was representative of the full quarter for individual patients. Hence, we only can estimate the total effects of SH on subsequent SH episodes, and cannot disentangle underlying intermediate effects due to physicians and patients' activities in responding the SH events (index SH) on subsequent SH episodes. Our observation that the greatest risk of recurrent SH occurred in the follow-up time windows closest to recent SH may only reflect a lag for physicians and patients to response the prior SH events (index SH). However, regardless of whatever explanations the greater risk on subsequent SH episodes in the first 3-month after index SH compared to those without occurrence of index SH suggests that the effect of recent SH occur immediately. If the lag to response the index SH can completely explain what we observed in this study our results would further demonstrate that a prompt and timely response after recent SH is crucial to prevent subsequent SH. Secondly, information on activities that affect the risk for SH, including physical activity and alcohol consumption, was not available quarterly, and we had to use annual collected information on physical activity and alcohol consumption as a surrogate for data for each quarterly visit. However, conventional risk factors for SH, including physical activity and alcohol consumption, were reported to be well under control in the DCCT because a careful review for these factors was performed for the patient once SH occurred in a patient during the trial [10]. Lastly, we should be cautious about extrapolating our findings to all patients with type 1 diabetes because of the selected trial population and the trial design of the DCCT.

Notwithstanding these limitations, we were able to utilize longitudinal information from the DCCT with 1,220 eligible patients who contributed to 22,207 quarterly intervals to illustrate the acute effects of SH over clinically relevant observation periods.

In summary, our study adds to the evidence for an acute effect of recent SH on subsequent episodes. It indicates that the immediate periods after occurrences of SH are crucial in clinical management of type 1 diabetes to prevent subsequent SH.

Table V-A 1: Distributions of some covariates by exposure status --occurrence of severe hypoglycemia (SH) on risk of subsequent SH in the 1st subsequent observation window in the DCCT

		Intensive Therapy (No. of patients=605)		Conventional Therapy (No. of patients=615)	
		Prior SH	No Prior SH	Prior SH	No Prior SH
Total contributed intervals*		1,179	9,920	384	10,724
Expected A1C levels (%)					
	<6%	26.5	18.2	10.0	4.1
	6-6.99%	25.2	26.3	13.8	6.8
	7-7.99%	23.8	23.9	16.1	10.7
	≥8%	24.5	31.6	60.1	78.4
Actual observed A1C (%)					
	<6%	10.2	8.3	6.7	1.4
	6-6.99%	44.9	40.9	18.4	6.1
	7-7.99%	35.8	35.9	28.3	16.8
	≥8%	9.1	14.8	46.7	75.8
Self-monitoring of blood glucose (%)					
	at least one per day	91.8	93.1	31.9	22.2
	Often done	7.6	6.0	40.4	50.6
	No use	0.6	1.0	27.7	27.2
Alcohol use (%)					
	more than 75 grams per week	11.6	10.3	7.3	10.7
	1-75 grams per week	23.0	24.4	32.6	27.8
	No use	65.4	65.3	60.2	61.5
Physical activities (%)					
	Strenuous	4.6	4.5	9.6	6.1
	Moderate	56.5	53.2	53.1	52.2
	Little	38.9	42.4	37.2	41.7
Meal plan adherence (%)					
	Yes	83.1	81.7	82.8	80.5
	No	16.9	18.3	17.2	19.5
Insulin use adherence (%)					
	Yes	75.1	77.3	87.5	87.4
	No	24.9	22.7	12.5	12.6

* One patient would contribute multiple observation quarterly intervals.

Table V-A 2: Risk (transition probabilities) of severe hypoglycemia (SH) in three subsequent observation windows in the DCCT

Treatment Assignment	Observed window after index SH	No. of intervals with SH episodes	Total contributed intervals*	Transition probabilities for subsequent SH (TP, %)†	Differences of TP (prior SH minus no prior SH)
Intensive Therapy	Months 1-3				
	Prior SH	350	1,179	21.5	8.9
	No prior SH	844	9,920	12.6	0.0 (reference)
	Months 4-6				
	Prior SH	293	1,096	21.4‡	7.1
	No prior SH	842	9,398	14.3‡	0.0 (reference)
	Months 7-9				
	Prior SH	271	1,038	17.3	4.6
	No prior SH	792	8,852	12.7	0.0 (reference)
Conventional Therapy	Months 1-3				
	Prior SH	103	384	19.8	13.9
	No prior SH	282	10,724	5.9	0.0 (reference)
	Months 4-6				
	Prior SH	64	358	15.4‡	4.3
	No prior SH	301	10,135	11.1‡	0.0 (reference)
	Months 7-9				
	Prior SH	64	335	15.9	7.8
	No prior SH	282	9,543	8.1	0.0 (reference)

* One patient would contribute multiple observation intervals;

†Assigned following values for the covariates including the intercept in models to produce transition probabilities: intercept (1), occurrence of prior SH (yes or no), gender (female), baseline age (>29), duration of disease (> 10) and history of SH (no), most recent SH (no) and incidence rates of SH episodes up to the index SH (less or equal to the 90th percentiles of incidence rates in the corresponding treatment groups), expected A1C (6-6.9%), actual A1C (6-6.9%), alcohol use (no use), physical activities (moderate), meal plan (adherence) and insulin adherence (adherence), use of SMBG (often done), blood glucose variability (mean 3.89) and the follow-up time (24 months);

‡ Logit (link function) and binomial distribution were used to produce transition probabilities of SH with SAS GENMOD when the models for RR could not converge.

Table V-A 3: Relative risk of severe hypoglycemia (SH) on subsequent SH episodes in three subsequent observation windows in the DCCT

Treatment Assignment	Observed windows after index SH	Unadjusted RR (95% CI)*	Adjusted RR (95% CI) †
Intensive Therapy			
	Months 1-3		
	Prior SH	1.65(1.36,2.00)	1.70(1.48, 1.97)
	No prior SH	1.00(reference)	1.00(reference)
	Months 4-6		
	Prior SH	1.29(1.02, 1.63)	1.45(1.20, 1.74) ‡
	No prior SH	1.00(reference)	1.00(reference)
	Months 7-9		
	Prior SH	1.29(1.03, 1.61)	1.36(1.16, 1.61)
	No prior SH	1.00(reference)	1.00(reference)
Conventional Therapy			
	Months 1-3		
	Prior SH	3.88(2.33, 6.48)	3.38(2.21, 5.18)
	No prior SH	1.00(reference)	1.00(reference)
	Months 4-6		
	Prior SH	1.36(0.47, 3.99)	1.37(0.76,2.47) ‡
	No prior SH	1.00(reference)	1.00(reference)
	Months 7-9		
	Prior SH	1.69(0.82, 3.44)	1.95(1.33, 2.87)
	No prior SH	1.00(reference)	1.00(reference)

* Unadjusted RRs were the estimates which had been considered for a correlation within one patient;

†Adjusted following covariates in the models: Gender, baseline age, duration of disease and history of SH when patients entered the DCCT, most recent and incidence rates of SH episodes up to the occurrence of prior SH, expected and actual A1C, alcohol use, physical activities, meal plan and insulin adherence, use of self-monitoring blood glucose (SMBG), blood glucose variability (standard deviation calculated from 7-point blood glucose profiles for individual patient) and follow-up time since entry of the DCCT;

‡Incidence rate ratio was used to approximate RR when the models for RR could not converge.

B. Severe hypoglycemia and subsequent weight gain in patients with type 1 diabetes

1. Introduction

Intensification of insulin therapy in type 1 diabetes often results in weight gain [4]. In the DCCT, which randomized type 1 diabetic patients to either intensive or conventional therapy, the prevalence of overweight, defined as the BMI over 27.8kg/m^2 for men and 27.3kg/m^2 for women, reached 33% in the intensive therapy group compared with 19% in the conventional therapy group after an average 6.5 year follow-up [18].

A number of possible mechanisms have been described to explain weight gain on insulin therapy, including compensation for hypoglycemia [4]. The unpleasant symptoms and negative consequences of hypoglycemia may result in significant fear of hypoglycemia [17]. Because low blood glucose levels can be remedied by ingestion of glucose or food following a hypoglycemic event, patients may over-react by consuming or be instructed to consume more calories (e.g., frequent snacking) in response to the threat of subsequent hypoglycemia.

However, there is very limited direct evidence to support this hypothesis of compensation for hypoglycemia. An early DCCT study [25] using patients recruited in the first year reported that the 29 patients who experienced severe hypoglycemia (SH) gained more weight than those without SH in the intensive therapy arm, but not for the conventional therapy arm. Another study ,which re-analyzed data from a 26-week, randomized, open-label trial comparing insulin detemir with NPH insulin in 476 patients with type 2 diabetes [26], reported only *P* values for the correlations between the frequencies of hypoglycemia and weight gain and concluded that hypoglycemia was a predictor of weight gain with NPH insulin, but not with insulin detemir. However, the temporal relation between occurrence of

SH or hypoglycemia and weight gain was unclear in these studies because some of the weight gain may have occurred before the first SH or hypoglycemic episode.

The aims of this study are: 1) to examine the effects of SH on subsequent weight change/actual weight over various periods following SH episodes; 2) to estimate the effects of SH on the risk of substantial weight gain, overweight, and obesity in patients with type 1 diabetes.

2. Methods

Participants and study population

We used data from the DCCT, which randomized 1,441 patients between ages of 13 to 39 with duration of type 1 diabetes ranging 1-15 years to either intensive therapy or conventional therapy between 1983 and 1993. Patients were followed through quarterly visits over an average of 6.5 years [31]. In the trial, registered dietitians tailored meal plans and educational strategies to the need and life style of each individual to achieve and maintain ideal body weight with goals of 15-20% of energy from protein, 30-35% from fat, and 50-55% from carbohydrates. Patients in the intensive therapy group received more frequent and detailed instruction on individualized meal plans. Since weight gain with intensive therapy had been recognized, dietitians increased their emphasis and counseling on weight management for patients in the intensive therapy group, and greater attention was given to the relation between nutrient intake and insulin to try to achieve target glycemic levels without hypoglycemia or weight gain [31].

For our analyses, we restricted eligible patients to be 18 years or older when they entered the trial to avoid weight gain as a result of adolescent growth. In addition, we censored female patients at the time of their first pregnancy during follow-up based on their reported last menstrual period dates.

The public health institutional review board of University of North Carolina approved this study and all study participants in the DCCT have given written informed consent.

Definition of SH

SH was defined as an episode in which a patient required assistance of another person and the following conditions: 1) a blood glucose level of < 50mg/dl, or 2) prompt recovery following oral carbohydrate, intravenous glucose, or glucagons. Patients in the DCCT were instructed to report all episodes of suspected SH immediately, and all were interviewed to verify each episode [31].

Weight measurement

Weight was measured at baseline and each quarterly examination [27]. Weight (in kilograms) was measured with the patients in light clothing and stockings on the same balance-beam scale for the duration of the DCCT trial. Overweight was defined as BMI values ≥25.0, and obesity were defined as BMI values ≥30.0 [33].

Estimation of the effects of SH on subsequent weight gain over various periods

We estimated the effects of SH (index SH), occurring in a quarterly interval, on weight during the following one, two, three, four, eight, and twelve consecutive quarterly visits (intervals). Each patient could contribute multiple observations (figure 1). The different numbers of involved quarterly intervals approximated the observation periods with various durations from 3 months to 36 months after SH episodes. The comparison intervals were those without occurrence of index SH. Two continuous outcome variables were defined for each observation period: 1) the first outcome variable (weight change) was the difference between the weight measured at the end of the observation period and the weight at the beginning of the same observation period; 2) the second was the actual weight measured at the end of the observation period. Besides using various observation periods after index SH,

we also looked at the effects of index SH on weight change in three subsequent fixed-term time windows during the 1st, 2nd and 3rd quarterly interval following index SH (the observation period for each time window approximated a 3-month period in this analysis), and the outcome as the weight change in this analysis was the quarterly weight change for three intervals, respectively.

We controlled for confounding by including a set of *a priori* specified covariates in the models based on directed acyclic graph [38](Figure 7): 1) time-independent variables: gender, baseline age (3 categories "18-25, 26-29, and >29 years of old "), duration of type 1 diabetes (3 categories "1-5, 6-10 and >10 years") at the DCCT baseline and history of SH prior to the entry of the DCCT (dichotomized variable based on the DCCT baseline file); 2) time-dependent variables: recent SH (dichotomized variable to indicate the occurrence of SH in the quarterly interval prior to index SH), and rates of SH episodes from baseline to prior to the index SH (3 categories "no occurrence prior to the index SH, less or equal to the 90th percentile of the rates in corresponding treatment groups, and greater than 90th percentile"), physical activity (3 categories "little, moderate and strenuous" based on annual questionnaire), meal plan adherence (dichotomized variable for whether subjects claimed to have followed the meal plan almost all of the time in quarterly questionnaire), insulin adherence (dichotomized variable for whether subjects claimed to have followed the prescribed insulin dose in quarterly questionnaire), BMI prior to SH (a restricted quadratic spline with 3 knots at BMI 18.5, 25 and 30kg/m^2, corresponding to normal weight, overweight and obesity), quarterly daily insulin dose (a restricted quadratic spline with 3 knots at 5th , 50th and 95th percentiles) and follow-up time since entry of the DCCT (a restricted cubic spline with 5 knots at 5th , 10th , 25th , 75th and 95th percentiles). We categorized continuous covariates or created the knots for their splines based on established cut-points (e.g., BMI) or known conventions. Otherwise, we used empirical cut-

points based on the actual distributions from data themselves (e.g., the different distribution percentiles). We used multivariable linear regression to estimate the association between the index SH and subsequent weight gain. Because each patient could contribute multiple observations, we used GEE with a linear link, normal variance function, and exchangeable working correlation to allow for the dependence of observations within a person [28, 29].

Estimation of the effects of SH on substantial weight gain, overweight, and obesity

We used Cox proportional hazard models to estimate hazard ratio (HRs) for time to first substantial weight gain (defined as the 5, 10, 15 and 20% weight gain from baseline, respectively), becoming overweight, and becoming obese, comparing patients after they had their first SH episode with all patients who did not experience any SH episode at the same time during follow-up (they could have a SH episode later during follow-up) [30]. Hence, the exposure in the Cox models was a time-dependent variable: 1) the patient was defined as unexposed prior to the 1st SH episode; 2) after the 1st SH episode, the patient was defined as always exposed. We checked the proportional hazards assumption by adding an interaction term between the exposure and the follow-up time. We controlled for confounding by including a set of *a priori* specified covariates in the models based on a directed acyclic graph [38](Figure 8) : 1) time-independent variables: gender, baseline age (a restricted quadratic spline with 3 knots at 5th, 50th and 95th percentiles), duration of type 1 diabetes (a restricted quadratic spline with 3 knots at 5th , 50th and 95th percentiles), history of SH (same definition above) and BMI (continuous variable) at baseline; 2) time-dependent variables: average physical activity (dichotomized variable on whether a subject had strenuous activities in 80% of follow-up time or more, updated annually), average meal plan adherence (dichotomized variable on whether a subject had claimed to follow meal plan in 80% of follow-up time or more, updated quarterly) and average daily insulin dose (a restricted quadratic spline with 3 knots at 5th , 50th and 95th percentiles, updated quarterly).

Average daily insulin dose may be seen as a time-dependent confounder affected by prior SH (Figure 9). Under this assumption, the Cox model controlling for average daily insulin dose as well as a model not controlling for it would be biased [40]. As an ancillary analysis, we therefore used marginal structural models (MSM) that allow us to control for average daily insulin dose that may be affected by prior SH. We used weighted pooled logistic regression models to approximate the parameters of the MSM [13]. This involved three sets of models: 1) a pooled logistic model estimating the time-varying exposure propensity (the probabilities of having the first SH episode during the follow-up), 2) a pooled logistic model estimating the probability of censoring, and 3) the final model weighting exposed and unexposed by the weights obtained in steps 1) and 2) to obtain the estimates for the main exposure with robust standard errors for the confidence intervals. To estimate the time-dependent intercept for all three models in the MSM, we produced a restricted cubic spline for the follow-up time (the numbers of months since start of the trial) with the SAS macro RCSPLNE [42]. We also examined the distribution of the estimated weights to check whether the weights were well constructed (the well-constructed weights should have a mean around one and narrow ranges for the estimated weights) [41].

3. Results

We identified 1,218 eligible patients from both treatment arms in the DCCT who contributed 25,336 observation periods for final analysis (the exact eligible number of patients varied by observation periods with various durations, see footnotes in table 1). Periods with prior SH events had higher daily insulin doses than periods without SH in both treatment arms (Table V-B 1).

In the intensive therapy arm, the estimated effects of index SH on subsequent weight change and weight were close to the null effect (zero for the difference of the means for weight change or for difference of the means for weight) comparing to those without index

SH (table 2-3). In the conventional therapy arm, however, occurrence of SH appeared to be associated with a weight loss and less weight up to 12 months after the index SH (Table V-B 2,Table V-B 3). The effect sizes of weight loss for both outcome variables were quite constant over various periods following occurrence of SH: the adjusted differences of the means for weight change were -0.29 (95% CI: -0.51, -0.08) and -0.46 (-0.83, -0.09) in the observation period months 1-3 and 1-12 respectively, and the corresponding adjusted differences of the means for weight were -0.43 (-0.70, -0.16) and -0.43 (-0.77, -0.09). The results from the analysis for the effects of index SH on the quarterly weight change in three fixed-term time windows further showed that the observed weight loss in the conventional therapy arm happened only in the 1st 3-month period after index SH (Table V-B 4) but weight change were observed close to null effect in the 2nd time window (months 4-6) and the 3rd time window (months 7-9) following index SH. The adjusted differences of the means for weight change were -0.04 (-0.24, 0.17) and -0.04 (-0.23, 0.15) for the 2nd and 3rd time windows, respectively.

The estimated HRs for the occurrence of SH on substantial weight gain, becoming overweight or obese were also around the null effect (HR=1) in both treatment arms (Table V-B 5, 6-11). In the intensive therapy arm, the adjusted HRs were 0.94 (0.69, 1.30) and 1.07 (0.71, 1.64) for becoming overweight and obese following the first SH during follow-up, respectively. The corresponding HRs were 1.11 (0.71, 1.72) and 1.20 (0.52, 2.79) in the conventional therapy arm. The estimates obtained from Cox models and MSM were similar. We obtained well-behaved weights with the mean around one with a maximum weight of 5.82 in MSM models (Table V-B 12).

4. Discussion

Intensification of insulin therapy in type 1 diabetes often results in weight gain [4]. Furthermore, excessive weight gain in type 1 diabetes was found in DCTT to affect patients'

cardiovascular risk profile with adverse change in lipid and blood pressure levels [7]. Identifying risk factors contributing to excessive weight gain, especially modifiable factors may help to identify ways to improve clinical management of type 1 diabetes to prevent excessive weight gain in patients with type 1 diabetes on insulin therapy.

The results in this study do not provide evidence to support an association between SH episodes and subsequent weight gain using both weight change and actual weight as outcome variables. Instead, in the conventional therapy group, a small, short-term weight loss was found following SH episodes. Furthermore, our results also found little or no effect of occurrence of SH on substantial weight gain, becoming overweight or obese in the DCCT population.

We cannot readily explain why we observed a weight loss after occurrences of SH in patients in the conventional therapy arm. One plausible explanation for such weight loss could be due to a decrease of insulin dose in response to occurrences of SH. Although a slight decrease of insulin dose after occurrence of SH was observed in both treatment groups in the DCCT, occurrence of SH appeared to have greater effects on ≥10% decrease of insulin dose in the conventional therapy arm than in the intensive therapy (results not shown).

Because the occurrence of SH may lead to a decrease of insulin dose to raise patients' blood glucose levels, average daily insulin dose would be an intermediate covariate and confounder simultaneously. The similar estimates from the Cox and the MSM models seem to suggest that average daily insulin dose is not a major intermediate covariate between SH and weight gain in the DCCT. However, it should be noted that the insulin information was available only quarterly and based on self-report from patients in the DCCT data [27]. Therefore, any temporary changes on insulin dose in response to SH may not be captured by the quarterly insulin information.

This study has several limitations with regard to available information for our research questions and extrapolating our findings to other patients with type 1 diabetes. In the DCCT, no information on daily insulin dose is available, and we had to rely on quarterly data for insulin dose. We were only able to study severe cases of hypoglycemia and we do not know whether our findings would apply to less severe episodes. The availability of only quarterly information and limited sample size also impeded our attempt to further explore the relationship between the frequencies of SH and weight gain in this population. Because the DCCT is a clinical trial, patients in the DCCT likely had stricter diets and weight management compared with the patients in routine clinical care; the patients also received more dietary counseling that would occur in routine practice. Thus, patients were likely to be more adherent to their medical and diet regimen [31]. In our analysis, we cannot disentangle underlying causal mechanisms (overreaction to SH by consuming more calories vs. other unknown mechanisms) due to limited information on diet and physical activity. The DCCT study was conducted over 25 years ago and our results may not be generalizable to current patients with type 1 diabetes. However, the results in our study imply that adverse effects of SH on weight gain, if such association exists in other type 1 diabetic populations, may be avoidable under a strict diet and weight management.

Notwithstanding these limitations, we were able to utilize longitudinal information in the DCCT in our analysis. We also designed our study to address the temporality of SH episodes as a risk factor for subsequent weight gain. To our knowledge, our study is the first one to systematically test the association between the occurrence of SH and weight gain using various design and analytic methods including repeated observations, survival analysis and MSM models in a type 1 diabetic cohort.

In summary, our study did not find evidence to support an association between occurrence of SH and subsequent weight gain in patients with type 1 diabetes. Because the

DCCT is a clinical trial, we should be cautious about generalizing our findings to patients with type 1 diabetes in general. However, our findings imply that adverse effects of SH on weight gain, if such association exists in other type 1 diabetic populations, may be avoidable under a strict diet and weight management.

Table V-B 1: Number of available observation periods with various observation durations and the distributions of some covariates by exposure status --occurrence of severe hypoglycemia (SH) in the DCCT[a]

		Intensive Therapy		Conventional Therapy	
		SH	No SH	SH	No SH
Available observation periods					
	Months 1-3 [b]	1,308	11,275	403	12,350
	Months 1-6 [b]	1,231	10,748	383	11,756
	Months 1-9 [b]	1,175	10,204	366	11,164
	Months 1-12 [b]	1,117	9,664	352	10,570
	Months 1-24 [b]	892	7,532	289	8,239
	Months 1-36 [b]	639	5,525	217	5,971
Covariates for observation period months 1-3 [c]					
	Mean daily insulin dose (SD) [d]	54.0 (19.0)	50.8 (19.1)	50.6 (16.6)	46.4 (15.0)
	Mean BMI (SD)	25.2 (3.4)	25.3 (3.5)	24.6 (2.8)	24.6 (3.0)
	Physical activities (%)				
	Strenuous	5.3	5.3	11.2	6.7
	Moderate	59.1	53.6	50.3	53.1
	Little	35.6	41.1	38.5	40.2
	Meal plan adherence (%)				
	Yes	85.0	83.2	79.8	80.4
	No	15.0	16.8	20.2	19.6
	Insulin use adherence (%)				
	Yes	77.1	79.2	87.9	87.7
	No	22.9	20.8	12.1	12.3

[a] The numbers of eligible patients for the observation periods with various durations: months 1-3 (604 from intensive therapy [IT] and 614 from conventional therapy [CT]), months 1-6 (600 from IT and 609 from CT), months 1-9 (598 from IT and 608 from CT), months 1-12 (596 from IT and 603 from CT), months 1-24 (574 from IT and 603 from CT), and months 0-36 (553 from IT and 576 from CT);

[b] Durations were only approximated by number of composed consecutive quarterly intervals after occurrence of SH;

[c] The covariate distributions for other observation periods were similar to months 1-3;

[d] International unit for insulin.

Table V-B 2: Effects of severe hypoglycemia (SH) on subsequent weight change in kilogram in various observation periods in the DCCT

Treatment Assignment /durations after SH (months)	Unadjusted mean for weigh change (95% CI) by SH status [a]		Unadjusted difference of the means for weight change (95% CI) [c]	Adjusted difference of the means for weight change (95% CI) [c, d]
	Yes	No		
Intensive Therapy				
1-3[b]	0.27 (0.16, 0.29)	0.32 (0.29, 0.35)	-0.05 (-0.17, 0.06)	-0.04 (-0.16, 0.09)
1-6 [b]	0.58 (0.43, 0.73)	0.64 (0.58, 0.70)	-0.07(-0.22, 0.09)	-0.04 (-0.20, 0.12)
1-9 [b]	0.85 (0.63, 1.07)	0.96 (0.87, 1.05)	-0.11 (-0.33, 0.12)	-0.09 (-0.31, 0.14)
1-12 [b]	1.24 (1.00, 1.48)	1.25 (1.13, 1.37)	0.00 (-0.24, 0.22)	0.09 (-0.11, 0.3)
1-24 [b]	2.39 (2.00, 2.78)	2.51 (2.26, 2.76)	-0.12 (-0.45, 0.22)	0.00 (-0.31, 0.29)
1-36 [b]	3.27 (2.78, 3.76)	3.69 (3.32, 4.05)	-0.41 (-0.81, -0.02)	-0.33 (-0.70, 0.05)
Conventional Therapy				
1-3[b]	-0.15 (-0.35, 0.05)	0.16 (0.13, 0.19)	-0.31 (-0.52, -0.10)	-0.29 (-0.51, -0.08)
1-6 [b]	-0.06 (-0.30, 0.17)	0.28 (0.24, 0.32)	-0.35 (-0.59, -0.10)	-0.34 (-0.60, -0.09)
1-9 [b]	0.00 (-0.32, 0.32)	0.42 (0.36, 0.48)	-0.42 (-0.74, -0.09)	-0.41 (-0.73, -0.09)
1-12 [b]	-0.03 (-0.39, 0.33)	0.55 (0.46, 0.63)	-0.58 (-0.93, -0.22)	-0.46 (-0.83, -0.09)
1-24 [b]	0.62 (0.22, 1.02)	1.08 (0.91, 1.26)	-0.47 (-0.84, -0.09)	-0.14 (-0.52, 0.23)
1-36 [b]	1.06 (0.52, 1.59)	1.58 (1.32, 1.84)	-0.52 (-0.99, -0.05)	-0.18 (-0.64, 0.28)

[a] The estimates had been addressed the correlation within one patient but did not include other covariates;

[b] Observation durations were only approximated by number of composed consecutive quarterly intervals after occurrence of SH.

[c] The difference was the difference between the mean for weight change after occurrence of SH and the mean without SH for the corresponding observation period.

[d] Adjusted covariates included: Gender, baseline age, duration of disease, baseline history of SH, most recent and incidence rates of SH prior to index SH, BMI, daily insulin dose, exercise activities, insulin adherence, meal plan adherence and follow-time.

Table V-B 3: Effects of severe hypoglycemia (SH) on subsequent weight in kilogram in various observation periods in the DCCT

Treatment Assignment /durations after SH (months)	Unadjusted mean for weigh (95% CI) by SH status [a]		Unadjusted difference of the means for weight (95% CI) [c]	Adjusted difference of the means for weight (95% CI) [c, d]
	Yes	No		
Intensive Therapy				
1-3[b]	75.4 (74.3, 76.5)	75.1 (74.1, 76.2)	0.25 (-0.04, 0.54)	0.00 (-0.17, 0.17)
1-6 [b]	75.6 (74.5, 76.7)	75.4 (74.3, 76.4)	0.21 (-0.07, 0.50)	0.04 (-0.15, 0.23)
1-9 [b]	75.7 (74.6, 76.8)	75.6 (74.5, 76.6)	-0.10 (-0.17, 0.38)	0.01 (-0.19, 0.22)
1-12 [b]	75.9 (74.8, 77.0)	75.8 (74.9, 76.8)	0.11 (-0.16, 0.37)	0.13 (-0.08, 0.33)
1-24 [b]	76.5 (75.4, 77.6)	76.5 (75.4, 77.6)	0.00 (-0.30, 0.29)	0.09 (-0.15, 0.33)
1-36 [b]	77.1 (76.0, 78.3)	77.5 (76.3, 78.6)	-0.37 (-0.66, -0.08)	-0.08 (-0.38, 0.21)
Conventional Therapy				
1-3[b]	73.7 (72.7, 74.7)	73.8 (72.8, 74.8)	-0.06 (0.14, -0.34)	-0.43 (-0.70, -0.16)
1-6 [b]	73.8 (72.7, 74.8)	73.9 (72.9, 74.9)	-0.15 (-0.45, -0.14)	-0.43 (-0.72, -0.14)
1-9 [b]	73.8 (72.8, 74.8)	74.0 (73.0, 75.0)	-0.23 (-0.53, 0.07)	-0.43 (-0.75, -0.10)
1-12 [b]	73.8 (72.7, 74.8)	74.1 (73.1, 75.1)	-0.31 (-0.62, 0.00)	-0.43 (-0.77, -0.09)
1-24 [b]	74.4 (73.3, 75.4)	74.5 (73.5, 75.5)	-0.08 (-0.36, 0.20)	-0.10 (-0.36, 0.17)
1-36 [b]	74.8 (73.7, 75.9)	74.9 (73.9, 76.0)	-0.15 (-0.54, 0.25)	-0.11(-0.53, 0.31)

[a] The estimates had been addressed the correlation within one patient but did not include other covariates;

[b] Observation durations were only approximated by number of composed consecutive quarterly intervals after occurrence of SH.

[c] The difference was the difference between the mean for weight after occurrence of SH and the mean without SH for the corresponding observation period.

[d] Adjusted covariates included: Gender, baseline age, duration of disease, baseline history of SH, most recent and incidence rates of SH prior to index SH, BMI, daily insulin dose, exercise activities, insulin adherence, meal plan adherence and follow-time.

Table V-B 4: Effects of severe hypoglycemia (SH) on subsequent weight change in kilogram in three consecutive quarterly windows in the DCCT

Treatment Assignment /time window after SH (months)	Unadjusted mean for weigh change (95% CI) by SH status [a]		Unadjusted difference of the means for weight change (95% CI) [c]	Adjusted difference of the means for weight change (95% CI) [c, d]
	Yes	No		
Intensive Therapy				
1-3[b]	0.27 (0.16, 0.29)	0.32 (0.29, 0.35)	-0.05 (-0.17, 0.06)	-0.04 (-0.16, 0.09)
4-6 [b]	0.26 (0.16, 0.36)	0.30 (0.27, 0.33)	-0.04 (-0.15, 0.07)	-0.01 (-0.13, 0.12)
7-9 [b]	0.22 (0.09, 0.35)	0.29 (0.26, 0.32)	-0.07 (-0.21, 0.07)	-0.06 (-0.21, 0.10)
Conventional Therapy				
1-3[b]	-0.15 (-0.35, 0.05)	0.16 (0.13, 0.19)	-0.31 (-0.52, -0.10)	-0.29 (-0.51, -0.08)
4-6 [b]	0.04 (-0.19, 0.27)	0.14 (0.11, 0.17)	-0.10 (-0.34, 0.14)	-0.04 (-0.24, 0.17)
7-9 [b]	0.03 (-0.15, 0.22)	0.15 (0.12, 0.18)	-0.12 (-0.32, 0.08)	-0.04 (-0.23, 0.15)

[a] The estimates had been addressed the correlation within one patient but did not include other covariates;

[b] Observation windows were only approximated by quarterly intervals after occurrence of SH.

[c] The difference was the difference between the mean for weight change after occurrence of SH and the mean without SH for the corresponding time windows.

[d] Adjusted covariates included: Gender, baseline age, duration of disease, baseline history of SH, most recent and incidence rates of SH prior to index SH, BMI, daily insulin dose, exercise activities, insulin adherence, meal plan adherence and follow-time.

Table V-B 5: Summary of results for effects of severe hypoglycemia (SH) on risk of substantial weight gain, overweight and obesity in the DCCT

Treatment Assignment	Models with different outcomes	Adjusted HR (95% CI)	MSM[a] HR (95% CI)
Intensive Therapy			
	5% weight gain	0.99 (0.79, 1.24)	1.01 (0.79, 1.29)
	10% weight gain	1.09 (0.87, 1.36)[b]	1.12 (0.88, 1.43)
	15% weight gain	0.89 (0.68, 1.16)	0.91 (0.69, 1.2)
	20% weight gain	0.92 (0.66, 1.28) [b]	0.89 (0.63, 1.26)
	Overweight	0.94 (0.69, 1.3)	0.92 (0.64, 1.32)
	Obesity	1.07 (0.71, 1.64)	1.12 (0.72, 1.75)
Conventional Therapy			
	5% weight gain	1.15 (0.87, 1.53)	1.10 (0.82, 1.48)
	10% weight gain	1.06 (0.78, 1.46)	1.09 (0.76, 1.54)
	15% weight gain	1.19 (0.75, 1.90)	1.11 (0.65, 1.89)
	20% weight gain	1.11 (0.49, 2.51)	----[c]
	Overweight	1.11 (0.71, 1.72)	1.07 (0.69, 1.64)
	Obesity	1.20 (0.52, 2.79)	1.18 (0.46, 3.02)

[a] MSM stands for marginal structural model.

[b] *P* value<0.15 for Cox test for proportion assumption.

[c] MSM did not converge

Table V-B 6: Occurrence of severe hypoglycemia (SH) on 5% weight gain during the follow-up in the DCCT

Treatment Assignment		No. of Event	Quarterly Follow up intervals	Hazard Ratio		
				Unadjusted (95% CI)	Adjusted (95%CI)	MSM[a] (95% CI)
Intensive Therapy						
	SH	130	1248	1.07 (0.87, 1.33)	0.99 (0.79, 1.23)	1.01 (0.79, 1.29)
	No SH	403	2661	1	1	1
Conventional Therapy						
	SH	63	960	1.11 (0.84, 1.45)	1.15 (0.87, 1.53)	1.1 (0.82, 1.48)
	No SH	391	5397	1	1	1

[a] MSM stands for marginal structural model.

Table V-B 7: Occurrence of severe hypoglycemia (SH) on 10% weight gain during the follow-up in the DCCT

Treatment Assignment		No. of Event	Quarterly Follow up intervals	Hazard Ratio		
				Unadjusted (95% CI)	Adjusted (95%CI)	MSM[a] (95% CI)
Intensive Therapy						
	SH	160	3015	1.1 (0.89, 1.37)	1.08 (0.86, 1.35)[b]	1.12 (0.88, 1.43)
	No SH	244	4570	1	1	1
Conventional Therapy						
	SH	57	2027	1.15 (0.85, 1.56)	1.06 (0.78, 1.46)	1.09 (0.76, 1.54)
	No SH	197	8390	1	1	1

[a] MSM stands for marginal structural model.

[b] P value<0.15 for Cox test for proportion assumption

Table V-B 8: Occurrence of severe hypoglycemia (SH) on 15% weight gain during the follow-up in the DCCT

Treatment Assignment		No. of Event	Quarterly Follow up intervals	Hazard Ratio		
				Unadjusted (95% CI)	Adjusted (95%CI)	MSM[a] (95% CI)
Intensive Therapy						
	SH	152	5783	0.89 (0.69, 1.15)	0.89 (0.68, 1.16)	0.91 (0.69, 1.2)
	No SH	109	4313	1	1	1
Conventional Therapy						
	SH	28	2651	1.28 (0.82, 2.01)	1.21 (0.76, 1.92)	1.11 (0.65, 1.89)
	No SH	74	9720	1	1	1

[a] MSM stands for marginal structural model.

Table V-B 9: Occurrence of severe hypoglycemia (SH) on 20% weight gain during the follow-up in the DCCT

Treatment Assignment		No. of Event	Quarterly Follow up intervals	Hazard Ratio		
				Unadjusted (95% CI)	Adjusted (95%CI)	MSM[a] (95% CI)
Intensive Therapy						
	SH	81	5169	0.90 (0.66, 1.25)	0.92 (0.66, 1.28) [b]	0.89 (0.63, 1.26)
	No SH	83	6390	1	1	1
Conventional Therapy						
	SH	9	2902	1.17 (0.54, 2.57)	1.12 (0.51, 2.51)	----c
	No SH	23	10138	1	1	1

[a] MSM stands for marginal structural model.

[b] P value<0.15 for Cox test for proportion assumption

[c] MSM did not converge

Table V-B 10: Occurrence of severe hypoglycemia (SH) on becoming overweight during the follow-up in the DCCT

Treatment Assignment		No. of Event	Quarterly Follow up intervals	Hazard Ratio		
				Unadjusted (95% CI)	Adjusted (95%CI)	MSM[a] (95% CI)
Intensive Therapy						
	SH	80	2,538	0.97 (0.72, 1.31)	0.94 (0.69, 1.3)	0.92 (0.64, 1.32)
	No SH	165	3,284	1	1	1
Conventional Therapy						
	SH	27	1,276	0.92 (0.6, 1.4)	1.11 (0.71, 1.72)	1.07 (0.69, 1.64)
	No SH	151	5,099	1	1	1

[a] MSM stands for marginal structural model.

Table V-B 11: Occurrence of severe hypoglycemia (SH) on becoming obesity during the follow-up in the DCCT

Treatment Assignment		No. of Event	Quarterly Follow up intervals	Hazard Ratio		
				Unadjusted (95% CI)	Adjusted (95%CI)	MSM[a] (95% CI)
Intensive Therapy						
	SH	48	5200	0.82 (0.55, 1.21)	1.07 (0.71, 1.64)	1.12 (0.72, 1.75)
	No SH	69	6305	1	1	1
Conventional Therapy						
	SH	8	2685	1.09 (0.49, 2.43)	1.20 (0.52, 2.79)	1.18 (0.46, 3.02)
	No SH	37	9669	1	1	1

[a] MSM stands for marginal structural model.

Table V-B 12: Distributions of estimated weights in marginal structural models for outcomes as substantial weight gain, overweight and obesity in the DCCT

Treatment Assignment	Models with different outcomes	Estimated weights		
		Mean(SD)	Minimum	Maximum
Intensive Therapy				
	5% weight gain	0.99 (0.11)	0.62	2.15
	10% weight gain	1.00 (0.16)	0.56	3.16
	15% weight gain	1.00 (0.18)	0.21	4.08
	20% weight gain	1.00 (0.16)	0.45	3.64
	Overweight	1.00 (0.18)	0.44	2.88
	Obesity	1.00 (0.17)	0.24	2.44
Conventional Therapy				
	5% weight gain	1.01 (0.21)	0.3	4.74
	10% weight gain	1.00 (0.24)	0.35	5.78
	15% weight gain	1.00 (0.26)	0.26	5.76
	20% weight gain	1.00 (0.26)	0.32	5.82
	Overweight	0.99 (0.27)	0.37	5.43
	Obesity	1.00 (0.25)	0.46	5.51

C. Change in insulin dose in response to severe hypoglycemia in the Diabetes Control and Complication Trial

1. Introduction

We re-analyzed the Diabetes Control and Complication Trial (DCCT) data to estimate the quantitative association between occurrence of severe hypoglycemia (SH) and change of insulin dose as a supplementary analysis to assist interpretation of the results from study aim 1 and aim 2.

2. Methods

Study population

We used data from the Diabetes Control and Complications Trial (DCCT), which randomized 1,441 patients between ages of 13 to 39 with duration of type 1 diabetes 1-15 years to either intensive therapy or conventional therapy between 1983 and 1993.

We only included quarterly visits after the first year of follow-up in this analysis. We also restricted eligible patients who must be 18 years or older when they entered the trial, and censored female patients at the time of their first pregnancy during follow-up based on their reported last period dates.

Definition of SH

SH was defined as an episode in which a patient required assistance of another person and the following conditions: 1) a blood glucose level of < 50 mg/dl, or 2) prompt recovery following oral carbohydrate, intravenous glucose, or glucagons. Patients in the DCCT were instructed to report all episodes of suspected SH immediately, and all were interviewed to verify each episode [31]. The first SH was defined as the first SH episode

occurring during the DCCT follow-up, and the repeated SH was the any episodes after the first SH during the DCCT follow-up.

Definition of analytic unit for this analysis

Daily changes of therapy regimens in the DCCT were not centrally documented and were not accessible for analysis, and only typical daily insulin doses (TDID) for the past quarterly interval were recorded at each quarterly visit [31]. Thus, in the DCCT there is no way to tell whether the reported dose actually reflected the dose before occurrence of SH or after occurrence of SH if patients had occurred SH episodes in a quarterly interval. To account for this issue and conduct this supplementary analysis, new study analytic units were constructed and each analytic unit consisted of two consecutive quarterly visits to account for temporality of change of insulin dose following occurrence of SH (Figure 5). We defined two types of outcomes in each analytic unit: 1) continuous outcome (the difference of daily insulin doses between the end and beginning of the analytic unit); and 2) dichotomized outcome (≥ 10% dose reduction).

Statistical analyses

We used linear multivariable models to estimate the effects of SH occurring in the first composed quarterly interval in an analytic unit on the continuous outcome and used log-linear multivariable models to estimate the relative risk (RR) on the dichotomized outcome (≥ 10% dose reduction) in each analytic unit. We used generalized estimating equations to account for multi-observations per patient and to control relevant factors under working correlation structure “exchangeable”. Furthermore, for continuous outcome, we also used mixed models as a sensitivity analysis. However, negative variance components were reported when we used a random effect for the intercept for each subject by covariance structure as either variance component or unstructured in the mixed models. After serial

diagnostic tests, we omitted the random effect for the intercept, but correlation within the random effect was modeled by including covariance parameter in the residual variance matrix R with a toeplitz structure and toep (5) appeared a best fit.

3. Results

In the intensive therapy, the effect of SH appeared to be modified by whether the SH event was the first SH or the repeated SH for continuous outcome. For the first SH, the adjusted difference of mean change of insulin dose (DMID) was -0.25 units (95% CI: -1.90, 1.41), which was close to null effect (zero). The DMID for the repeated SH was -1.92 units (-2.82,-1.02) (Table V-C 1). Results from mixed models also showed the similar pattern. In the conventional therapy, a decrease of insulin dose was observed after the both first and repeated SH (Table V-C 1).

In terms of RR for the effect of SH on ≥ 10% dose reduction (dichotomized outcome), although the RRs seemed different numerically (RR with 1.19 for the first SH vs. 1.50 for repeated SH, Table V-C 2) in the intensive therapy, the heterogeneity test did not reach a statistical significance. In the conventional therapy, RRs of SH on ≥ 10% dose reduction for the first SH and repeated SH were similar. Furthermore, comparing to those without occurrence of SH, the effect of SH on ≥ 10% dose reduction in conventional therapy appeared greater than that in the intensive therapy (RR 2.45 for the first SH and 2.64 for the repeated SH in the conventional therapy compared to the corresponding RRs 1.19 and 1.50 in the intensive therapy).

4. Discussion

In the DCCT, no information on daily insulin dose is available, and we had to rely on quarterly self-reported insulin dose to assume that it was representative of the full quarter for individual patients. Furthermore, we had to create new analytic unit by combining two

consecutive intervals to estimate the association between the SH and change of insulin dose in the DCCT. Otherwise, the temporality between SH and subsequent change of insulin dose cannot be determined.

The seemingly different response to the first and repeated SH in the intensive therapy appears to coincide with the clinical trial design of the DCCT. The primary goal of the intensive therapy in the DCCT was to keep A1C as close to normal as possible. Although the DCCT did state the need to minimize the risk of SH, clinical staff were required to raise blood glucose and A1C target only when the intensive therapy resulted in repeated SH. Therefore, the relatively small effect of SH on ≥ 10% dose reduction compared to those without SH events in the intensive therapy may reflect that patients in the intensive therapy were striving to achieve their glycemic targets whenever they could.

In summary, the overall reduction of insulin dose following SH appears small in the DCCT. In the intensive therapy, no apparent change was observed after the first SH. Greater effects of SH on ≥ 10% dose reduction were observed in the conventional therapy than the effects observed in the intensive therapy.

Table V-C 1: Occurrence of severe hypoglycemia (SH) on changes of daily insulin doses in analytic units by treatment assignments in the DCCT [1]

		Intensive Therapy Difference of mean changes (95% CI)	Conventional Therapy Difference of mean changes (95% CI)
1st SH			
	GEE	-0.25 (-1.90, 1.41)	-3.85 (-5.80, -1.90)
	Mixed	0.30 (-0.89, 1.48)	-2.58 (-3.45, -1.71)
Repeated SH			
	GEE	-1.92 (-2.82, -1.02)	-1.79 (-3.23, -0.35)
	Mixed	-1.07 (-1.83, -0.30)	-1.66 (-2.50, -0.83)
P value for Heterogeneity Test[2]			
	GEE	0.09	0.10
	Mixed	0.06	0.13

[1] Covariates in GEE and mixed models: 1) occurrence of SH in current analytic unit, an indicator variable for the past experiences of SH during the trial up to but not including the SH occurred in current analytic unit, and their interaction term; 2) DCCT baseline variables gender, age, duration of type 1 diabetes, and history of hypoglycemia before entry of the DCCT; 3) ways to administrate insulin (injection /pump); 4) BMI at the beginning of the analytic unit; 5) HbA1c and exercise levels at the beginning of the analytic units; and 6) a restricted cubic spline with 5 knots for follow-time (months since beginning of the DCCT).

[2] A priori significant level of 0.10 was pre-set for the interaction term between the occurrences of SH in current analytic units and the past SH experience during the trials as a statistical heterogeneity test.

Table V-C 2: Occurrence of severe hypoglycemia (SH) on effect ≥10% decrease of daily insulin doses in analytic units by treatment assignments in the DCCT[1]

		Intensive Therapy	Conventional Therapy
1st SH			
	RR of reduction (95% CI)	1.19(0.92, 1.53)	2.45 (1.84, 3.25)
Repeated SH			
	RR of reduction (95% CI)	1.50 (1.28, 1.74)	2.64 (1.99, 3.50)
P value for Heterogeneity Test[2]		0.13	0.68

[1] Covariates in GEE model included: 1) occurrence of SH in current analytic unit, an indicator variable for the past experiences of SH during the trial up to but not including the SH occurred in current analytic unit, and their interaction term; 2) DCCT baseline variables gender, age, duration of type 1 diabetes, and history of hypoglycemia before entry of the DCCT; 3) ways to administrate insulin (injection /pump); 4) BMI at the beginning of the analytic unit; 5) HbA1c and exercise levels at the beginning of the analytic units; and 6) a restricted cubic spline with 5 knots for follow-time (months since beginning of the DCCT).

[2] A priori significant level of 0.10 was pre-set for the interaction term between the occurrences of SH in current analytic units and the past SH experience during the trials as a statistical heterogeneity test.

VI.CONCLUSIONS

A. Overview findings

Hypoglycemia and weight gain are two primary adverse events during insulin therapy among patients with type 1 diabetes [1-4]. Hypoglycemia has been documented to be the leading limiting factor of intensive diabetes management among patients with type 1 diabetes mellitus [5, 6]. Hypoglycemia also causes recurrent physical and psychological morbidity, higher risk of mortality and impairs defenses against subsequent hypoglycemia [1]. Excessive weight gain can not only increase diabetic morbidity and mortality when weight gain becomes a barrier to the intensification of insulin treatment, but also adversely affect cardiovascular risk profiles as well [4, 7].

Although prior studies have consistently reported prior SH as a risk factor in general for recurrent subsequent SH [9-16], very few studies provide epidemiologic evidence to demonstrate the acute effects of SH on subsequent SH in a clinically relevant time range, and the magnitude of such effects from population levels in patients with type 1 diabetes is not completely clear. The unpleasant symptoms and negative consequences of hypoglycemia may result in significant fear of hypoglycemia [17]. Because low blood glucose levels can be remedied by ingestion of glucose or food following a hypoglycemic event, patients may over-react or be instructed by consuming more calories in response to the threat of subsequent hypoglycemia. However, there is very limited direct evidence to support this hypothesis of compensation for hypoglycemia.

We conducted a secondary data analysis using data from the DCCT trial study, and the overall objectives of this study are: 1) in a clinically relevant time range, to estimate and

illustrate the acute effects of SH on risk of subsequent SH episodes in patients with type 1 diabetes; 2) to provide insight into the interplay of two primary adverse events (SH and weight gain) in patients with type 1 diabetes.

The first aim of this study was to estimate the effects of SH (index SH) on subsequent SH in three subsequent time windows at months 1-3, 4-6 and 7-9 after index SH in patients with type 1 diabetes. The highest absolute risks (transition probabilities) for subsequent SH episodes after index SH were observed in the first 3-month observation window in both treatment arms in the DCCT. In the conventional therapy, the risks to develop subsequent SH episodes in the first 3 months, months 4-6 and months 7-9 after index SH were 19.8%, 15.4% and 15.9%, respectively; the corresponding risks were 21.5%, 21.4% and 17.3% in the intensive therapy group. In both treatment arms, the greatest adjusted RRs on the risk of subsequent SH episodes were also observed in the first 3 months after the index SH compared to those without occurrence of the index of SH: in the conventional therapy group, the RRs and the 95% CI in the first 3 months, months 4-6 and 7-9 were 3.38 (2.21, 5.18), 1.37 (0.76, 2.47) and 1.95 (1.33, 2.87) respectively, and the corresponding RRs were 1.7(1.48, 1.97), 1.45 (1.2, 1.74) and 1.36 (1.16, 1.61) in the intensive therapy group. Similar to RRs, the greatest risk differences (RDs) for the effects of index SH on subsequent episodes (calculated by transition probabilities) were also found in the 1st observation window after the index SH in both treatment groups. In summary, our results add to the empirical evidence for an acute effect of recent SH on subsequent episodes. It also indicates that the immediate periods after occurrences of SH are crucial in clinical management of type 1 diabetes to prevent subsequent SH.

The first two parts of the study aim 2 was to estimate the effects of SH (index SH) on subsequent weight change/actual weight in various observation periods from 3, 6, 9, 12, 24 and 36 months after index SH, and on weight change in other three fixed-term time windows

during months 1-3, 4-6 and 7-9 after index SH, respectively. In the intensive therapy arm, the estimated effects of index SH on subsequent weight change and weight appeared to be close to the null effect (zero for the difference of the means for weight change or for difference of the means for weight) comparing to those without index SH. However, in the conventional therapy group, a small weight loss was observed in the 1st 3-month period following SH (adjusted differences of the means for weight change were -0.29 (-0.51, -0.08)), but the estimated differences of the means for weight change were close to null in the subsequent 2nd time window (months 4-6) and the 3rd time window (months 7-9) following index SH. The last part of the study aim 2 was to estimate the HRs of occurrence of SH on time to substantial weight gain (defined as the 5, 10, 15 and 20% weight gain from the DCCT baseline), on becoming overweight, or on becoming obese, respectively. The estimated HRs of SH on time to substantial weight gain, becoming overweight or becoming obese were also found around the null effect (HR=1) in both treatment arms. In the intensive therapy group, the adjusted HRs were 0.94 (0.69, 1.3) and 1.07 (0.71, 1.64), respectively, for becoming overweight, and becoming obese following the first SH during follow-up; the corresponding HRs were 1.11 (0.71, 1.72) and 1.20 (0.52, 2.79) in the conventional therapy arm. The HRs obtained from Cox models and marginal structural models (MSM) were similar. We used MSM as an ancillary analysis to allow us to control for average daily insulin dose, which may be seen as a time-dependent confounder affected by prior SH. In summary, our study did not find evidence to support an association between occurrence of SH and subsequent weight gain in patients with type 1 diabetes.

B. Strengths

Our study utilized longitudinal information from the DCCT study accounting for repeated observations to estimate the acute effects of SH on risk of subsequent SH episodes in a clinically relevant time range. This approach has not been mentioned in any

relevant prior studies in literature. The similar designs were also used for the estimations of the association between the occurrence of SH and subsequent weight gain in various periods in this study.

We designed our study to reserve the temporality of SH as a risk factor for subsequent weight gain, which has not been addressed by any previous studies either. As an ancillary analysis, we used marginal structural models (MSM) to control for average daily insulin dose, which may be seen as a time-dependent confounder affected by prior SH. To our knowledge, our study is the first one to systematically test the association between the occurrence of SH and subsequent weight gain using various study designs and analytic methods including the design for repeated observations, survival analysis and MSM models in a type 1 diabetic cohort.

Last but not least, our study data (the DCCT data) were from one of the largest and longest follow-up type 1 diabetes cohorts up to today. The DCCT had well defined information for SH, which is the exposure and outcome variable for our study aim 1 and the exposure definition for the study aim 2. To be matter of fact, American Diabetes Association Workgroup on hypoglycemia adopted a same definition for SH as that in the DCCT [20].

C. Limitations

The daily changes of therapy regimens in the DCCT were not centrally documented and were not accessible for analysis. We only had quarterly data available for patients in our study and assumed that it was representative of the full quarter for individual patients. Information on activities that affect the risk for SH, including physical activity and alcohol consumption, was not available even quarterly, and we had to use annual collected information on physical activity and alcohol consumption as a surrogate for data for each quarterly visit. However, conventional risk factors for SH, including physical activity and

alcohol consumption, were reported to be well under control in the DCCT because a careful review for these factors was performed for the patient once SH occurred in a patient during the trial [10]. Because of limited information in the DCCT, we only can estimate the total effects of SH on subsequent SH episodes, and cannot disentangle underlying intermediate effects due to physicians and patients' activities in responding the SH events (index SH) on subsequent SH episodes, and similarly, we also cannot disentangle underlying causal mechanisms (overreaction to SH by consuming more calories vs. other unknown mechanisms) due to limited diet and physical activity information for the association between SH and subsequent weight gain. Furthermore, in term of association between occurrence of hypoglycemia and weight gain, we were only able to study severe cases of hypoglycemia and we do not know whether our findings would apply to less severe episodes.

We should be cautious about extrapolating our findings to all patients with type 1 diabetes because of the selected trial population and the trial design of the DCCT. Because the DCCT is a clinical trial, patients in the DCCT likely had greater medical surveillance, stricter diets and weight management compared with the patients in routine clinical care, and patients were likely to be more adherent to their medical and diet regiments as well [31].

D. Future directions

1. Public health and clinical practice implication in study aim 1

Our findings for acute effects of recent SH provide some evidence to support an American Diabetes Association (ADA) suggestion. According to this ADA suggestion, patients with recent SH episodes may benefit from at least a short-term relaxation of glycemic targets to reduce risk of recurrent SH [43]. However, this suggestion was mostly based on the studies which only provided a proof of principle to use relaxation of glycemic targets as a potential intervention to reduce subsequent SH events, and they were

conducted in relatively small-scales under only a couple of patients and had insufficient follow-up duration to directly measure differences in SH [19]. Thus, more studies in future should be needed to examine the magnitudes and durations of various interventions in the time period immediately after recent SH (e.g., relaxation of glycemic targets and/or increasing glucose monitoring etc.) to assess optimal benefit and harm profiles to reduce both subsequent SH and other diabetic complications (e.g., micro-macrovascular diseases).

2. Future research for study aim 2

In our analysis, we cannot disentangle underlying causal mechanisms (overreaction to SH by consuming more calories vs. other unknown mechanisms) due to limited diet information in the DCCT. Future studies should also consider examining the relationship between the occurrence of SH/hypoglycemia and subsequent diet change to directly address the assumed mechanism for the hypothesis of compensation for hypoglycemia.

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