CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research question:

Is post-prandial blood glucose monitoring better than pre-prandial blood glucose monitoring in term of improving glycemic control in type 2 diabetic subjects treated with insulin?

3.2 Research objectives:

Primary objective:

To compare the effect on glycemic control between pre-prandial and post-prandial blood glucose monitoring in insulin treated type 2 diabetes subjects.

Secondary objectives:

a) To compare the effect of pre-prandial and post-prandial self-monitoring of glucose on body weight, total daily dose of insulin, lipid profiles in insulin treated type 2 diabetes patients.

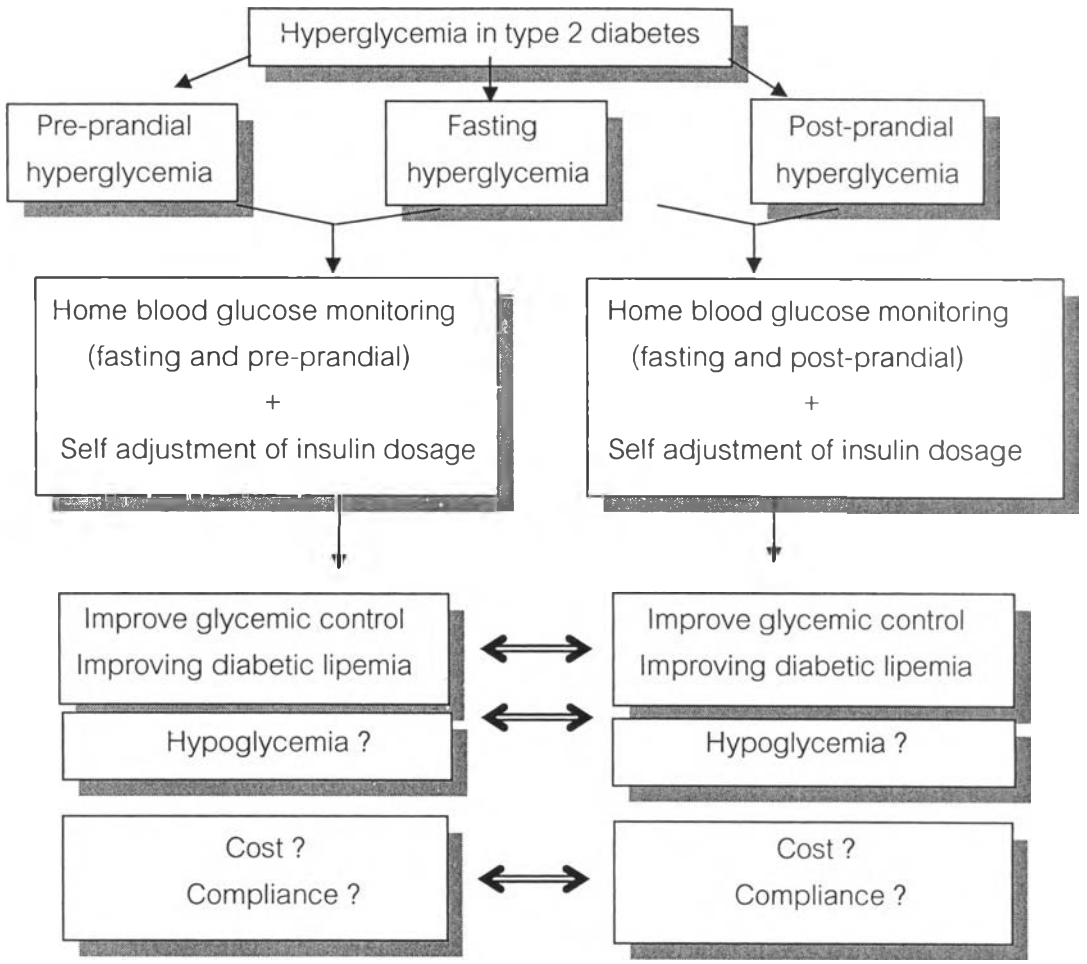
b) To compare the incidence of hypoglycemic episodes between pre-prandial and post-prandial self-monitoring of glucose

c) To compare the cost of pre-prandial and post-prandial blood glucose monitoring.

3.3 Hypothesis

In insulin treated type 2 diabetes subjects, post-prandial blood glucose monitoring with self insulin adjustment leads to a better glycemic control compared to pre-prandial blood glucose monitoring.

3.4 Conceptual framework



3.5 Keywords:

Type 2 diabetes mellitus, preprandial, postprandial, self-blood glucose monitoring, glycemic control.

3.6 Operational definition:

Preprandial state: state or time period between 4-6 hours after the last meal

Postprandial state: state or time period within 4 hours after the meal

Fasting state: state or time peroid more than 6 hours after the meal

Self monitoring of blood glucose: fingertip blood glucose testing at home by the subject using a portable glucometer.

3.7 Research design

This study was a randomized un-blinded parallel group study design, with 16 weeks follow-up period to assess efficacy/effectiveness of pre- and post-prandial self monitoring of blood glucose at home in type 2 diabetes patients who are currently treated with insulin alone or in combination oral hypoglycemic agents. Duration of the entire study from first patient enrolled to last patient visit is estimated to be 6 months. Enrollment is estimated to take 2 months.

A run-in period of 2-4 weeks was required to screen for inclusion and exclusion criteria and to assess the eligibility. After signing the inform consent, the enrolled subjects was asked to perform self blood glucose monitoring at home with a fixed scheduled regimen including; daily fasting glucose, pre-prandial (prelunch, predinner) and post-prandial (postbreakfast, postlunch, postdinner) as well as bedtime blood glucose. The investigators contacted the subjects and adjusted the insulin dosage by phone during this two-week period.

Subject was then randomized to either pre-prandial monitoring or post-prandial monitoring group by using block randomization with the block size of 4. Subject was asked to perform home blood glucose monitoring according to the assignment with a fixed-schedule regimen (twice daily, daily fasting plus one preset pre-prandial or post-prandial blood glucose testing) for 8 weeks. Then the subject was asked to perform home blood glucose monitoring with a flexible-regimen (at least 7 times per week or 1 time/day, but not more than 28 times per weeks or 4 times/day). It was recommended that approximately half of the tests would be fasting and half of the tests would be any time points (pre-prandial, post-prandial or bedtime glucose measurement). For safety concern, the subjects were allowed to measure the blood glucose at any time when hypoglycemic symptoms are highly suspected. Subjects were also instructed to self-adjust the dose of insulin by 1-2 units, according to the blood glucose testing results. (see table 15)

Table 12 Study design

← Run-in period →		← Study period →		
Screening & Eligibility assessment	Training and insulin dosage adjustment	Randomization	8 weeks fixed-scheduled pre or post regimen	8 weeks liberated-scheduled pre and/or post regimen
Days -28 to -15	Days -14 to -1	Day 0	Day 1-56	Day 57-112
Visit 1	Visit 2	Visit 3	Visit 5	Visit 6

While the primary efficacy endpoint was a change in fructosamine and HbA_{1c} level at the end of 8 and 16-week period of treatment, the treatment effect was also evaluated on body weight changes, total amount of insulin, hyper- and hypo-glycemic episodes, lipid profiles and changing of self care behaviors such as dietary habit and activities and number of self-blood glucose testing.

Memory-based portable glucose meters were used to measure blood glucose levels. Compliance was assessed by reviewing of patients' monitoring record as well as the meter's memory record. Comparison of both records was done for reliability of the results.

3.8 Population

Target Population

Insulin treated type 2 diabetes subjects

Study population

Insulin treated type 2 diabetic subjects who attend the diabetic clinic at King Chulalongkorn Memorial Hospital

Inclusion and exclusion criteria

Inclusion criteria

Diabetic subjects, of either sex, age 30 to 70 years with a documented history of type 2 diabetes mellitus according to WHO criteria.

1. Subjects currently treated with insulin (two shots per day) or insulin combined with metformin for at least 6 months
2. HbA_{1C} 6.5 – 10.0%
3. Fasting plasma glucose (FPG) $\geq$ 100 mg/dl to $\leq$ 180 mg/dl).
4. Body Mass Index (BMI) of 20 to 30 Kg/m^2
5. Not having received any investigational drug within 30 days prior to enrolment in the study.
6. Written informed consent to participate in the study
7. Ability to communicate, and comply with all study requirements, e.g., report for scheduled study visits between 7:00 and 10:00 a.m.

Exclusion Criteria

1. Pregnant or lactating female.
2. History of type 1 diabetes mellitus, diabetes that is a result of pancreatic injury, or secondary forms of diabetes, e.g., Cushing's syndrome and acromegaly.
3. History of acute metabolic diabetic complications such as ketoacidosis or hyperosmolar nonketotic coma within 6 months.
4. Previous treatment with investigational drugs within 30 days prior to the study.
5. Any of the following significant medical conditions:
    i) A myocardial infarction (MI), coronary artery surgery, ventricular tachycardia, ventricular fibrillation or stroke within the past 6 months.

ii) Patients with decompensated heart failure (NYHA class III and IV) or diagnosis of unstable angina or uncontrolled hypertension (BP ≥ 190/105 mmHg).

iii) Known unawareness of hypoglycemia.

iv) Liver disease such as cirrhosis or chronic active hepatitis or persistent alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase three times greater than the upper limit of normal (ULN).

v) Acute infections which may affect blood glucose control within the past 4 weeks.

vi) Concurrent medical conditions that may interfere with the interpretation of efficacy and safety data during the study.

vii) Current treatment with systemic corticosteroid or any other medication known to affect glucose homeostasis (e.g. non selective beta blocker, thiazide diuretics).

viii) A disease state that judged by the investigator, could interfere with study participation or study evaluation.

3.9 Sample size estimation:

By using the formula (see appendix 1)

$$n = \frac{\sigma^2_e [Z_{1-\alpha/2} + Z_{1-\beta}]^2}{\Delta^2}$$

where n = number randomly allocated to each group

σ^2_e = standard deviation of HbA1c

α = type 2 error

β = type 1 error

Δ = expected mean difference of HbA1c

By assuming that an expected mean difference of absolute HbA1c level 0.4% of with standard deviation of 0.05 would be achieved, a sample size of 36 per group was

needed for a two-sided significance level of 5% and 90% power. To accommodate for the possible 10% dropout rate and noncompliance, approximately 40 subjects per group should be a randomized.

3.10 Observation and Measurement:

Efficacy and effectiveness assessments

All subjects recruited were requested to complete a questionnaire investigating SMBG practice, the presence and severity of diabetes complications and co-morbidities. In particular, the performance of SMBG was assessed by questioning frequency of testing. This information was cross-checked with the data obtained from the glucose meter's memory record, to reassure the reliability of the information. Patients were also asked to report the frequency of hypoglycemic symptoms (sweating, weakness, trembling) on a five-point scale ranging from ≥1 time per week to never.

Plasma glucose was assessed at the endocrinology laboratory employing a glucose oxidase method. Fructosamine and HbA_{1c} were assessed by Immunoassay method using Cobras Mira Integra analyzer (Roche Diagnostic, USA). Lipid profiles, including total cholesterol, triglycerides, HDL-cholesterol were assessed at the central laboratory by standard enzymatic method.

Assessment of fructosamine and HbA_{1c}

Fructosamine and HbA_{1c} were assessed at randomization, and at the end of 8^{th} and 16^{th} week of each monitoring program.

For primary efficacy assessment and change in HbA_{1c} levels at 8^{th} and 16^{th} week, only patients who have completed per protocol study were included.

Patients who are evaluated for the per protocol efficacy analyses are as listed below:

- Patients who fulfill inclusion and exclusion criteria, unless otherwise agreed by the study group.

- Patients who have complied with treatment, according to the compliance assessment:
  - For patients who take less than 80% or more than 120% of planned numbers of glucose testing, the evaluability were considered. The distribution and missed performing would be taken into consideration.
- Patients who have complied with the protocol in general and do not meet any withdrawal criteria.

7-point blood glucose profiles (optional)

Six subjects from each group were randomly selected and asked to perform a 7-point blood glucose profile (fasting, before and after each meal, and at bedtime on the same day) one day during the 8^{th} and 16^{th} week, on a typical weekday.

Assessment of safety

Hypoglycemic episodes were recorded by the subject in his/her diary throughout the trial. The recording included date, time, symptoms and blood glucose level.

Safety assessments were based on history especially of hypoglycemia, clinical examination including vital signs, laboratory tests. Abnormal laboratory tests were listed by subject. Adverse events (AEs) were summarized by event frequency and frequencies of patients with events.

3.11 Intervention

Treatment assignment

Patients were randomized to either one of the monitoring program for the first 8 weeks period. Randomization was performed by the investigator using a pre-assignment randomization numbers.

For the pre-prandial blood glucose monitoring program assignment: subjects were asked to perform a self blood glucose monitoring at home only in the morning (fasting) before breakfast, before lunch and dinner time (within 30 minutes before meal) and record in the record book provided.

For the post-prandial blood glucose monitoring program assignment: subjects were asked to perform a self blood glucose monitoring at home only in the morning (fasting), within1-2 hr period after breakfast, lunch or dinner time and record in the record book provided.

All subjects were prescribed a diet of 30 to 35 kcal/kg of ideal body weight per day. Calorie intake and food choices were taught by a dietitian at the beginning of the study and were asked to keep constant throughout the study period.

Concomitant therapy

All treatment being undertaken by the patient on entry to the study and any subsequent treatment given during the study, in addition to the study medication, was regarded as concomitant treatment.

Any changes in concomitant medications were recorded at each visit. Prohibited concomitant therapy includes oral corticosteroids or any other medication known to affect glucose homeostasis (e.g. non selective beta blocker, thiazide diuretic, corticosteroids, etc.).

Treatment Compliance

The memory-based portable glucose meters were dispensed to the subjects after signing the inform consent. Compliance and performance of the blood glucose monitoring were checked at each visit. The number, time and results of the blood glucose testing from the glucose meter's memory record were checked to see that they were consistent with the numbers that were recorded in the record book. The percentage of compliance was calculated at the end of the study. Patients who monitor their blood glucose with an average of less than 4 times/week or more than 14 times/week during the whole period of the study were considered as "non-compliance" However these patients would be included in the intention to treat and safety analyzes.

Table 13 Self-adjustment of insulin dosage guideline for pre-prandial monitoring

	Blood glucose (mg/dl)	Action
Fasting plasma glucose	<60	decrease morning insulin dosage by 2 units *contact investigator*
	60 -100	decrease morning insulin dosage by 1 unit
	100- 140	inject the same dose
	140 -180	increase morning insulin dosage by 1 unit
	180 - 220	increase morning insulin dosage by 2 units
	220 - 280	increase morning insulin dosage by 3 units
	>280	increase morning insulin dosage by 4 units *contact investigator*
Pre-prandial plasma glucose (lunch)	<60	have meal earlier, *contact investigator*
	60 -160	no action
	160 -280	add short acting insulin 2 units before meal
	> 280	add short acting insulin 3 units before meal
Pre-prandial plasma glucose (dinner)	<60	have meal earlier, *contact investigator*
	60-160	inject the same dose
	160 -280	increase morning insulin dosage by 1 unit
	>280	increase morning insulin dosage by 2 units
Bedtime plasma glucose	<60	drink one cup of juice, *contact investigator*
	60 - 160	no action
	160 -280	add short acting insulin 2 units before meal
	> 280	add short acting insulin 3 units before meal

Table 14 Self-adjustment of insulin dosage guideline for post-prandial monitoring

	Blood glucose *(mg/dl)*	Action
Fasting plasma glucose	<60	decrease morning insulin dosage by 2 units *contact investigator*
	60 -100	decrease morning insulin dosage by 1 unit
	100- 140	inject the same dose
	140 -180	increase morning insulin dosage by 1 unit
	180 - 220	increase morning insulin dosage by 2 units
	220 - 280	increase morning insulin dosage by 3 units
	>280	increase morning insulin dosage by 4 units *contact investigator*
Post-prandial plasma glucose (breakfast)	<60	drink fruit juice, *contact investigator*
	60 - 180	no action
	180 -280	inject short acting insulin 2 units
	> 280	inject short acting insulin 4 units
Post-prandial plasma glucose (lunch)	<60	drink fruit juice, *contact investigator*
	60 - 180	no action
	180 - 280	inject short acting insulin 2 units before meal
	> 280	inject short acting insulin 4 units before meal
Post-prandial plasma glucose (dinner)	<60	drink fruit juice, *contact investigator*
	60 - 180	no action
	180 - 280	inject short acting insulin 2 units before meal
	> 280	inject short acting insulin 4 units before meal
Bedtime plasma glucose	<60	drink one cup of juice, *contact investigator*
	60 - 160	no action
	160 -280	add short acting insulin 2 units before meal
	> 280	add short acting insulin 3 units before meal

Table 15 Sample of the self blood glucose monitoring record

A: Example of twice daily rotating schedule

Date	Insulin type	Dosage		Blood glucose						
		morning	evening	Breakfast		Lunch		Dinner		bedtime
				pre	post	pre	post	pre	post	
1/4/02	Mixtard30	16	8	110	135					
2/4/02	Mixtard30	16	8	96		122				
3/4/02	Mixtard30	17	8	118			142			
4/4/02	Mixtard30	16	8	150				138		
5/4/02	Mixtard30	16	8	124					160	
6/4/02	Mixtard30	16	8	110						140
7/4/02	Mixtard30	16	8	124	188					

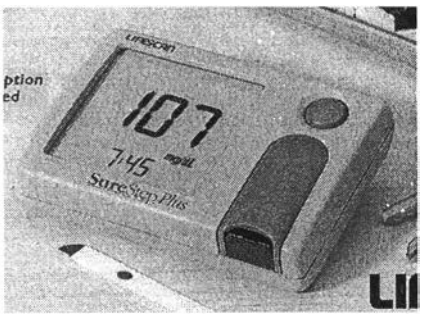
B: Example of twice daily random schedule

Date	Insulin type	Dosage		Blood glucose						
		morning	evening	Breakfast		Lunch		Dinner		bedtime
				pre	post	pre	post	pre	post	
1/4/01	Mixtard30	16	8	96				122		
2/4/01	Mixtard30	17	8			118	142			
4/4/01	Mixtard30	16	8					138		150
5/4/01	Mixtard30	16	8	124		160				
7/4/01	Mixtard30	16	8	110			124			140
8/4/01	Mixtard30	16	8		188				160	
11/4/01	Mixtard30	16	8	100		118				

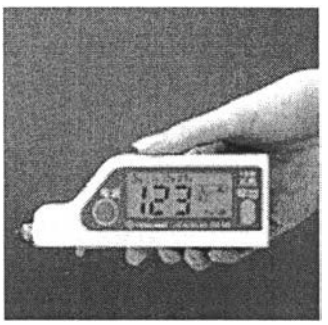
C: Example of insulin dosage adjustment

Date	Insulin type	Dosage		Blood glucose (Pre/Post)				Note
		morning	evening	fasting	breakfast	Lunch	Dinner	
1/4/01	Mixtard30	16	8	110				
2/4/01	Mixtard30	16	8			130		
4/4/01	Mixtard30	17	8	150			140	
5/4/01	Mixtard30	16	8					
7/4/01	Mixtard30	16	8	110		188		RI 2 units before lunch

Figure 8 Example of Memory-based portable glucose meters



SureStep



Medisafe

Precision QID

Glucotrend

Table 16 Visit schedule and assessments

	Run-in period		Randomization	Study period	
	Screening& Eligibility Assessment	Insulin dose titration & SMBG training		Pre / Post monitoring	Liberated monitoring
Day	-28 to -15	-14 to -1	0	56	112
Visit week	-3 to -1	-1	0	8	16
Visit number	1	2	3	4	5
Informed consent	X				
Inclusion/ exclusion criteria	X	X			
Medical history and diabetic history	X				
Concomitant medications/ illness	X	X	X	X	X
Height	X				
Physical examination	X	X+	X+	X+	X
Weight and vital signs	X	X	X	X	X
Urinalysis	X				X
Clinical chemistry	X	(X)*			X
HbA1c	X	(X)*		X	X
Fasting Plasma glucose	X	(X)*	X	X	X
Adverse events			X	X	X
Glucometer dispensed		X			
Compliance assessment				X	X
7-point CBG				X	X

* Review of test results only + Brief physical examination

3.12 Data Management

Data collection

The primary outcome of this study was the glycemic control during the 8-week period of each monitoring program which is represented by fructosamine and HbA1c level. The second parameter was the 7-points home blood glucose profiles. The third parameter was the glycemic control during 8^{th} -16^{th} –week period.

The secondary outcomes of the study were the number of hypoglycemic and hyperglycemic episodes, the biochemical profiles such as serum lipoproteins, free fatty acid, etc. Body weight and total number of blood testing would also be recorded.

3.13 Data analysis

Statistical analysis of the outcomes

Data were summarized with respect to demographic and baseline characteristics, efficacy observations and measurements, safety observations and measurements.

All demographic, clinical history and baseline characteristics of patients included into the study were presented. The comparability between the two treatment groups regarding demographic and other baseline data were assessed using appropriate statistical methods such as Student's t-test..

The change in HbA_{1c} from baseline at 8-week and 16-week was calculated for each group of patients. Mean change of HbA_{1c} of each arm of treatment were compared using Student's t-test.

The primary efficacy variable, the HbA1c values was analyzed using a linear model, in tables and graphs by treatment group and study duration. The percent change or an absolute change (in %) from baseline (premeal) to the endpoint on 8^{th} week and 16^{th} week were also described by summary statistics.

The secondary efficacy variables (mean reduction of pre-prandial and 2-h post-prandial plasma glucose level, lipid profiles, body weight) were analyzed in a

similar way as the primary efficacy variable. All other efficacy variables were analyzed descriptively.

Economical analysis of the outcomes

The total cost of self blood glucose monitoring of both pre-prandial and post-prandial program were calculated. Comparing the of different frequency of monitoring was also calculated.

3.14 Ethical consideration and Good Clinical Practice

This study was carried out in compliance with the protocol and adhered to Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996

2. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, were reviewed and approved by the Chulalongkorn's Institutional Review Board/Ethics Committee.

Informed consent

The Investigator explained to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it might entail. Each subject was informed that participation in the study was voluntary and that he/she might withdraw from the study at any time and that withdrawal of consent would not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent was given by means of a standard written statement, written in non-technical language. The subject was given a copy of the signed document. No patient entered the study before his/her informed consent had been obtained.

3.15 Limitation

Some limitations are expected in this study. First; the generalizablity of the study results are probably limited. The subjects enrolled in this study were type 2 diabetes subjects currently using insulin. The results could not apply for the type 2 diabetes subjects who were not having insulin therapy. Secondly: the duration of study is 16 weeks which could be considered as too short in terms of long term benefits or too long in terms of adaptive behavioral changes. Most diabetic subjects will improve their glycemic control when initiating the home monitoring especially the first few weeks.

3.16 Expected Benefit of the study and Application

The results from this study could be applied to the clinical practice in daily care of type 2 diabetic patients. If the benefit of pre or post-prandial monitoring was proven, specific home blood glucose monitoring program should be strongly recommended for insulin treated type 2 diabetic subjects.

3.17 Obstacles and Strategies to solve the problems

Several possible obstacles were expected in this study

1. Compliance:

This study protocol needs a good co-operation from the subjects on a day-to-day basis. To assure the compliance, telephone call and home visit by nurse educators were set up on a regular basis.

2. Co-intervention, contamination and migration:

Since this study is un-blinded, subjects were aware of the outcome by means of the self-monitoring results. Violation of the protocol such as measuring both pre- and post-prandial blood glucose or adjusting insulin dose regimen more or less than the recommendation could happen at anytime. A thoroughly explanation of the design was

done before randomization. A closed follow up and communication by telephone was done to ensure the compliance and adherence to the protocol. Results from the self record and the meter's memory were compared at each visit.