EFFECT OF INSULIN DOSE ADJUSTMENT FOR GLYCAEMIC CONTROL ON BODY MASS INDEX: A RETROSPECTIVE COHORT STUDY OF TYPE 1 DIABETES PATIENTS AT THE KALAFONG DIABETIC CLINIC BETWEEN 2009 AND 2014

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Effect of insulin dose adjustment for glycaemic control on body mass index: a retrospective cohort study of type 1 diabetes patients at the Kalafong Diabetic Clinic between 2009 and 2014

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DECLARATION

I hereby declare that this dissertation presented to the University of Pretoria for the Master of Science in Epidemiology degree is my own work and has not been previously presented to any other tertiary institution for any degree.

AUTHORSHIP

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EXECUTIVE SUMMARY

Type 1 diabetes mellitus (T1DM), an autoimmune disease in which the insulin-producing pancreatic *β*-cells are destroyed, results in the inability of the pancreas to produce insulin to regulate blood glucose levels, an accumulation of glucose in the blood and cell starvation. Elevated glycated haemoglobin (HbA1c) levels, a metabolic marker of glucose control, are characteristic of T1DM. Chronic exposure to high blood glucose levels leads to microvascular and macrovascular complications. Disease management requires regular blood glucose monitoring and daily exogenous insulin administration to maintain fasting and post-prandial blood glucose levels within near to the normal range of 3.9 to 5.6 mmol/L. However, T1DM patients on daily insulin replacement therapy have been observed to experience weight-gain over time, regardless of the level of glycaemic control achieved. The study aimed to determine the effects of quarterly adjusted total daily doses of twice-daily biphasic insulin and basal NPH plus prandial regular insulin to achieve optimum glycaemic control, on body mass index (BMI) in T1DM patients. Secondarily, dosage regimens that achieved optimum glycaemic control, without increasing BMI, as well as gender differences in BMI and HbA1c outcomes, were also explored.

All available clinic records of T1DM patients who attended the Kalafong Hospital Diabetes clinic between 2009 and 2014, and not on metformin and/or acarbose, were reviewed (n=493) and all eligible patients included in the study (n=211, mean±SD=43±14.4 years, 51% female, duration of T1DM ≥2 years). Baseline and quarterly BMI levels were calculated from initial and quarterly height and weight measurements obtained from clinic records, respectively. Prescribed total daily insulin dosage and regimen at each visit and measurements of other clinically important covariates of interest were also recorded.

Baseline characteristics stratified by gender indicated no significant differences in the mean age distribution, number of years with T1DM, number of years of observation in the study, proportions on the basal NPH plus prandial regular

insulin regimen and number of clinic visits. However, females had a statistically significant higher baseline BMI than males and more males were current smokers than females. Although females had a statistically and clinically significant higher baseline HbA1c level than males, they were prescribed similar average twice-daily biphasic insulin doses.

On multivariate multilevel mixed-effects linear regression analysis, time-varying BMI was significantly increased by exposure to any insulin regimen. Higher baseline HbA1c and BMI levels were predictive of an increase in BMI. However, males experienced significant comparative reductions in BMI on exposure to the adjusted twice-daily biphasic regimen, the regimen prescribed for 85% of patients and equally spread by gender. Poor glycaemic control during insulin therapy was associated with a reduction in BMI, and vice versa, regardless of regimen.

The study concluded that exposure to adjusted doses of insulin to achieve optimum glycaemic control in T1DM patients resulted in a statistically significant increase in BMI. However, this relationship seemed to be more prominent in female patients and in patients at higher baseline HbA1c levels and BMI categories, respectively. In addition, increasing BMI was consistent with improvements in blood glucose control.

Key words: T1DM, HbA1c, BMI, insulin dose adjustment, glycaemic control, multilevel mixed-effects linear regression.

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LIST OF ABBREVIATIONS

AGEs : Advanced glycation end-products
AIC : Akaike Information Criterion
ANOVA : Analysis of variance
BIC : Bayesian Information Criterion
BMI : Body mass index
DKA : Diabetic ketoacidosis
eGDR : Estimated glucose disposal ratio
GAD-AB : Glutamic acid decarboxylase antibodies
GOF : Goodness-of-fit
HbA1c : Glycated haemoglobin
HBGI : High blood glucose index
HLA : Human leukocyte antigen
IIT : Intensive insulin therapy
LBGI : Low blood glucose index
LOCF : Last observation carried forward
MAR : Missing at random
MCAR : Missing completely at random
MLE : Maximum Likelihood Estimation
MPG : Mean plasma glucose
NKHHC : Non-ketotic hyperglycaemic-hyperosmolar coma
NMAR : Not missing at random
NPH : Neutral protamine Hagedorn
T1DM : Type 1 diabetes mellitus
T2DM : Type 2 diabetes mellitus
WHO : World Health Organization

CHAPTER 1

1.1 PURPOSE OF THE STUDY

Currently, the literature indicates that increases in BMI occur with insulin therapy in most study settings, regardless of the level of glycaemic control achieved. The purpose of the study was therefore to determine the effects of quarterly adjusted total daily insulin doses to achieve optimum glycaemic control, on the BMI of T1DM patients. The secondary purpose was to also establish dosage regimens that achieve optimum glycaemic control without increasing BMI in T1DM patients at the Kalafong Hospital Diabetes Clinic. Outcome differences in BMI and blood glucose control by gender were also investigated.

1.2 AIMS

The study sought to determine the magnitude of change in BMI as a result of insulin treatment in the population of study. As sub-objectives, the study also sought to determine:

1. Whether there were any gender differences in the change in body weight on exposure to any insulin to control blood glucose to optimum levels,
2. How well the optimum quarterly dose increases of insulin (in units/kg) safely improved and maintained blood glucose control, without increasing body weight, if at all,
3. Whether there were any gender differences in how well blood glucose control was achieved,
4. The effect of baseline HbA1c on the degree of weight-gain during the study period.

1.3 STUDY DESIGN

The current study was a retrospective cohort study using secondary data from clinic records collected over 5 years in a cohort of T1DM patients at the Kalafong

Diabetes Clinic. Patients were seen at the clinic at least quarterly between 2009 and 2014.

1.3.1 Study inclusion criteria

Patients were included in the study if they met the following criteria:

i. Diagnosed with T1DM.
ii. Seen at the Kalafong Diabetes Clinic for at least 2 years.
iii. At least 18 years of age on first encounter in the diabetes clinic.
iv. Not on concomitant metformin and/or acarbose therapy, for at least two years from the start of therapy.
v. Patients with a maximum of only 2 consecutive quarters of interrupted study observation (defined as a no-show for clinic appointments, as well as absence of measurements in the prescription and clinic records), during an actual observation period of two years or more.
vi. Patients with a maximum of only 3 non-consecutive quarters of interrupted study observation during an actual observation period of two years or more.

1.3.2 Study exclusion criteria

Patients were excluded from the study cohort if they were diagnosed with type 2 diabetes mellitus (T2DM); were younger than 18 years on first encounter at the clinic; were diagnosed with T1DM, but were on both insulin and metformin and/or acarbose at any time during the observation period; had more than 2 consecutive quarters of interrupted study observation during an observation period of two years or more; or had more than 3 non-consecutive quarters of interrupted study observation during an observation period of two years or more.

1.3.3 Measurements

The following quarterly measurements, except for semi-annually measured HbA1c level, were extracted from clinic records by means of an MSExcel (Windows XP 2005 version) spreadsheet pre-populated with non-personal identifiers such as hospital numbers, of patients selected for the study (see Appendix II):

a. Quarterly prescribed daily insulin dosages (IU/kg/day);
b. Type of insulin regimen prescribed (twice-daily biphasic or basal-bolus insulin regimens):

Regimen	Generic name	Trade name	Supplier
Twice-daily biphasic	30% regular insulin plus 70% NPH insulin	Humulin 30/70	Eli Lilly
		Actraphane HM	Novo Nordisk
		Insuman Comb	Sanofi-Aventis
Basal	NPH insulin	Humulin N	Eli Lilly
		Protaphane	Novo Nordisk
Bolus	Regular insulin	Humulin R	Eli Lilly
		Actrapid	Novo Nordisk

c. Height and time-varying weight (from which time-varying BMI was calculated);
d. Height and baseline weight (from which baseline BMI was calculated);
e. Baseline HbA1c level (%) measured at first visit;
f. Time-varying HbA1c level (%) measured semi-annually during the observation period;
g. Gender;
h. Age at first seen at the clinic;
i. Time-varying age during the study;
j. Smoking history;
k. Number of clinic visits; and
l. Time spent in the study.

1.4 ETHICAL CONSIDERATIONS

Ethical approval to conduct this secondary data analysis study was obtained from the University of Pretoria, Faculty of Health Sciences Ethics Committee (Ethics Approval Certificate No. 94/2015, see Appendix V). No conflict of interest with regard to the study topic is hereby declared. At their first visit to the clinic, patients were routinely requested to sign a notification that informed them that routinely collected information at the clinic may be used for research purposes, that all information used would be strictly confidential and that no patient identifiers would be made known by the researcher. Consent to access routinely collected patient information for this study was obtained from the Kalafong Hospital Chief Executive Officer as the custodian of the clinic data (see Appendix I). All patient information was used anonymously and all personal patient identifiers were concealed during analysis.

CHAPTER 2

2.1 LITERATURE REVIEW

T1DM, previously known as juvenile-onset diabetes or insulin-dependent diabetes is a chronic medical condition that is physiologically characterised by too little or no insulin produced by the pancreatic islet β-cells.[1] Without insulin, body cells cannot access blood glucose, which then accumulates in the blood. Chronic hyperglycaemia in poorly controlled diabetes leads to chronic diabetic complications such as nephropathy, retinopathy, peripheral neuropathy and foot ulcers.[2] Most laboratories consider 6% as the normal value of HbA1c,[3] which represents a regression-estimated mean plasma glucose (MPG) level of 7.6 mmol/L and an approximate clinically relevant MPG level of 7.5 mmol/L, during the preceding 120 days.[4]

Various factors are postulated to cause T1DM. These factors are mainly genetic inheritance, through the human leukocyte antigen (HLA) complex and, as yet unknown, infectious environmental exposures.[5] A study by Pirie et al.[7] found several HLA alleles associated with T1DM in 47 South African Zulu patients, using healthy blood donors as controls. Another study by Panz et al.[8] reported the role of pancreatic islet β-cell autoimmunity, mediated by glutamic acid decarboxylase antibodies (GAD-AB), in the pathogenesis of T1DM in black South Africans.

T1DM typically appears during childhood or adolescence, but can also develop in adulthood due to various environmental risk exposures that predispose to islet β-cell autoimmunity.[6] Although there is currently no known cure for the condition, diabetes can be managed through administration of different formulations of insulin preparations at doses initially calculated according to body weight and later adjusted to obtain better physiologic glycaemic control.[5] Intensive insulin therapy aims to achieve a fasting plasma glucose level of between 3.9 and 5.6 mmol/L and a post-prandial (2 hours after meals) level of less than 7.8 mmol/L. The third objective of insulin therapy is to achieve an HbA1c level of less than 7%.[3] Well-

controlled blood glucose levels delay the onset, and reduce the risk, of the development of complications in T1DM patients. Diabetic complications have to be screened for regularly, and monitored during therapy. These complications account for major morbidity and mortality associated with T1DM.[5] Insulin therapy can also be accompanied by an over-replacement of insulin, leading to increased food-intake to counter-act hypoglycaemia, with resultant weight-gain.[9]

A study by Nansel et al.[10] investigated the cross-sectional and longitudinal associations of body mass index (BMI, in kg/m^2) with glycaemic control in youth with T1DM (n = 340, 12.5±1.7 years, 49% female, duration of T1DM ≥1year), participating in a 2-year multicenter intervention study targeting family-based diabetes management. The results of the study indicated that, patients with lower baseline HbA1c levels ≤8.0% had a smaller increase in BMI on follow-up (mean±SE=1.38±0.17 kg/m^2) than those with a higher baseline HbA1c level >8.0% (1.82±0.12 kg/m^2, p=0.04). Patients with lower baseline HbA1c levels had a greater subsequent increase in HbA1c (0.73±0.1% versus 0.28±0.1%, p=0.002). The authors ventured that these results implied that, whilst insulin therapy did lower blood glucose levels in patients with a high baseline HbA1c, there was also a subsequent increase in body weight during insulin treatment. Furthermore, this could be as a result of upwardly-adjusted insulin doses to achieve optimal blood glucose control leading to increased food-intake to counter possible episodes of hypoglycaemia. Similarly, patients with a lower baseline BMI achieved a significantly higher subsequent rate of increase in BMI compared to patients with a higher baseline BMI (p=0.01) during insulin therapy.[10] BMI was significantly negatively related to HbA1c (%) and significantly positively related to insulin dose after multilevel linear regression analysis. Therefore, in the cohort of patients studied, a high observed baseline HbA1c level, due to poor glycaemic control, predicted a lower subsequent BMI. A higher average daily dose of insulin, which may intuitively predispose to episodes of hypoglycaemia and hence an increased carbohydrate-intake, predicted a higher BMI. Similarly, in the treatment group, BMI was positively related to the insulin pump regimen (β±SE=0.18±0.08, p=0.02).[10]

2.1.1 The prevalence of T1DM internationally and in South Africa:

T1DM occurs in all regions of the world, affects all age-groups, and appears to have strong associations with both genetic and environmental aetiological factors.[6] According to the WHO Fact Sheet No. 312 reviewed in October 2013,[12] at least 347 million people worldwide had T1DM or type 2 diabetes (T2DM), and an estimated 3.4 million people died from diabetic complications in 2004. Also, more than 80% of these deaths occurred in low- and middle-income countries. The WHO projected that by 2030 diabetes will be the 7th leading cause of death in the world. Similarly, Cobas et al.[13] noted that the incidence of T1DM has been increasing in most regions of the world. For instance, the authors cited the state of São Paulo in Brazil where the average annual incidence of T1DM between 1978 and 1991 was 7.6 per 100 000 people, but increased 9.6 times to 73 per 100 000 people between 1986 and 2006 in the city of Bauru located within the same state, especially among children of low socio-economic status between 5 and 9 years of age.

According to the American Diabetes Association,[14] the number of people developing T1DM has been increasing annually in the United States. Eisenbarth[15] estimated that T1DM accounted for 10% of all diabetes cases in the United Sates before 2004, with 30 000 cases occurring each year by 2004 and T1DM affecting 1:300 children, and not less than 1:100 adults, during their life-time. Globally, the figure was approximated at 10-20 million people, with about 40% of cases at younger than 20 years of age.[14]

A systematic review and meta-analysis of the prevalence of diabetes mellitus stratified by sex in eastern, middle, and sub-Saharan Africa also found considerable variations in the prevalence of diabetes mellitus among the adult population in the regions investigated, despite the relatively high prevalence of 5.7% from the combined studies.[16] The high and increasing value was ascribed to the epidemiologic transition from communicable to non-communicable diseases that much of the developing world is facing. The study also found sex-linked differences in the prevalence of diabetes mellitus in women and men, which were

postulated to be related to gender-based differences in lifestyle and obesity (both known risk factors for diabetes mellitus). The authors also noted that the increasing risk factors for diabetes mellitus appear to coincide with the increasing life-expectancies in the region, which necessitates intensification of diabetes prevention efforts.[16]

2.1.2 The need for exogenous insulin therapy in T1DM and the reasons for optimal glycaemic control:

The impairment of glucose metabolism that is characteristic of T1DM leads to hyperglycaemia, which has a number of effects on the micro- and macrovascular system and multiple organs.[17] These effects are thought to be mediated by advanced glycation end-products (AGEs) implicated in the modification of long-lived macromolecules,[27] the polyol metabolic pathway implicated in diabetic retinopathy,[28] the hexosamine pathway, which is a relatively minor branch of the glycolytic pathway implicated in insulin resistance in uncontrolled T1DM,[29] as well as chronic activation of protein kinase C implicated in disruption of vascular cell homeostasis.[30] Late T1DM complications include retinopathy, nephropathy, atherosclerotic coronary and peripheral arterial disease, as well as peripheral and other neuropathies.[3] As a result, T1DM is a key factor in end-stage renal disease, blindness, amputation, and also a major risk factor in cardiovascular disease and premature death in affected patients.[16] The diabetic syndrome is also associated with risks of diabetic ketoacidosis (DKA) or non-ketotic hyperglycaemic-hyperosmolar coma (NKHHC).[3] Clinical signs and symptoms of T1DM may be sudden and include increased thirst and frequent urination, extreme hunger, weight loss, fatigue and blurred vision.[12] The primary diagnostic criterion for asymptomatic diabetes mellitus is hyperglycaemia, which is defined as a fasting plasma glucose level greater or equal to 7.0 mmol/L.[3]

The control of blood glucose levels to near normoglycaemic ranges in T1DM patients requires life-long insulin replacement. While it is accepted that achieving optimum blood glucose control to avoid the two extremes of hypo- and hyperglycaemia is the primary therapeutic target for patients, clinical guidelines

differ with respect to recommended blood glucose targets.[19] A review of 12 studies (n=2230) by Fullerton et al.[18] assessed the effects of intensive versus conventional glycaemic targets in patients with T1DM, in terms of long-term complications and whether very low, near normoglycaemic values, are of additional benefit. The participants were followed-up for a mean duration of 6.5 years. Assessed study endpoints included microvascular complications of retinopathy, neuropathy and nephropathy, as well as macrovascular complications such as stroke and myocardial infarction. While the study found that intensive glucose control predisposes to severe hypoglycaemia, further analysis according to baseline HbA1c values, determined that the risk was possibly only increased in patients who started the study with "relatively low values of less than 9.0%".[18] In addition, the review demonstrated evidence that tight blood glucose control reduced the risk of developing diabetic complications, particularly in younger patients at the early stages of the disease. However, the effects of tight blood glucose control appeared to be weaker once complications had already occurred in older patients, or in patients with established macrovascular disease.[18]

2.1.3 Challenges of insulin administration for optimal glycaemic control:

According to Angamo et al.,[19] achieving good glycaemic control is a challenge in diabetes patients due to several factors associated with individual patient circumstances. The authors conducted a hospital-based cross-sectional study of diabetes patients in Ethiopia and found that, after multivariate logistic regression analysis of the data, body weight of more than 70 kg, a lower total daily insulin dose, total daily insulin dose variations without evidence of blood glucose levels, knowledge deficits about signs and symptoms of hyperglycaemia, and poor adherence to dietary management plans were independent predictors of poor glycaemic control in the patient population studied.

Danne et al.[20] reported a study that investigated the effect of aggregate mean HbA1c level feedback from 21 international paediatric diabetes centres on average metabolic control, rate of severe hypoglycaemia and insulin therapy per centre between 1995 and 1998. Although the aggregate mean HbA1c levels remained

unchanged between the two time periods (8.62±0.03% in 1995 and 8.67±0.04% in 1998), the study found profound differences in average HbA1c concentrations among the reporting centres, which persisted even after adjustment for sex, age and diabetes duration. The study also found a lack of association between upward adjustments in average insulin dose and better levels of glycaemic control at different ages. There was a positive association between BMI, age and duration of diabetes. Therefore, although feedback on HbA1c levels resulted in an increase in average insulin doses, there was no corresponding improvement in mean glycaemic control among the centres. At the same time, average BMI increased with age and mean duration of diabetes.

2.1.4 The risk factors for weight-gain in T1DM patients:

In a longitudinal study to determine the prevalence and incidence of overweight and obesity in a cohort of 589 T1DM patients, followed up over at least 18 years, Conway et al.[21] found an increase of 47% in overweight and of 700% in obesity from baselines of 28.6% and 3.4%, respectively. Of the total cohort, 7% were on intensive insulin therapy (IIT), defined as ≥3 insulin injections per day or on insulin pump, at baseline. Eight to 11 years later the number on IIT had increased to 82%. Analysis of the data revealed that IIT and a higher baseline HbA1c were predictors of weight-gain in these patients, while symptomatic autonomic neuropathy and overt nephropathy predicted weight loss. The findings of IIT-associated weight-gain were corroborated by those of the Diabetes Control and Complications Trial (DCCT), cited in the review by Kaufman,[9] which demonstrated the association between excess weight-gain and IIT. The risk factors suggested by the review include oral glucose ingestion to offset hypoglycaemia where the fear of nocturnal hypoglycaemia, especially in children, results in night-time snacking.

The anabolic effect of insulin on skeletal muscle mass was another mechanism suggested.[9] According to Dimitriadis et al.,[37] insulin has major effects on muscle and adipose tissue by increasing the rate of glucose transport across cell membranes and the activity of hexokinase and 6-phosphofructokinase, leading to increased glycolysis. Insulin also stimulates glycogenesis and decreases the rate

of glycogen breakdown. Insulin is also involved in the alteration of the regulators of lipolysis and lipogenesis,[9] by lowering plasma fatty acid levels after decreasing the rate of lipolysis in adipose tissue; stimulating fatty acid and triacylglycerol synthesis in tissues; increasing the uptake of circulating triglycerides into adipose tissue and skeletal muscle, and decreasing the rate of fatty acid oxidation in muscle and the liver.[37] Insulin modifies protein metabolism by increasing the rate of transport of some amino acids into tissues; increasing the rate of protein synthesis in tissues and decreasing the rate of protein degradation. Altogether, these anabolic actions result in the synthesis of carbohydrate, fat and protein in patients.[37]

In a study of 45 Brazilian women with type 1 diabetes mellitus (36±9 years; BMI 24.6±4.4 kg/m^2), whose body composition and insulin resistance were determined by dual-energy X-ray absorptiometry and estimated glucose disposal ratio (eGDR), respectively, Momesso et al.[38] found that 45% of the subjects had metabolic syndrome according to the WHO criteria, and that central fat deposition was related to metabolic syndrome and insulin resistance. Consistent with the observation by Nansel et al.[10] that fat and fat-free weight-gain have different health implications in type 1 diabetes mellitus patients, the authors concluded that body fat composition analysis could be used to identify those patients at increased risk of metabolic syndrome at baseline.

Conservation of calories in previously poorly-controlled diabetes patients was also hypothesised as another mechanism of weight-gain in T1DM. According to this hypothesis, conservation of ingested calories occurred when glycosuria was resolved with improved glycaemic control during IIT to blood glucose levels below the renal secretion threshold.[9,39]

2.1.5 Balancing glycaemic control with prevention of weight-gain in T1DM patients:

In a 2010 review, Hahr and Molitch[22] investigated the optimization of insulin therapy to achieve glycaemic control while minimizing weight-gain and

hypoglycaemia. The authors observed that optimal glycaemic control in T1DM patients depended on an insulin regimen that closely mimicked endogenous insulin secretion. This was best achieved with long-acting basal insulin, to maintain normal fasting glucose levels and multiple daily injections of short or rapidly-acting insulin (pre-prandial rapid-acting analogue insulin) with meals. Furthermore, the choice of regimen depended on the individual needs and circumstances of the patient, as well as the treating health professional's judgement.[22] This assertion was consistent with Fullerton et al.[18] that there is currently (2014) no firm evidence for specific blood glucose targets. As a result, there was a need to individualize therapeutic goals, that take into account the patient's age, disease progression, macrovascular risk, lifestyle and disease management capabilities.[18]

The reviewers concluded that patient-specific regimens to optimise glycaemic control had been made possible by the development of insulin analogues, which increased flexibility and control in the treatment of T1DM. Also, because IIT with basal and prandial regular insulin required multiple daily injections, it was crucial that health workers communicate therapy-related issues with patients. These issues included lifestyle adjustments and exercise, meal intake and its effects, proper self-administration and the effects of dose-adjustments not based on reliable ambulatory blood glucose readings.[22]

2.1.6 The dosage range at which optimal glycaemic control is negated by an increase in body weight:

Based on the literature reviewed for this study, there does not seem to be a dosage range at which optimal glycaemic control can be easily achieved without an increase in body weight. Rather, optimal glycaemic control through IIT, based on measured HbA1c levels, appears to be a consistent and independent predictor of an increase in body weight. In turn, HbA1c levels and dosage regimens required for glycaemic control depend on individual patients' circumstances, as described by Fullerton et al.[18] and Hahr and Molitch,[22] amongst others.

CHAPTER 3

3.1 DATA MANAGEMENT AND ANALYSIS

3.1.1 Data security

All study data was electronically stored on the server hard-drive of the Department of Internal Medicine at Kalafong Hospital. The server and hard-drive were located in the Server Room of the Klinikala building and all data was, and still is, password protected.

3.1.2 Data management

3.1.2.1 Baseline data management

Relevant baseline measurements, indicated in section 1.3.3 above, was identified from clinic records and entered into an MSExcel spreadsheet (Windows XP 2005 version). Baseline characteristics were then compared using relevant statistical analytical methods referred to in section 3.1.3.1 below.

3.1.2.2 Time-varying data management

Time-varying measurements were also identified from clinic records and similarly entered into an MSExcel spreadsheet. Relevant measurements, indicated in section 1.3.3 above, routinely captured in clinic records during administrative and clinical management of patients were entered into the MSExcel spreadsheet in long form to allow for panel data analysis during the data regression analysis phase. Patient hospital numbers were used as the 'panel' variable per patient. The unique number of visits and variable measurements per patient were used as the 'time' variable. This allowed for the first visit and/or measurements to be allocated the number '1', the second visit and/or measurements to be allocated the number '2' and so forth. This data management method allowed ease of use of the "xtset panel time" data set-up command in STATA.

3.1.3 Data analysis

3.1.3.1 Baseline data analysis

Baseline patient characteristics were summarized using means and standard deviations for continuous variables and frequencies for count variables. The t-test and χ^2 test (or Fisher's exact when insufficient cell numbers for chi-square and Welch test where the equal variance assumption was not satisfied), were used as appropriate to evaluate differences in baseline characteristics by HbA1c levels and gender.

Characteristics compared by baseline HbA1c levels (≤ or >8.0%, 64 mmol/mol) were, age at first clinic visit, gender, baseline BMI, height in cm, baseline weight in kg, duration of T1DM at last visit in years, mean insulin dose stratified by regimen, years of observation and smoking status. The less stringent cut-off HbA1c level of 8% was chosen for this cohort of patients, in whom the goal of optimum glycaemic control is difficult to attain despite diabetes self-management education and other measures, in line with recommendations of the American Diabetic Association.[36] Differences in the other baseline characteristics were stratified by six BMI categories of ≤19, 20–25, 25.1–30, 30.1–35, 35.1–40 and >40 kg/m² used at the Kalafong Diabetes Clinic. Differences in the numbers of patients in each BMI category by each baseline characteristic were evaluated for significance by one-way ANOVA (or the Kruskal-Wallis rank test, where assumptions of equal variance were not satisfied).

3.1.3.2 Time-varying data analysis

The long form panel data was then exported to the STATA version 12 programme for multilevel mixed-effects linear regression analysis. The "xtset panel time" STATA command was used for analysis, in line with analytical methods used by Nansel et al.,[10] in a similar study design investigating the cross-sectional and longitudinal associations of glycaemic control and weight, in a sample of children and adolescents with T1DM assessed at multiple times over 2 years. The last

observation carried forward (LOCF) imputation method was employed where there were missing measurements for subsequent visits in order to preserve the power of the study. This multiple imputation method was employed only for missing quarterly prescribed insulin dose measurements, which were in any case observed to be constant over time, with very few exceptions where the dose was only temporarily increased and then brought back to the usual optimum blood glucose control dose.

The longitudinal relationship of time-varying BMI with time-varying glycaemic control (HbA1c level), adjusted for the main effect of daily insulin dose exposure and other clinically important covariates, was examined by multilevel mixed-effects linear regression analysis, which accounted for correlated repeated measurements within subjects. The model allowed for baseline variation in BMI by including a random effects parameter, while also allowing the relationship of time and BMI to vary between subjects by including a random coefficient for the time variable (delta=1 incremental unit of consecutive time measurements). The use of maximum likelihood estimation (MLE) allowed for varying observed time intervals between outcome measurements and different numbers of observations at each time point. The analysis included the time-invariant covariates of gender, baseline BMI, baseline HbA1c, years of observation and smoking status. Time-varying covariates included subsequent age, BMI (in kg/m^2), HbA1c (in %), quarterly prescribed insulin regimen and daily insulin dose (in IU/kg).

All ten variables were first subjected to univariate regression analysis. The data analysis plan involved excluding variables with $p>0.25$ in the univariate models from multivariate analysis. Thereafter covariates were removed from multivariate regression models one-at-a-time, starting with the variable with the highest p-value, until the only variables left were those significant at $p=0.05$. The variables were initially chosen for univariate analysis due to the biological plausibility of influencing the exposure-outcome relationship of insulin and both HbA1c and BMI. The final and reduced multivariate regression models were evaluated for goodness-of-fit of the data by means of the regression model information criteria

statistics, AIC and BIC. A model with a lower value for the AIC or BIC is considered to be a better model.[32] Potential effect modification, where warranted, was examined using multiplicative interaction terms or stratified analyses. Collinearity between time-varying covariates, where suspected, was assessed using a correlation matrix. A significant correlation coefficient at p=0.05 indicated collinearity, prompting a decision to drop one of the covariates from the analysis.

CHAPTER 4

4.1 RESULTS

Patient flow, exclusion and inclusion were performed as per Figure 1 below.

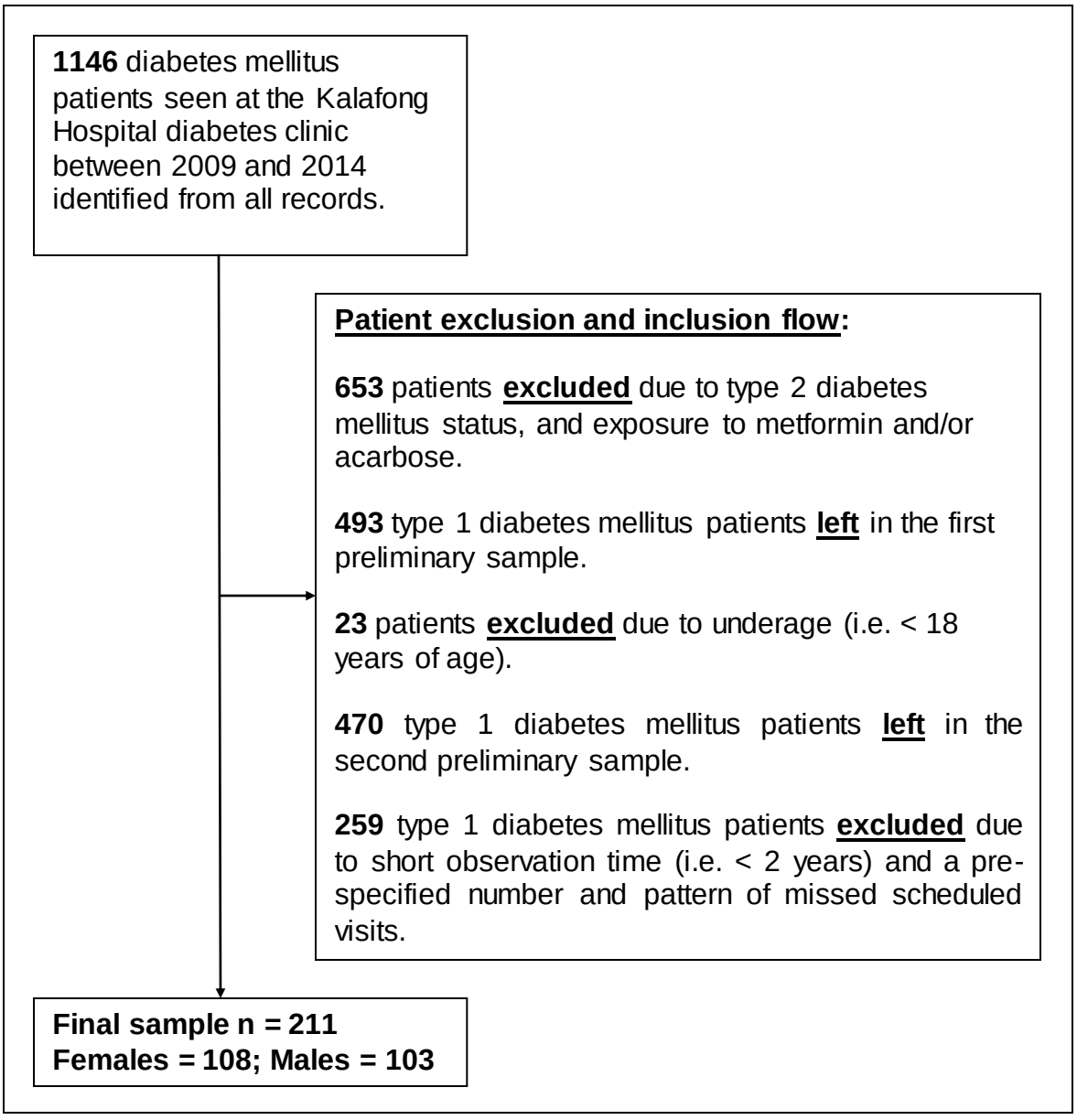

Figure 1: Study sample selection and exclusion flow diagram

The final sample consisted of 211 T1DM patients (108 females and 103 males). The mean±SD age and range were 43.1±14.4 and 18 to 78 years, respectively.

The mean baseline BMI and HbA1c for the full sample were 27.8±5.5 kg/m^2 and 10.2±3.5%, respectively (see Table 1). Most patients were at more than 8% HbA1c levels at baseline (66%) and most were prescribed twice-daily biphasic insulin regimen (85%). At baseline, mean (±SD) daily insulin prescribed was 59.8±36.7 IU and 49.4±17.1 IU for the basal NPH plus prandial regular insulin and the twice-daily biphasic insulin, respectively.

Table 1: Baseline characteristics

	Full sample n=211 [mean±SD, n (%)]
Age (years)	43.1±14.4
Height (cm)	165.4±9.2
Weight (kg)	75.5±15.0
BMI (kg/m^2)	27.8±5.5
HbA1c (%, mmol/mol)	10.2±3.5, 88±36
BMI category (kg/m²):	
< 19	10 (5)
20 – 25	66 (31)
25.1 – 30	73 (35)
30.1 – 35	44 (21)
35.1 – 40	14 (7)
> 40	4 (2)
HbA1c level in % (mmol/mol):	
≤ 8.0 (≤ 64)	72 (34)
> 8.0 (> 64)	139 (66)
Insulin Regimen daily dose:	
Basal NPH + Prandial regular insulin (IU/day)	59.8±36.7 [32 (15)]
Twice-daily Biphasic insulin (IU/day)	49.4±17.1 [179 (85)]
Smoking status:	
0 = Never smoked	135 (64)
1 = Stopped > 1 year ago	32 (15)
2 = Stopped < 1 year ago	7 (3)
3 = Currently smoking	37 (18)

Table 1a: Other full sample characteristics

	Full sample (n=211), [mean±SD, n (%)]
*Duration of type 1 diabetes mellitus (years):	15.5±7.7 [131(62)]
Number of clinic visits	20.7±6.7
Time in study (years)	4.7±1.6

*Only 62% of all patients had measurements for duration of type 1 diabetes mellitus

4.1.1 Baseline characteristics by gender

There were no statistically significant gender differences in the mean age distribution, number of years with T1DM, number of years of observation in the study, proportions on the twice-daily biphasic insulin or basal NPH plus prandial regular insulin regimens and number of clinic visits. However, females had a statistically significant higher baseline BMI than males (mean±SD) 28.9±6.2 versus 26.5±4.4 kg/m², p=0.002. Males were, as expected, significantly taller compared to females (171.3±7.8 versus 159.8±6.7 cm, p<0.001), but not significantly heavier (77.5±13.4 versus 73.6±16.2 kg, p=0.06). There was no statistically significant gender difference in the dosage of any insulin regimen prescribed. Females had a higher mean baseline HbA1c level than males (10.8±3.8% versus 9.5±3.0%, p=0.01), and more males were current smokers than females (28% versus 7%, p<0.001), see Table 2.

4.1.2 Baseline characteristics stratified by baseline HbA1c level

There were significant differences in the distributions of duration of T1DM, number of clinic visits, years spent in the study, exposure to the twice-daily biphasic insulin dosage regimen or basal NPH plus prandial regular insulin regimen and smoking status by stratified baseline HbA1c categories of ≤8.0% and >8.0%. However, baseline characteristics showed no significant differences in the distributions of age, gender and baseline BMI by baseline HbA1c category, see Table 3.

Table 2: Baseline characteristics stratified by gender

Variables	Female [mean±SD, n (%)] [108 (51)]	Male [mean±SD, n (%)] [103 (49)]	p
Age (years)	41.9±16.2	44.3±12.1	0.222
Height (cm)	159.8±6.7	171.3±7.8	<0.001
Weight (kg)	73.6±16.2	77.5±13.4	0.063
BMI (kg/m^2)	28.9±6.2	26.5±4.4	0.002
HbA1c (%)	10.8±3.8	9.5±3.0	0.010
BMI category (kg/m²):			**0.012**
< 19	4 (4)	6 (6)	
20 – 25	30 (28)	36 (35)	
25.1 – 30	31 (29)	42 (41)	
30.1 – 35	28 (26)	16 (16)	
35.1 – 40	11 (10)	3 (3)	
> 40	4 (4)	0	
HbA1c level in % (mmol/mol):			**0.407**
≤ 8.0 (≤ 64)	34 (31)	38 (37)	
> 8.0 (> 64)	74 (69)	65 (63)	
Insulin Regimen daily dose			
Basal NPH + Prandial regular insulin (IU/day):	65.9±47.5 [17(16)]	52.9±25.1 [15(15)]	0.350
Twice-daily Biphasic insulin (IU/day):	48.8±17.7 [91(84)]	50.1±16.6 [88(85)]	0.614
Smoking status:			**<0.001**
0 = Never smoked	93 (86)	41 (40)	
1 = Stopped > 1 year ago	5 (5)	27 (26)	
2 = Stopped < 1 year ago	2 (2)	5 (5)	
3 = Currently smoking	8 (7)	29 (28)	

Table 2a: Other characteristics stratified by gender

Variables	Female [mean±SD, n (%)] [108 (51)]	Male [mean±SD, n (%)] [103 (49)]	p
*Duration of type 1 diabetes mellitus (years):	16.1±7.9 [60(56)]	15.1±7.6 [71(69)]	0.457
Number of clinic visits	20.1±6.9	21.3±6.4	0.218
Time in study (years)	4.6±1.7	4.9±1.5	0.182

*Only 62% of all patients had measurements for duration of type 1 diabetes mellitus

Table 3: Baseline characteristics stratified by baseline HbA1c level

	Baseline HbA1c (%) [mean±SD, n (%)]		
	≤8.0 [72(34)]	>8.0 [139(66)]	p
Age	44.9±16.0	42.1±13.4	0.1989
Height (cm)	166.3±9.3	164.9±9.1	0.3108
Weight (kg)	78.8±13.7	73.8±15.4	0.0227
BMI (kg/m²)	28.6±5.2	27.2±5.7	0.0790
Gender:			**0.4072**
Female	34 (31)	74 (69)	
Male	38 (37)	65 (63)	
Insulin Regimen daily dose			
Basal NPH + Prandial regular insulin (IU/day):	57.1±25.0	65.4±33.0	<0.001
Twice-daily Biphasic insulin (IU/day)	49.4±17.7	55.6±17.7	<0.001
Smoking status:			**0.045**
0 = Never smoked	41 (30)	94 (70)	
1 = Stopped > 1 year ago	18 (56)	14 (44)	
2 = Stopped < 1 year ago	2 (29)	5 (71)	
3 = Currently smoking	11 (30)	26 (70)	

Table 3a: Other characteristics stratified by baseline HbA1c level

	Baseline HbA1c (%) [mean±SD, n (%)]		
	≤8.0 [72(34)]	>8.0 [139(66)]	p
*Duration of type 1 diabetes mellitus at last visit (years):	13.5±7.4	16.9±7.7	0.0142
Number of clinic visits	22.4±6.1	19.8±6.8	0.0067
Time in study (years)	5.2±1.5	4.5±1.7	0.0033

*Only 62% of all patients had measurements for duration of type 1 diabetes mellitus

4.1.3 Baseline gender characteristics stratified by baseline HbA1c level

Except for the number of clinic visits (p=0.037) and number of years in the study (p=0.015), where higher values for both variables were associated with lower baseline HbA1c levels of ≤8%, there were no other statistically significant differences in baseline characteristics stratified by HbA1c level in females, see Table 4. In males, however, higher BMI individuals were associated with lower baseline HbA1c levels, while those male patients who had had T1DM for longer were associated with a higher baseline HbA1c level of >8%, see Table 5.

Table 4: Baseline female characteristics stratified by baseline HbA1c level [n=108 (51%)]

	Baseline HbA1c (%) [mean±SD, n (%)]		
	≤8.0 [34(31)]	**>8.0 [(74(69)]**	**p**
Age	45.2±18.6	40.4±15.0	0.167
Height (cm)	159.6±5.5	160.0±7.2	0.766
Weight (kg)	75.2±14.1	72.9±17.2	0.503
BMI (kg/m^2)	29.6±5.5	28.7±6.3	0.482
BMI category (kg/m²):	**34 (31)**	**74 (69)**	**0.437**
< 19	1 (3)	3 (4)	
20 – 25	6 (18)	24 (32)	
25.1 – 30	13 (38)	18 (24)	
30.1 – 35	9 (26)	19 (26)	
35.1 – 40	4 (12)	7 (9)	
> 40	1 (3)	3 (4)	
Insulin Regimen daily dose			
Basal NPH + Prandial regular insulin (IU/day):	72.5±33.7 [n=4 (12)]	74.7±48.7 [n=12 (16)]	0.936
Twice-daily Biphasic insulin (IU/day):	46.8±18.7 [n=29 (85)]	50.0±17.3 [n=61 (82)]	0.425
Smoking status:	**34 (31)**	**74 (69)**	**0.108**
0 = Never smoked	28 (82)	65 (88)	
1 = Stopped > 1 year ago	4 (12)	1 (1)	
2 = Stopped < 1 year ago	0	2 (3)	
3 = Currently smoking	2 (6)	6 (8)	

Table 4a: Other female characteristics stratified by HbA1c level **[n=108 (51%)]**

	Baseline HbA1c (%) [mean±SD, n (%)]		
	≤8.0 [34(31)]	**>8.0 [74(69)]**	**p**
*Duration of type 1 diabetes mellitus at last visit (years):	8.7±8.0 [24 (22)]	10.1±7.6 [36 (33)]	0.496
Number of clinic visits	22.2±6.5	19.2±6.9	0.037
Time in study (years)	5.1±1.5	4.3±1.7	0.015

*Only 62% of all patients had measurements for duration of type 1 diabetes mellitus

Table 5: Baseline male characteristics stratified by baseline HbA1c level [n=103 (49%)]

	Baseline HbA1c (%) [mean±SD, n (%)]		
	≤8.0 [38(37)]	**>8.0 [65(63)]**	**p**
Age	44.6±13.7	43.8±11.5	0.744
Height (cm)	172.3±7.8	170.6±7.7	0.278
Weight (kg)	82.0±12.7	74.8±13.2	0.009
BMI (kg/m^2)	27.8±4.8	25.7±4.1	0.024
BMI category (kg/m²):	**38 (37)**	**65 (63)**	**0.049**
< 19	3 (8)	3 (5)	
20 – 25	8 (21)	28 (43)	
25.1 – 30	17 (45)	25 (38)	
30.1 – 35	7 (18)	9 (14)	
35.1 – 40	3 (8)	0	
> 40	0	0	
Insulin Regimen daily dose			
Basal NPH + Prandial regular insulin (IU/day):	43.0±9.9 [n=2 (5)]	57.2±25.0 [n=11 (17)]	0.458
Twice-daily Biphasic insulin (IU/day):	48.0±17.0 [n=36 (95)]	51.5±16.4 [n=52 (80)]	0.337
Smoking status:	**38 (37)**	**65 (63)**	**0.334**
0 = Never smoked	13 (34)	28 (43)	
1 = Stopped > 1 year ago	14 (37)	13 (20)	
2 = Stopped < 1 year ago	2 (5)	3 (5)	
3 = Currently smoking	9 (24)	20 (31)	

Table 5a: Other male characteristics stratified by HbA1c level [n=103 (49%)]

	Baseline HbA1c (%) [mean±SD, n (%)]		
	≤8.0 [38(37)]	**>8.0 [65(63)]**	**p**
*Duration of type 1 diabetes mellitus at last visit (years):	5.6±6.8 [29(28)]	10.9±7.3 [42(41)]	0.003
Number of clinic visits	22.6±5.7	20.5±6.6	0.102
Time in study (years)	5.2±1.4	4.7±1.5	0.101

*Only 62% of all patients had measurements for duration of type 1 diabetes mellitus

4.1.4 Baseline characteristics stratified by baseline BMI category

Baseline characteristics stratified by baseline BMI category showed no significant differences in prescriptions of any insulin regimen and in baseline HbA1c. However, baseline BMI increased significantly with baseline age (p<0.001). There were also no significant differences in the number of clinic visits and years under observation in the study, p=0.823 and p=0.688, respectively. There was a significant difference in the distributions of males and females by baseline BMI category. There were more males in the classification category ≤19 to <30 kg/m^2 than females and there were more females in classification category >30 to >40 kg/m^2 than males (p=0.001), see Table 6.

Table 6: Baseline characteristics stratified by baseline BMI category

	Baseline BMI category in kg/m² [mean±SD, n (%)]						
	≤19	**20–25**	**25.1 – 30**	**30.1 – 35**	**35.1 – 40**	**> 40**	**p**
Age (years)	27.8±7.7	36.9.6±12.3	44.9±13.3	49.4±14.6	52.5±14.5	51.6±11.6	<0.001
Height (cm)	169.2±9.1	167.8±9.8	166.2±8.5	161.5±8.2	159.4±7.2	165.3±7.1	0.002
Weight (kg)	51.3±8.3	64.3±8.0	77.2±9.0	84.6±10.0	95.0±7.6	120.7±8.7	<0.001
HbA1c (%)	10.8±5.0	10.7±3.6	9.8±3.5	9.9±3.5	9.4±2.9	10.7±4.9	0.597
Gender:	**10 (5)**	**66 (31)**	**63 (30)**	**44 (21)**	**14 (7)**	**4 (2)**	**0.001**
Female	4 (4)	30 (28)	31 (29)	28 (26)	11 (10)	4 (4)	
Male	6 (6)	36 (35)	42 (41)	16 (16)	3 (3)	0	
Insulin Regimen daily dose							
Basal NPH insulin plus Prandial regular insulin (IU/day):	54.0±13.1	51.7±28.1	72.3±26.0	68.7±17.4	84.0±33.9	0	0.158
Twice-daily biphasic insulin (IU/day):	50.4±40.5	48.9±18.4	55.4±17.0	55.3±19.3	55.6±17.7	52.7±17.4	0.601
Total insulin dose at last visit (IU/day):	54.9±30.8	48.2±22.8	56.4±20.8	59.7±21.9	56.7±23.1	52.7±17.4	0.146
Smoking status:	**8 (3.8)**	**64 (30.3)**	**70 (33.2)**	**45 (21.3)**	**18 (8.5)**	**6 (2.8)**	**0.005**
0=Never smoked	6 (75)	39 (61)	32 (46)	34 (76)	14 (78)	5 (83)	
1=Stopped>1 year ago	1 (12.5)	5 (8)	26 (37)	5 (11)	2 (11)	1 (17)	
2=Stopped<1 year ago	0	3 (5)	2 (3)	1 (2)	0	0	
3=Currently smoking	1 (12.5)	17 (27)	9 (13)	5 (11)	2 (11)	0	

Table 6a: Other characteristics stratified by baseline BMI category

	Baseline BMI category in kg/m² [mean±SD, n (%)]						
	≤ 19	**20 – 25**	**25.1 – 30**	**30.1 – 35**	**35.1 – 40**	**> 40**	**p**
Number of clinic visits	20.6±7.2	20.0±7.3	21.2±6.0	20.6±6.5	22.3±6.0	18.8±10.0	0.823
Time in study (years)	4.5±1.8	4.6±1.6	4.8±1.5	4.5±1.6	5.1±1.7	4.0±2.3	0.688

4.1.5 Temporal relationship between baseline BMI and BMI measured at last clinic visit

In the 4.7±1.6 years from baseline to last BMI measured, there was a net migration of 7 individuals from lower BMI categories of ≤19 kg/m^2 (-2 patients), 20–25 kg/m^2 (-2 patients) and 25.1–30 kg/m^2 (-3 patients), to higher BMI categories of 30.1–35 kg/m^2 (+1 patients), 35.1–40 kg/m^2 (+4 patients) and >40 kg/m^2 (+2 patients), (see Table 7 and Figure 2). A paired t-test analysis of BMI measurements between baseline and the last BMI indicated a significant mean(±SD) increase in BMI, from 27.8±5.5 to 28.4±3.1 kg/m^2 (p=0.003).

Of interest, a related analysis of the mean difference between baseline HbA1c and the last HbA1c measured indicated that there was a statistically significant mean(±SD) reduction in HbA1c, from 10.2±3.5 to 8.8±2.4% (p<0.001), over the same time of observation in the study.

Table 7: Cross-tabulation of numbers of patients between baseline and final BMI category (BMI at last visit)

	BMI at last visit (kg/m^2)						
Baseline BMI (kg/m^2)	**≤19**	**20-25**	**25.1-30**	**30.1-35**	**35.1-40**	**>40**	**Total**
≤19	3	7	0	0	0	0	**10**
20-25	4	43	16	3	0	0	**66**
25.1-30	1	13	43	15	1	0	**73**
30.1-35	0	0	11	25	8	0	**44**
35.1-40	0	1	0	2	8	3	**14**
>40	0	0	0	0	1	3	**4**
Total	**8**	**64**	**70**	**45**	**18**	**6**	**211**

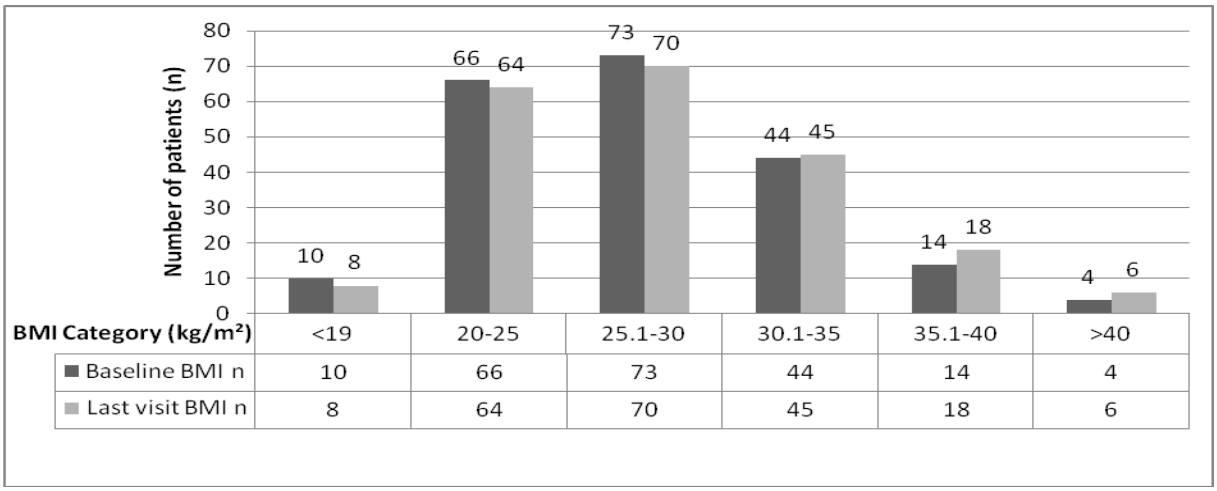

Figure 2: Change in numbers of patients between baseline and BMI at last visit

4.1.6 Trending of prescribed daily insulin dosages and last measured HbA1c levels stratified by last measured BMI category

Table 8: Equality of means (in selected variables) stratified by increasing final BMI category (BMI at last visit)

	Final BMI category in kg/m²						
	≤19	**20-25**	**25.1-30**	**30.1-35**	**35.1-40**	**>40**	**p**
Last-measured HbA1c (%)	9.81±2.2	9.51±2.8	8.62±2.0	8.28±2.4	7.96±1.3	7.62±1.1	**0.02**
Total exposure to any insulin Regimen (IU/day)	54.8±30.8	48.2±22.8	56.4±20.8	59.7±21.9	56.7±23.1	52.7±17.4	**0.15**
Basal NPH + Prandial regular insulin dose (IU/day)	60.7±8.1	51.7±28.2	66.1±26.2	72.1±18.7	85.0±32.5	0	**0.22**
Twice-daily biphasic insulin dose (IU/day)	51.2±39.9	49.4±18.1	54.5±17.1	55.9±18.8	55.9±17.8	52.7±17.4	**0.70**

There was a statistically significant decline in last measured HbA1c levels as last measured BMI increased. There were no statistically significant differences in the mean prescribed daily insulin doses stratified by the last measured BMI category, see Table 8.

4.1.7 Univariate association of subsequent (time-varying) BMI with insulin regimens and other independent variables

Unadjusted exposures to the twice-daily biphasic insulin regimen, when compared to the basal NPH plus prandial regular insulin regimen, was associated with a large and significant increase in time-varying BMI levels (β±SE=2.644±0.257 kg/m^2, p<0.001). Unadjusted total exposure to insulin regardless of regimen also resulted in an increase in BMI. That is, every 1 IU/day increase in any insulin exposure was associated with a 0.029 kg/m^2 increase in BMI over the period of the study, see Table 9. In general, when not adjusted by other variables, an additional visit to the clinic, a unit increase in baseline and subsequent HbA1c, being male and current smoking status, were associated with varying decreases in subsequent BMI levels (weight loss). Also, every 1 kg/m^2 increase in baseline BMI predicted a significant increase of 0.983 kg/m^2 in subsequent BMI. Increasing age also predicted an increase of 0.142 kg/m^2 in BMI per year, see Table 9.

Table 9: Univariate regression models by time-varying BMI value

	β±SE	p
Age	0.142±0.007	<0.001
Baseline BMI	0.983±0.009	<0.001
Baseline HbA1c	- 0.151±0.031	<0.001
Time-varying HbA1c	- 0.333±0.061	<0.001
Number of clinic visits	- 0.046±0.018	0.010
Time in study	0.022±0.014	0.128
Gender:		
Female	Reference	
Male	- 3.262±0.203	<0.001
Insulin Regimen:		
Basal NPH + Prandial regular insulin	Reference	
Twice-daily Biphasic insulin	2.664±0.257	<0.001
Total insulin exposure	0.029±0.005	<0.001
Smoking Status:		
0 = Never smoked	Reference	
1 = Stopped > 1 year ago	- 0.011±0.286	0.970
2 = Stopped < 1 year ago	1.337±0.744	0.072
3 = Currently smoking	- 2.221±0.299	<0.001

4.1.8 Univariate association of subsequent (time-varying) HbA1c level with insulin regimens and other independent variables

Unadjusted exposure to the twice-daily biphasic insulin regimen was associated with a reduction in time-varying HbA1c level of 0.433% (better glycaemic control) when compared to the unadjusted exposure to the basal NPH plus prandial regular insulin regimen. Unadjusted additional visit to the clinic, a unit (1 kg/m^2) increase in subsequent BMI, an additional year spent in the study, being male, stopped smoking for more than 1 year, every 1 year increase in age, were also associated with varying decreases in subsequent HbA1c levels. However, unadjusted total exposure to insulin regardless of regimen was associated with an increase in HbA1c. Specifically, every 1 IU/day increase in total insulin was associated with a 0.011% (p<0.001) average increase in time-varying HbA1c level (poorer glycaemic control), see Table 10.

Table 10: Univariate Regression Models by Time-varying HbA1c Level

	β±SE	p
Age (years)	- 0.038±0.005	<0.001
Time-varying BMI	- 0.072±0.013	<0.001
Baseline BMI	- 0.081±0.013	<0.001
Baseline HbA1c	0.414±0.018	<0.001
Number of clinic visits	- 0.054±0.011	<0.001
Time in study (years)	- 0.046±0.010	<0.001
Gender:		
0 = Female	Reference	
1 = Male	- 0.313±0.141	0.026
Insulin Regimen:		
Basal NPH + Prandial regular insulin	Reference	
Twice-daily Biphasic insulin	- 0.433±0.173	0.012
Total insulin exposure (IU/day)	0.011±0.003	<0.001
Smoking Status:		
0 = Never smoked	Reference	
1 = Stopped > 1 year ago	- 0.822±0.186	<0.001
2 = Stopped < 1 year ago	0.351±0.469	0.454
3 = Currently smoking	- 0.037±0.194	0.850

4.1.9 Multivariate association of subsequent (time-varying) BMI with insulin regimens and other covariates

Multilevel mixed-effects linear regression modeling was used to explore the relationship between time-varying BMI and baseline BMI, HbA1c, age, insulin dose and time in the study, as well as gender, smoking history, time-varying HbA1c, age and insulin dose adjustments. Stepwise hierarchical backwards multilevel mixed effects linear regression was carried out using STATA (version 12), (see section 3.1.3.2 above). The variables of baseline BMI, baseline and time-varying HbA1c and age, were modeled as continuous variables. Insulin regimen exposure, gender and smoking history were modeled as categorical variables. The variable smoking history was categorized into 4 descriptive classes. The specification for age, albeit not statistically significant, gave the best goodness of fit characteristics and the lowest information criteria statistics and was included in the full and final model,[32]

(see Table 11). A STATA output of the modeling process is attached as Appendix III.

Table 11: Multivariate regression model by time-varying BMI value

	β±SE	p
Age	**- 0.004±0.006**	**0.557**
Baseline BMI (kg/m^2)	0.978±0.016	<0.001
Time-varying HbA1c (%)	- 0.157±0.031	<0.001
Baseline HbA1c (%)	0.091±0.026	<0.001
Time in study	- 0.111±0.052	0.003
Gender:		
0 = Female	Reference	
1 = Male	- 0.609±0.180	0.001
Insulin regimen		
0 = Basal NPH + Prandial regular insulin (IU/day):	Reference	
1 = Twice-daily Biphasic insulin (IU/day):	0.498±0.191	0.009
Total insulin exposure (IU/day)	0.009±0.003	0.008
Smoking Status:		
0 = Never smoked	Reference	
1 = Stopped > 1 year ago	- 0.446±0.223	0.045
2 = Stopped < 1 year ago	- 0.849±0.518	0.101
3 = Currently smoking	- 0.414±0.232	0.075
Constant	2.036±0.668	0.002

Model information criteria statistics:
Model AIC=5627.19 (versus 5693.39 without the "age" variable in the full model)
Model BIC=5693.26 (versus 5754.52 without the "age" variable in the full model)

Due to signficant collinearity between the number of patient visits to the clinic and the number of years of follow-up in the study, the number of patient visits to the clinic was excluded from the model. Exposure to the twice-daily biphasic insulin regimen, adjusted for other variables including total insulin exposure, was associated with a 0.50 kg/m^2 increase in subsequent BMI in comparison to the basal-bolus insulin regimen. After adjustment for other variables, the following covariables: an additional year of observation, every 1% increase in HbA1c (poor glycaemic control), being male, stopped smoking for more than a year, were

associated with a decrease in subsequent BMI, by 0.11, 0.16, 0.61 and 0.45 kg/m^2, respectively. Also, every 1 kg/m^2 increase in baseline BMI predicted a 0.98 kg/m^2 increase in subsequent BMI. And, every 1% increase in baseline HbA1c predicted a 0.09 kg/m^2 increase in subsequent BMI, see Table 11.

In general, for patients in this cohort, time-varying BMI was statistically significantly increased by exposure to any insulin regardless of regimen (β±SE=0.01±0.003, p=0.008), as well as higher baseline HbA1c and BMI levels, both at p<0.001. The multivariable multilevel mixed-effects linear regression model indicated that time-varying BMI, regressed on insulin regimen adjusted for important covariates, was consistently positively associated with exposure to daily insulin doses, adjusted quarterly, for optimum blood glucose control.

4.1.10 Multivariate association of subsequent (time-varying) HbA1c level with insulin regimens and other covariates

Multilevel mixed-effects linear regression modeling was used to explore the relationship between time-varying HbA1c and baseline BMI, HbA1c, age, insulin dose and time in the study, as well as gender, smoking history, time-varying BMI, age and insulin dose adjustments. Stepwise hierarchical backwards multilevel mixed effects linear regression was carried out using STATA version 12, (see section 3.1.3.2 above). The variables of baseline HbA1c, baseline and time-varying BMI and age, were modeled as continuous variables. Insulin regimen exposure, gender and smoking history were modeled as categorical variables. The variable smoking history was categorized into 4 descriptive classes.

In the full model, exposure to the twice-daily biphasic insulin regimen, adjusted for other variables including total insulin exposure, was non-significantly associated with a 0.16% decrease in subsequent HbA1c in comparison to the basal-bolus insulin regimen (p=0.366), see Appendix IV. Meaning, there was no main effect of insulin regimen on HbA1c levels. In any case, on GOF diagnosis, the model was found to have higher information criteria statistics than when regimen category was dropped by stepwise backward deletion, see Table 12. Therefore, the

reduced model gave the best goodness of fit characteristics and the lowest information criteria statistics.[32] A STATA (version 12) output of the post-modeling process is attached as Appendix IV.

Table 12: Multivariate regression model by time-varying HbA1c level

	β±SE	**p**
Age	- 0.010±0.005	0.044
Baseline BMI (kg/m^2)	0.108±0.030	<0.001
Time-varying BMI (kg/m^2)	- 0.135±0.026	<0.001
Baseline HbA1c (%)	0.379±0.021	<0.001
Time in study	- 0.114±0.048	0.017
Insulin regimen:		
Total insulin exposure (IU/day)	0.011±0.003	0.001
Smoking Status:		
0 = Never smoked	Reference	
1 = Stopped > 1 year ago	- 0.401±0.187	0.032
2 = Stopped < 1 year ago	- 0.139±0.468	0.767
3 = Currently smoking	- 0.207±0.201	0.303
Constant	6.972±0.562	<0.001

Model information criteria statistics:
Model AIC=5422.79 (versus 5423.98 with the insulin regimen explanatory variable in the full model)
Model BIC=5478.70 (versus 5484.97 with the insulin regimen explanatory variable in the full model)

Also, an additional year of observation in the study, increasing subsequent BMI, increasing age and stopped smoking for more than a year were associated with statistically significant decreases in HbA1c in the observed patients. Higher baseline HbA1c and BMI levels predicted increased subsequent HbA1c in patients, both at p<0.001, (see Table 12). There were no gender differences in how well blood glucose control was achieved after full or reduced regression modeling in this population (p>0.05).

4.1.11 Effect modification of BMI on insulin exposure for glycaemic control

Potential interactions between baseline BMI or last measured BMI and any insulin dosage regimen or total daily insulin dose on glycaemic control was explored by linear regression using multiplicative interaction terms and stratified analyses, where warranted. The interaction terms generated were not statistically significant ($p>0.05$). For instance, the interaction between the 6 baseline BMI categories delineated above and insulin dosage exposure was explored by linear regression and was non-significantly positively related to the last measured HbA1c level (β±SE) 0.0007±0.007 ($p=0.915$). The interaction between the 6 last measured BMI categories and insulin dosage exposure was also explored by linear regression and was non-significantly positively related to the last measured HbA1c level 0.004±0.006 ($p=0.519$). However, when an interaction term was created between different BMI categories and total daily insulin to assess effect modification between the total daily insulin dose and the time-varying BMI it was statistically significant ($p=0.049$). There was therefore a borderline significant interaction between the total daily insulin dose and time-varying BMI. This interaction confirmed the consistently positive longitudinal association of insulin exposure and BMI found in the “Results” section of this report.

CHAPTER 5

5.1 DISCUSSION

The present study describes longitudinal associations of time-varying BMI with exposure to quarterly prescribed daily insulin dosage regimens in an attempt to achieve optimum blood glucose control, in a sample of T1DM patients at the Diabetes Clinic of Kalafong Hospital, near Pretoria in the Gauteng Province of South Africa. The study also attempted to explore the relationship between prescribed insulin regimens and blood glucose control, as well as baseline characteristics that predicted optimum blood glucose control and weight-gain responses to insulin therapy in the sample of T1DM patients observed. Gender differences in outcomes of insulin therapy were also investigated.

In relation to background prevalence of overweight and obesity, South Africa has a high prevalence of overweight (BMI >25) and obesity (BMI >30). Age, gender, demographics, ethnicity and socio-economic status influence the prevalence of obesity in this setting.[11] More than 29% of men and 56% of women are classified as overweight or obese, with nearly 30% of women aged between 30 and 59 years classified as obese. In a sample of 7 726 women aged 15-95 years old, black women had the highest prevalence of overweight and obesity at 58.5%.[11]

Baseline characteristics by gender:

As expected, females had a statistically significant higher baseline BMI than males.[11] However, the sample for this study exhibited a different distribution of overweight and obesity by gender compared to the general distribution in the South African population. The distribution of baseline overweight and obesity between males and females was observed to be 60 and 69%, respectively. The departure from the almost 2-fold margin between prevalence in males (29%) and females (56%) cited above[11] can be explained by selection bias with regard to the current sample being uniformly exposed to insulin therapy, which has shown a consistently positive association with weight-gain after multilevel linear regression

analysis in most settings.[9,10,18,21,25,26] Although there was no statistically significant gender difference in the baseline dosage of any insulin regimen prescribed, females had a higher baseline HbA1c level than males. This result could be explained by the finding that there was no significant difference of HbA1c levels by baseline BMI (p=0.079) in this sample. The current study also found that more males were current smokers than females. Cigarette smoking has been shown to decrease BMI in numerous cross-sectional studies due to its effects on human metabolic efficiency and rate, as well as decreasing caloric absorption, leading to reduction in appetite.[31]

Baseline sample characteristics stratified by baseline HbA1c level:

When stratified by baseline HbA1c level and also compared to a lower mean baseline weight (mean±SD=73.8±15.4 kg), a higher mean baseline weight (78.8±13.7 kg) was associated with baseline HbA1c levels at or below 8.0% (p=0.02). However, as mentioned before, there was no significant difference of HbA1c levels by baseline BMI. This was adjudged to be consistent with good clinical management practices of considering only BMI, and not weight by itself, in evaluating exogenous insulin needs for blood glucose control in T1DM patients. Also, when compared to baseline HbA1c levels at or below 8%, higher baseline HbA1c levels (above 8%) predicted longer durations of T1DM when such duration is measured up to the time point of last visit to the clinic (16.9±7.7 versus 13.5±7.4 years, p=0.01). This relationship could suggest deterioration in blood glucose control over time the longer the duration of T1DM in this sample of patients.

As was to be expected, when comparing patients with lower baseline HbA1c levels (at or below 8%) with patients at higher baseline HbA1c levels (above 8.0%), patients at higher baseline HbA1c levels were prescribed higher doses of both the twice-daily biphasic insulin (55.6±17.7 versus 49.4±17.7 IU/day, p<0.001) and the basal NPH plus prandial regular insulin (65.4±33.0 versus 57.1±25.0 IU, p<0.001). Patients at lower baseline HbA1c levels were associated with more clinic visits than those at levels above 8.0% (22.4±6.1 versus 19.8±6.8 clinic visits, p=0.01), indicating the independent value of regular clinic visits in controlling blood glucose

levels to within the normal range. Consistent with the unadjusted number of clinic visits variable, patients with more years of observation (5.2±1.5 versus 4.5±1.7 years, p=0.003) were associated with lower baseline HbA1c levels.

Baseline characteristics stratified by baseline BMI category:

Significant differences were found by baseline BMI category in the distribution of age, gender, number of clinic visits, years of observation in the study and smoking status. Baseline BMI increased significantly with baseline age (p=0.001). There was a significant difference in the distributions of males and females by baseline BMI category. There were more males in the BMI category ≤19 to <30 kg/m^2 than females and there were more females in BMI category >30 to >40 kg/m^2 than males (p=0.001). When stratified by baseline BMI, there were no statistically significant differences in the number of clinic visits, as well as the number of years spent in the study. This could indicate that the degree of adherence to scheduled clinic visits was independent of baseline BMI category during the period of observation of this cohort of diabetes patients. That is, patients attended the clinic at more or less the same frequency regardless of baseline BMI status.

Relationship between baseline BMI and BMI measured at last visit (final BMI):

In the 4.7±1.6 years from baseline to last BMI measured, there was a migration of 7 individuals from the lower BMI categories of ≤19 – 30 kg/m^2 to the higher categories of 30.1 to >40 kg/m^2. The migration increased the last mean BMI measurement for the whole sample by a small and highly variable but statistically significant amount of 0.7±3.1 kg/m^2 (p=0.003). The related mean decrease in the last measured HbA1c was an also small and highly variable but statistically significant -1.4±3.4% (p<0.001). In other words, as BMI marginally increased, HbA1c levels marginally decreased.

The finding that BMI trended upwards while HbA1c levels trended downwards was consistent with Nansel et al.,[10] that weight-gain is an often observed outcome of improved glycemic control in adults with T1DM. Nansel et al. also observed that,

multilevel mixed-effects linear regression models have indicated significant inverse relationships of time-varying BMI with time-varying HbA1c in most settings.

Trends of prescribed daily insulin dosages and last measured HbA1c levels stratified by last measured BMI category:

As the last measured BMI category increased, last measured mean HbA1c levels declined by an increasing and statistically significant margin. According to the Diabetes Control and Complications Trial Report,[34] a 1% decline in HbA1c level resulted in a ≥30% reduction of microvascular complications. The authors concluded that a 1% reduction in HbA1c can therefore be considered a clinically meaningful improvement. For this sample, there was a 0.3-0.34% improvement in HbA1c between the 19 to 35 kg/m^2 BMI categories. However, the most clinically significant improvements of >1% in HbA1c occurred between the first two and the last two BMI categories, see Table 8. Interestingly, at the same time there was no statistically significant increase in the mean prescribed daily insulin dose. The latter finding may indicate confounding by dietary advice and/or physical exercise. Exercise can improve HbA1c outcomes and fat metabolism in previously physically inactive adults, without weight-loss.[35] In any case, both variables were not measured and therefore their specific interaction with exogenous insulin exposure for glucose control cannot be known for this particular sample of patients.

Univariate association of subsequent (time-varying) BMI with insulin regimens and other independent variables:

Consistent with findings from other similar studies cited above, unadjusted exposure to any insulin regimen, as well as the twice-daily biphasic insulin compared to the basal NPH plus prandial regular insulin, were statistically significantly associated with weight-gain, both at p<0.001. Also, when not adjusted for other variables, an additional visit to the clinic, an increase in baseline and subsequent HbA1c, being male and current smoking status, were associated with lower subsequent BMI levels. BMI was reduced by 0.046 kg/m^2 for every additional visit to the clinic, by 3.26 kg/m^2 if male and by 2.22 kg/m^2 for current

smokers. In addition, higher baseline BMI levels predicted an increase in subsequent BMI. Increasing age also predicted an increase of 0.142 kg/m^2 in BMI per year. In the sample, observed BMI was also reduced by 0.333 kg/m^2 with every 1% increase in subsequent HbA1c. This loss of weight has been attributed to the possibility of non-compliance with prescribed insulin dosing in other settings,[33] a possibility which should be explored further in this cohort. Another possibility for this type of negative longitudinal association between BMI and HbA1c may have to do with worsening glycaemic control leading to symptomatic autonomic neuropathy and overt nephropathy, which are known predictors of weight-loss in uncontrolled T1DM patients.[21]

Univariate association of subsequent (time-varying) HbA1c with insulin regimens and other independent variables:

When compared to the basal NPH plus prandial regular insulin regimen, unadjusted exposure to the twice-daily biphasic insulin regimen was associated with a reduction in time-varying HbA1c level (better glycaemic control). This meant that, patients on the twice-daily biphasic insulin regimen experienced an unadjusted 0.433% reduction in HbA1c levels on average than patients on a basal NPH plus prandial regular insulin regimen.

Also, when not adjusted by other variables, an additional visit to the clinic, a unit (1 kg/m^2) increase in subsequent BMI, an additional year spent in the study, being male, stopped smoking for more than 1 year and every 1 year increase in age, were associated with varying decreases in subsequent HbA1c levels (better glycaemic control). HbA1c was reduced by 0.054% with every additional visit to the clinic, by 0.046% with every additional year spent in the study, by 0.313% if male, by 0.822% after stopping smoking for more than a year and by 0.038% with every 1 year increase in age. Similarly, every 1% increase in baseline HbA1c predicted a 0.414% higher subsequent HbA1c (poor glycaemic control). Every 1 kg/m^2 increase in baseline BMI predicted a 0.081% decrease in subsequent HbA1c (better glycaemic control). And, HbA1c was decreased by 0.072% with every 1 kg/m^2 increase in time-varying BMI ($p<0.001$). Again, this was consistent

with the observations by Williams et al.,[25] Nansel et al.[10] and others, that weight-gain is often associated with improved glycaemic control in adult T1DM patients in most settings.

Unadjusted total exposure to insulin regardless of regimen was, however, associated with an increase in HbA1c. Every 1 IU/day increase in total daily insulin dose was associated with a 0.011% average increase in time-varying HbA1c level (poorer glycaemic control). The effect size was consistent even after multivariate regression analysis. However, it should be noted that this result was from an unstratified insulin exposure analysis of the association. Therefore the effect size was most probably modified by the inclusion of the 15% of patients who were exposed to the basal NPH plus prandial regular insulin regimen only.

Multivariate association of BMI or HbA1c with insulin regimens and other covariates:

On multilevel mixed-effects linear regression analysis, a number of covariates were associated with BMI in a statistically significant insulin regimen-dependant manner. For instance, and also consistent with Nansel et al.[10] and Williams et al.,[25] the observation that a higher baseline HbA1c predicted a significant increase in subsequent BMI was observed in patients exposed to the twice-daily biphasic insulin dosage regimen in this study population when compared to the basal NPH plus prandial regular insulin regimen group. This finding was consistent with findings by Kulenović et al.,[40] in a study comparing metabolic control indicators in patients with T1DM treated either conventionally (twice-daily biphasic insulin) or by the intensified insulin regimen (IIT). The study found a significantly higher mean value of BMI in the twice-daily biphasic insulin regimen group when compared to the IIT group. The twice-daily biphasic insulin regimen group had a higher BMI outcome value of 23.2±2 kg/m^2 when compared to the IIT group (21.2±1.2 kg/m^2, $p<0.01$). The study also found that the proportion of overweight in the twice-daily biphasic insulin regimen group was significantly higher (27.3% versus 0%, $p=0.012$). In contrast, and as cited in section 2.1.4 above, Conway et al.[21] found an increase of 47% in overweight (BMI >25) and of 700% in obesity (BMI >30), from baselines of 28.6% and 3.4%, respectively, in patients on IIT (≥3 insulin injections

per day or on insulin pump). Although the Conway et al. study did not compare IIT to other regimens, the findings highlight the practical difficulty of reconciling optimum glycaemic control with maintenance of appropriate weight levels in patients on either insulin regimen as currently used in the public health sector. This difficulty was demonstrated in a meta-analysis of randomized controlled trials study by Wang et al.[41] The study evaluated the effects of the biphasic insulin regimen compared with the basal-bolus insulin regimen on weight, glycaemic control, total daily insulin requirements, risk of hypoglycaemia, and quality of life in insulin naïve type 2 diabetes mellitus patients. The authors found that weight, as well as total daily insulin requirements, was increased with both regimens. It should be noted though that the potential confounding of weight outcomes by concomitant exposure to oral antiglycaemic medicines was not reported by the authors. Therefore, in terms of effect size and mechanisms of weight-gain, these findings may not be completely relatable to weight outcomes seen in T1DM patients on insulin replacement therapy alone.

Consistent with the Williams et al.[25] study, a lower baseline BMI was predictive of improvements in glycaemic control when adjusted for exposure to any insulin regardless of regimen and other important covariates ($\beta \pm SE = 0.108 \pm 0.030$, $p < 0.001$). This finding, examined together with the finding that a statistically significant majority of patients at elevated HbA1c levels >8.0% were prescribed higher twice-daily biphasic dosage regimens over the course of the study (65%, mean±SD=55.6±17.7 IU/day) compared to those at HbA1c levels ≤8.0% (35%, 49.4±17.7 IU/day, $p < 0.001$), suggested increased clinical efforts in this cohort of patients to attain optimum blood glucose control in T1DM patients already at elevated BMI levels at baseline.

Also partly consistent with Nansel et al.,[10] in the multilevel mixed-effects linear regression models, when adjusted by exposure to any insulin regardless of regimen, as well as to the twice-daily biphasic insulin regimen specifically, the models indicated significant inverse relationships of time-varying BMI with time-varying HbA1c. Consistent with findings by Conway et al.,[21] that adjusted insulin

therapy to control blood glucose and a higher baseline HbA1c were predictors of weight-gain in T1DM patients, the current study also found significant positive associations between BMI and baseline HbA1c, specifically on exposure to the twice-daily biphasic insulin regimen (when compared to the basal NPH plus prandial regular insulin) and generally on exposure to any insulin regardless of regimen.

The findings of insulin-associated weight-gain are also consistent with those of the Diabetes Control and Complications Trial (DCCT), cited in the review by Kaufman.[9] The trial demonstrated the association between excess weight-gain and adjusted insulin therapy. According to Nansel et al.,[10] these findings support the hypothesis that the inverse association consistently observed between blood glucose control and BMI may be partly attributable to increased insulin administration, independent of regimen.

A previous study by Domargard et al.[24] reported a lack of a significant relationship between baseline BMI and subsequent change in HbA1c. However, the current study found a consistently significant positive relationship ($\beta \pm SE = 0.108 \pm 0.03$, $p < 0.001$), when adjusted for total insulin exposure (see Table 12). According to Nansel et al.,[10] such differences of associations between studies could be attributed to differences in sample characteristics, study designs and statistical analytical methods. Furthermore, Nansel et al. also found the association between BMI and glycaemic control to be complex, highlighting the need for longitudinal study designs and appropriate analyses to account for the time-dependant direction of causation of the observed association. The current study design has attempted to characterise that complex relationship in the observed sample of patients. The curious finding in the current study of a statistically significant association between total insulin exposure and HbA1c level ($\beta \pm SE = 0.011 \pm 0.003$, $p = 0.001$), meaning no improvements in subsequent HbA1c (see Table 12), is consistent with findings by Danne et al.,[20] that there was no corresponding improvement in mean glycaemic control even when average insulin doses were increased in their study.

Effect modification of BMI on insulin exposure for glycaemic control:

When potential effect modification of BMI on insulin exposure for glycaemic control was explored by multiplicative interaction terms, there was no statistically significant interaction between insulin dosage and baseline BMI or last measured BMI. However, the interaction between the 6 baseline BMI categories and insulin dosage, explored by multilevel mixed-effects linear regression, was non-significantly positively related to the last measured HbA1c level. The interaction between the 6 last measured BMI categories and insulin dosage was also explored by multilevel mixed-effects linear regression and was also non-significantly positively related to the last measured HbA1c level. No other statistical or potential clinical interactions were explored further.

The risk factors for weight-gain in T1DM patients:

The current study identified insulin exposure, high baseline HbA1c and baseline BMI to be significant predictors of weight-gain in the cohort of patients observed. These findings are consistent with those of Conway et al.[21] from a longitudinal study to determine the prevalence and incidence of overweight and obesity in a cohort of 589 T1DM patients, followed up over at least 18 years. Analysis of the data revealed that IIT and a higher baseline HbA1c were predictors of weight-gain in these patients. The Diabetes Control and Complications Trial (DCCT), cited in the review by Kaufman,[9] also demonstrated the association between excess weight-gain and IIT.

CHAPTER 6

6.1 LIMITATIONS AND CONCLUSIONS

6.1.1 Limitations

This was an observational study of primary data routinely collected for clinical management of T1DM patients at the Diabetes Clinic of Kalafong Hospital. The data was therefore not collected explicitly for research purposes. As a result, there were a number of missing measurements, either not recorded or not measured. That state of affairs is expected to introduce some bias in the data set, which is difficult to account for. For instance, the effect of age of T1DM onset on the degree of weight originally stated as an objective could not be investigated because 80 patients or 38% out of a full sample of 211 patients had missing data on the time of first T1DM diagnosis. Also, compliance with prescribed doses, frequency and timing of dosing could not be verified in all patients due to the observational (uncontrolled) design of this study.

Missing measurements in this dataset were manually observed to follow the so-called 'item non-response' distribution (i.e. data missing in some cases, for some variables), as opposed to 'unit non-response', where there would be data missing on all variables for some cases.[23] The effects of missing data were mitigated by including in the study only those patients with a maximum of 2 missing consecutive visits and a maximum of 3 missing random non-consecutive visits and measurements.

Current statistical methods and software presume that all variable measurements in a specified model are accounted for. In an instance where that is not the case, conventional statistical software simply deletes cases with missing data on the variables of interest by 'complete case analysis' or by 'listwise deletion'.[23] Where the deletion affects a large proportion of the sample, it results in loss of statistical power to detect significant differences in effect sizes, a Type II error. Therefore, the last observation carried forward (LOCF) multiple imputation method was used for missing quarterly prescribed insulin dose data in order to preserve some statistical power in the study. In any case, prescribed insulin doses were observed

to be fairly constant over time with very few exceptions, thus providing confidence that the last observation carried forward was as close to reality as possible. Also, one other affected variable was weight measurements at each quarterly visit, which followed an 'item non-response' missingness distribution defined above. This variable was, however, not imputed for randomly missing data, but was rather analysed as is without the 27.6% missing measurements, to avoid possible measurement bias. As a result, it is highly probable, based on the literature, that the observed magnitude of BMI increases in this sample was underestimated by the missing 27.6% quarterly-repeated weight measurements in the sample.

In addition, no patients in this study were on analogue insulins. These include the rapid-acting aspart, lispro and glulisine analogues, the long-acting basal glargine and detemir analogues, as well as the premixed insulin analogue formulations. These types of insulin have been formulated to more closely mimic a normal insulin release profile.[26,39] However, the diabetes treatment protocol at Kalafong Hospital is similar to that in other South African government hospitals in that, only human insulins are available and not the more physiologically compatible analogue insulins. These analogues may be much better than the traditional human insulin currently used at the Kalafong Diabetes Clinic. Successful management of T1DM relies on how closely the prescribed regimen mimics normal physiological insulin release patterns.[26,39] A question for further research is: whether the analogue insulins will have the same increase in BMI, as well as the same non-impressive improvements in HbA1c seen in this sample.

Furthermore, findings from the current study are limited to a single ethnic demographic and may not be generalizable beyond those confines. As with the Nansel et al. study,[10] another limitation was that no diet or physical activity data were available to determine their contribution to time-varying BMI in particular. Also, the relative contributions of fat and fat-free mass to change in BMI could not be determined as body composition measurements were not recorded. However, and in line with findings by Nansel et al., the current study indicated no significant difference in baseline BMI according to baseline HbA1c level ≤ or > 8% (p=0.079), suggesting that the marginally higher baseline BMI at HbA1c ≤8% (28.6±5.2 kg/m^2

vs. 27.2±5.7 kg/m^2) may be attributed to increased fat mass. As a consequence, Nansel et al. advise that measures of body composition, as well as blood lipids, be recorded for future longitudinal studies of this association between BMI and HbA1c in T1DM patients on insulin therapy, given the different health implications of fat and fat-free mass.[10]

6.1.2 Conclusions

Baseline characteristics indicated obvious differences in age, height, weight, exposure to the twice-daily biphasic regimen and baseline HbA1c when stratified by BMI category, but other covariates were equally distributed. Exposure to any insulin regimen to control blood glucose levels in T1DM appeared to significantly increase BMI to varying extents. An increase in HbA1c level during insulin therapy appears to reduce BMI particularly in the twice-daily biphasic regimen group.

There was no evidence of optimum quarterly prescribed daily dosage increases of insulin that safely improved and maintained blood glucose control, without increasing body weight. Both regimens consistently and independently increased weight in particularly female patients, without clinically significantly improving blood glucose control. The study also found that an increase in baseline BMI and HbA1c predicted weight-gain regardless of insulin regimen.

There were significant gender differences in the change in body weight on exposure to any insulin to control blood glucose to optimum levels. Males tended to experience reductions in time-varying BMI on exposure to the twice-daily biphasic regimen, the regimen prescribed for 85% of patients in this cohort and equally spread by gender. However, the study concluded that exposure to adjusted doses of insulin to achieve optimum glycaemic control in T1DM patients resulted in a statistically significant increase in BMI. This relationship seemed to be more prominent in female patients and in patients at higher baseline HbA1c levels and BMI categories, respectively. Conclusions can also be drawn that increasing BMI is consistent with improvements in blood glucose control in clinical

settings. Strategies should therefore be found to control weight-gain through physical exercise, dietary and lifestyle advice as part of clinical management.

The unadjusted number of clinic visits was marginally but significantly positively associated with a reduction in both BMI and HbA1c in the cohort observed, reflecting the potential value of regular clinic check-ups in monitoring the safety and efficacy outcomes of insulin therapy. Based on the literature[35] and the unadjusted observed association of clinic visits with a reduction in both BMI and HbA1c, it is feasible that dietary and physical exercise advice during clinic visits also had a role in keeping treatment-related BMI increases in check in this sample. The same could be said for the observed improvements in blood glucose control. Stopping smoking for more than a year was also observed to have a positive effect on BMI and HbA1c outcomes after multilevel mixed-effects linear regression analysis. It could be ventured that the motivation to stop smoking was just an indicator of general healthy-state seeking behaviours such as, perhaps, a healthier diet and some physical exercise, hence the marginal significance of the association in relation to the observed improvements. The study also found that time-varying HbA1c levels were positively associated with exposure to insulin after regression analysis. The latter association could be intuitively clarified by the fact that insulin doses would naturally be increased in patients who are experiencing increasing HbA1c levels during clinical management of T1DM.

The strength of this study was mostly based on the longitudinal design and a fairly large, if not geographically and ethnically homogenous, sample. These study characteristics can be considered as supportive of the external validity of the findings to black T1DM patients in the observed age groups (18 to 78 years) attending the Kalafong Hospital Diabetes Clinic. In using both cross-sectional and longitudinal statistical regression analytic methods, also adjusting for clinically important covariates, the internal validity of the findings was also strengthened.

6.2 RECOMMENDATIONS

According to findings of the current study, high baseline BMI and HbA1c levels predicted increases in subsequent BMI and HbA1c levels. Such patients should therefore be monitored with extra vigilance to prevent further worsening of their condition during intensive therapy. The therapeutic strategy should also involve intensive counseling regarding known risk-mitigating dietary and physical exercise interventions. Therefore, regular attendance of scheduled check-ups, which encompasses dietary and physical exercise advice, may be advisable, especially in females and patients presenting with elevated BMI and HbA1c levels on first clinic encounter. It could also be advised that an increase in subsequent BMI may not necessarily be bad for HbA1c control, which further highlights the need for health delivery strategies of treatment and compliance monitoring that minimize weight-gain while optimizing blood glucose control in T1DM patients.

6.3 ADDITIONAL COMMENTS

Although not by any means the objective of this study, the study has highlighted the need for measures to be put in place to design routine clinical data-collection systems and processes that are deliberately geared toward scientific data mining methods in public hospitals. This approach will assist to promote and facilitate sound observational medical research in those settings. It is however recognized that such systems may be resisted at the various workplaces due to work pressures and health system weaknesses. In that case, the management challenge will then have to be in finding ways of educating and integrating scientific approaches to routine administrative and medical data-collection practices at the workplace that do not add an additional burden on already overworked health care staff.

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Appendix I Site Permission to conduct research letter

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

KALAFONG HOSPITAL
PRIVATE BAG X396
PRETORIA
0001
6 MARCH 2015

ENQUIRIES : DR D. UBOMBA
TEL : 012 318 6503
FAX : 012 373 9021

To Mr Sehloho

RE: PERMISSION TO CONDUCT RESEARCH

Title: Effect of insulin dose adjustment for glycaemic control on body mass index: a retrospective cohort study of adult type1 diabetes patients at the Kalafong diabetes clinic between 2009 and 2014.

Permission is hereby granted for the research to be conducted at Kalafong Hospital. This approval is given on the condition that ethics clearance will be obtained from the training institution ethics committee.

Upon completion of the project. Please send a copy of the research report to my office.

DR D. UBOMBA
MEDICAL MANAGER
KALAFONG HOSPITAL

NG PROVINCIAL GOVERNMENT
KALAFONG HOSPITAL / HOSPITAAL
06 MAR 2015
PRIVATE BAG / PRIVAATSAK X396 PRETORIA 0001
NTENDENT'S OFFICE

Appendix II

Model MSExcel data collection form:

Page 1 and …

Panel/id. (Hosp. no.)	Time (Δ=1)	Clinic visit/ Script no.	Baseline Wt (kg)	Wt (kg)	Ht (cm)	Baseline BMI (kg/m^2)	BMI (kg/m^2)
11111111	1	1					
11111111	2	2					
11111111	3	3					
11111111…	4…	4…					
22222222	1	1					
22222222	2	2					
22222222…	3…	3…					
33333333	1	1					
33333333…	2…	2…					

Page 1 extended...

Baseline HbA1c (%)	HbA1c (%)	Sex	Smoking status	Age	Basal dose	Biphasic dose	Prandial dose

Page 1 extended.

Total dose							

Appendix III:

1. Time-varying BMI full model (AIC=5627.19; BIC=5693.26)

```
. xtmixed bmi basebmi hba1c basehba1c obstime i.gender i.smoking age totaldose i.dummyregimen

Mixed-effects ML regression                     Number of obs      =      1191

                                                Wald chi2(11)      =   5378.10
Log likelihood = -2800.5934                     Prob > chi2        =    0.0000
```

bmi	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
basebmi	.9780737	.0163259	59.91	0.000	.9460755	1.010072
hba1c	-.1571431	.0312012	-5.04	0.000	-.2182963	-.0959898
basehba1c	.0913229	.0256073	3.57	0.000	.0411335	.1415122
obstime	-.1112205	.0521899	-2.13	0.033	-.213511	-.0089301
1.gender	-.608802	.1796101	-3.39	0.001	-.9608313	-.2567728
smoking						
1	-.4461955	.2229546	-2.00	0.045	-.8831785	-.0092126
2	-.8493956	.5180948	-1.64	0.101	-1.864843	.1660515
3	-.4140842	.2322508	-1.78	0.075	-.8692874	.0411189
age	-.0035535	.0060525	-0.59	0.557	-.0154163	.0083093
totaldose	.0092661	.0034888	2.66	0.008	.0024282	.016104
1.dummyreg~n	.4976655	.1905964	2.61	0.009	.1241035	.8712275
_cons	2.036243	.6677207	3.05	0.002	.7275348	3.344952

Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
sd(Residual)	2.540918	.0520619	2.4409	2.645034

```
. estat ic
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
.	1191	.	-2800.593	13	5627.187	5693.26

Note: N=Obs used in calculating BIC; see **[R] BIC note**

2. Time-varying BMI reduced model (AIC=5693.39; BIC=5754.52)

```
. xtmixed bmi basebmi hba1c basehba1c obstime i.gender i.smoking totaldose i.dummyregimen

Mixed-effects ML regression                     Number of obs      =      1205

                                                Wald chi2(10)      =   5386.11
Log likelihood = -2834.6935                     Prob > chi2        =    0.0000
```

bmi	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
basebmi	.9727674	.0147798	65.82	0.000	.9437995	1.001735
hba1c	-.1552423	.0309833	-5.01	0.000	-.2159686	-.0945161
basehba1c	.0910975	.025391	3.59	0.000	.0413321	.1408629
obstime	-.1108314	.0517788	-2.14	0.032	-.212316	-.0093469
1.gender	-.6115922	.177664	-3.44	0.001	-.9598072	-.2633772
smoking						
1	-.4712153	.2207386	-2.13	0.033	-.903855	-.0385756
2	-.8589421	.5184145	-1.66	0.098	-1.875016	.1571317
3	-.4791855	.2268076	-2.11	0.035	-.9237202	-.0346509
totaldose	.0094743	.0034465	2.75	0.006	.0027192	.0162294
1.dummyreg~n	.4514128	.1875464	2.41	0.016	.0838286	.818997
_cons	2.047756	.6630462	3.09	0.002	.7482088	3.347302

Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
sd(Residual)	2.543406	.0518092	2.443862	2.647005

```
. estat ic
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
.	1205	.	-2834.694	12	5693.387	5754.518

Note: N=Obs used in calculating BIC; see **[R] BIC note**

Appendix IV:

1. Time-varying HbA1c full model (AIC=5423.98; BIC=5484.97)

```
. xtmixed hba1c bmi basebmi basehba1c obstime i.smoking age totaldose i.dummyregimen

Mixed-effects ML regression                     Number of obs      =      1191

                                                Wald chi2(10)      =    484.78
Log likelihood =  -2699.988                     Prob > chi2        =    0.0000
```

hba1c	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
bmi	-.133539	.0262177	-5.09	0.000	-.1849247	-.0821532
basebmi	.1073527	.0298616	3.60	0.000	.0488251	.1658804
basehba1c	.3799304	.0207767	18.29	0.000	.3392089	.4206519
obstime	-.1123392	.0478981	-2.35	0.019	-.2062177	-.0184606
smoking						
1	-.3784442	.1885967	-2.01	0.045	-.748087	-.0088014
2	-.1163845	.468685	-0.25	0.804	-1.03499	.8022211
3	-.1993939	.2009601	-0.99	0.321	-.5932685	.1944807
age	-.010379	.0055339	-1.88	0.061	-.0212253	.0004673
totaldose	.0110242	.0031982	3.45	0.001	.0047558	.0172926
1.dummyreg~n	-.1577344	.1746584	-0.90	0.366	-.5000586	.1845898
_cons	6.998558	.5616926	12.46	0.000	5.897661	8.099455

Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
sd(Residual)	2.335098	.0478447	2.243182	2.43078

```
. estat ic
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
.	1191	.	-2699.988	12	5423.976	5484.967

Note: N=Obs used in calculating BIC; see **[R] BIC note**

.

2. Time-varying HbA1c reduced model (AIC=5422.71; BIC=5478.70)

```
. xtmixed hba1c bmi basebmi basehba1c obstime i.smoking age totaldose

Mixed-effects ML regression                     Number of obs      =      1191

                                                Wald chi2(9)       =    483.63
Log likelihood = -2700.3956                     Prob > chi2        =    0.0000
```

hba1c	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
bmi	-.1352109	.0261612	-5.17	0.000	-.1864859	-.0839359
basebmi	.1077064	.0298693	3.61	0.000	.0491638	.1662491
basehba1c	.37882	.0207473	18.26	0.000	.3381559	.419484
obstime	-.1141159	.0478741	-2.38	0.017	-.2079474	-.0202845
smoking						
1	-.4010196	.1869967	-2.14	0.032	-.7675265	-.0345128
2	-.1388617	.4681839	-0.30	0.767	-1.056485	.7787619
3	-.2069441	.2008549	-1.03	0.303	-.6006125	.1867242
age	-.0110517	.0054854	-2.01	0.044	-.021803	-.0003005
totaldose	.0109529	.0031983	3.42	0.001	.0046843	.0172215
_cons	6.972439	.5611396	12.43	0.000	5.872625	8.072252

Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
sd(Residual)	2.335897	.0478611	2.24395	2.431613

```
. estat ic
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
.	1191	.	-2700.396	11	5422.791	5478.699

Note: N=Obs used in calculating BIC; see **[R] BIC note**

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Appendix V

Copy of the Faculty of Health Sciences Ethics Approval Certificate (Ref. No. 94/2015), dated 29 April 2015. The signed copy is available on request.