5-14-2014

Injection Site Massage to Improve the Pharmacokinetics of Aspart in Obese Adolescents with Diabetes

Hiwot Ketema Girma
hiwot.ketema@gmail.com

INJECTION SITE MASSAGE TO IMPROVE THE PHARMACOKINETICS OF

ASPART IN OBESE ADOLESCENTS WITH DIABETES

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

May 14, 2014

Hiwot Ketema Girma, PA-SII   **Dr. Eda Cengiz, MD, MHS, FAAP**
Class of 2014   **Assistant Professor of Pediatrics**
Yale Physician Associate Program   **Department of Endocrinology**

Table of Contents

LIST OF TABLES

ABSTRACT

The invention of rapid-acting insulin analogs, such as aspart, was a great step forward in achieving optimal control of blood glucose in patients with type 1 diabetes. Aspart's action resembles the physiologic endogenous post-meal insulin action; however, the slow rate of absorption through subcutaneous tissue leads to a delay in the time to peak levels and action of pre-meal insulin injection and suboptimal control of postprandial blood glucose excursions. We propose that massaging the site of aspart injection will significantly accelerate insulin action and mitigate postprandial blood glycemic excursions. The study will investigate the effect of injection site massage on the pharmacokinetics and pharmacodynamics of subcutaneously administered aspart in overweight and obese adolescents with type 1 diabetes who are at high-risk for impaired insulin action. Massage will offer a cost effective solution to the undesired postprandial glycemic excursions that directly and indirectly contribute to mortality and morbidity associated with diabetes.

CHAPTER I: INTRODUCTION

<u>**Background**</u>

Type 1 diabetes mellitus (T1DM) is a common metabolic disorder that is a result of multifactorial interactions including genetic, environmental and immunologic factors. It is characterized by an absolute insulin deficiency due to an autoimmune destruction of beta cells of the pancreas. It accounts for 5-10% of the world's diagnosed diabetes, and its incidence has been gradually increasing 2-5% annually.[1-3] There are approximately 15,600 children, inducing adolescents, diagnosed with T1DM every year.[4] The majority of these children with T1DM are not achieving target blood sugar levels and overall glycemic control with only 32% reaching the age specific target hemoglobin A1c (HbA1c) set by the American Diabetes Association (ADA).[5,6] This challenge of achieving an optimal glycemic control is particularly emphasized in the adolescent population as demonstrated by data from the T1DM Exchange Clinic Registry. Out of all the participants in the registry, children between the ages of 13 and 26 years old had the highest HbA1c at 8.7%.[7] Additionally, data collected from the same cohort showed that only 21% of adolescent participants (ages 13-20 years old) met the HbA1c target of < 7.5% set by the ADA and International Society for Pediatric and Adolescent Diabetes (ISPAD).[5,6,8]

A few physiological and behavioral factors suggest why T1DM adolescents have higher mean hemoglobin A1c (HbA1c) values compared to the rest of the pediatric population and adult counterparts.[9] The main physiologic factor that contributes to poor glycemic control in adolescents is the significantly reduced insulin sensitivity secondary to pubertal changes, which in turn impairs insulin-stimulated glucose metabolism.[10-12] In healthy (non-diabetic) individuals, when a meal intake is initiated, the pancreas

immediately starts rapid production of insulin. Within 30-45 minutes, the insulin in the bloodstream reaches its maximal concentration.[13] By binding to receptors in target peripheral tissues and activating the trafficking of glucose transporters onto cell surfaces, this insulin facilitates the effective uptake of postprandial blood glucose (PPG), which reaches its peak level at ~60-75 minutes.[3,14,15] The hepatic production of glucose is also reduced by direct and indirect insulin action. This physiologic mechanism is impaired in patients with T1DM resulting in an ineffective metabolism of ingested mixed meals.[16] In the pubertal population, the increased insulin resistance (IR) leads to an increased and long-lasting PPG levels compared to healthy individuals.

These abnormally high and prolonged glucose elevations seen in the T1DM adolescent population are referred to as postprandial blood glucose excursions (PPGEs), or postprandial hyperglycemia (PPH). According to the ADA, PPGEs are glucose values defined as the change from baseline blood glucose levels or increments above the pre-meal blood glucose levels.[14] These PPGEs in the adolescent population are of a clinical significance because they are major contributors to overall glycemic control as well as independent risk factors of vascular complications associated with T1DM[17-20].

Besides the distinct physiological factor in adolescents that contributes to their poor glycemic control, this pubertal age group is also considered fragile in terms of some behavioral factors. The T1DM Exchange clinic registry data reports a poor adherence of the pediatric population to the recommended regular continuous glucose monitoring in order to adjust pre-meal insulin therapy accordingly[7]. In addition, as T1DM children reach their teenage years, there is a struggle to find a balance between their need to gain independence from parents and the vitality of continual supervision and assistance in

regards to insulin therapy decision-making.[21] This challenge becomes even more

pertinent considering the reported low adherence of patients with diabetes to the

recommended injection of rapid-acting insulin analogs (RAIAs) 15-30 minutes pre-meal

for optimal control of PPGEs.[22,23]

      RAIA therapy provides the most effective and safe insulin therapy that closely

resembles the endogenous secretion of insulin.[24] Relative to the previously used human

insulin, these RAIAs have a much faster onset and shorter duration of action. However,

even with an improved pharmacokinetic (PK) and pharmacodynamic (PD) profile,

RAIAs still have shortcomings that interfere with the goal of tight glycemic control in

T1DM management. Per guidelines, RAIAs are subcutaneously (SC) injected before a

meal in order to reproduce the instantaneous physiologic increase in insulin secretion that

occurs after carbohydrate intake in those without diabetes. However, due to the slow rate

of absorption through the SC tissue, there is a delay in the time to peak action of these

analogs. It is known that SC injected analogs require up to 1hr to reach maximum

concentration in the bloodstream and up to 90-120 min to reach a maximal glucose

lowering effect[16]. This delay is associated with the incidence of PPH in children, with

PPG excursions reaching over 300 mg/dl[25] above the recommended PPG level of <

180mg/dl.[5]

      The contribution of overall glycemic control to the micro and macro-vascular

complications seen over time in T1DM patients has long been understood.[9,26-28] Over the

past two decades, many prospective studies and epidemiological data have demonstrated

the significant contribution of glycemic variability independent of long-term glycemic

control to the development of these complications.[18,19,29,30] Daily fasting glucose

fluctuations significantly contribute to acute and long-term glycemic variability. Contrary to previous belief, PPG spikes also contribute to vascular complications.[17-20] Therefore, it is crucial to find ways such as an ultrafast-acting insulin to prevent postprandial hyperglycemic states by hastening the absorption of SC administered RAIAs and mimicking the function of pancreatic beta cells.[31] This is especially important in the obese and overweight adolescent population, as they constitute a high-risk group within the pediatric population of T1DM.

According to the American Academy of Pediatrics and Center for Disease Control (CDC)'s sex and age-specific growth ranking charts, overweight and obesity in the pediatric population ages 2-19 years are defined as a BMI percentile that is $\geq$ 85% and < 95% and $\geq$ 95% and < 99% respectively.[32,33] Currently, 21% of pediatric patients newly diagnosed with T1DM are obese or overweight,[34] and this has been significantly rising since the 1980s.[35] Besides the aforementioned physiological and behavioral factors, this particular group of obese and overweight adolescents has an added obstacle in achieving optimal glycemic control. Primarily, the increase in fat mass observed in this group contributes to the already high IR of body tissue seen in adolescents.[11] Secondly, the increased injection site SC fat is negatively correlated to the rate of absorption of SC injected RAIAs.[36] Lastly, it is well known that this group is at a higher risk of developing cardiovascular diseases than the lean counterpart.[37] All these factors place the adolescent population at the frontline to benefit from an intervention that will provide improved insulin absorption and action.

There are various approaches that have been investigated in an attempt to increase the rate of absorption and thus, action of SC injected insulin analogs. These include, the

manipulation of the insulin formulation, delivery method and injection site stimulations to increase blood flow. Results from many studies investigating such methods have been promising (discussed in detail in Chapter II).[16,38-42] However, there is not yet one universally accepted practical solution to mitigate the PPGEs seen in T1DM patients.

Massage is one approach that was introduced in the 1980s with some promising preliminary results suggesting an improved SC insulin absorption and action in T1DM patients. There is only one massage study that was done in T1DM patients, and it was a poorly controlled 1983 trial. The study used local skin massage 15 minutes post-insulin administration in lean, well-controlled, insulin-dependent patients and showed a significantly higher insulin concentration and reduced serum glucose level 45 minutes after SC injection of a mixed regular and intermediate-acting insulin.[38] The overall metabolic control of these patients as well as an additional 18 participants after 3-6 and 8-12 months of therapy was also significantly improved from baseline. This study, even with many limitations, had illustrated the potential of a simple, yet practical solution to the unquestionable need for an ultrafast-acting insulin therapy. Thus, there is an obvious need for well-controlled trials to investigate the benefit of local skin massage specifically using RAIAs in T1DM patients.

<u>Statement of the Problem</u>:

Rapid-acting insulin analog therapy has improved overall glycemic control in T1DM patients. Compared to regular human insulin these analogs have a faster and higher serum insulin peak and a much shorter duration of action. This has allowed for significantly reduced and shorter PPGEs as well as reduced incidence of hypoglycemia.[24,43-46] RAIAs, however, are not fast-acting enough to overcome the

glycemic control challenges faced by the overweight and obese adolescent T1DM patients. There is an increasing prevalence of overweight and obesity at the time of T1DM diagnosis[7,34,37]. Overweight and obese adolescents face additional obstacles in diabetes treatment because of a higher insulin resistance associated with increased body mass[11] and pubertal hormonal changes[10,47] as well as a delayed absorption of insulin analogs due to increased SC fat.[36] Therefore, this population will significantly benefit from an acceleration of insulin absorption and action to achieve good glycemic control by specifically targeting PPGEs.

The discontinued interest in massage as a potential solution to this obstacle was surprising to find in the literature considering the fact that massage, if proven efficacious, does not require many resources besides educating patients on how to apply a standardized massaging technique. Besides the risk of hypoglycemia suggested in studies that were not undertaken in T1DM patients,[48,49] there are no reported adverse effects associated with the use of massage therapeutically. Additionally, local skin massage will not add cost to the already burdensome expenses of T1DM management, and this, as well as its non-invasive nature, makes it an interesting and practical solution to mitigate PPH.

Goals and Objectives:

The goal of this study is to evaluate the effect of a standardized local skin massage at the site of SC aspart injection on insulin action in overweight and obese adolescents with T1DM at the Yale Pediatric Diabetes Clinic. Massage has the potential to accelerate SC administered insulin absorption and action to moderate the PPG levels that are elevated and prolonged in overweight and obese adolescents. Faster insulin action by massaging will reduce the PPGEs in this population leading to an improved

6

short-term glycemic variability as well as long-term glycemic control, which in turn might decrease the risk of developing micro and macrovascular complications associated with T1DM.

The objective of this proposed study is to gather preliminary data regarding differences in the onset, peak and duration of action of aspart by comparing mean values after treatment with SC abdominal (10cm from the umbilicus) aspart injection followed by a 3-minute standardized local skin massage with the control treatment, identical dose of SC aspart injection without massaging. These data will be collected using the gold standard insulin action test, the euglycemic clamp technique[50] (refer to Chapter III). This study would also provide a pilot protocol that other studies can adopt and modify to establish an optimal standardized insulin infusion site massage protocol to accelerate insulin absorption and action.

Hypothesis:

The primary hypothesis of this proposal is that overweight and obese type 1 diabetes mellitus patients ages 12-18 years old, who massage the site of injection of the rapid-acting insulin analog, aspart, for 3 minutes after SC abdominal injection will have a statistically significant increase in the rate of insulin absorption and action. The measure of insulin absorption is defined as time to maximal serum insulin concentration (T-INSmax) and insulin action is defined as time to maximal glucose infusion rate (T-GIRmax) during an insulin action study. We expect that the mean time to peak serum insulin concentration between the two intervention groups will differ by at least 33 minutes. This will be a clinically significant difference provided that it is longer than the

7

~20 min delay of aspart in reaching maximal serum concentration as compared to the physiologic insulin peak time.

We also expect a difference in change in our secondary outcomes between the two study days where participants receive either SC aspart injection or a 3-min local skin massage post-SC aspart injection. The secondary pharmacokinetic outcomes will include, the peak insulin levels (C-INSmax) and the total area under the curve for change in insulin levels (AUCINS). The secondary pharmacodynamic outcomes will include the maximal glucose infusion rate (GIRmax) and the total area under the curve for the change in glucose infusion rate (AUCGIR 0-300min).

<u>Definitions</u>:

- Overweight adolescents: BMI percentile that is $\geq 85\%$ and $< 95\%$

- Obese adolescents: BMI percentile that is $\geq 95\%$

<u>Abbreviations:</u>

T1DM: Type 1 diabetes mellitus

RAIAs: Rapid-acting insulin analogs

SC: Subcutaneous

PPG: Postprandial glucose

PPGEs: Postprandial glucose excursions

PPH: Postprandial hyperglycemia

PK: Pharmacokinetics

PD: Pharmacodynamics

IMI: Injection-meal interval

HbA1c (Hemoglobin A1c): glycated hemoglobin

IR: Insulin resistance

RHI: Regular human Insulin

Min: Minute(s)

References:

1.	American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* Jan 2014;37 Suppl 1:S81-90.
2.	Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinology and metabolism clinics of North America.* Sep 2010;39(3):481-497.
3.	Powers AC. Diabetes Mellitus. *Harrison's Principles of Internal Medicine.* 18th ed. New York: McGraw-Hill; 2012.
4.	NIDDK. National Diabetes Statistics. 2011; http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.aspx - NewCasesDD.
5.	American Diabetes A. Standards of medical care in diabetes--2014. *Diabetes Care.* Jan 2014;37 Suppl 1:S14-80.
6.	Wood JR, Miller KM, Maahs DM, et al. Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care.* Jul 2013;36(7):2035-2037.
7.	Beck RW, Tamborlane WV, Bergenstal RM, et al. The T1D Exchange clinic registry. *The Journal of clinical endocrinology and metabolism.* Dec 2012;97(12):4383-4389.
8.	Federation ID. Global IDF/ISPAD Guidlines for Diabetes in Childhood and Addolescence. 2011: http://www.idf.org/global-idfispad-guideline-diabetes-childhood-and-adolescence.
9.	Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. *The Journal of pediatrics.* Aug 1994;125(2):177-188.
10.	Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV. Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *The New England journal of medicine.* Jul 24 1986;315(4):215-219.
11.	Cree-Green M, Triolo TM, Nadeau KJ. Etiology of insulin resistance in youth with type 2 diabetes. *Curr Diab Rep.* Feb 2013;13(1):81-88.
12.	Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). *Diabetes Care.* Mar 2012;35(3):536-541.
13.	Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *The Journal of clinical investigation.* Feb 1988;81(2):442-448.
14.	American Diabetes A. Postprandial blood glucose. American Diabetes Association. *Diabetes Care.* Apr 2001;24(4):775-778.
15.	Luijf YM, van Bon AC, Hoekstra JB, Devries JH. Premeal injection of rapid-acting insulin reduces postprandial glycemic excursions in type 1 diabetes. *Diabetes Care.* Oct 2010;33(10):2152-2155.
16.	Heinemann L, Muchmore DB. Ultrafast-acting insulins: state of the art. *J Diabetes Sci Technol.* Jul 2012;6(4):728-742.

17. Ceriello A. The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes/metabolism research and reviews.* Mar-Apr 2000;16(2):125-132.

18. Chiasson JL, Josse RG, Gomis R, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA : the journal of the American Medical Association.* Jul 23 2003;290(4):486-494.

19. Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA : the journal of the American Medical Association.* Apr 12 2006;295(14):1681-1687.

20. Wu D, Gong CX, Meng X, Yang QL. Correlation between blood glucose fluctuations and activation of oxidative stress in type 1 diabetic children during the acute metabolic disturbance period. *Chinese medical journal.* Nov 2013;126(21):4019-4022.

21. Silverstein J, Klingensmith G, Copeland K, et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. *Diabetes Care.* Jan 2005;28(1):186-212.

22. Cobry E, McFann K, Messer L, et al. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. *Diabetes technology & therapeutics.* Mar 2010;12(3):173-177.

23. Overmann H, Heinemann L. Injection-meal interval: recommendations of diabetologists and how patients handle it. *Diabetes research and clinical practice.* Feb 1999;43(2):137-142.

24. Garg SK. New insulin analogues. *Diabetes technology & therapeutics.* Oct 2005;7(5):813-817.

25. Boland E, Monsod T, Delucia M, Brandt CA, Fernando S, Tamborlane WV. Limitations of conventional methods of self-monitoring of blood glucose: lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes. *Diabetes Care.* Nov 2001;24(11):1858-1862.

26. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *The New England journal of medicine.* Sep 30 1993;329(14):977-986.

27. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes.* Aug 1995;44(8):968-983.

28. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* Sep 12 1998;352(9131):854-865.

29. Prince CT, Becker DJ, Costacou T, Miller RG, Orchard TJ. Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC). *Diabetologia.* Nov 2007;50(11):2280-2288.

30. Weber C, Schnell O. The assessment of glycemic variability and its impact on diabetes-related complications: an overview. *Diabetes technology & therapeutics.* Oct 2009;11(10):623-633.

31. Cengiz E. Undeniable need for ultrafast-acting insulin: the pediatric perspective. *J Diabetes Sci Technol.* Jul 2012;6(4):797-801.

32. Krebs NF, Jacobson MS, American Academy of Pediatrics Committee on N. Prevention of pediatric overweight and obesity. *Pediatrics.* Aug 2003;112(2):424-430.

33. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital and health statistics. Series 11, Data from the national health survey.* May 2002(246):1-190.

34. Kaminski BM, Klingensmith GJ, Beck RW, et al. Body mass index at the time of diagnosis of autoimmune type 1 diabetes in children. *The Journal of pediatrics.* Apr 2013;162(4):736-740 e731.

35. Libman IM, Pietropaolo M, Arslanian SA, LaPorte RE, Becker DJ. Changing prevalence of overweight children and adolescents at onset of insulin-treated diabetes. *Diabetes Care.* Oct 2003;26(10):2871-2875.

36. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. *Diabetologia.* // 1994;37(4):377-380.

37. Liu LL, Lawrence JM, Davis C, et al. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. *Pediatr Diabetes.* Feb 2010;11(1):4-11.

38. Dillon RS. Improved serum insulin profiles in diabetic individuals who massaged their insulin injection sites. *Diabetes Care.* // 1983;6(4):399-401.

39. Cengiz E, Weinzimer SA, Sherr JL, et al. Acceleration of insulin pharmacodynamic profile by a novel insulin infusion site warming device. *Pediatric Diabetes.* // 2013;14(3):168-173.

40. Engwerda EE, Abbink EJ, Tack CJ, de Galan BE. Improved pharmacokinetic and pharmacodynamic profile of rapid-acting insulin using needle-free jet injection technology. *Diabetes Care.* Aug 2011;34(8):1804-1808.

41. Gupta J, Felner EI, Prausnitz MR. Rapid pharmacokinetics of intradermal insulin administered using microneedles in type 1 diabetes subjects. *Diabetes technology & therapeutics.* Apr 2011;13(4):451-456.

42. Hompesch M, Muchmore DB, Morrow L, Vaughn DE. Accelerated insulin pharmacokinetics and improved postprandial glycemic control in patients with type 1 diabetes after coadministration of prandial insulins with hyaluronidase. *Diabetes Care.* Mar 2011;34(3):666-668.

43. Home PD. The pharmacokinetics and pharmacodynamics of rapid-acting insulin analogues and their clinical consequences. *Diabetes, obesity & metabolism.* Sep 2012;14(9):780-788.

44. Howey DC, Bowsher RR, Brunelle RL, Woodworth JR. [Lys(B28), Pro(B29)]-human insulin. A rapidly absorbed analogue of human insulin. *Diabetes.* Mar 1994;43(3):396-402.

45.   Lindholm A, McEwen J, Riis AP. Improved postprandial glycemic control with insulin aspart. A randomized double-blind cross-over trial in type 1 diabetes. *Diabetes Care.* May 1999;22(5):801-805.

46.   Owens D, Vora J. Insulin aspart: a review. *Expert opinion on drug metabolism & toxicology.* Oct 2006;2(5):793-804.

47.   Swan KL, Weinzimer SA, Dziura JD, et al. Effect of puberty on the pharmacodynamic and pharmacokinetic properties of insulin pump therapy in youth with type 1 diabetes. *Diabetes Care.* Jan 2008;31(1):44-46.

48.   Berger M, Cuppers HJ, Hegner H, Jorgens V, Berchtold P. Absorption kinetics and biologic effects of subcutaneously injected insulin preparations. *Diabetes Care.* Mar-Apr 1982;5(2):77-91.

49.   Linde B. Dissociation of insulin absorption and blood flow during massage of a subcutaneous injection site. *Diabetes Care.* Nov-Dec 1986;9(6):570-574.

50.   DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *The American journal of physiology.* Sep 1979;237(3):E214-223.

CHAPTER II: LITERATURE REVIEW

Introduction:

This proposal is based on a literature review conducted between December 2013 and May 2014. Databases used to gather information relevant to the proposal include Medline, PubMed, Embase and Cochrane Library. The search was restricted to articles published from 1973 to present. References from retrieved publications were also used to expand literature search. Only clinical trials in humans were included. Search terms or keywords used include, type 1 diabetes mellitus, obesity, puberty, massage, rapid-acting insulin analogs, insulin absorption, insulin resistance, postprandial glycemic excursion, microneedles, warming device and hyaluronidase.

Review of Empiric Studies:

Prevalence and Implications of Postprandial Hyperglycemia

Glycemic management in type 1 and 2 diabetes mellitus is based on the overall glycemic control as measured by glycated hemoglobin (HbA1c). This focus of management in T1DM patients is based on the outcomes of a prospective study, the Diabetes Control and Complications Trial (DCCT), which showed that HbA1c, a measure that reflects plasma glucose concentration over the preceding 2-3 months, is directly associated with the late-stage macrovascular and microvascular complications of T1DM (including retinopathy, nephropathy and neuropathy).[1,2] Based on findings from the DCCT, the American Association of Diabetes to this day recommends a tight glycemic control in all non-pregnant adults with an HbA1c of $< 7\%$.[3] However, more recent studies indicate that HbA1c values are merely a fragment of the multiple risk factors of complications associated with T1DM. Acknowledging the immense role of the

DCCT in the improved management of diabetic patients, Hirsch and Brownlee in 2010 illustrated that the contribution of HbA1c and duration of diabetes to the risk of development of retinopathy seen on this trial was overall very low at 11%.[4] This commentary suggested that there are many other factors that must be contributing to the variation in risk for microvascular complications.

Various epidemiologic as well as experimental studies over the years have shown that overall long-term and short-term glycemic variability around a stable mean baseline HbA1c also plays a significant role in predicting microvascular and macrovascular complications in the diabetic population.[5-14] This glycemic variability is associated with the vascular complications of diabetes both as an independent risk factor[5,9,14-16] and indirectly by affecting the overall glycemic control (HbA1c).[17,18]

Glycemic variability was initially thought to be solely a result of the daily variations in fasting glucose levels. However, many studies have established the contribution of PPGEs to long-term and acute glucose fluctuations (glycemic variabilities) seen in patients with diabetes.[7,9,11] Boland et al. first emphasized the prevalence and possible clinical implication of PPGEs in their study of continuous glucose monitoring system in a cohort of 56 children ages < 18. They demonstrated that PPG values were significantly elevated above the target value of < 180 mg/dl in ~90% of the children after a 3-day monitoring trial. Out of these, 50% were above 300 mg/dl despite having HbA1c values within the target range.[8] This study suggested that even in those T1DM patients with a relatively optimal HbA1c and pre-meal glucose values, marked glycemic excursions post-meal are of a significant existence.

Monnier et al. further asserted these findings in 2003 by comparing the relative contribution of fasting and postprandial plasma glucose excursions to the overall hyperglycemia of patients with type 2 diabetes mellitus (T2DM).[17] They found that both were major contributors to the overall glycemic control of diabetes patients, but PPH had a significant contribution in those patients with an overall good ("mild-moderate") metabolic control as determined by an HbA1c ≤ 8.4% compared to those who have poor glycemic control. At HbA1c levels < 7.3%, PPH contributed ~70% to the HbA1c value, thus suggesting the importance of effective PPG management for an optimal long-term metabolic control in diabetes patients. Three years later, Slama et al. also reported that in their sample population of T2DM patients, PPGEs accounted for an average of 40% to the total "abnormal hyperglycemia", which in turn contributed to about 1% absolute rise in HbA1c values.[13] They also emphasized the contribution of PPGEs to the overall glycemic control of patients with a more controlled HbA1c. These studies point to the importance of controlling PPGEs and not only fasting glucose levels in patients with diabetes to maintain a good glycemic control and avoid morbidity and mortality associated with the disease.

Besides its contribution towards the overall glucose control, PPH was also shown to be an independent risk factor for macrovascular complications associated with diabetes by the STOP-NIDDM (Noninsulin-Dependent Diabetes Mellitus) trial in 2003.[9] The study demonstrated that an alpha-glycosidase inhibitor, acarbose, which is an anti-diabetic drug that interferes with the metabolism of carbohydrates and thus normalizes postprandial plasma glucose concentrations, reduced the incidence of cardiovascular diseases (CVD) and hypertension. Patients with impaired glucose intolerance who

received treatment with acarbose had a significantly lower risk of developing CV events including coronary heart disease, cardiovascular death, congestive heart failure, cerebrovascular events and peripheral vascular disease with a hazard ratio (HR) of 0.51 (95% CI, 0.28-0.95; P =0.03) when compared to those receiving placebo. These patients also had a significantly lower risk of developing hypertension (HR = 0.66; 95% CI, 0.49-0.89; P = 0.006).

In addition, both Ceriello and Home described the role of PPH as a risk factor for microvascular changes in their review articles.[7,11] Their theory behind the association of PPH and vascular events is that it has a modifying effect on most of the cardiovascular risk factors such as LDL oxidation, thrombosis activation, and endothelial dysfunction. Increased oxidative stress or the overproduction of the reactive free radical molecule superoxide secondary to hyperglycemia is described to be the unifying mechanism to all of these risk factors.[19-21] Monnier et al. in 2006[15] and recently (2013) Wu and associates[16] showed that acute glucose fluctuations have a direct correlation in the activation of oxidative stress in T2DM patients and newly diagnosed children with T1DM, respectively. These studies even more signify the deleterious effect of PPH and its contribution to the vascular complications associated with diabetes. Therefore, more studies targeting interventions to moderate unnecessarily elevated and prolonged PPGEs, which contribute to glucose variability in both type 1 and 2 diabetic patients, are needed.

Shortcoming of Current Management of T1DM with Rapid-Acting Insulin Analogs: Postprandial Hyperglycemia

Insulin therapy has been the key player in the control of hyperglycemia in patients with T1DM and at the later stages of T2DM since the early 1920s.[22] However, until the

discovery of RAIAs, an insulin therapy that most closely mimics the fast physiologic insulin response seen in healthy individuals was not available. What was considered to be the best insulin therapy at the time, regular human insulin (RHI), did not reach peak serum levels until 2-3hrs after subcutaneous injection, which is significantly delayed in relation to the time to peak postprandial serum glucose levels.[23] RAIAs, on the other hand, reach maximal concentration in the blood in about 1hr and have a peak glucose lowering effect ~90-120min after SC injection.

One of the first studies in 1996 comparing RAIAs, specifically lispro to RHI by Heinemann et al. showed that after a carbohydrate-rich meal in T1DM patients, there was a significantly less prandial glucose excursion with insulin lispro vs. RHI (9.9 ± 1.4mmol/l vs. 11.9 ± 2.8mmol/l; $P < 0.05$).[24] Also, there was a rapid peak plasma insulin concentration at 68 ± 18min to a level of 369 ± 73pmol/l after SC injection of lispro vs. the slow insulin rise to 263 ± 59pmol/l within 116 ± 27min with RHI injection ($p < 0.01$). This study along with others[23,25-31] have demonstrated that RAIAs have a significantly faster absorption rate, peak plasma concentration, faster peak glucose lowering effect and shorter duration of action. These properties markedly improved the coverage of prandial blood glucose spikes with close resemblance to the mealtime physiology seen in people without diabetes. The shorter duration of action of RAIAs also minimized the incidence of postprandial hypoglycemia that was significant with RHI. RAIAs, therefore, have been incorporated and are routinely used as part of the standard of care for patients with T1DM.

Currently there are three kinds of FDA approved RAIAs available for treatment of diabetes: insulin lispro, aspart and glulisine. A review article by Home P.D. examined the

possible clinically significant differences in efficacy, tolerability, safety and treatment satisfaction that might exist between these three RAIAs. After a systematic analysis of 19 articles, the author recognized a slightly faster onset of action for insulin glulisine. Nevertheless, no other significant differences between the three RAIAs in terms of their pharmacokinetic and pharmacodynamic profiles were identified. The faster onset of action of glulisine has not been shown to have any clinically significant benefit in terms of overall glucose control or treatment satisfaction.[32]

As part of the standard of care for T1DM, RAIAs have offered an immense clinical benefit in optimizing postprandial glucose excursions. However, the need for an even better control with an injection-meal interval (IMI) was recommended by follow-up studies, as postprandial blood glucose levels were still above target ranges.[33,34] Compared to the normal physiologic conditions, the relatively delayed absorption of SC injected RAIAs is recognized as the obstacle. A randomized crossover trial by Luijf and associates has shown that insulin aspart administered 15min before a meal has a significantly lower PPG and peak glucose value when compared to a 0min and -30min treatment arms (P = 0.038).[34] Another study by Cobry et al. has demonstrated a similar reduction in postprandial excursions with use of RAIAs, specifically glulisine.[33] When administered 20min before a standardized meal in patients with T1DM, glulisine showed a significantly lower glycemic excursion 60 and 120 minutes after meal initiation than when administered immediately before or 20min after the meal. (At 60min - 180.3 ± 66.4mg/dL vs. 222.0 ± 58.9mg/dL [P = 0.0029] and 235.7 ± 46.6mg/dL [P = 0.001]; at 120min - 176.3 ± 70.7mg/dL vs. 207.7 ± 48.5mg/dL [P = 0.0294] and 205.8 ± 50.7mg/dL [P = 0.0408], respectively). The pre-meal injection group also had significantly lower

blood glucose levels under the postprandial goal of < 180mg/dl compared to the at-meal and post-meal injection group (P < 0.0001 for both).[33] These studies, beyond demonstrating the benefit of RAIAs in reducing PPG, emphasized the need for an appropriate injection-meal interval (IMI) to compensate for the delay between subcutaneous (SC) injection of insulin and its onset of action.

Patients are therefore strongly advised to take their pre-meal insulin injections on an average of 5-15 minutes before mealtime. The current administration guidelines in the U.S. recommend aspart to be injected 5-10min before a meal, lispro 15min before or immediately after a meal and glulisine 15min before a meal or within 20min after starting a meal.[35-37] Even though these guidelines are in place, many T1DM patients do not adhere to these recommendations in their daily life due to impracticality.[38] This IMI is also potentially detrimental as it could cause hypoglycemia in situation where meal is delayed after the pre-meal administration of an RAIA (e.g. in a restaurant setting). Such pre-meal dosing practices will be even more dangerous if patients have pre-meal blood sugars in the low side of the normoglycemic range.[39]

Additional Challenges in Controlling Post-meal Blood Glucose: Overweight and Obese Adolescent with T1DM

In the last 10-15 years the prevalence and clinical implications of the vastly increasing overweight and obese children and adolescents with T1D have become a focus of many studies. A retrospective study by Libman and associates in 2003 reported that the prevalence of being overweight and obese in African American and Caucasian children at the onset of T1DM has tripled from 12.6% in the 1980s to 36.8% in the 1990s (P=0.0003).[40] According to their analysis at the time, this prevalence rate went hand in

hand with the increasing prevalence of obesity in the general population. Two articles

that described the BMI distribution of a diverse group of T1DM children and adolescents

in comparison with the general population identified that currently there are about 11-

13% overweight and about 21-22% obese (total of about 34%) children and adolescents

with T1DM compared to ~33% of youth without diabetes. [41,42]

The challenge of achieving optimal PPGEs is even more pronounced in this

increasingly obese and overweight pediatric population with T1DM. Besides all the other

factors that interfere with the effective absorption of SC injected insulin analogs in non-

obese individuals, this population will have an added obstacle of increased subcutaneous

fat. It has been shown that increased subcutaneous fat thickness has a negative correlation

with the absorption of SC injected insulin analogs.[43] de Galan et al. comparing the

efficacy of needle-free jet RAIA injection with a conventional insulin pen in overweight

and obese individuals without diabetes demonstrated that higher body weight indices are

associated with a delayed insulin absorption and onset of action when administered

through a conventional insulin pen.[44] Also, obese and overweight individuals have an

increased insulin resistance impairing exogenously injected insulin's action,[45] which

further contributes to the difficulty of maintaining normoglycemia.

Part of the pediatric population is the adolescent children in their prime time of

puberty. This particular group has been shown to have an increased insulin resistance and

the DCCT has demonstrated that near-normalization of blood glucose levels was more

difficult in adolescent subjects as compared to adult subjects with T1DM.[46] As expected,

the HbA1c levels for the adolescent group was higher than the adult subjects with

T1DM.[46-48] According to the 2012 review article by Cree-Green et al.,[45] the surge in

pubertal hormones such as estrogen, testosterone and growth hormone as well as the

greater fat mass in the adolescent population plays a big role in the decreased insulin

sensitivity seen in T1DM as well as non-diabetics in the pubertal age. The obese

adolescent population therefore seems to require a larger pre-meal insulin dose to avoid a

higher and longer PPH and attain a good overall glycemic control.[47]

Approaches Currently in Clinical Development to Control Postprandial Glycemic

Excursions

Faster acting insulin will mimic physiologic insulin response better and will lower

blood sugar immediately. Several investigators have developed interest to find such

ultrafast-acting insulin and many studies have been underway since before the discovery

of RAIAs. Currently, there are several mechanical and insulin formulation based

approaches that are at different stages of clinical development. Some of the current

mechanical approaches include the use of a warming device to increase local blood flow,

use of needle-free jet injectors or application of an enzyme to spread the insulin into a

wider area in the SC tissue and the use of microneedles to inject insulin into a more

vascularized layer, the dermis. The formulation approach comprises studies that play with

the insulin formulation to find "novel insulin analogs" by adding excipients that either

increase blood flow to the area or promote the breakdown of the hexameric RAIAs.[49] Out

of all these approaches, the warming device, the enzyme, recombinant human

hyaluronidase (rHuPH20), and microneedles have so far shown some promising results.

(Refer to Table 1 for some studies reviewed).

InsuPatch (InsuLine Medical. Petach-Tikva, Israel) is a small warming patch

devised and approved for use in Israel. It is supposed to warm the local skin injection site

to a temperature of 38 °C and increase blood flow to the SC tissue by a mechanism of vasodilation. This device has been proven to increase the absorption of subcutaneously injected insulin analogs by various studies. [49-52] It is also safe and well tolerated by patients. Cengiz et al. demonstrated that the bioavailability of insulin aspart was improved by 28% with the use of InsuPatch and showed a significantly enhancement in aspart's pharmacokinetic profile (Cmax, $P=0.04$ & Tmax, $P=0.03$).[52] This study also showed a significant (TGIRmax; $P=0.002$) improvement in the time to peak action of aspart. However, there was no clinically significant change in the peak action of the insulin analog between the two groups. The authors attribute this lack of pharmacodynamics improvement to the specific characteristic of the population used, as adolescents in the pubertal age have been shown to have a greater insulin resistance, which might have "blunted" the expected results.[52] This theory is supported by the study of Raz and associates in 2009, who performed a meal tolerance study in an adult population investigating the effect of InsuPatch in the action of RAIAs.[50] There was a significant enhancement in insulin Cmax ($P<0.05$) and a reduction in the Tmax ($P<0.05$) demonstrating an improved PK profile. This study did not use the euglycemic glucose clamp technique to analyze the PD profile of RAIAs, but by sampling the plasma glucose after a meal challenge, they were able to determine the PPG concentrations. There was a significant reduction (~40%) in the peak glucose excursion 90min after the meal (74 ± 48mg/dl vs. 121 ± 43mg/dl; $P<0.005$) in the group using InsuPatch vs. the group not using it.

The other mechanism that has shown good PK and PD improvements in the currently available RAIAs is recombinant human hyaluronidase. Hyaluronidase is an

enzyme that transiently disrupts hyaluronan, which is "the component that confers a gel-like consistency to" the SC extracellular matrix, "limiting the spread of injected materials to the process of diffusion."[49] This product is approved by the U.S. Food and Drug Administration (FDA) and has been utilized as an additive in other injectable medical fluids for its absorptive properties. Hompesch and associates, using an individually optimized dose of RAIA or RAIA plus hyaluronidase, performed a liquid meal challenge study in T2DM adults. Their study showed the effectiveness of hyaluronidase in reducing PPG within 2 hours after a meal challenge (159 ± 31 vs. 138 ± 32mg/dl; P=0.0098) in those who received lispro with rHuPH20 vs. just lispro.[53] A similar study in T1DM adults showed a significant reduction in the peak of total glycemic excursions (P=0.002) in those who had hyaluronidase coadministered with lispro vs. those without hyaluronidase.[54]

Microneedles are short-length needles that directly infuse insulin into the dermis without reaching the SC tissue. Since the dermis is a more vascularized space compared to the SC tissue, by injecting insulin intradermally, Gupta and colleagues hypothesized that insulin absorption will be accelerated and postprandial hyperglycemia will be well controlled.[55] This hypothesis has since been proven true by a few studies[55,56]. Even though they had a small sample size of only 5 T1DM patients (2 adults, 1 adolescents and a child), Gupta et al. were the first to demonstrate a significantly faster absorption of insulin (P=0.01) as well as a markedly improved post-meal excursion of glucose (with an unspecified significance) in those who received lispro with a microneedle compared to those with SC insulin infusion. There were no significant changes seen in the peak insulin level (C-INSmax) and bioavailability of the insulin (AUCINS).[55]

Even though most of the above mentioned approaches and more have shown promising results towards finding ultra-fast acting insulin, there is not yet one approach that is approved and incorporated into the daily management of T1DM patients in most parts of the world. Even when approved, the complexity of most of these approaches might make them costly and not as easily accessible to the majority of the population who needs it. This will especially be evident in developing countries where even the most up-to-date RAIAs are hard to locate. Therefore, more studies are needed to look for alternative approaches that are more convenient and easily accessible.

Table 1. Insulin pharmacokinetic and pharmacodynamic profiles following RAIA administration with or without a specific intervention.

**All values of statistical significance ($p < 0.05$) are in bold. I = individualized

Study (First Author)	Intervention	Population (N)	Insulin Type	PK Parameters		PD Parameters	
				Cmax	Tmax	GIR max	TGIR max
	Hyaluronidase						
	RAIA			(pmol/l)			
Hompesch		T2DM adults (21)	Lispro/ I	**3329±33**	**74±36**		
Hompesch		T1DM adults (22)	Lispro/ I	**~4000**	**49**		
	RAIA + rHuPH20						
Hompesch		T2DM adults (21)	Lispro/ I	**5662±24**	**43±16**		
Hompesch		T1DM adults (22)	Lispro/ I	**~2900**	**30**		
	Warming device						
	RAIA			(uU/ml)			
Cengiz		TIDM adolescents (13)	Aspart/ 0.2u/kg	**81±8**	**67±10**	7.2±.7	**125±8**
Raz		T1DM adults (9)	Lispro/ 0.15u/kg	**86±16**	**78±35**		
	RAIA + Device						
Cengiz		TIDM adolescents (13)	Aspart/ 0.2u/kg	**106±11**	**41±4**	8.0±1	**90±6**
Raz		T1DM adults (17)	Lispro/ 0.15u/kg	**118±35**	**45±28**		
	Microneedles						
	RAIA			(μU/ml)			
Gupta		T1DM (5)	Lispro/ I	24.6±7.5	**57±20**		
McVey		T1DM adults (22)	Lispro/ I		**51.6**		
	RAIA+ Microneedle						
Gupta	0.9mm needle	T1DM (5)	Lispro/ I	32.2±14	**27±13**		
McVey		T1DM adults (22)	Lispro/ I		**36**		

Local Skin Massage as an alternative approach to mitigate PPG

Massaging the local insulin injection site is one promising approach that was explored before the invention of RAIAs.[57-59] Until today, to our knowledge, only one study has investigated and described the potential benefit of massaging SC insulin injection site on the PK and PD profiles of insulin, specifically in patients with T1DM. This study was done by Dillon in 1983, and it used as an intervention a 3-minute massage 15 minutes after the administration of insulin vs. no massage to assess the benefit of massaging on the bioavailability of SC injected insulin.[57] The study was done on 8 lean insulin-dependent diabetic individuals (1 adolescent and 7 adults) who had a relatively well-controlled glucose profiles and were using low doses of mixtures of regular and intermediate insulin. The patients were in a fasting state throughout the study during which blood glucose, free and total plasma insulin levels were sampled for four 15-minute-intervals on both the control (no intervention) and intervention days. A significant increase from baseline in plasma free insulin level was seen 30 minute after massage (45min after SC injection) on the day of the intervention vs. no-intervention (4.5 ± 1.8 vs. 14.7 ± 3.7 microU/ml; $P < 0.05$). At the same time, a significant ($p < 0.05$) fall in serum glucose level was seen with an 8.3% lower blood glucose change from baseline in those who received the massage intervention suggestive of more potent insulin action after massaging the insulin injection site.

Even though this study has contributed vital information for its time, it has many limitations that make it less generalizable and inconclusive regarding the current issues with RAIA therapy discussed in this chapter. First and foremost, the study had a very small number of participants, 8 subjects. This, as it is for any randomized controlled

studies, blunts any significant changes that might have been detected. Secondly, the data

collection for glucose and insulin levels was done in a very wide time interval. This also

might have reduced the chance of identifying a much faster insulin peak and glucose

lowering effect. Thirdly, the patients were taking their own home dose insulin mixture of

intermediate and regular insulin and the increase in free and total insulin absorption

observed in the study could be of either insulin. This makes it hard to discern if regular

insulin or intermediate insulin should have been the target of further investigation

probably accounting for the reason why other studies have not been done in this area.

Considering that regular insulin takes up to 2-3 hours to reach peak levels, and longer to

show glucose reducing effect, the study also failed to assess full PK profile of the injected

insulin as samples were taken only for 45 minutes after SC injection.

Dillon's study speculates that the mechanism behind improved insulin

pharmacokinetics seen in T1DM patients is the increase in blood flow to the area of

injection as well as improved insulin delivery to the circulation through the lymphatic

system.[57] In their recent review article Heinemann and Muchmore also discuss this

probable increase in local blood flow following massage just as seen with increased

ambient skin temperature[43,50,60] A review article discussing the physiology behind the

therapeutic effects of massage also supports this theory and discusses that massaging

results in the vasodilation of superficial blood vessels and increases blood flow to the

area of the massage.[61] For light pressure massages, the vasodilatation is thought to be a

result of local axonal reflexes. However, for deeper massages histamine release plays a

big role in the vascular response that is achieved. These authors also discuss the

"uncomplicated" mechanism in regards to the theory of improved lymphatic drainage

associated with massaging.[61] Massage compresses superficial lymph veins promoting their drainage, and this in turn, opens up space in the vessels for interstitial fluid to drain into. This improved lymphatic draining by itself could be one contributing factor for the fast absorption of insulin, but additionally, the decreased pressure in the interstitial space is indicated to reduce arterial congestion further improving perfusion in the area.[58,61]

This variety of possible mechanisms of action makes massage an appealing approach to pursue in the attempt to find a faster acting insulin analog. Even though Dillon's work has many limitations, we speculate that with new controlled studies particularly utilizing RAIAs, massaging local injection site could provide a cost-effective, non-invasive, practical and easily accessible approach to facilitate insulin absorption and improve PPH in T1DM patients. To the best of our knowledge, there are no data on the benefit of this approach in overweight and obese T1DM adolescents in regards to RAIA pharmacokinetics and pharmacodynamics. In the high-risk overweight and obese adolescent population, such an intervention that could accelerate exogenous insulin action will have paramount role. Not only will it improve the daily or acute plasma glucose fluctuations that have a significant contribution to the late-stage complications associated with T1DM, but also improve the overall glycemic control (HbA1c) of this population.

Review of Relevant Methodology

Study design, randomization and blinding

This proposed study will be a randomized, controlled, single-blinded, crossover euglycemic clamp study. As illustrated by the studies reviewed in this chapter[50,52-56], crossover trials are the standard for testing the time-action profiles of rapid-acting insulin

analogs. Crossover study designs, if done with randomization of intervention orders, are known to be the strongest possible approaches to control for participant characteristics at a study design level. However, this approach should not be used if a carry-over effect is expected from one intervention to the next (control to active intervention or vice versa)[62]. In our study, we do not suspect any carry-over effects from the control intervention to the massage intervention as the SC infused insulin has a transient effect wearing off in the 5-8 hour clamp study duration. In regards to carry-over effects from the massage intervention, we believe it is unlikely, but since there are no recent studies that validated the exact mechanism behind the effect of local skin massaging on the insulin absorption and action profiles, we will allow for at least a week of washout period between the two intervention study days.

Another limitation of a crossover design is a history threat to the internal validity of the study. This is any external event that could potentially occur simultaneously with the independent variable and alter the outcome of the study. The event could differentially affect the subjects during the two intervention orderings, therefore, it is crucial to keep controlled and similar settings on both study days. Since temperature has been shown to hasten SC injected insulin absorption, we will perform the clamp study in a temperature-controlled room (22-24°C).[50,51,60] Additionally, the same conventional pen will be used for both injection days for each subject to avoid any variability.

We propose to use the euglycemic clamp technique, first described by Defronzo et al.[63], to determine our PK and PD end points described in Chapter III of this proposal. Even though there are other approaches that have been validated to measure insulin sensitivity including the insulin tolerance test (ITT), the oral glucose tolerance test

(OGTT), and frequently sampled intravenous glucose tolerance test (FSIVGTT), the euglycemic clamp technique is considered the gold standard test for insulin action in our population.[63,64] Besides the recent review article that reconfirmed this, many studies have utilized this test to determine insulin time-action profiles in diverse populations with optimal results.[30,52,65] The technique uses an approach to measure the true tissue sensitivity to insulin by maintaining a steady-state euglycemia throughout the study.[63] At steady plasma glucose levels, the glucose infusion rate following the exogenous insulin infusion will reflect the actual glucose uptake by peripheral tissues.

In the proposed study, allocation of intervention days will be randomized in order to prevent selection bias. Due to the nature of the intervention, massaging cannot be concealed from the study subjects and investigators in the room. However, data-analysts will be blinded to the treatment allocation. Additionally, the study outcomes, both primary and secondary, are objective measures of RAIA PK and PD profiles and should not be influenced by blinding.[66]

Study population, sampling and recruitment

The target population for this study is overweight and obese adolescents between the ages of 12-18 years who have a diagnosis of T1DM for ≥ 1 year at the time of enrollment. Inclusion and exclusion criteria for enrollment are listed in chapter III of this proposal and are based on literature reviewed in this Chapter. The age range for the study participants, 12-18 years, is chosen to avoid variability due to insulin sensitivity differences among study subjects. It has been shown that teenagers in puberty have an increased insulin resistance (IR) compared to the rest of the pediatric population.[45,48,67] Swan et al.[48] showed that there is an ~37% delay in the PD response of SC injected

RAIAs, specifically aspart, in the pubertal age compared to the pre-pubertal age group. The pre-pubertal group had a significantly higher glucose infusion rate requirement (1,326 ± 131mg/kg vs. 964 ± 65mg/kg; P<0.01) indicating better insulin sensitivity. A longitudinal study of insulin sensitivity also demonstrated that during puberty there is an ~50% decrease in insulin sensitivity[67]. The rise in this IR begins around age 7, peaks before Tanner stage II and normalizes at the end of puberty.[68] Therefore, we will only include adolescents between Tanner stages III-V.

Participants will be recruited from the Yale Pediatric Diabetes Clinic. Since the study subjects will be sampled from a single-center, it was important for us to determine the availability of the source population. Combining data collected from a pediatric endocrinologist at the clinic and data from the T1D registry,[69,70] we have identified a minimum of 168 obese and overweight 12-18 year-old adolescents, which is approximately 12% of the total pediatric patients at the clinic. Even with a limited source population, we do not anticipate any obstacles, as our needed sample size is relatively small (discussed later in this chapter).

Intervention and Safety

SC injection of aspart, one of the three FDA approved RAIAs, is the only proposed intervention for the control day of the study. The same insulin analog and dose, in addition to the 3-minute massage, will be used for the intervention day. In 2012, a systematic review of 19 articles by Home P.D. reported an equal efficacy and safety of all three RAIAs.[32] However, these insulin analogs exhibit small clinically insignificant variability in their PK and PD profiles including peak insulin concentration, time to maximal concentration, onset of action, and duration of action.[35-37] Thus, in this proposed

study we will only use one type of RAIA, aspart, to avoid any inter and intra-subject variability in insulin absorption and action. In addition, even though not significantly different, aspart has a faster onset and peak action, as well as a shorter duration of action.

The prescription information for aspart lists potential adverse effects of hypoglycemia, hypersensitivity and local injection site reactions, lipodystrophy, rash and pruritus[35]. In 1998, a randomized double-blinded crossover trial in T1DM patients comparing the glycemic control benefit of insulin aspart with RHI also identified a few adverse events, including, hypoglycemia episodes, fatigue, anorexia, vomiting and pyrexia.[26] Mild to moderately severe hypoglycemia and headache were also noted in another randomized double-blinded crossover trial comparing RHI with aspart[27]. These events however were noted consistently between both RHI and aspart. Also, recent trials that used aspart in their study do not report any such adverse effects.[44,52,65] Throughout the study, we will keep in mind these potential adverse events that might present associated with aspart use. We will also cautiously perform a pre and post-study history and physical exam in all our participants.

In choosing the site of SC insulin injection, we looked at two studies in particular along with recent trials that utilized the euglycemic clamp technique. Mudaliar et al., comparing the abdominal site to the deltoid and thigh, showed a significantly shorter duration of glucose lowering action (~34min shorter; $P < 0.001$) in healthy, non-diabetic men who received aspart vs. RHI in a randomized crossover euglycemic study[28]. This study also demonstrated a 10-14% lower total glucose infusion rate (AUC$_{GIR}$) when aspart was injected abdominally vs. in the thigh or deltoid region. In addition to this study, a randomized crossover trial using lispro[71] has also shown some PK property

differences between the abdomen and other two sites, though not significant. Based on these data suggesting an optimal insulin action for RAIAs injected abdominally, we have chosen the abdomen for SC aspart administration.

In regards to the euglycemic clamp study, as suggested by Brown and Yanovski,[64] skilled investigators who have extensive experience and clinical judgment in adjusting the glucose infusion rates will be utilized in the study. In addition, a validated computer algorithm will be available to assist with the decision-making. Other personnel involved in the study will also be experienced physician assistants. They will continually monitor the patency of the IV lines to avoid infiltration, and also for any patient symptoms and signs of hypoglycemia.

Massage

Due to lack of prior clinical trials that determined an optimal rate and pressure of massage associated with the study's dependent variables, a standard slow rate of 30cycles/minute and firm pressure will be used to standardize our intervention. This is adopted based on the information in the review article of manual massage techniques by Goats.[72] This article states that a slow yet firm pressured massage technique known as effleurage is identified to increase blood and lymph flow to massaged tissue.

There are no reported adverse effects associated with the use of massage for the purpose of facilitating medication absorption. The only study of massage in T1DM patients reports no adverse effects.[57] But, it is not clear if this is because there were none observed or if the effects were not simply measured as part of the original plan. Another study that performed a 30 minute long massage in non-diabetes patients also reports no adverse effects.[58] This study obviously used a much longer massage period from that of

the proposed period on this study. We are, therefore, confident to say that we don't

expect any major adverse effects associated with our standardized massage intervention.

Potential confounders and inclusion and exclusion criteria:

It is important to consider injection site subcutaneous fat thickness as an inter-

and intra subject confounding variable to the proposed study. The negative correlation of

SC fat with insulin absorption has been discussed previously on this chapter[43,44] and

considering our sample population's body weight, it's likely that most of our subjects will

have a substantial amount of abdominal fat, and this will vary between subjects and also

from one site to another in a subject. Therefore, the same abdominal injection site will be

used on both intervention days and this will be 10cm horizontal to the umbilicus on either

side. This will exclude the intrasubject confounding effect of SC fat. The thickness of SC

fat layer at the injection site will be estimated for each subject using an ultrasonography

and if a significant variability is noted we will include SC fat subgroup analysis to

account for a possible intersubject variability.

Insulin dosing among the study subjects will be determined based on their body

weight. Insulin concentration, however, will be kept the same among all subjects at

0.2U/kg. This is to avoid any intersubject variability of end point measures, as studies by

Sindelka et al. and others have shown a delayed insulin absorption and reduced serum

insulin concentration associated with higher concentrations of insulin administered

SC.[43,73] Although our obese population might require high insulin doses that could show

a modest prolongation of action of aspart, studies assert the insignificance of this as a

possible confounder.[74,75]

Strenuous exercise has an effect on the insulin sensitivity of peripheral tissues,

most significantly the muscle tissue. Acutely, during and right after an intense exercise, there is an enhanced utilization of muscle glycogen stores, which facilitates insulin-assisted muscle glucose uptake[76,77]. This has a significant influence on the glucose lowering effect of insulin therapy. Long-term training also determines insulin action profiles, as there is an overall improved glucose metabolism.[78] Therefore, exercising is one possible confounding variable for our study, especially since adolescents might be more motivated to enroll in strenuous training programs. We have eliminated the short-term effect of a strenuous exercise by including a no-exercise inclusion criterion at least 48 hours before the study dates. However, it is difficult to eliminate any modifying effect a long-term exercise routine might have in our study design.

Many studies have demonstrated the effect of ambient and local skin temperature on the rate of insulin absorption. With increased surrounding temperature whether as a result of sauna[60], or local heat application to site of injection[50-52], the absorption and action of insulin is significantly increased due to increased blood flow to the skin. Therefore, if not adequately controlled for, room temperature could differentially affect subjects in the two intervention orderings. Our study will be performed in the same temperature-controlled room on both intervention days.

Lastly, any medications and behaviors that interfere with insulin action, glucose metabolism or skin blood and lymphatic circulation such as corticosteroids[79], smoking[80], caffeine intake[81] and more are controlled for through our inclusion and exclusion criteria.

Sample size calculation

Sample size (SS) is determined based on the expected difference between the treatment and control groups on the measure of our PK primary outcome - rapid-acting

insulin analog absorption rate (T-INSmax). Since there are no prior clinical trials specifically examining the efficacy of local site massage on the PK and PD properties of RAIAs in overweight and obese T1DM adolescents, the expected effect size for our study was extrapolated based on data from previous relevant studies.

First, we evaluated a randomized crossover trial investigating the efficacy of a local site warming device, a mechanistically similar approach to that of massage, on the absorption of SC injected insulin aspart in normal weight and overweight T1DM adolescents. In this study, an absolute difference of 26 min and a relative reduction of ~39% in the time to maximal insulin concentration (T-INSmax) was observed between the group with the activation of the warming device at the SC injection site and without the warming device.[52] Moreover, a randomized, controlled crossover study showed that the absorption of SC injected insulin aspart using a conventional pen is delayed among overweight healthy adults when compared to their non-overweight counterparts by ~30min (95% CI 13.7 – 48.5; P < 0.012), which represents a relative delay of ~37.5%. [44] This delay in aspart absorption in this population is consistent with that of the obese population. The aspart insulin prescription information for obese healthy individuals describes the median time to maximal insulin concentration is ~85min compared to ~60min in non-obese individuals.[35] In addition, a randomized, controlled crossover trial comparing the PK and PD properties of glulisine and aspart showed a delayed absorption of aspart in obese adults with type 2 diabetes, with a T-INSmax of 93min.[82]

Based on this data, we estimate that the smallest relative reduction of time to maximal insulin concentration for our study population would be 39%. To be conservative, our sample size calculation assumes the lowest possible absolute difference

in the time to peak insulin concentration between our intervention and the standard of care. Therefore, we estimated we will need 19 subjects to detect an absolute mean difference of effect of 33 ± 41minutes to achieve 90% power and 2-tailed significance level of 5%. The absolute difference is deduced from a 39% reduction of effect from 85 minute. The standard deviation is adapted from Cengiz et al.'s randomized, controlled crossover trial[52]. Based on this same study, an attrition rate of 7.7% was determined, but to be conservative, we have accounted for a loss of 20% of patients/data. This will add 4 more subjects to our sample size resulting in a total of 23 participants.

Conclusion:

Based on the literature analyzed in this chapter, there is strong evidence that the obese and overweight adolescents have a significant challenge in achieving good glycemic control and also avoiding daily glycemic variability. Unfortunately, there have not been many studies that investigated the benefit of potentially faster-acting insulin, with an accelerated absorption and action, on this particular population. PPH being one main contributor to the morbidity and mortality associated with T1DM, we will seek to investigate injection site massage as a potential solution by improving the PK and PD profile of RAIAs.

References:

1. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *The New England journal of medicine.* Sep 30 1993;329(14):977-986.
2. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes.* Aug 1995;44(8):968-983.
3. American Diabetes A. Standards of medical care in diabetes--2014. *Diabetes Care.* Jan 2014;37 Suppl 1:S14-80.
4. Hirsch IB, Brownlee M. Beyond hemoglobin A1c--need for additional markers of risk for diabetic microvascular complications. *JAMA : the journal of the American Medical Association.* Jun 9 2010;303(22):2291-2292.
5. Prince CT, Becker DJ, Costacou T, Miller RG, Orchard TJ. Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC). *Diabetologia.* Nov 2007;50(11):2280-2288.
6. Kilpatrick ES, Rigby AS, Atkin SL. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. *Diabetes Care.* Nov 2008;31(11):2198-2202.
7. Ceriello A. The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes/metabolism research and reviews.* Mar-Apr 2000;16(2):125-132.
8. Boland E, Monsod T, Delucia M, Brandt CA, Fernando S, Tamborlane WV. Limitations of conventional methods of self-monitoring of blood glucose: lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes. *Diabetes Care.* Nov 2001;24(11):1858-1862.
9. Chiasson JL, Josse RG, Gomis R, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA : the journal of the American Medical Association.* Jul 23 2003;290(4):486-494.
10. Hirsch IB, Brownlee M. Should minimal blood glucose variability become the gold standard of glycemic control? *Journal of diabetes and its complications.* May-Jun 2005;19(3):178-181.
11. Home P. Contributions of basal and post-prandial hyperglycaemia to micro- and macrovascular complications in people with type 2 diabetes. *Current medical research and opinion.* Jul 2005;21(7):989-998.
12. Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. *JAMA : the journal of the American Medical Association.* Apr 12 2006;295(14):1707-1708.
13. Slama G, Elgrably F, Sola A, Mbemba J, Larger E. Postprandial glycaemia: a plea for the frequent use of delta postprandial glycaemia in the treatment of diabetic patients. *Diabetes & metabolism.* Apr 2006;32(2):187-192.

14. Weber C, Schnell O. The assessment of glycemic variability and its impact on diabetes-related complications: an overview. *Diabetes technology & therapeutics.* Oct 2009;11(10):623-633.

15. Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA : the journal of the American Medical Association.* Apr 12 2006;295(14):1681-1687.

16. Wu D, Gong CX, Meng X, Yang QL. Correlation between blood glucose fluctuations and activation of oxidative stress in type 1 diabetic children during the acute metabolic disturbance period. *Chinese medical journal.* Nov 2013;126(21):4019-4022.

17. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care.* Mar 2003;26(3):881-885.

18. Woerle HJ, Neumann C, Zschau S, et al. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes Importance of postprandial glycemia to achieve target HbA1c levels. *Diabetes research and clinical practice.* Aug 2007;77(2):280-285.

19. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes.* Jun 2005;54(6):1615-1625.

20. Son SM, Whalin MK, Harrison DG, Taylor WR, Griendling KK. Oxidative stress and diabetic vascular complications. *Curr Diab Rep.* Aug 2004;4(4):247-252.

21. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care.* Mar 1996;19(3):257-267.

22. Rosenfeld L. Insulin: discovery and controversy. *Clinical chemistry.* Dec 2002;48(12):2270-2288.

23. Howey DC, Bowsher RR, Brunelle RL, Woodworth JR. [Lys(B28), Pro(B29)]-human insulin. A rapidly absorbed analogue of human insulin. *Diabetes.* Mar 1994;43(3):396-402.

24. Heinemann L, Heise T, Wahl LC, et al. Prandial glycaemia after a carbohydrate-rich meal in type I diabetic patients: using the rapid acting insulin analogue [Lys(B28), Pro(B29)] human insulin. *Diabetic medicine : a journal of the British Diabetic Association.* Jul 1996;13(7):625-629.

25. Trautmann ME. Effect of the insulin analogue [LYS(B28),PRO(B29)] on blood glucose control. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme.* Dec 1994;26(12):588-590.

26. Home PD, Lindholm A, Hylleberg B, Round P. Improved glycemic control with insulin aspart: a multicenter randomized double-blind crossover trial in type 1 diabetic patients. UK Insulin Aspart Study Group. *Diabetes Care.* Nov 1998;21(11):1904-1909.

27. Lindholm A, McEwen J, Riis AP. Improved postprandial glycemic control with insulin aspart. A randomized double-blind cross-over trial in type 1 diabetes. *Diabetes Care.* May 1999;22(5):801-805.

28. Mudaliar SR, Lindberg FA, Joyce M, et al. Insulin aspart (B28 asp-insulin): a fast-acting analog of human insulin: absorption kinetics and action profile

compared with regular human insulin in healthy nondiabetic subjects. *Diabetes Care.* Sep 1999;22(9):1501-1506.

29. Perriello G, Pampanelli S, Porcellati F, et al. Insulin aspart improves meal time glycaemic control in patients with Type 2 diabetes: a randomized, stratified, double-blind and cross-over trial. *Diabetic medicine : a journal of the British Diabetic Association.* May 2005;22(5):606-611.

30. Becker RH, Frick AD, Burger F, Potgieter JH, Scholtz H. Insulin glulisine, a new rapid-acting insulin analogue, displays a rapid time-action profile in obese non-diabetic subjects. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association.* Sep 2005;113(8):435-443.

31. Garg SK. New insulin analogues. *Diabetes technology & therapeutics.* Oct 2005;7(5):813-817.

32. Home PD. The pharmacokinetics and pharmacodynamics of rapid-acting insulin analogues and their clinical consequences. *Diabetes, obesity & metabolism.* Sep 2012;14(9):780-788.

33. Cobry E, McFann K, Messer L, et al. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. *Diabetes technology & therapeutics.* Mar 2010;12(3):173-177.

34. Luijf YM, van Bon AC, Hoekstra JB, Devries JH. Premeal injection of rapid-acting insulin reduces postprandial glycemic excursions in type 1 diabetes. *Diabetes Care.* Oct 2010;33(10):2152-2155.

35. A/S NN. Novolog (Prescription Information). Novo Nordisk A/S, Denmark, 2013. 2013.

36. Company ELa. Humalog (Prescription Information). Lilly USA, LLC, Indianapolis, 2013. 2013.

37. Sanofi-aventis UL. Apidra (Prescription Information). Sanofi-Aventis, US LLC, Bridgewater, 2013. 2013.

38. Overmann H, Heinemann L. Injection-meal interval: recommendations of diabetologists and how patients handle it. *Diabetes research and clinical practice.* Feb 1999;43(2):137-142.

39. Berger M, Cuppers HJ, Hegner H, Jorgens V, Berchtold P. Absorption kinetics and biologic effects of subcutaneously injected insulin preparations. *Diabetes Care.* Mar-Apr 1982;5(2):77-91.

40. Libman IM, Pietropaolo M, Arslanian SA, LaPorte RE, Becker DJ. Changing prevalence of overweight children and adolescents at onset of insulin-treated diabetes. *Diabetes Care.* Oct 2003;26(10):2871-2875.

41. Kaminski BM, Klingensmith GJ, Beck RW, et al. Body mass index at the time of diagnosis of autoimmune type 1 diabetes in children. *The Journal of pediatrics.* Apr 2013;162(4):736-740 e731.

42. Liu LL, Lawrence JM, Davis C, et al. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. *Pediatr Diabetes.* Feb 2010;11(1):4-11.

43. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. *Diabetologia.* // 1994;37(4):377-380.

44. de Galan BE, Engwerda EE, Abbink EJ, Tack CJ. Body mass index and the efficacy of needle-free jet injection for the administration of rapid-acting insulin analogs, a post hoc analysis. *Diabetes, obesity & metabolism.* Jan 2013;15(1):84-86.

45. Cree-Green M, Triolo TM, Nadeau KJ. Etiology of insulin resistance in youth with type 2 diabetes. *Curr Diab Rep.* Feb 2013;13(1):81-88.

46. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. *The Journal of pediatrics.* Aug 1994;125(2):177-188.

47. Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV. Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *The New England journal of medicine.* Jul 24 1986;315(4):215-219.

48. Swan KL, Weinzimer SA, Dziura JD, et al. Effect of puberty on the pharmacodynamic and pharmacokinetic properties of insulin pump therapy in youth with type 1 diabetes. *Diabetes Care.* Jan 2008;31(1):44-46.

49. Heinemann L, Muchmore DB. Ultrafast-acting insulins: state of the art. *J Diabetes Sci Technol.* Jul 2012;6(4):728-742.

50. Raz I, Weiss R, Yegorchikov Y, Bitton G, Nagar R, Pesach B. Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion. *Clinical Therapeutics.* // 2009;31(5):980-987.

51. Freckmann G, Pleus S, Haug C, Bitton G, Nagar R. Increasing local blood flow by warming the application site: beneficial effects on postprandial glycemic excursions. *Journal of diabetes science and technology.* // 2012;6(4):780-785.

52. Cengiz E, Weinzimer SA, Sherr JL, et al. Acceleration of insulin pharmacodynamic profile by a novel insulin infusion site warming device. *Pediatric Diabetes.* // 2013;14(3):168-173.

53. Hompesch M, Muchmore DB, Morrow L, Ludington E, Vaughn DE. Improved postprandial glycemic control in patients with type 2 diabetes from subcutaneous injection of insulin lispro with hyaluronidase. *Diabetes technology & therapeutics.* Mar 2012;14(3):218-224.

54. Hompesch M, Muchmore DB, Morrow L, Vaughn DE. Accelerated insulin pharmacokinetics and improved postprandial glycemic control in patients with type 1 diabetes after coadministration of prandial insulins with hyaluronidase. *Diabetes Care.* Mar 2011;34(3):666-668.

55. Gupta J, Felner EI, Prausnitz MR. Rapid pharmacokinetics of intradermal insulin administered using microneedles in type 1 diabetes subjects. *Diabetes technology & therapeutics.* Apr 2011;13(4):451-456.

56. McVey E, Hirsch L, Sutter DE, et al. Pharmacokinetics and postprandial glycemic excursions following insulin lispro delivered by intradermal microneedle or subcutaneous infusion. *J Diabetes Sci Technol.* Jul 2012;6(4):743-754.

57. Dillon RS. Improved serum insulin profiles in diabetic individuals who massaged their insulin injection sites. *Diabetes Care.* // 1983;6(4):399-401.

58. Linde B. Dissociation of insulin absorption and blood flow during massage of a subcutaneous injection site. *Diabetes Care.* Nov-Dec 1986;9(6):570-574.

59. Linde B, Philip A. Massage-enhanced insulin absorption--increased distribution or dissociation of insulin? *Diabetes research.* Aug 1989;11(4):191-194.

60. Koivisto VA. Sauna-induced acceleration in insulin absorption from subcutaneous injection site. *British medical journal.* Jun 14 1980;280(6229):1411-1413.

61. Goats GC. Massage--the scientific basis of an ancient art: Part 2. Physiological and therapeutic effects. *British journal of sports medicine.* Sep 1994;28(3):153-156.

62. Polit DF, Beck CT. *Nursing Research: Generating and Assessing Evidence for Nursing Practice.* 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2012.

63. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *The American journal of physiology.* Sep 1979;237(3):E214-223.

64. Brown RJ, Yanovski JA. Estimation of insulin sensitivity in children: methods, measures and controversies. *Pediatr Diabetes.* Apr 23 2014.

65. Engwerda EE, Abbink EJ, Tack CJ, de Galan BE. Improved pharmacokinetic and pharmacodynamic profile of rapid-acting insulin using needle-free jet injection technology. *Diabetes Care.* Aug 2011;34(8):1804-1808.

66. Wood L, Egger M, Gluud LL, et al. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. *Bmj.* Mar 15 2008;336(7644):601-605.

67. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. *Pediatric research.* Dec 2006;60(6):759-763.

68. Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). *Diabetes Care.* Mar 2012;35(3):536-541.

69. Beck RW, Tamborlane WV, Bergenstal RM, et al. The T1D Exchange clinic registry. *The Journal of clinical endocrinology and metabolism.* Dec 2012;97(12):4383-4389.

70. Arshad SH, Cengiz E. Prevalence of Hypertension (HT) and pre-Hypertension (p-HT) in 721 Children with Type 1 Diabetes (T1D): findings from the Yale Pediatric Diabetes Cohort. 2013.

71. ter Braak EW, Woodworth JR, Bianchi R, et al. Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin. *Diabetes Care.* Dec 1996;19(12):1437-1440.

72. Goats GC. Massage--the scientific basis of an ancient art: Part 1. The techniques. *British journal of sports medicine.* Sep 1994;28(3):149-152.

73. Heinemann L, Weyer C, Rave K, Stiefelhagen O, Rauhaus M, Heise T. Comparison of the time-action profiles of U40- and U100-regular human insulin and the rapid-acting insulin analogue B28 Asp. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association.* 1997;105(3):140-144.

74. Becker RH, Frick AD, Nosek L, Heinemann L, Rave K. Dose-response relationship of insulin glulisine in subjects with type 1 diabetes. *Diabetes Care.* Oct 2007;30(10):2506-2507.

75. Nosek L, Roggen K, Heinemann L, et al. Insulin aspart has a shorter duration of action than human insulin over a wide dose-range. *Diabetes, obesity & metabolism.* Jan 2013;15(1):77-83.

76. Kang J, Robertson RJ, Hagberg JM, et al. Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabetes Care.* Apr 1996;19(4):341-349.

77. Peter R, Luzio SD, Dunseath G, et al. Effects of exercise on the absorption of insulin glargine in patients with type 1 diabetes. *Diabetes Care.* Mar 2005;28(3):560-565.

78. Yki-Jarvinen H, DeFronzo RA, Koivisto VA. Normalization of insulin sensitivity in type I diabetic subjects by physical training during insulin pump therapy. *Diabetes Care.* Nov-Dec 1984;7(6):520-527.

79. Hirsch IB, Paauw DS. Diabetes management in special situations. *Endocrinology and metabolism clinics of North America.* Sep 1997;26(3):631-645.

80. Gerber PA, Locher R, Schmid B, Spinas GA, Lehmann R. Smoking is associated with impaired long-term glucose metabolism in patients with type 1 diabetes mellitus. *Nutrition, metabolism, and cardiovascular diseases : NMCD.* Feb 2013;23(2):102-108.

81. Whitehead N, White H. Systematic review of randomised controlled trials of the effects of caffeine or caffeinated drinks on blood glucose concentrations and insulin sensitivity in people with diabetes mellitus. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association.* Apr 2013;26(2):111-125.

82. Bolli GB, Luzio S, Marzotti S, et al. Comparative pharmacodynamic and pharmacokinetic characteristics of subcutaneous insulin glulisine and insulin aspart prior to a standard meal in obese subjects with type 2 diabetes. *Diabetes, obesity & metabolism.* Mar 2011;13(3):251-257.

CHAPTER III: STUDY METHODS

Study Design:

      This proposed study will conduct a single-centered, data analyst-blinded randomized crossover trial to investigate the pharmacokinetics and pharmacodynamics of aspart with or without a 3-minute abdominal SC injection site massage. All participants will undergo two euglycemic glucose clamp experiments in an inpatient, controlled setting where they will be admitted for a 24-hour period at a time. On the first day of the study, subjects will be randomly assigned by a computer program to begin with either SC aspart injection (control intervention) or SC aspart injection plus a 3-minute massage at an abdominal injection site, 10cm horizontally from the umbilicus (active intervention). On the second day of admission, participants will switch interventions. The insulin aspart dose that will be injected during each admission will be identical. There will be a washout period of at least 1 week between the two clamp studies. Female subjects will be tested at 4-week intervals to ensure that experiments take place during corresponding periods of the menstrual cycle.

Study Population, Sampling and Recruitment:

      A total sample of 23 male and female overweight and obese adolescents with T1DM will be recruited from the Yale Children's Diabetes Clinic for the purpose of this study. The study will be announced to all the on-site clinicians through a meeting where detailed description of the study will be communicated. These clinicians will in turn approach patients who meet the inclusion criteria and provide them with the study description, risk and benefits, consent procedures and privacy protection policy. Patients who agree to participate will then be prescreened for exclusion criteria eligibility by

participating investigators. Once eligibility is confirmed, investigators will go through the

consent process with participants again to ensure that they have been well informed.

During this process, investigators will explain the nature of the intervention, the need for

hospitalization for 24 hours (the period of the study) on two different occasions and

address any concerns the subjects might have.

Subjects will be eligible to participate in the study if they fulfill the inclusion and

exclusion criteria listed below:

Inclusion Criteria:

- A clinical diagnosis of T1DM for ≥ 1 year
- On insulin therapy for at least 3 months
- Age between 12 and 18 years old
- BMI percentile that is ≥ 85%
- Hemoglobin A1c (HbA1c) < 10.0%
- Non-smoker
- Able to abstain from alcohol, coffee and strenuous physical exercise at least 48hrs prior to study date
- The ability to comprehend both written and spoken English

Exclusion Criteria:

- Any other chronic disease besides T1DM
- On any medication that might interfere with the absorption of insulin or affect glycemic control such as glucocorticoid therapy
- On any new medication affecting blood glucose metabolism such as oral anti-diabetic drugs, or antipsychotic drugs in the last month
- Pregnancy or breast-feeding
- Any medical or psychosocial condition that might compromise the adolescent's or parent's ability to make an informed consent
- Any skin diseases that affect skin blood flow
- HbA1c > 10% to eliminate the effect of poorly controlled diabetes

Subject Protection and Confidentiality:

Parents of all participants (age < 18yrs) will be given written informed consents

(Appendix A) prior to the beginning of the study. We will also obtain an informed assent

from all the adolescent subjects (Appendix B). To protect the confidentiality of the study

participants, the proposed study will first be submitted to the Pediatric Protocol Review Committee (PPRC). Once it's approved, the study will proceed according to the guidelines and regulation of the Human Investigation Committee (HIC) and the Health Insurance Portability and Accountability Act (HIPAA). Subject identifying information will be kept confidential during and after study completion per Yale University Research Regulation. All collected data will be de-identified with the use of specific subject identification numbers assigned to each individual during enrollment. Data entry and analysis by data analysts and investigators will be performed and stored in secure databases on Yale's encrypted computer systems. Access to research data will only be available to investigators and analysts who have special passwords. The principal investigator will be responsible for identifying this personnel and protecting subject's personal information.

<u>Study Variables and Measures</u>:

The independent variable is a 3-minute injection site massage after SC injection to the abdomen 10cm on either side of the umbilicus. The control variable is an abdominal SC aspart injection also 10cm from the umbilicus.

The primary pharmacokinetic end point (dependent variable) is the time to peak serum insulin level (T-INSmax). This is a measure of the rate of absorption of insulin corresponding to the time when insulin plasma concentration reaches its maximum level after SC injection. The primary pharmacodynamic end point is the time to maximal glucose infusion rate (T-GIRmax), which corresponds to the time the SC injected inulin reaches its maximum glucose lowering effect.

The secondary end points (dependent variables) are also used to expand on the assessment of the PK and PD properties of SC injected aspart. The secondary pharmacokinetic outcomes will include, the peak insulin levels (C-INSmax) and the total area under the curve for change in insulin levels from baseline (AUC$_{INS}$). The secondary pharmacodynamic outcomes will include the maximal glucose infusion rate (GIRmax) and the total area under the curve for the glucose infusion rate (AUC$_{GIR\ 0-300min}$), which is the same as the total amount of glucose administered. All of these parameters will be measured and expressed as means ± SEM.

Demographic data including their mean daily insulin dose, mean glycemic control and duration of diabetes for each subject will be collected at baseline and reported in a table.

Study Protocol/Intervention:

Each subject will be admitted to the Yale Hospital Research Unit on two occasions for a 24 hour period to undergo a clamp study, which will allow us to demonstrate the insulin action properties of SC injected aspart insulin with and without injection site massage.

All participants will be admitted the afternoon prior to the study day after abstaining from caffeine, alcohol use and strenuous exercise for at least 48hrs. The experiments will be performed in a temperature-controlled room (22-24°C). Subjects will be asked to lie in a supine position in a comfortable bed set up for the purpose of the study. Two intravenous catheters will be placed in an antecubital vein of the arms, one for blood sampling and the other for the purpose of exogenous glucose infusion. An

insulin infusion set will also be inserted in the anterior abdomen on the opposite side of the umbilicus from where the insulin bolus will be injected the next day.

After an hour of equilibration period, we will begin sampling blood glucose levels hourly throughout the night. Using the new insulin infusion set, basal insulin infusion will be adjusted as needed to achieve glucose levels between 80 and 120mg/dl at the start of the clamp study the next morning. Subjects will fast overnight and continue until the end of the 5-hr euglycemic clamp procedure the next day.

Insulin Action (Clamp) Study

On the morning of the clamp study, participants will receive 0.2unit/kg body weight bolus of SC aspart insulin at a previously marked abdominal injection site using a conventional pen (NovoPen III, Novo Nordisk). Based on their random assignment for that study day, subjects will receive the SC abdominal aspart injection with or without massage. The administration of insulin begins the 5-hr clamp study and the basal insulin infusion will be suspended immediately. Plasma glucose will be maintained at euglycemic levels between 90-100mg/dl ± 5% during the study by a variable infusion of 20% dextrose based on plasma glucose measurements at 5-minute intervals. Blood for plasma insulin levels will be collected every 10 minute for the first 90 minutes and then every 30 minutes for the remainder of the time. This same procedure will be repeated on the second euglycemic clamp study day.

Massage

A physician assistant (PA) participating in the study as a co-investigator will be trained to perform a standardized massage after the SC abdominal injection of aspart at 10cm from the umbilicus. The massage will be performed using three fingers (2nd-4th

digits). The PA doing the massage will be trained on human subjects and will adapt a

standard rate and pressure that will be consistently applied to all study participants. To

minimize traumatizing friction against the skin, personnel will be trained to massage the

skin without lifting off fingers to avoid a back and forth movement on the skin. Rather,

the PA will keep all fingers on the skin and use a circular motion covering a 3-5cm

diameter from the injection site. A firm pressure adopted from the effleurage manual

technique of massage will be applied at a rate of 30 cycles/minute. To limit inter and

intrasubject variability only one person will be responsible for performing the massage.

<u>Methodology Consideration</u>:

Blinding:

Even thought double-blinded controlled trials are the best in avoiding information

bias, it is difficult to blind an intervention such as massaging from the study subjects and

the data-collecting investigator in the room. Therefore, intervention assignments will only

be concealed from personnel analyzing the data. Data analysts will not be present

throughout the study, and participants will be instructed not to discuss their group

assignments with anyone outside of the study room in order to avoid unexpected

interaction during their inpatient stay.

Assignment of intervention:

Patients will be assigned to a random order of intervention and control day using a

computer algorithm.

Monitoring of adverse events:

Before the beginning of the study, all participants will undergo a thorough history

and physical examination by the principal investigator or other PA co-investigators on the

study. Patients, who have been using a rapid-acting insulin analog different from aspart before this study, will especially be monitored for any potential adverse events reported by the producing company including hypoglycemia, hypersensitivity reactions, local injection site reactions, lipodystrophy, rash and pruritus. In addition to this, on the day of the intervention (local site massaging), subjects will be monitored for any local site adverse reaction from the massage such as erythema, edema, and rash. In the unlikely case that any of these adverse events occur acutely, intervention will be discontinued immediately and patients will pursue an appropriate level of care. The identified event will be reported to the Human Investigation Committee.

Data Collection:

During the study, plasma glucose will be measured every 5 minutes using the Yellow Springs Instrument (YSI) Glucose Analyzer (YSI Life sciences incorporated, Yellow Springs, OH, USA). Blood for plasma insulin levels will be collected every 10 minutes for the first 90 minutes and then every 30 minutes for the remainder of the time. Plasma insulin will be determined by the Mercodia iso-insulin ELISA test, with a reported cross-reactivity of 80% with insulin aspart (MercodiaAB, Uppsala, Sweden). All these insulin and glucose infusion rate data points will be used to calculate the mean values for all the PK and PD parameters for each of the 23 subjects.

Sample Size Calculation:

The number of subjects (N) needed to test out primary hypothesis that local skin massage will significantly reduce the T-INSmax and T-GIRmax of SC injected aspart with a 90% power and 2-tailed significance level of 5% is N=23. For the given effect size mean difference of 33 and SD = 41, a sample size of 19 pairs and alpha of 0.05, the

power is 90% to reject the null hypothesis that the difference between groups = 0. Based on another euglycemic clamp study, we determined an approximately 7.7% attrition rate, but to be conservative, we have accounted for a 20% attrition rate. This will bring our N to 23 pairs. The Power Analysis and Sample Size (PASS) software, version 2008 was used to calculate the sample size (Appendix C).

Data Analysis:

The study subjects will be their own controls as the study design is a randomized crossover, controlled trial. Therefore, there is no need to compare subject baseline characteristics between control and intervention group descriptively. However, for the purpose of our subgroup analysis demographic data will be collected and categorized.

Pharmacodynamic parameters will be derived from the exogenous glucose infusion rate (GIR) profiles. Pharmacokinetic parameters will be derived from the serum aspart concentrations. All data will be expressed as means ± standard deviation and as continuous variables. Distribution of all outcome variables will be evaluated for normality. If normally distributed, a parametric test of paired *t*-test will be used for bivariate analysis. Alternative statistical approaches such as the Wilcoxon signed-rank test (a non-parametric test) will be used when appropriate if a skewed distribution is noted. PK and PD parameters will be analyzed in subgroups defined by baseline characteristics such as sex, race and BMI (overweight vs. obese).

Timeline:

Recruitment of participating adolescents from the Yale Children's Diabetic Clinic will begin in January 2015. All patients who meet the inclusion criteria should be contacted by their clinician by the end of the month and will have until the end of March

2015 to decide if they want to enroll in the study. This will allot a total recruitment time

of 3 months. Once enough subjects are recruited, participants will be screened for

exclusion criteria and those who qualify will go through the consent process between

April – May 2015. After this, the duration of intervention and data analysis will

approximately be 6-8 months.

Table 2. Study timeline of proposed study

Study Phase	Estimated Start Date	Estimated End Date
Submission for PPRC and HIC Review	May 14, 2014	--------------------
Participant Recruitment	January, 2015	March 31, 2015
Screening/Enrollment	April 1, 2015	May 30, 2015
Study Implementation and Data Collection	June 1, 2015	November, 2015
Data analysis	December, 2015	January, 2016

CHAPTER IV: CONCLUSION

Strengths and Advantages

This study has numerous strengths in regards to the study design, methodology and clinical implications. Primarily, it is going to address an important issue by introducing a non-invasive practical intervention to accelerate insulin action. As discussed in prior chapters, a few other approaches are in clinical development to address the shortcomings of RAIAs in the T1DM population. However, none are as practical as massage. Another important strength is our subject group. We are targeting the high-risk group of patients (obese, overweight adolescents) who will benefit the most from this intervention. Moreover, insulin injection site massaging has never been studied in the pediatric patient population, and T1DM is the most common type of diabetes in this population[1].

The greatest methodological strength of this study is its randomized, single-blinded and controlled crossover design. This is in contrast to the only study that sought to look at the benefits of our proposed intervention in T1DM patients and the few others done in healthy individuals. The inpatient nature of our clamp study will allow us to control for variables that could confound the efficacy of local skin massage. Also, even though it is impossible to blind the intervention from the study subjects and investigators, we will blind the personnel who will be analyzing our collected data. Our study is also superior to other studies as we plan to use a larger sample size, and it will be the first study that will utilize the validated, gold standard clamp technique to demonstrate the potential of insulin injection site massage after insulin bolus delivery in accelerating insulin absorption and action.

55

The homogeneity of our selected T1DM population is another strength of this study. Even though this is usually perceived as a limitation, our study has a specific goal and targets only those who we believe will significantly benefit from an uncomplicated intervention that has a potential to control PPG levels – the obese and overweight adolescents. Additionally, the inclusion criteria for an HbA1c < 10% was specifically included as studies have suggested patients with moderately elevated HbA1c values above target will benefit more from an optimal control of PPGEs.[2,3] The other advantage of the homogeny of our subjects is to allow us to accurately determine the effect of our intervention. At this preliminary stage, we cannot assume that the efficacy of massaging will be consistent among different groups. Therefore, the narrower the differences amongst our subjects, the more focused our inferences will be.

Another strength of this study is the use of a novel standardized massage protocol among all our participants. We cannot attest to this protocol being the most optimal, however, this pilot protocol will serve as the basis for further investigations. As it is the first study that uses a specific but simple standard massage, we suspect more trials will be need to determine the most efficient length, timing, depth, and rate of massage. Last, but definitely important to recognize is Yale's experience in performing such studies with a skilled clinical team. As suggested by Brown and Yanovski[4] this is an asset to the safety and productivity of clamp studies.

Limitations and Disadvantages

The major limitation of this study is that the euglycemic clamp will only assess insulin action properties in a fasting state. Therefore, it will not allow us to directly assess postprandial glucose control. We chose not to include a meal challenge test in our design

because the goal for this initial study is to serve as the proof-of-concept that massage in

our particular population can achieve an accelerated insulin absorption and inulin action.

Additionally, to include a meal study such as one by Hompesch's group[5] (reviewed in

Chapter II) would require longer study duration, as patients would need to have

additional visits to determine an individualized optimal insulin dose. This might have

been difficult to achieve in the 2-year period of time available for this proposed study.

Nonetheless, as a follow-up to our trial, we strongly recommend the pursuit of a meal

study to investigate the efficacy of insulin injection site massage in real-life management

of diabetes.

The novelty of our proposed pilot protocol for local skin massage might also pose

a minor limitation to our study, as it may not illustrate the full potential of the

intervention. However, this is very unlikely as we are proposing a well-controlled and

sufficiently powered study that will detect the smallest significant difference between our

control and intervention groups. We have also standardized the massaging technique

among all our subjects and will only have one investigator performing the massage.

Clinical and Public Health Significance

The incidence of T1DM and obesity in children are both on the rise.[6,7] As

demonstrated by the recent T1DM Exchange Clinic data, the majority of children with

T1DM have poor glycemic control.[6,7] By increasing tissue insulin resistance, obesity

poses an independent risk factor for impaired insulin action.[8] This presents an additional

challenge to controlling postprandial blood glucose levels in children with T1DM, and

thus, results in poor glycemic control. This is despite the clinical advances that have

provided multiple interventions such as RAIAs and the continuous glucose monitoring

system. This study will provide important clinical information about the benefit of insulin injection site massage in improving RAIA absorption and action to match that of the physiologic insulin response. If demonstrated to be effective, insulin injection site massage combined with the already available approaches can potentially mitigate the risk factors that lead to late-stage progression of microvascular and macrovascular complications in this already high-risk population.

A development of such an intervention is not only imperative from a clinical aspect, but also from a public health standpoint. According to the American Diabetes Association, the estimated national medical cost associated with diabetes in 2012 was $176 billion. Out of this, 17% comes from the cost of antidiabetic agents and diabetes supplies.[9] This high economic cost of diabetes has increased hand in hand with the increasing prevalence of diabetes[10]. Therefore, any non-costly and easily accessible intervention that will improve glycemic control and reduce the short and long-term complications of diabetes will be essential to alleviate the extensive economic burden of disease management. Ideally, this study will provide promising results that will eventually lead to the incorporation of insulin injection site massage in the standard of care.

References:

1. Powers AC. Diabetes Mellitus. *Harrison's Principles of Internal Medicine*. 18th ed. New York: McGraw-Hill; 2012.
2. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care.* Mar 2003;26(3):881-885.
3. Slama G, Elgrably F, Sola A, Mbemba J, Larger E. Postprandial glycaemia: a plea for the frequent use of delta postprandial glycaemia in the treatment of diabetic patients. *Diabetes & metabolism.* Apr 2006;32(2):187-192.
4. Brown RJ, Yanovski JA. Estimation of insulin sensitivity in children: methods, measures and controversies. *Pediatr Diabetes.* Apr 23 2014.
5. Hompesch M, Muchmore DB, Morrow L, Ludington E, Vaughn DE. Improved postprandial glycemic control in patients with type 2 diabetes from subcutaneous injection of insulin lispro with hyaluronidase. *Diabetes technology & therapeutics.* Mar 2012;14(3):218-224.
6. Beck RW, Tamborlane WV, Bergenstal RM, et al. The T1D Exchange clinic registry. *The Journal of clinical endocrinology and metabolism.* Dec 2012;97(12):4383-4389.
7. Wood JR, Miller KM, Maahs DM, et al. Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care.* Jul 2013;36(7):2035-2037.
8. Cree-Green M, Triolo TM, Nadeau KJ. Etiology of insulin resistance in youth with type 2 diabetes. *Curr Diab Rep.* Feb 2013;13(1):81-88.
9. American Diabetes A. Economic costs of diabetes in the U.S. in 2012. *Diabetes Care.* Apr 2013;36(4):1033-1046.
10. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Population health metrics.* 2010;8:29.

APPENDIX A: HIC INOFORMED CONSENT FORM

**PARENTAL CONSENT FOR CHILD/ADOLESCENT PARTICIPATION IN A
RESEARCH PROJECT
YALE UNIVERSITY SCHOOL OF MEDICINE -
YALE NEW HAVEN HOSPITAL – SAINT RAPHEL CAMPUS**

Study Title: *INJECTION SITE MASSAGE TO IMPROVE THE PHARMACOKINETICS
OF ASPART IN OBESE ADOLESCENTS WITH DIABETES*

Principal Investigator: *Hiwot K. Girma, PA-SII; Eda Cengiz, MD, MHS, FAAP*

Funding Source: *Yale University School of Medicine, Department of Endocrinology*

Invitation to Participate and Description of Project

We are inviting your child to participate in a research study designed to look at the
efficacy of injection site massage to accelerate the absorption and action of insulin. Your
child has been asked to participate because he/she has type I diabetes for ≥ 1 year and has
been identified by his/her endocrinologist as a good fit for this study based on the study
inclusion criteria.

In order to decide whether or not you wish your child to be part of this research
study you should know enough about its risks and benefits to make an informed decision.
This permission form gives you detailed information about the research study, which a
member of the research team will discuss with you. This discussion should go over all
aspects of this research: its purpose, the procedures that will be performed, any risks of the
procedures, and possible benefits. Once you understand the study, you will be asked if you
wish your child to participate; if so, you will be asked to sign this form.

Description of procedures

If you agree for your child to participate in this study, you will be asked to provide
demographic and medical information about your child such as their age, gender, ethnicity,
weight, past medical history, duration of diabetes, daily insulin dose, and previous insulin
therapy. Your child will also be required to give 20ml of blood to assess the baseline
HbA1c. This information will provide us with the details we need to determine if your child
is eligible to enroll in the study. All information provided will be completely confidential.

Following this initial assessment, if your child is eligible for the study and you wish
for him/her to participate, he/she will be enrolled into the study. The study will be
performed in two separate days that are at least 1 week apart for boys and 4 weeks apart
for girls. A computer program will be used to randomly assign participants to receive either
the intervention or control treatment in different orderings for the two test days. Once your
child is randomly assigned to the interventions, a member of our investigation team will sit
down with you to give you details entailing each test day and procedures.

On one of the test days, your child will receive an injection of Aspart, which is the standard of care for diabetes management, on the abdomen. If you are not familiar with this drug, it is an insulin therapy that belongs to the class of drugs that are commonly known as short or rapid-acting inulin analogs. Its other name is NovoLog and it is in the same class as Lispro (Humalog) and Glulisine (Apidra). It is approved by the Food and Drug Administration (FDA) for glucose control in patients with type I diabetes. On the other test day, your child will receive an injection of this same insulin followed by a 3 minute standardized injection site massage. The 3min massage is the intervention that is being investigated.

On the days of the study, each participant will be admitted to the Yale Hospital Research Unit for a 24 hours period. Your child will be admitted the afternoon prior to the study day after abstaining from caffeine, alcohol and strenuous exercise for at least 48hrs. Before the beginning of the study, all participants will undergo a thorough medical interview and physical examination by the principal investigator or other physician assistants who will be co-investigators on the study. Following this, the participants will be asked to lie flat on their back on a comfortable bed that we will provide for the length of the study. Two intravenous (IV) lines will be placed in your child's arms, one for blood sampling and the other for the purpose of glucose infusion during the study. An insulin infusion set will also be inserted on the participant's abdomen on the opposite side of the belly from where Aspart will be injected the next day.

Throughout the night before the study day, we will monitor your child's blood glucose by sampling blood every hour. Based on his/her glucose level we will adjust the inulin that we will be infusing through the set that is placed on your child's abdomen. Our goal is to achieve a good glucose level in preparation for the main procedure the next day. Participants will fast overnight and continue until the end of the procedure the next day.

Insulin Action Study Procedure
On the morning of the study, based on your child's random assignment he/she will receive a subcutaneous injection of Aspart at a previously marked abdominal injection site with or without a 3-min massage. After this, we will continually monitor your child's blood glucose by sampling blood every 5 minutes from the IV line that was inserted the day before. Blood insulin levels will also be collected every 10min for the first 90min of the study and every 30min for the remainder of the study period. The total study period will be 5 hours on each test day.

Risks and Inconveniences

Participants in this study may experience hypoglycemia, hypersensitivity reactions, local injection site reactions, lipodystrophy, rash and itching associated with the use of Aspart. However, the risk of your child experiencing these adverse effects is very rare, and we will minimize the possibility by the careful administration of Aspart and appropriate monitoring post-administration. Your child will be in the hands of very experienced Yale University investigators, PAs and nurses.

There are no known risks or side effects associated with the 3-minute local skin massage. Trained personnel will perform this intervention and we will be monitoring the massage site for any adverse reactions during and after the procedure.

Even though very rare, you should also be aware of the risks associated with IV line placements and blood draws that will take place during the study. Trained clinical staff will perform blood draws for the initial assessment of HbA1c levels and also place the IV lines on the day of your child's admission. General discomfort, excessive bleeding, feeling of lightheadedness, bruising from a small amount of bleeding under the skin, and minor infections are the possible, but rare side effects associated with these two procedures. The discomfort is transient and our trained clinicians will do their best to make your child comfortable. Excessive bleeding and lightheadedness are uncommon, but are easily treatable if necessary. A bruise will also go away once the IV line is removed, which we will do immediately if one is detected. Infections can also be treated, but our team will use sterile equipment and procedures to avoid any chance of an infection.

Benefits

Your child's participation in this study will provide very critical information regarding the potential benefit of an injection site massage on the improvement of insulin absorption and action. Better insulin absorption and action will improve obese and overweight adolescents' post-meal blood glucose values, which will have long-term clinical benefits. Additionally, if proven to positively affect insulin properties, your child's participation in this study might in the future provide a cost-effective, and non-invasive intervention such as massage.

In Case of Injury

If your child is injured as a result of his/her participation in this study, treatment will be provided. You or your insurance provider will be expected to pay for the cost of this treatment. No additional financial compensation for injury or lost wages is available.

You do not give up any of your child's legal rights by signing this form.

Economic Considerations

All materials and procedures to be used in this study will come at no cost to you, your child or healthcare insurer. Your child will be offered a meal after the end of the procedure and can utilize the research unit's facilities for personal hygiene needs. Otherwise, your child will not be offered any financial compensation for participating in the study.
Confidentiality

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S.

or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. All identifying patient information obtained during the recruitment and study will remain confidential in accordance with Health Insurance Portability and Accountability Act (HIPAA) guidelines. Data entry and analysis by our team will be performed on encrypted computers that will only be accessible by a special password. This will remain confidential after the completion and publication of our work.

When the results of the research are published or discussed in conferences, no information will be included that would reveal your child's identity unless your specific consent for this activity is obtained.

Representatives from the Yale Human Research Protection Program, the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects) may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

Voluntary Participation and Withdrawal

Your child's participation in this study is voluntary. You are free to choose not to have your child take part in this study. Refusing to participate will not involve any penalty or benefits to which your child is otherwise entitled, such as health care outside the study, the payment for your child's health care, and health care benefits. However, you will not be able to enroll in this study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study.

If your child does become a subject, you are free to stop and withdraw from this study at any time during its course. You can alert any of the investigators who will be in the study room that you no longer want your child to take part.

The researchers may withdraw your child from participating in the research if necessary. You may be withdrawn for reasons relating to your health status, progression of underlying disease or treatment, or non-adherence to the study protocol.

If you choose for your child to not participate or if you withdraw it will not harm your relationship with your child's doctors at Yale Pediatrics Diabetes Clinic or with Yale New Haven Hospital. Your child will continue to receive a standard of care therapy without any complications.

Questions

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the consent form carefully – as long as you feel is necessary – before you make a decision. If you have any questions

about this project, you may contact the investigator *Hiwot Girma, PA- SII through email at Hiwotkg@ynhh.edu*

<u>Authorization</u>

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my child's involvement

and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject:_____

Signature:_____

Relationship:_____

Date:_____

_____   _____
Signature of Principal Investigator   Date

                        or

_____   _____
Signature of Person Obtaining Consent   Date

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator, contact information can be provided by your research associate. If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919.If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

THIS FORM IS NOT VALID UNLESS THE FOLLOWING BOX
HAS BEEN COMPLETED BY THE HIC OFFICE

> THIS FORM IS VALID ONLY THROUGH:
> _____.
>
> INITIALED:
> _____

Appendix B: HIC Informed Child/Adolescent Assent Form

**CHILD/ADOLESCENT ASSENT FOR CHILD/ADOLESCENT
PARTICIPATION IN A RESEARCH PROJECT
YALE UNIVERSITY SCHOOL OF MEDICINE -
YALE NEW HAVEN HOSPITAL – SAINT RAPHEL CAMPUS**

Title: INJECTION SITE MASSAGE TO IMPROVE THE PHARMACOKINETICS OF ASPART IN OBESE ADOLESCENTS WITH DIABETES

Why am I here?

We are asking you to take part in a research study because we are trying to learn more about how to make the insulin injections that type I diabetes children take get absorbed faster and work quicker. We plan to do this by massaging your insulin injection site for 3 minutes after we inject your insulin on your belly. We are inviting you to be in the study because you have type I diabetes and already use an insulin injection.

What will happen to me?

You will come into the hospital the afternoon before we do the study and stay overnight. We will ask you not to eat starting from when you come into the hospital until we finish the study the next day. We will have a comfortable bed for you to lie on when we do the test. Once you are comfortable, an experienced nurse will place an intravenous (IV) line on both of your arms. An IV line is a straw-like plastic that we put in your arm using a small needle to help us get some of your blood or inject medication. Another similar tube will also be inserted on your belly so we can use it to inject insulin to control you blood sugar levels until we start the study.

On the morning of the study, you will get an injection of insulin on your belly using a pen that will look very similar to what you have been using at home. Depending on what you were assigned to receive by our computer for that day, you will either get a 3 minute massage following the insulin injection or no massage. If you don't get a massage that first test day, you will get it on the next test day you come into the hospital. After this, we will start drawing a small amount of blood from the IV lines that we had placed the night before every 5-10 minutes. The test day should take 5 hours and you will be offered a lunch when we are done with the study.

Will the study hurt?

The initial IV placement in your arms might hurt a small amount, but the nurses, who place the IV, are very well trained and will try their best to make you comfortable.

66

Will the study help me?

The study will not immediately help you at the time of the test. However, our goal, if the massage gives us good results, is that it will in the future help make your and other children's blood sugar levels more controlled.

What if I have any questions?

You can ask any questions that you have about the study. If you have a question later that you didn't think of now, you can call me (917 638 8273) or ask me next time.

Do my parents know about this?

This study was explained to your parents and they agreed that you could be in it. You can talk this over with them before you decide.

Do I have to be in the study?

You do not have to be in the study. No one will be upset if you don't want to do this. If you don't want to be in this study, you just have to tell us. You can say yes now and change your mind later. It's up to you.

Agreement to be in the Study:
Writing your name on this page means that that you agree to be in the study, and know what will happen to you. If you decide to quit the study all you have to do is tell the person in charge.

_____   _____

Signature of Child   Date

_____   _____

Signature of Researcher   Date

THIS FORM IS NOT VALID UNLESS THE FOLLOWING

BOX HAS BEEN COMPLETED IN THE HIC OFFICE


```
+--------------------------------------------------+
|                                                  |
|  THIS FORM IS VALID ONLY THROUGH:                |
|  _____.               |
|                                                  |
|                                                  |
|  HIC PROTOCOL #:                                 |
|  _____                   |
|                                                  |
|                                                  |
|  INITIALED:                                      |
|  _____                |
|                                                  |
+--------------------------------------------------+
```

Appendix C: Sample Size Calculation

Using a statistical program (Power and Precision Version 4 Software), sample size was calculated using the values listed below:

Effect Size: 33min
Standard Deviation (SD): 41
α (two-tailed) = 0.05
$\beta = 1 - 0.912 = 0.088$

Sample size required to compare means of continuous variables using a Paired t-test analysis and considering alpha and beta errors: 19 pairs

Total participants/pairs required = 19
+ a conservative 20% dropout/data loss rate = **23 pairs**.

Attrition rate was calculated from another euglycemic clamp study (Cengiz, 2013) using the formula:

$$\text{Attrition rate} = \frac{\text{Average number of subjects lost}}{\text{Average Number of subjects enrolled}} \times 100$$

$$= 1/13 \times 100 = 7.69\%$$

Appendix D: Sample Letter of recruitment to Clinicians at YPDC

<div align="right">(Date)</div>

Dear (Name of Clinician),

      We would like to let you know about a research study that the Yale Pediatric Endocrinology Department in coordination with Yale New Haven Hospital is planning to conduct within the next two years. We hope this letter followed by our planned information meeting next month will give you enough information to interest you in the study. We ask you to consider referring your patients for possible participation.

The title of the study is:

<div align="center">

INJECTION SITE MASSAGE TO IMPROVE THE PHARMACOKINETICS OF ASPART IN OBESE ADOLESCENTS WITH DIABETES

</div>

      The study we are conducting is a single-blinded, randomized controlled crossover trial to assess the efficacy of injection site massage in improving the pharmacokinetics and pharmacodynamics of aspart in overweight and obese adolescents with type I diabetes mellitus (T1DM).

      There is an increasing incidence of obesity at the time of T1DM diagnosis in children, and the majority of children with T1DM have poor glycemic control. Obesity being an independent risk factor for impaired insulin action; it presents an additional challenge to controlling postprandial blood glucose with the use of pre-meal rapid-acting insulin analogs. Postprandial blood glucose excursions are known to have a strong correlation with the vascular morbidities associated with T1DM both directly and indirectly by affecting overall glycemic control. Therefore, an intervention that improves insulin action, to mitigate this post-meal glucose variability will be of critical value. We suspect that massage will have a beneficial role in accelerating subcutaneously administered insulin absorption and action to moderate the PPG levels that are elevated and prolonged in overweight and obese adolescents. Therefore, we wish to study the hypothesis that a standardized abdominal skin massage at the site of subcutaneous (SC) aspart injection will significantly increase in the rate of insulin absorption and action.

Patients that meet the following criteria may be eligible for the study:

Inclusion Criteria:

- A clinical diagnosis of T1DM for $\geq$ 1 year.
- On insulin therapy for at least 3 months
- Age between 12 and 18 years old
- BMI percentile that is $\geq$ 85% and < 99%
- Hemoglobin A1c (HbA1c) < 10.0%
- Non-smoker

- Able to abstain from alcohol, coffee and strenuous physical exercise at least 48hrs prior to study date
- The ability to comprehend both written and spoken English

Exclusion Criteria:

- Any other Chronic Disease besides T1D
- On any medication that might interfere with the absorption of insulin or blood glucose metabolism.
- Pregnancy or breast-feeding
- Any medical or psychosocial condition that might compromise the adolescent's or parent's ability to make an informed consent
- Any skin diseases that might affect skin blood flow
- HbA1c > 10% to eliminate the effect of poorly controlled diabetes

We invite you to a participate in a meeting at Yale Pediatric Diabetic Clinic on January __, 2015 at __PM to provide you with further details in regards to this study and recruitment process. We hope you can make it, but please feel free to contact us beforehand or after the meeting if you are not able to attend. You can reach as at (917) 638 8273 or hiwot.girma@yale.edu

We look forward to speaking with you and hope you are interested in working with us.

Thank you for your time and consideration.

Sincerely,

Hiwot Ketema Girma, PA-SII   Dr. Eda Cengiz, MD, MHS, FAAP
Yale Physician Associate Program Assistant Professor of Pediatrics
Hiwot.girma@yale.edu   Department of Endocrinology

Bibliography:

1. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *The New England journal of medicine.* Sep 30 1993;329(14):977-986.
2. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. *The Journal of pediatrics.* Aug 1994;125(2):177-188.
3. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes.* Aug 1995;44(8):968-983.
4. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* Sep 12 1998;352(9131):854-865.
5. Federation ID. Global IDF/ISPAD Guidlines for Diabetes in Childhood and Addolescence. 2011: http://www.idf.org/global-idfispad-guideline-diabetes-childhood-and-adolescence.
6. A/S NN. Novolog (Prescription Information). Novo Nordisk A/S, Denmark, 2013. 2013.
7. American Diabetes A. Postprandial blood glucose. American Diabetes Association. *Diabetes Care.* Apr 2001;24(4):775-778.
8. American Diabetes A. Economic costs of diabetes in the U.S. in 2012. *Diabetes Care.* Apr 2013;36(4):1033-1046.
9. American Diabetes A. Standards of medical care in diabetes--2014. *Diabetes Care.* Jan 2014;37 Suppl 1:S14-80.
10. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* Jan 2014;37 Suppl 1:S81-90.
11. Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV. Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *The New England journal of medicine.* Jul 24 1986;315(4):215-219.
12. Arshad SH, Cengiz E. Prevalence of Hypertension (HT) and pre-Hypertension (p-HT) in 721 Children with Type 1 Diabetes (T1D): findings from the Yale Pediatric Diabetes Cohort. 2013.
13. Beck RW, Tamborlane WV, Bergenstal RM, et al. The T1D Exchange clinic registry. *The Journal of clinical endocrinology and metabolism.* Dec 2012;97(12):4383-4389.
14. Becker RH, Frick AD, Burger F, Potgieter JH, Scholtz H. Insulin glulisine, a new rapid-acting insulin analogue, displays a rapid time-action profile in obese non-diabetic subjects. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association.* Sep 2005;113(8):435-443.
15. Becker RH, Frick AD, Nosek L, Heinemann L, Rave K. Dose-response relationship of insulin glulisine in subjects with type 1 diabetes. *Diabetes Care.*

Oct 2007;30(10):2506-2507.

16. Berger M, Cuppers HJ, Hegner H, Jorgens V, Berchtold P. Absorption kinetics and biologic effects of subcutaneously injected insulin preparations. *Diabetes Care.* Mar-Apr 1982;5(2):77-91.

17. Boland E, Monsod T, Delucia M, Brandt CA, Fernando S, Tamborlane WV. Limitations of conventional methods of self-monitoring of blood glucose: lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes. *Diabetes Care.* Nov 2001;24(11):1858-1862.

18. Bolli GB, Luzio S, Marzotti S, et al. Comparative pharmacodynamic and pharmacokinetic characteristics of subcutaneous insulin glulisine and insulin aspart prior to a standard meal in obese subjects with type 2 diabetes. *Diabetes, obesity & metabolism.* Mar 2011;13(3):251-257.

19. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Population health metrics.* 2010;8:29.

20. Brown RJ, Yanovski JA. Estimation of insulin sensitivity in children: methods, measures and controversies. *Pediatr Diabetes.* Apr 23 2014.

21. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes.* Jun 2005;54(6):1615-1625.

22. Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. *JAMA : the journal of the American Medical Association.* Apr 12 2006;295(14):1707-1708.

23. Campbell MS, Schatz DA, Chen V, et al. A contrast between children and adolescents with excellent and poor control: the T1D Exchange clinic registry experience. *Pediatr Diabetes.* Mar 2014;15(2):110-117.

24. Cengiz E. Undeniable need for ultrafast-acting insulin: the pediatric perspective. *J Diabetes Sci Technol.* Jul 2012;6(4):797-801.

25. Cengiz E, Weinzimer SA, Sherr JL, et al. Acceleration of insulin pharmacodynamic profile by a novel insulin infusion site warming device. *Pediatric Diabetes.* // 2013;14(3):168-173.

26. Ceriello A. The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes/metabolism research and reviews.* Mar-Apr 2000;16(2):125-132.

27. Charkoudian N. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clinic proceedings.* May 2003;78(5):603-612.

28. Chiasson JL, Josse RG, Gomis R, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA : the journal of the American Medical Association.* Jul 23 2003;290(4):486-494.

29. Cobry E, McFann K, Messer L, et al. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. *Diabetes technology & therapeutics.* Mar 2010;12(3):173-177.

30. Company ELa. Humalog (Prescription Information). Lilly USA, LLC, Indianapolis, 2013. 2013.

31. Cree-Green M, Triolo TM, Nadeau KJ. Etiology of insulin resistance in youth

with type 2 diabetes. *Curr Diab Rep.* Feb 2013;13(1):81-88.

32.   de Galan BE, Engwerda EE, Abbink EJ, Tack CJ. Body mass index and the efficacy of needle-free jet injection for the administration of rapid-acting insulin analogs, a post hoc analysis. *Diabetes, obesity & metabolism.* Jan 2013;15(1):84-86.

33.   DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *The American journal of physiology.* Sep 1979;237(3):E214-223.

34.   Dillon RS. Improved serum insulin profiles in diabetic individuals who massaged their insulin injection sites. *Diabetes Care.* // 1983;6(4):399-401.

35.   Engwerda EE, Abbink EJ, Tack CJ, de Galan BE. Improved pharmacokinetic and pharmacodynamic profile of rapid-acting insulin using needle-free jet injection technology. *Diabetes Care.* Aug 2011;34(8):1804-1808.

36.   Freckmann G, Pleus S, Haug C, Bitton G, Nagar R. Increasing local blood flow by warming the application site: beneficial effects on postprandial glycemic excursions. *Journal of diabetes science and technology.* // 2012;6(4):780-785.

37.   Galli-Tsinopoulou A, Stergidou D. Insulin analogues for type 1 diabetes in children and adolescents. *Drugs of today.* Dec 2012;48(12):795-809.

38.   Gan MJ, Albanese-O'Neill A, Haller MJ. Type 1 diabetes: current concepts in epidemiology, pathophysiology, clinical care, and research. *Current problems in pediatric and adolescent health care.* Nov-Dec 2012;42(10):269-291.

39.   Garg SK. New insulin analogues. *Diabetes technology & therapeutics.* Oct 2005;7(5):813-817.

40.   Gerber PA, Locher R, Schmid B, Spinas GA, Lehmann R. Smoking is associated with impaired long-term glucose metabolism in patients with type 1 diabetes mellitus. *Nutrition, metabolism, and cardiovascular diseases : NMCD.* Feb 2013;23(2):102-108.

41.   Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care.* Mar 1996;19(3):257-267.

42.   Goats GC. Massage--the scientific basis of an ancient art: Part 2. Physiological and therapeutic effects. *British journal of sports medicine.* Sep 1994;28(3):153-156.

43.   Goats GC. Massage--the scientific basis of an ancient art: Part 1. The techniques. *British journal of sports medicine.* Sep 1994;28(3):149-152.

44.   Golden SH, Sapir T. Methods for insulin delivery and glucose monitoring in diabetes: summary of a comparative effectiveness review. *Journal of managed care pharmacy : JMCP.* // 2012;18(6 Suppl):S1-17.

45.   Gupta J, Felner EI, Prausnitz MR. Rapid pharmacokinetics of intradermal insulin administered using microneedles in type 1 diabetes subjects. *Diabetes technology & therapeutics.* Apr 2011;13(4):451-456.

46.   Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. *Pediatric research.* Dec 2006;60(6):759-763.

47.   Hansen TI, Kristensen JH. Effect of massage, shortwave diathermy and ultrasound upon 133Xe disappearance rate from muscle and subcutaneous tissue in the human calf. *Scandinavian journal of rehabilitation medicine.*

1973;5(4):179-182.

48.   Heinemann L, Heise T, Wahl LC, et al. Prandial glycaemia after a carbohydrate-rich meal in type I diabetic patients: using the rapid acting insulin analogue [Lys(B28), Pro(B29)] human insulin. *Diabetic medicine : a journal of the British Diabetic Association.* Jul 1996;13(7):625-629.

49.   Heinemann L, Muchmore DB. Ultrafast-acting insulins: state of the art. *J Diabetes Sci Technol.* Jul 2012;6(4):728-742.

50.   Heinemann L, Weyer C, Rave K, Stiefelhagen O, Rauhaus M, Heise T. Comparison of the time-action profiles of U40- and U100-regular human insulin and the rapid-acting insulin analogue B28 Asp. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association.* 1997;105(3):140-144.

51.   Heptulla RA, Allen HF, Gross TM, Reiter EO. Continuous glucose monitoring in children with type 1 diabetes: before and after insulin pump therapy. *Pediatr Diabetes.* Mar 2004;5(1):10-15.

52.   Heurtault B, Reix N, Meyer N, et al. Extensive study of human insulin immunoassays: promises and pitfalls for insulin analogue detection and quantification. *Clinical chemistry and laboratory medicine : CCLM / FESCC.* Mar 2014;52(3):355-362.

53.   Hirsch IB, Brownlee M. Should minimal blood glucose variability become the gold standard of glycemic control? *Journal of diabetes and its complications.* May-Jun 2005;19(3):178-181.

54.   Hirsch IB, Brownlee M. Beyond hemoglobin A1c--need for additional markers of risk for diabetic microvascular complications. *JAMA : the journal of the American Medical Association.* Jun 9 2010;303(22):2291-2292.

55.   Hirsch IB, Paauw DS. Diabetes management in special situations. *Endocrinology and metabolism clinics of North America.* Sep 1997;26(3):631-645.

56.   Hirsch IB, Paauw DS. Diabetes management in special situations. *Endocrinology and metabolism clinics of North America.* Sep 1997;26(3):631-645.

57.   Home P. Contributions of basal and post-prandial hyperglycaemia to micro- and macrovascular complications in people with type 2 diabetes. *Current medical research and opinion.* Jul 2005;21(7):989-998.

58.   Home PD. The pharmacokinetics and pharmacodynamics of rapid-acting insulin analogues and their clinical consequences. *Diabetes, obesity & metabolism.* Sep 2012;14(9):780-788.

59.   Home PD, Lindholm A, Hylleberg B, Round P. Improved glycemic control with insulin aspart: a multicenter randomized double-blind crossover trial in type 1 diabetic patients. UK Insulin Aspart Study Group. *Diabetes Care.* Nov 1998;21(11):1904-1909.

60.   Hompesch M, Muchmore DB, Morrow L, Ludington E, Vaughn DE. Improved postprandial glycemic control in patients with type 2 diabetes from subcutaneous injection of insulin lispro with hyaluronidase. *Diabetes technology & therapeutics.* Mar 2012;14(3):218-224.

61.   Hompesch M, Muchmore DB, Morrow L, Vaughn DE. Accelerated insulin pharmacokinetics and improved postprandial glycemic control in patients with type 1 diabetes after coadministration of prandial insulins with hyaluronidase.

Diabetes Care. Mar 2011;34(3):666-668.

62.   Howey DC, Bowsher RR, Brunelle RL, Woodworth JR. [Lys(B28), Pro(B29)]-human insulin. A rapidly absorbed analogue of human insulin. *Diabetes.* Mar 1994;43(3):396-402.

63.   Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). *Diabetes Care.* Mar 2012;35(3):536-541.

64.   Kaminski BM, Klingensmith GJ, Beck RW, et al. Body mass index at the time of diagnosis of autoimmune type 1 diabetes in children. *The Journal of pediatrics.* Apr 2013;162(4):736-740 e731.

65.   Kang J, Robertson RJ, Hagberg JM, et al. Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabetes Care.* Apr 1996;19(4):341-349.

66.   Kilpatrick ES, Rigby AS, Atkin SL. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. *Diabetes Care.* Nov 2008;31(11):2198-2202.

67.   Koivisto VA. Sauna-induced acceleration in insulin absorption from subcutaneous injection site. *British medical journal.* Jun 14 1980;280(6229):1411-1413.

68.   Krebs NF, Jacobson MS, American Academy of Pediatrics Committee on N. Prevention of pediatric overweight and obesity. *Pediatrics.* Aug 2003;112(2):424-430.

69.   Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital and health statistics. Series 11, Data from the national health survey.* May 2002(246):1-190.

70.   Laakso M, Cederberg H. Glucose control in diabetes: which target level to aim for? *Journal of internal medicine.* Jul 2012;272(1):1-12.

71.   LaPorte RE, Matsushima M, Chang Y-F. Prevalence and Incidence of Insulin-Dependent Diabetes. *Diabetes in America*. 2nd ed. Bethesda, MD: National Diabetes Data Group of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

72.   Libman IM, Pietropaolo M, Arslanian SA, LaPorte RE, Becker DJ. Changing prevalence of overweight children and adolescents at onset of insulin-treated diabetes. *Diabetes Care.* Oct 2003;26(10):2871-2875.

73.   Linde B. Dissociation of insulin absorption and blood flow during massage of a subcutaneous injection site. *Diabetes Care.* Nov-Dec 1986;9(6):570-574.

74.   Linde B, Philip A. Massage-enhanced insulin absorption--increased distribution or dissociation of insulin? *Diabetes research.* Aug 1989;11(4):191-194.

75.   Lindholm A, McEwen J, Riis AP. Improved postprandial glycemic control with insulin aspart. A randomized double-blind cross-over trial in type 1 diabetes. *Diabetes Care.* May 1999;22(5):801-805.

76.   Liu LL, Lawrence JM, Davis C, et al. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. *Pediatr Diabetes.* Feb 2010;11(1):4-11.

77.   Luijf YM, van Bon AC, Hoekstra JB, Devries JH. Premeal injection of rapid-acting insulin reduces postprandial glycemic excursions in type 1 diabetes. *Diabetes Care.* Oct 2010;33(10):2152-2155.

78. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinology and metabolism clinics of North America.* Sep 2010;39(3):481-497.

79. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinology and metabolism clinics of North America.* Sep 2010;39(3):481-497.

80. McVey E, Hirsch L, Sutter DE, et al. Pharmacokinetics and postprandial glycemic excursions following insulin lispro delivered by intradermal microneedle or subcutaneous infusion. *J Diabetes Sci Technol.* Jul 2012;6(4):743-754.

81. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care.* Mar 2003;26(3):881-885.

82. Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA : the journal of the American Medical Association.* Apr 12 2006;295(14):1681-1687.

83. Morello CM. Pharmacokinetics and pharmacodynamics of insulin analogs in special populations with type 2 diabetes mellitus. *International journal of general medicine.* 2011;4:827-835.

84. Mortensen HB, Robertson KJ, Aanstoot HJ, et al. Insulin management and metabolic control of type 1 diabetes mellitus in childhood and adolescence in 18 countries. Hvidore Study Group on Childhood Diabetes. *Diabetic medicine : a journal of the British Diabetic Association.* Sep 1998;15(9):752-759.

85. Muchmore DB, Vaughn DE. Accelerating and improving the consistency of rapid-acting analog insulin absorption and action for both subcutaneous injection and continuous subcutaneous infusion using recombinant human hyaluronidase. *J Diabetes Sci Technol.* Jul 2012;6(4):764-772.

86. Mudaliar SR, Lindberg FA, Joyce M, et al. Insulin aspart (B28 asp-insulin): a fast-acting analog of human insulin: absorption kinetics and action profile compared with regular human insulin in healthy nondiabetic subjects. *Diabetes Care.* Sep 1999;22(9):1501-1506.

87. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA : the journal of the American Medical Association.* Oct 8 2003;290(14):1884-1890.

88. NIDDK. National Diabetes Statistics. 2011; http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.aspx#NewCasesDD.

89. Nosek L, Roggen K, Heinemann L, et al. Insulin aspart has a shorter duration of action than human insulin over a wide dose-range. *Diabetes, obesity & metabolism.* Jan 2013;15(1):77-83.

90. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA : the journal of the American Medical Association.* Feb 26 2014;311(8):806-814.

91. Overmann H, Heinemann L. Injection-meal interval: recommendations of diabetologists and how patients handle it. *Diabetes research and clinical practice.* Feb 1999;43(2):137-142.

92. Owens D, Vora J. Insulin aspart: a review. *Expert opinion on drug metabolism & toxicology.* Oct 2006;2(5):793-804.

93. Perriello G, Pampanelli S, Porcellati F, et al. Insulin aspart improves meal time glycaemic control in patients with Type 2 diabetes: a randomized, stratified, double-blind and cross-over trial. *Diabetic medicine : a journal of the British Diabetic Association.* May 2005;22(5):606-611.

94. Peter R, Luzio SD, Dunseath G, et al. Effects of exercise on the absorption of insulin glargine in patients with type 1 diabetes. *Diabetes Care.* Mar 2005;28(3):560-565.

95. Polit DF, Beck CT. *Nursing Research: Generating and Assessing Evidence for Nursing Practice.* 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2012.

96. Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *The Journal of clinical investigation.* Feb 1988;81(2):442-448.

97. Powers AC. Diabetes Mellitus. *Harrison's Principles of Internal Medicine*. 18th ed. New York: McGraw-Hill; 2012.

98. Prince CT, Becker DJ, Costacou T, Miller RG, Orchard TJ. Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC). *Diabetologia.* Nov 2007;50(11):2280-2288.

99. Raz I, Weiss R, Yegorchikov Y, Bitton G, Nagar R, Pesach B. Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion. *Clinical Therapeutics.* // 2009;31(5):980-987.

100. Rosenfalck AM, Thorsby P, Kjems L, et al. Improved postprandial glycaemic control with insulin Aspart in type 2 diabetic patients treated with insulin. *Acta diabetologica.* Mar 2000;37(1):41-46.

101. Rosenfeld L. Insulin: discovery and controversy. *Clinical chemistry.* Dec 2002;48(12):2270-2288.

102. Sanofi-aventis UL. Apidra (Prescription Information). Sanofi-Aventis, US LLC, Bridgewater, 2013. 2013.

103. Silverstein J, Klingensmith G, Copeland K, et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. *Diabetes Care.* Jan 2005;28(1):186-212.

104. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. *Diabetologia.* // 1994;37(4):377-380.

105. Slama G, Elgrably F, Sola A, Mbemba J, Larger E. Postprandial glycaemia: a plea for the frequent use of delta postprandial glycaemia in the treatment of diabetic patients. *Diabetes & metabolism.* Apr 2006;32(2):187-192.

106. Son SM, Whalin MK, Harrison DG, Taylor WR, Griendling KK. Oxidative stress and diabetic vascular complications. *Curr Diab Rep.* Aug 2004;4(4):247-252.

107. Swan KL, Weinzimer SA, Dziura JD, et al. Effect of puberty on the pharmacodynamic and pharmacokinetic properties of insulin pump therapy in youth with type 1 diabetes. *Diabetes Care.* Jan 2008;31(1):44-46.

108. ter Braak EW, Woodworth JR, Bianchi R, et al. Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin. *Diabetes Care.* Dec 1996;19(12):1437-1440.

109. Trautmann ME. Effect of the insulin analogue [LYS(B28),PRO(B29)] on blood glucose control. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme.* Dec 1994;26(12):588-590.

110. Uzu T, Harada T, Sakaguchi M, et al. Glucocorticoid-induced diabetes mellitus: prevalence and risk factors in primary renal diseases. *Nephron. Clinical practice.* 2007;105(2):c54-57.

111. Walchli C, Saltzwedel G, Kruerke D, et al. Physiologic Effects of Rhythmical Massage: A Prospective Exploratory Cohort Study. *Journal of alternative and complementary medicine.* May 23 2013.

112. Weber C, Schnell O. The assessment of glycemic variability and its impact on diabetes-related complications: an overview. *Diabetes technology & therapeutics.* Oct 2009;11(10):623-633.

113. Weissberg-Benchell J, Wolpert H, Anderson BJ. Transitioning from pediatric to adult care: a new approach to the post-adolescent young person with type 1 diabetes. *Diabetes Care.* Oct 2007;30(10):2441-2446.

114. Whitehead N, White H. Systematic review of randomised controlled trials of the effects of caffeine or caffeinated drinks on blood glucose concentrations and insulin sensitivity in people with diabetes mellitus. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association.* Apr 2013;26(2):111-125.

115. Woerle HJ, Neumann C, Zschau S, et al. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes Importance of postprandial glycemia to achieve target HbA1c levels. *Diabetes research and clinical practice.* Aug 2007;77(2):280-285.

116. Wood JR, Miller KM, Maahs DM, et al. Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care.* Jul 2013;36(7):2035-2037.

117. Wood L, Egger M, Gluud LL, et al. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. *Bmj.* Mar 15 2008;336(7644):601-605.

118. Writing Team for the Diabetes C, Complications Trial/Epidemiology of Diabetes I, Complications Research G. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA : the journal of the American Medical Association.* Oct 22 2003;290(16):2159-2167.

119. Wu D, Gong CX, Meng X, Yang QL. Correlation between blood glucose fluctuations and activation of oxidative stress in type 1 diabetic children during the acute metabolic disturbance period. *Chinese medical journal.* Nov 2013;126(21):4019-4022.

120. Yaturu S. Insulin therapies: Current and future trends at dawn. *World journal of diabetes.* Feb 15 2013;4(1):1-7.

121. Yeh HC, Brown TT, Maruthur N, et al. Comparative effectiveness and safety of methods of insulin delivery and glucose monitoring for diabetes mellitus: A systematic review and meta-analysis. *Annals of Internal Medicine.* // 2012;157(5):336-347.

122. Yki-Jarvinen H, DeFronzo RA, Koivisto VA. Normalization of insulin sensitivity in type I diabetic subjects by physical training during insulin pump therapy. *Diabetes Care.* Nov-Dec 1984;7(6):520-527.