Diplomarbeit

DER HYDRAULISCHE GEWEBSWIDERSTAND AM ABGABEORT EINER INSULINPUMPE

Bestimmung des Zusammenhanges zwischen Hydraulischem Gewebswiderstand, Insulinabsorption und Tragedauer in Menschen mit Typ 1 Diabetes mellitus

eingereicht von

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zur Erlangung des akademischen Grades

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Graz, 06. Juni 2018

Eidesstattliche Erklärung

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Vorwort

Diese Diplomarbeit ist aus der Mitgestaltung und Mitarbeit an einer Studie entstanden. Die Studie HYDRA-01 (EudraCT-Nummer 2013-001791-38) wurde im Rahmen des EU-Projektes „Artificial Pancreas at Home (AP@Home)“ gefördert und wurde im Zeitraum September 2013 bis November 2014 im Clinical Research Center (CRC), einer Einrichtung der Medizinischen Universität Graz und der klinischen Abteilung für Endokrinologie und Diabetologie am LKH-Universitätsklinikum Graz durchgeführt. Die Erstellung der Diplomarbeit erfolgte im Zeitraum von März 2017 bis Mai 2018 unter der Anleitung von Herrn DI Dr. techn. Werner Regittnig. Die Hauptschrift liegt in englischer Sprache vor.

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Deutschsprachige Kurzfassung

Für die Insulinpumpentherapie bei Typ 1 Diabetes mellitus (T1DM) wird von Herstellern ein Katheterwechsel alle zwei bis drei Tage empfohlen. Studien haben gezeigt, dass sich die Insulinabsorption zum dritten Tag der Tragedauer eines Katheters verbessert und bei einer Vielzahl von Menschen die empfohlene Tragedauer problemlos überschritten werden kann. Bislang finden sich wenige Forschungsergebnisse über die Änderung der Insulinabsorption über den dritten Tag hinaus. Ein einfach messbarer Indikator für die Effizienz der Insulinabsorption wäre der hydraulische Gewebswiderstand (TR; Widerstand, den die Insulinflüssigkeit beim Einströmen in das Gewebe erfährt).

Um TR und die Insulinabsorption über eine verlängerte Tragedauer zu beobachten, wurde eine klinische Studie im Rahmen des EU-Projektes „AP@home“ durchgeführt. Ziel dieser Diplomarbeit war die statistische Auswertung der erhobenen Druck- und Glukosedaten, sowie die Beschreibung der Ergebnisse. Zur Bestimmung des TR während der Tragedauer erfolgten Druckmessungen mittels eines zwischen Reservoir und Katheter platzierten Drucksensors während der Bolusabgabe. Der TR wurde unmittelbar nach dem Setzen eines neuen Infusionskatheters (basal TR) und an jedem weiteren Tag bestimmt, bis TR den basalen Wert um das mindestens Zehnfache überstieg. Zur Bestimmung eines Zusammenhanges zwischen TR und Insulinabsorption innerhalb einer Probandin/eines Probanden wurde jeweils zu Beginn (nach Setzen eines neuen Katheters) und am Ende der Tragedauer ein oraler Glukosetoleranztest (OGTT; Trinklösung mit 75g Glukose) durchgeführt. Zu Beginn eines jeden OGTTs wurde über den Studienkatheter eine standardisierte Insulindosis verabreicht.

Die durchschnittliche Tragedauer des Katheters betrug 10,4 (6-13) Tage. Die durchschnittlichen maximal gemessenen Druck-Werte stiegen über die Tragedauer von 24 auf 59 kPa ($p<0,01$) und der durchschnittliche Gewebswiderstand von 123 auf 3670 Pa×s/mm ($p<0,01$) an. Der mittlere Plasmaglukoseanstieg über den Ausgangswert betrug während des OGTTs am ersten Tag 113 mg/dl und während des OGTTs am letzten Tag 155 mg/dl ($p<0,05$).

Die Ergebnisse weisen darauf hin, dass ein Anstieg des Gewebswiderstandes über die Tragedauer mit einer Reduktion der Insulinabsorption einher geht. Sollte sich dieser inverse Zusammenhang zwischen Gewebswiderstand und Insulinabsorption in weiteren Studien bestätigen, könnte der Gewebswiderstand als Indikator für die optimale Kathetertragedauer bei der Insulinpumpentherapie Anwendung finden.

Abstract in English

In the therapy of type 1 diabetes mellitus (T1DM) with an insulin pump, manufacturers recommend changing the infusion catheter every 2 to 3 days. Studies have shown that insulin absorption is improving over the first three days of catheter wear. Further studies have shown that in a large group of patients the duration of catheter wear can be safely prolonged far beyond the recommended 2-3-day period. There are only limited results regarding the change of the insulin absorption beyond day three. An easily obtainable indicator of the efficiency of insulin absorption from the infusion site could potentially be the hydraulic tissue resistance (TR; defined as the resistance exerted by the tissue upon the insulin solution entering the tissue).

To assess TR and the insulin absorption over a prolonged catheter wear-time, a clinical study has been conducted within the EU project “AP@home”. The aim of this diploma thesis was the statistical analysis of the collected pressure and glucose data as well as the graphical representation and description of the results. To determine the TR during catheter wear, pressure measurements were performed during bolus delivery using a sensor interposed between reservoir and infusion catheter. TR measurements were performed shortly after the insertion of a new catheter (basal TR) and on each consecutive day until TR reached values that exceeded more than ten times the basal value. To assess the relationship between TR and insulin absorption within a subject, oral glucose tolerance tests (OGTT; drinking solution containing 75g glucose) were performed directly after insertion as well as at the end of catheter wear. At the beginning of each OGTT, an insulin bolus of equal size was administered by an insulin pump.

The average catheter wear-time observed in 10 patients with T1DM was 10.4 (6 to 13) days. Average pressure values increased during the catheter wear-time from 24 to 59 kPa ($p<0.01$). Average TR values increased from 123 to 3670 Pa×s/mm ($p<0.01$). The average increase in plasma glucose concentration above basal values was 113 mg/dl during the OGTT on day one and 155 mg/dl during the OGTT on the last day ($p<0.05$).

The results indicate that an increase in the TR is associated with a reduction in the insulin absorption. If this inverse relationship between TR and extent of insulin absorption is confirmed in further studies, TR could be used as an indicator of the efficiency of insulin absorption and applied in future insulin pumps for the determination of the optimal wear-time of an infusion catheter.

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Abbreviations

°C ..... *Celsius/Centigrade temperature scale*

ΔP_{Smax} ..... *Maximum hydraulic pressure by the system alone*

ΔP_{Smean} ..... *Mean hydraulic pressure by the system*

ΔP_T ..... *Pressure Difference caused by resistance to flow in tissue*

A ..... *area*

AM ..... *ante meridiem, Latin for "before midday"*

AUC ..... *Area Under the Curve*

AUC_{System} ..... *Area under the curve during the infusion through the system alone*

$AUC_{Tissue+System}$ .... *Area under the curve obtained during the infusion into the tissue*

BMI ..... *body mass index*

CGM ..... *Continuous Glucose Monitoring*

CRC ..... *Clinical Research Center*

CSII ..... *Continuous Subcutaneous Insulin Infusion*

DM ..... *Diabetes mellitus*

DNA ..... *Deoxyribonucleic acid*

e.g. ..... *From Latin e.g., an abbreviation of exempli gratia ("for example").*

ELISA ..... *Enzyme-linked immunosorbent assay*

FR ..... *flow rate*

g ..... *gram*

G_{mean} ..... *Average plasma glucose concentration during the OGTT*

HbA1c ..... *glycated hemoglobin A1c*

i.e. ..... *From Latin i. e., an abbreviation of id est (“that is”).*

IDF ..... *International Diabetes Federation*

IDS ..... *Insulin diluting solution*

ISF ..... *Interstitial Fluid*

IVGTT ..... *Intravenous Glucose Tolerance Test*

MDI ..... *Multiple Daily Injection Regimen*

ml ..... milliliter

mm^2 ..... *square millimeter*

mm^3 ..... *cubic millimeter*

n ..... *number*

OGTT ..... *Oral Glucose Tolerance Test*

Pa ..... *Pascal*

PM ..... *for Latin post meridiem, meaning "after midday"*

P_{max} ..... *Maximum hydraulic pressure*

P_{mean} ..... *Mean hydraulic pressure*

RIA ..... *Radioimmunoassay*

R_{SYS} ..... *System resistance*

s ..... *second*

sTR ..... *Specific tissue resistance*

t ..... *Time*

T1DM ..... *Type 1 Diabetes mellitus*

T2DM ..... *Type 2 Diabetes mellitus*

TR ..... *Tissue resistance*, Tissue Resistance

U ..... unit

Vd ..... *Volume difference*

WHO ..... *World Health Organization*

µl ..... *microliter*

1 Introduction

This chapter aims to depict current shortcomings in the insulin pump treatment of patients with type 1 diabetes mellitus. To define the terminology and methodology used for this diploma thesis, a general overview of diabetes mellitus and its treatment will be provided. The tissue resistance, a method to assess the hydraulic properties of the tissue at the site of an infusion, will be presented. At the end of this introduction, the aims of the underlying clinical study performed during this thesis as well as the aims of this thesis itself will be presented.

1.1 Pancreatic Insulin Secretion and Glucose Homeostasis in Healthy People

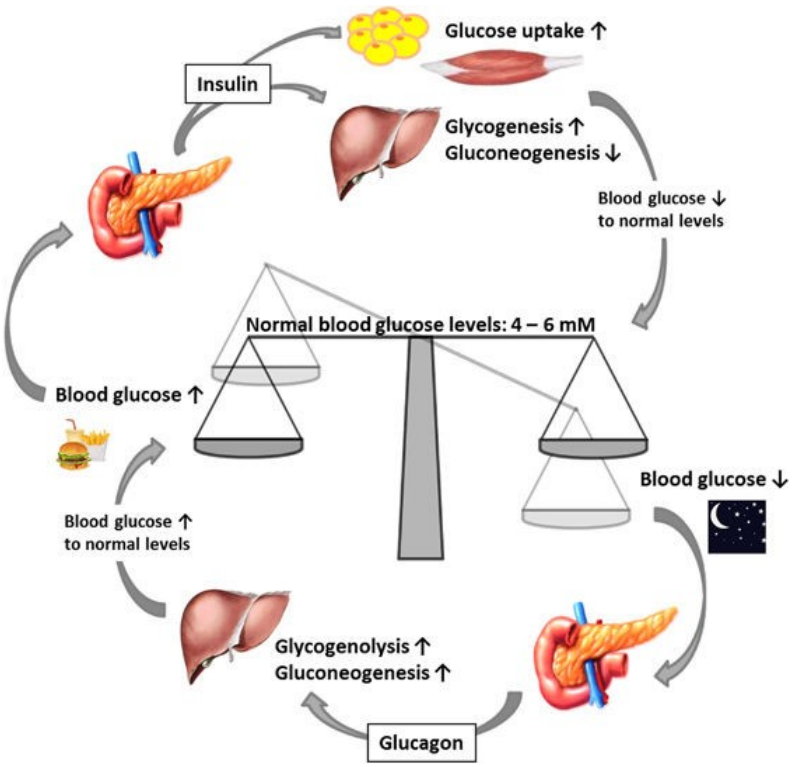
In healthy individuals, the body keeps tight control of its blood glucose levels (1). The hormones primarily involved in glucose homeostasis (i.e., maintaining blood glucose concentration within a normal range) are glucagon and insulin (1,2). Glucagon increases blood glucose concentration, whereas insulin lowers blood glucose concentration, see Figure 1 (1,2). In addition to the glucose concentration-lowering effects, insulin also promotes metabolic, pathways, e.g., glycogenesis and lipogenesis (1,2). Within the endocrine pancreas, beta cells of the islets of Langerhans produce and store insulin (1–3). Beta cells store the insulin in hexameric form (i.e., six insulin molecules forming a unit) within specialized vesicles, i.e., secretory granules (2–5).

The pancreas releases its hormones directly into the bloodstream, i.e., endocrine release (1,3,6). The islets are highly vascularized, and their capillary network contains so-called fenestrated capillaries, facilitating diffusion of molecules, like insulin (3,6,7). In the fasting state, small amounts of insulin are secreted throughout the day to maintain a normal blood glucose concentration (normoglycemia), i.e., basal insulin secretion (2,5,8). The beta cells continuously sense changes in the blood glucose concentration (4,9). An increase in blood glucose concentration, e.g., following carbohydrate consumption, triggers additional (i.e., bolus) insulin release (1,2,5,10). In this way, blood glucose concentration is kept mainly within the narrow range of 4 to 6 mmol/l (approximately 70 to 110 mg/dl) in the fasting state and up to 7.8 mmol/l (140 mg/dl) two hours after a meal, see Figure 2 (1,11).

Beta cells release insulin into the pancreatic effluent to the portal vein by the fusion of secretory granules with the plasma membrane, i.e., exocytosis (3–6,12,13). Within the bloodstream, the insulin hexamers rapidly dissociate into biologically active monomers, and then the insulin is distributed throughout the body (4,5). Many tissues, e.g., muscular, hepatic and adipose tissue possess insulin receptors with an extracellular binding domain on the outer cellular membrane (14). The binding of insulin to the insulin receptor at the target tissue activates a signal cascade resulting in the cellular glucose uptake by glucose transporter type 4 (2).

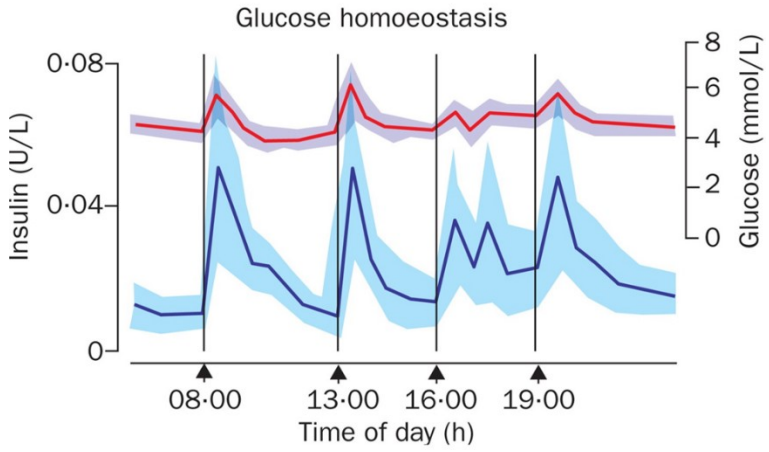
The insulin is mainly cleared from the body by cellular uptake, processing and enzymatic degradation (15). The liver and the kidneys perform the clearance of insulin, but “all insulin-sensitive cells remove and degrade the hormone” (15). Within the circulation, insulin’s half-life is only about four to seven minutes (2,5,15).

Figure 1: Homeostasis of blood glucose levels by insulin and glucagon



When blood glucose levels are low, the pancreas secretes glucagon, which increases blood glucose levels through glycogenolysis. After a meal, when blood glucose levels increase, insulin is released to trigger glucose uptake into insulin-dependent muscle and adipose tissues as well as to promote glycogenesis. Reprinted with permission from (1), licensed under CC BY-NC-ND 4.0 (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Figure 2: Plasma glucose and plasma insulin profiles in healthy individuals



N=10. Mean values with 95% CI; Blue: insulin; Red: Glucose; Bottom arrows (in order of appearance): Breakfast, Lunch, Snack, Dinner. Reprinted from the Lancet, with permission from Elsevier (16).

1.2 Diabetes Mellitus

Diabetes mellitus (DM) is the collective term for a group of heterogeneous chronic metabolic diseases, characterized by elevated blood glucose concentration, i.e., hyperglycemia (17,18). Hyperglycemia is caused by either defective insulin production, or defective action of the hormone insulin, or both (17,18).

Common symptoms of hyperglycemia are, e.g., polyuria, polydipsia, and weight loss, Figure 3 (17). The chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs (17). For complications, both acute and chronic, see chapter 1.2.4.

There are several classification systems established for the classification of DM. The World Health Organization (WHO) bases its current classification primarily on disease etiology (11,17,19). It comprises four main groups, shown in Table 1.

Approximately 90 to 95% of all patients with DM have type 2 diabetes mellitus (2,5,17). Type 2 diabetes mellitus (T2DM) is characterized by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (17). T2DM typically manifests itself in patients older than 40 to 50 years of age, which is the reason for its synonymously used name “adult-onset diabetes mellitus” (2,17).

With 5 to 10% patient share, the second largest group of patients has type 1 diabetes mellitus, i.e., T1DM (2,17,20). It is a condition caused by the autoimmune or idiopathic destruction of the pancreatic beta cells, resulting in an absolute deficiency of insulin secretion and, therefore the mandatory need to substitute insulin for survival (17,21,22). T1DM commonly occurs in childhood and adolescence but may occur at any age (21). Other types of DM include disorders of the glucose metabolism by other specific mechanisms, diseases, as well as the gestational diabetes mellitus (17,18,23).

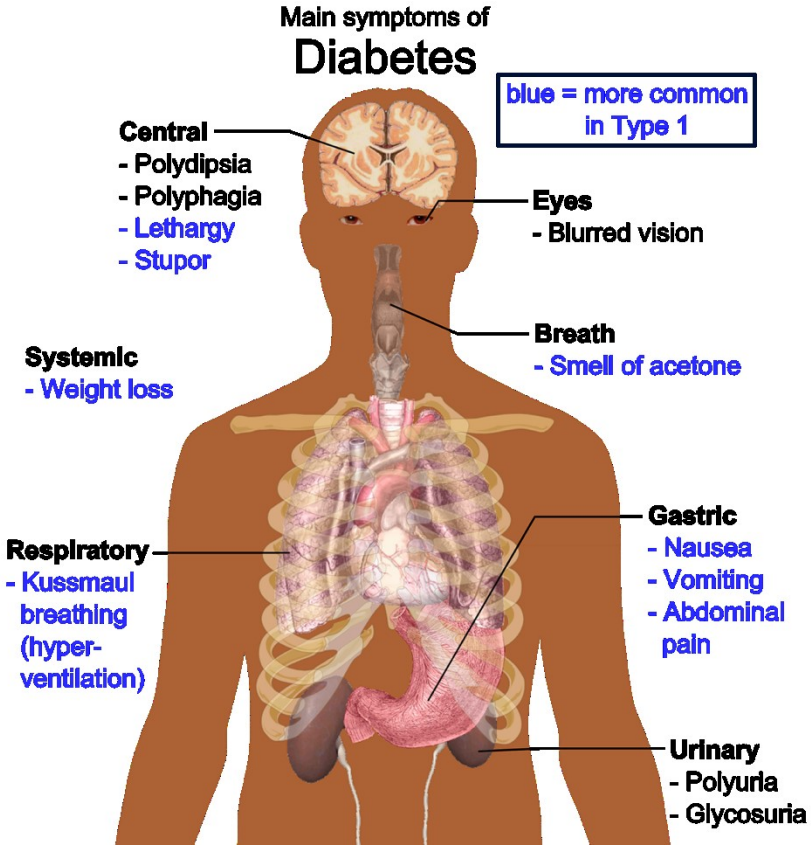
The global prevalence of DM amounts to approximately 8.5% (24). According to the International Diabetes Federation (IDF), there were 415 million adults living with DM in 2015 (25). This number is expected to increase to over 640 million by 2040 (25). Furthermore, it is projected that the treatment of diabetes and its complications currently account for approximately 12% of the global health expenditure 2015 (25).

Table 1: Etiologic classification of diabetes mellitus

I.	Type 1 diabetes mellitus (autoimmune or idiopathic)
II.	Type 2 diabetes mellitus
III.	Other specific types
	Genetic disorders of beta cell function
	Genetic disorders of insulin action
	Diseases of the pancreas
	Endocrinopathies
	Drug-induced
	Infections
	Uncommon immunological forms
	Genetic syndromes
IV.	Gestational diabetes mellitus

Adapted from (17,19,23).

Figure 3: Main symptoms of diabetes mellitus



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1.2.1 Insulin Replacement for the Treatment of Type 1 Diabetes Mellitus

Patients with T1DM lack sufficient pancreatic insulin production and secretion. Therefore insulin must be provided from an external source, i.e., exogenous insulin (5,21,27). The primary goal in the treatment of T1DM is to maintain blood glucose concentration within a normal range by mimicking the physiological pattern of insulin secretion as present in the healthy pancreas (5).

Therapeutic insulins became available in the 1920s (21,28). The first therapeutic insulins were extracted from animals (29–31). Recombinant DNA technology uses bacteria to produce insulins today (31–33). Most insulins are pharmacologically prepared as insulin solutions for subcutaneous administration (16,32,34).

Patients learn to use a technique called carbohydrate counting (i.e., grams of carbohydrates consumed) to estimate the insulin dose needed to maintain normoglycemia (20,21,35). Moreover, to guide adjustments in insulin dosage, food consumption and physical activity, the patients self-monitor their blood glucose

concentration by measuring the glucose in blood obtained by finger-pricking (33,36,37). These measurements are usually performed before meals as well as at bedtime. To prevent hypoglycemia or prolonged hyperglycemia, it is recommended to perform additional measurements, e.g., during periods of illness, increased activity or an uncertain amount of carbohydrates eaten (37).

The actual insulin requirement in a patient varies from day to day (38). Potential reasons for day-to-day variability are, e.g., changes in diet, physical activity, and illness. Patients are additionally challenged to consider the timing of insulin administration, possible error in dose calculation and dose administration, as well as variability in insulin absorption (38).

There are several methods for metabolic optimization via insulin therapy (21). Most patients administer insulin in the form of multiple daily injections (MDI; also known as basal-bolus therapy regimen) (5). The patients use an insulin pen or disposable syringe (5,39,40), see Figure 5, panel A. In this therapy, the patient self-administers long-acting insulin to mimic basal endogenous pancreatic insulin secretion whereas rapid-acting insulin is injected to lower blood glucose concentration after carbohydrate intake, i.e., prandial endogenous insulin secretion (5,39).

As an alternative treatment strategy to MDI, insulin pump therapy, also known as continuous subcutaneous insulin infusion (CSII), was introduced in the late 1970s (28,41,42). Its use has increased substantially over the past decade (5,21). In this treatment, a portable pump is used to better mimic insulin secretion patterns of healthy individuals (5,33,39), see Figure 5, panel B.

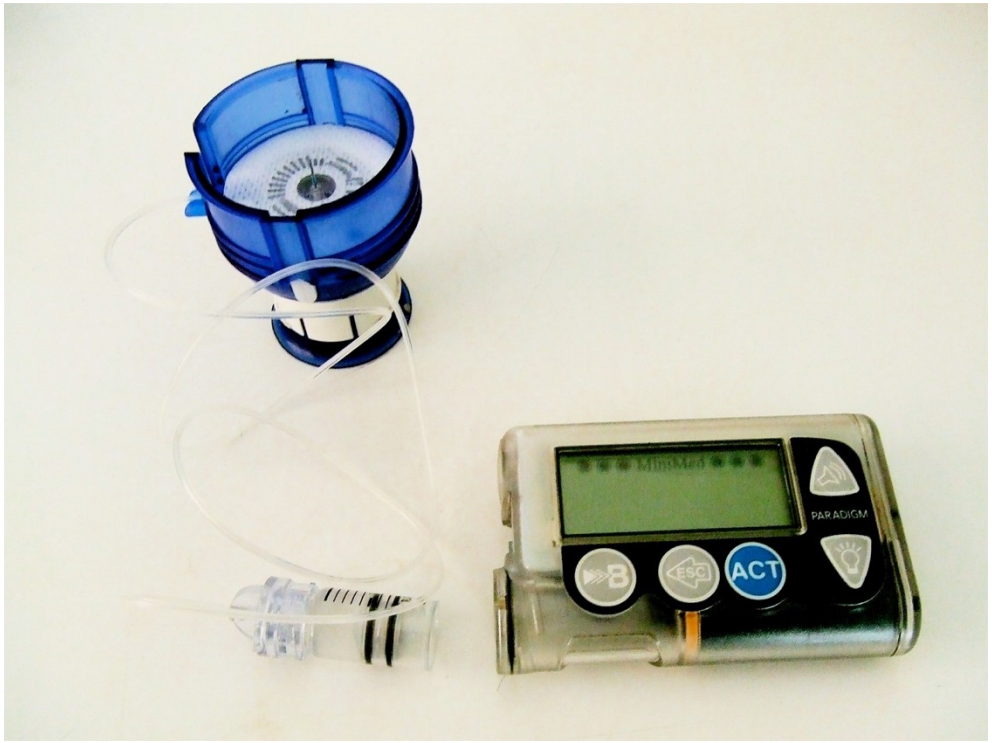
The insulin pump usually comprises a motor, an energy source, a disposable reservoir for up to 300 units of short-acting insulin, and a programmable interface for adjusting the insulin delivery rate, see Figure 4 (33,39,41). Most current insulin pumps also offer an on-board automated bolus calculator (33,39,41). The pump is connected to the patient through flexible tubing to a subcutaneously set infusion cannula (also known as catheter or infusion set), either made of metal or polytetrafluoroethylene, i.e., Teflon (42).

The basal pancreatic secretion is replaced by a slow, but variable infusion (basal insulin delivery) of rapid-acting insulin (39,41–43). At mealtimes and in case of hyperglycemia correction, the patient administers insulin as a bolus at a higher rate using the insulin pump (bolus insulin delivery) (39,41–43). This bolus insulin

delivery, in contrast to basal insulin delivery, is usually delivered as macro-pulses with a high repetition frequency of the pulses (pulse intervals equal to or smaller than four seconds) (44). It is furthermore possible to adjust the basal infusion rate at any time to meet changes in insulin requirements, e.g., occurring during recreational activities, illness (33,39,41). With CSII, the increased flexibility of insulin delivery improves glycemic control in patients who were suboptimal controlled by MDI therapy previously (39,42). Currently, depending on national health policy, CSII is used by up to 25% of patients with T1DM (39).

Alternatives to injectable insulin are, e.g., inhalable insulins (5). They are subject to a substantial research effort, but only a small number of patients is currently treated with these alternatives in the course of clinical trials (5).

Figure 4: An insulin pump, a reservoir, and a cannula with an insertion device



The blue object is a spring-loaded insertion device to insert the metal needle (which is surrounded by a plastic cannula) beneath the skin. The metal needle is then removed, leaving the cannula in place.
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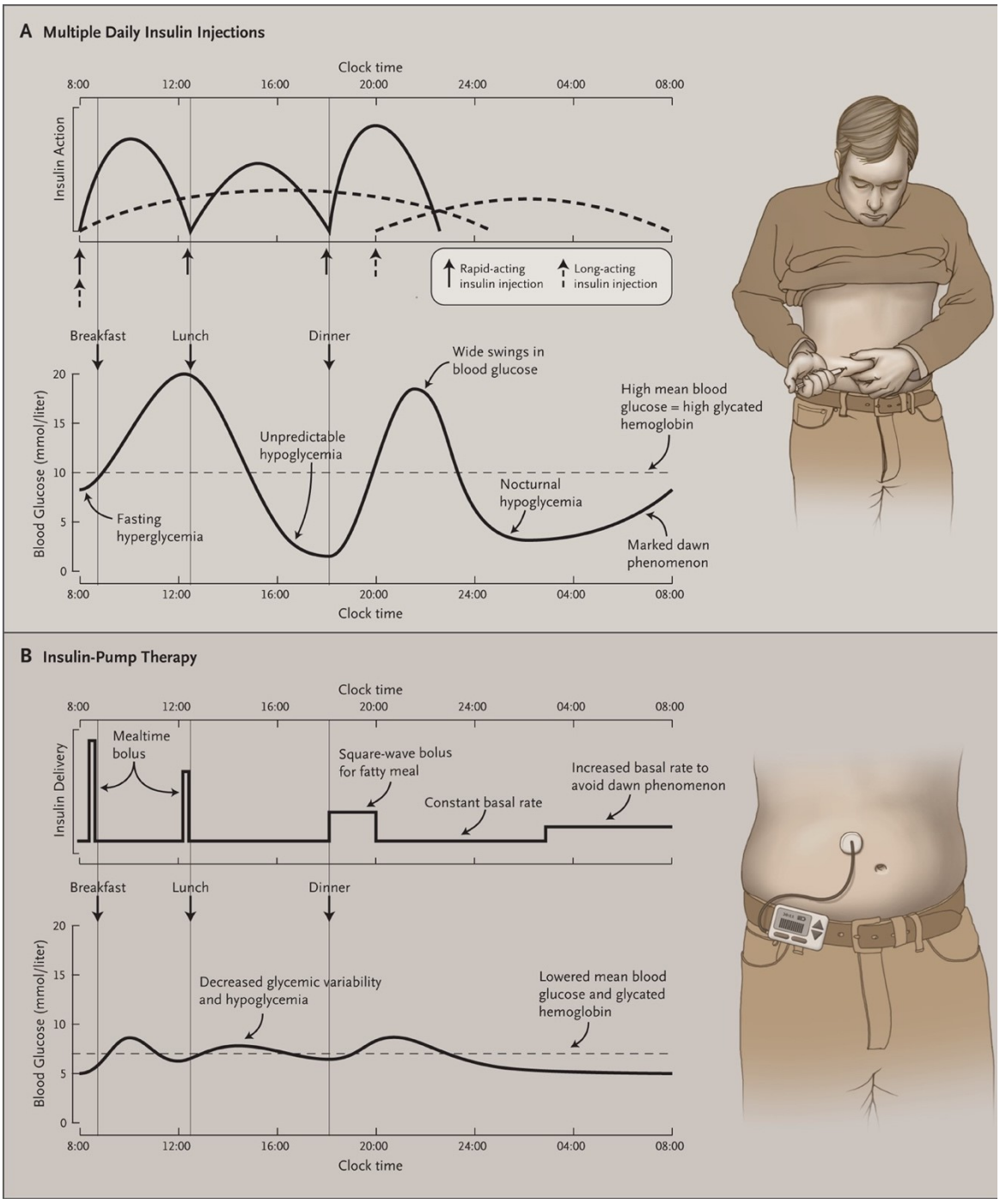
Figure 5: Multiple daily insulin injections (MDI) and Insulin-pump (CSII) therapy

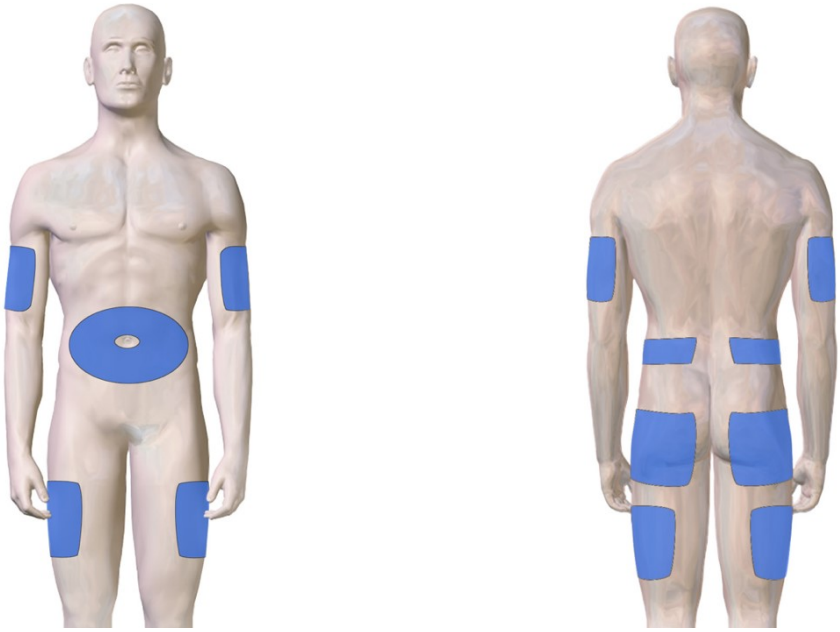


*MDI therapy **(A)** involves the injection of rapid-acting insulin before main meals and the injection of long-acting insulin once or twice daily (upper graph). Some patients have poor glycemic control (lower graph). CSII therapy **(B)** consists of a constant but variable basal infusion of insulin from a portable pump. The rate can be preset to increase or decrease on the basis of the patient's insulin requirements at that time of the day. Patient-activated insulin boluses at meals can be administered over a short period of time or as an extended square wave. Reproduced with permission from (41), Copyright Massachusetts Medical Society.*

1.2.2 The Administration Site of Insulin and Methods for Determining Insulin Absorption

The site most commonly used for insulin administration is the anterior abdominal wall (41,43). Less common sites are the outer thighs, arms, hips, and buttocks (41,43). Figure 6 shows these common and less-common injection sites.

Figure 6: Common sites for insulin administration



Left: anterior injection sites. Right: posterior injection sites.
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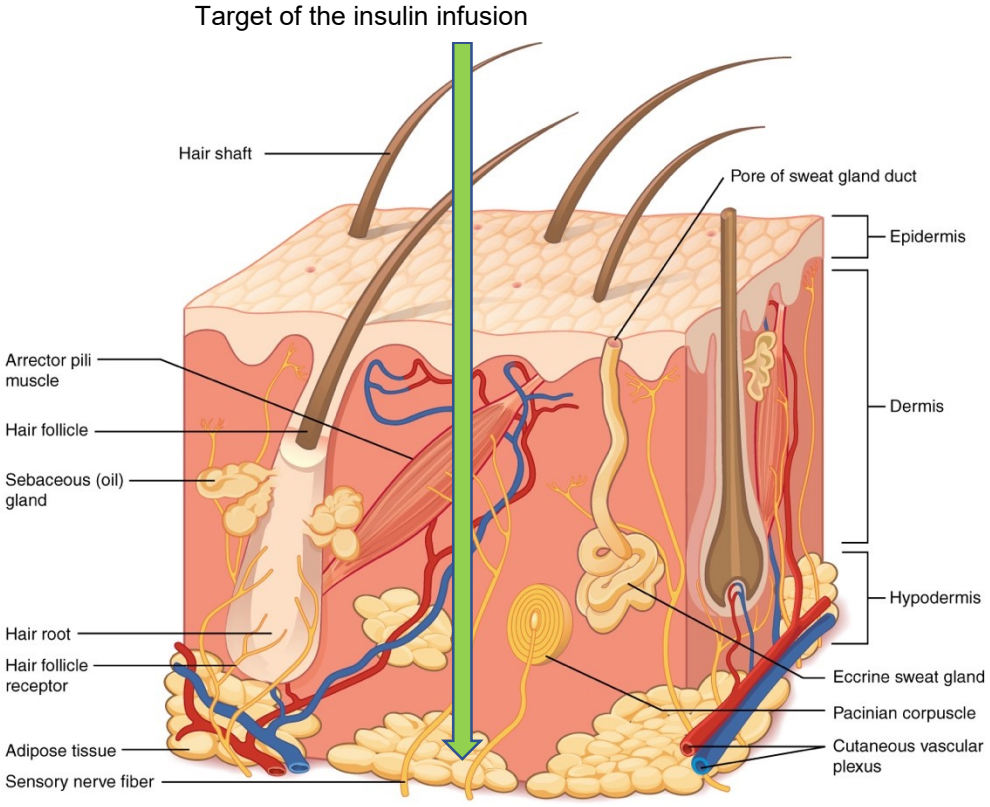
Patients with T1DM treated with CSII infuse the therapeutic insulin solution into the subcutaneous tissue (i.e., the lowest layer of the skin, also known as hypodermis), see Figure 7. This tissue layer is mostly composed of adipocytes kept together by a connective tissue capsule. The vasculature consists of capillaries of the of the so-called continuous type (45).

Absorption is the first process of pharmacokinetics. The other processes comprise the distribution throughout the body, the metabolism, and the elimination. Following subcutaneous administration, a subcutaneous depot of insulin is built within the interstitial fluid (ISF, i.e., the fluid that fills spaces between the cells) around the tip of the infusion cannula (41,46). The insulin solution is distributed within the ISF and across the capillary walls by convective and diffusive transport (44). Once absorbed,

the distribution and metabolism of insulin follow that of endogenous insulin. The insulin circulates through the bloodstream and diffuses into other compartments, and is mainly degraded within the liver and the kidneys (27).

Figure 7: Layers and structure of the skin and target of the infusion



The brackets at the right side depict the primary layers of the skin. The green arrow shows the target region (i.e., subcutaneous tissue/hypodermis) of the insulin infusion. Licensed and adapted from Wikimedia Commons, the free media repository under CC BY 3.0, https://creativecommons.org/licenses/by/3.0/deed.en.

The continuous capillaries within the subcutaneous tissue have, compared to the fenestrated pancreatic capillaries, slower diffusion capabilities, limiting the speed of subcutaneous insulin absorption (45). Within the common insulin administration sites, the anterior abdominal wall has the insulin absorption rate closest to normal (34,47).

The absorption is furthermore affected by many factors, e.g., the depth of injection, ambient temperature, local blood flow and catheter wear-time (27,38). Experimental

methods to assess insulin absorption measure either the disappearance of insulin from the administration site, the appearance of insulin within the bloodstream (i.e., pharmacokinetics) or the metabolic response to the insulin (i.e., pharmacodynamics).

The disappearance from the administration site is determined by labeling insulin with 125I radioiodine and placing an external scintillation counter above the site of administration (27,47,48).

The appearance of the insulin in the bloodstream can be assessed with either the radioimmunoassay (RIA) technique or the enzyme-linked immunosorbent assay (ELISA). Both methods use marker molecules binding to insulin (47).

Probably due to the technical ease of the measurement, almost all methods assessing the metabolic response monitor the glucose-lowering effects of insulin (49).

As such a method, the oral glucose tolerance test (OGTT) was introduced in the 1930s (49). It was originally designed as a provocation test to indirectly examine the efficiency of the body to release insulin and thereby to lower blood glucose concentration (19). A standardized amount of glucose, typically 75 to 100g in solution, is administered orally. In a mixed meal test, a standardized meal is provided instead of the glucose drink in the OGTT. To account for intraindividual day-to-day variability in enteral glucose absorption, the intravenous glucose tolerance test (IVGTT) was developed as an alternative to the OGTT (49). Instead of administering the glucose solution orally, a standardized amount of glucose infusion is given. To assess the blood glucose-lowering effects of insulin in patients with DM, a pre-determined amount of exogenous insulin is administered. The profile of the changes in the blood glucose concentration over the time course of the test (area under the curve, AUC) resembles the action of insulin (48,50,51).

The euglycemic glucose clamp “provides the most precise characterization of insulin action on carbohydrate metabolism in vivo” (49). At first, a variable intravenous insulin infusion is used to reach and maintain a specific plasma glucose target, e.g., 100 mg/dl (5.6 mmol/l). Once this target is reached and stabilized, a pre-determined amount of the test insulin is given, while the variable insulin infusion is tapered. From the onset of action (defined as the time point of lowering the plasma glucose level from the initial clamp target level) of the test insulin, a variable glucose infusion is

used to maintain target plasma glucose levels. The profile of the glucose infusion rate resembles the glucose-lowering effects of the administered insulin (48,49).
These experimental methods require extensive resources and are impractical in the day-to-day life of the patients. An easily-measurable surrogate parameter for the determination of the variable insulin absorption capabilities would be desirable.

1.2.3 Shortcomings of the Treatment of Type 1 Diabetes Mellitus

In healthy individuals, the pancreas releases the right amount of insulin at the right time. The current treatment of T1DM shows shortcomings of both, timing and amount.
Due to the slower absorption of insulin from the subcutaneous tissue, patients need to factor in a delay between injection and food intake (5). Like endogenous pancreatic insulin within the secretory granules, therapeutic insulin formulations mainly exist in their hexameric structure as well and, therefore cannot pass the continuous capillary walls (31). After administration, these hexamers start to dissolve slowly within the ISF and then pass the capillary walls (5,31). Pharmaceutical companies have re-engineered insulin formulations to dissolve more quickly from hexamer to monomer to allow better insulin absorption from the subcutaneous tissue (5,52,53). Despite those developments, kinetics of exogenous insulin still does not match with the kinetics of physiologic insulin secretion and therefore cause higher variability of glucose level excursions (5).
Due to changes in the physiologic insulin requirements the administration of the correct amount of basal insulin may be challenging (54). In addition to basal requirements, patients need to dose their bolus insulin according to their current blood glucose concentration as well as the number of carbohydrates consumed (i.e., carbohydrate counting) and their current or planned activity level (20). Carbohydrate counting is prone to error, as not all nutritional data are always at hand (55). Self-monitoring of blood glucose concentration by finger pricking is laborious, indiscreet and may be painful too. In addition to resulting possible discomfort, it does not contain any prospective information on rate and direction of change of blood glucose levels, making it harder for the patient to estimate the correct insulin dose. Therefore, continuous glucose monitoring systems (CGM) have been developed to

provide this complimentary information (48). These systems are expensive and, thus often not paid for by health insurance providers (37).

The insulin administration as present in MDI cannot closely mimic the profile as needed. Thus, some patients may encounter poor glycemic control (41). CSII can better mimic pancreatic secretion but comes with a host of shortcomings itself.

Potential problems with the CSII therapy regimen are mostly either related to the infusion catheter 1) or to the infusion site (i.e., skin) 2):

Catheter-related problems are leakage, occlusion and kinking of the cannula, resulting in inadequate insulin dose reaching the tissue (56,57).

Site-related problems may be caused by the tissue trauma itself, or by the tissue adhesive. The trauma following cannula insertion at an insulin infusion site causes a local inflammatory response and wound healing (58–60). Over time, this may lead to changes in tissue architecture (i.e., remodeling), like encapsulation, thus leading to changes in rate and extent of insulin absorption (61). The trauma also bears the risk of infection. Additionally, tissue adhesives may cause skin irritation or contact dermatitis (42). In rare cases, these skin-related problems are the reason for discontinuation of CSII therapy (62).

Catheter-related problems, especially catheter leakage, can be identified by carefully monitoring the catheter during bolus administration and treated by using a new infusion set. Inflammatory reactions on the outer skin may be identified easily by visual inspection and the occurrence of a burning sensation. Other infusion site-related problems under the surface, however, are not as easy to assess.

To address these potential problems during CSII treatment, a general recommendation of drug, insulin pump, and infusion set manufacturers, as well as the current literature, is to change the infusion site every two to three days (56,63,64).

Nevertheless, surveys on the catheter wear-time among patients treated with CSII have shown that many patients successfully extend the catheter wear-time beyond the recommended three-day period and the number of complications seems to have generally decreased (43,57).

1.2.4 Complications Derived from the Shortcomings

The shortcomings of the current treatment result in complications which account for significant morbidity and mortality, see Figure 8 (40). The wide range of complications can be divided into short-term and long-term complications.

Short-term complications derive from inadequate insulin dose. Too high doses result in low blood glucose concentration (i.e., hypoglycemia) accompanied by symptoms like temporary cognitive impairment, possible loss of consciousness or seizures, causing, e.g., vehicle accidents or falls with fractures (5,40,65). Severe hypoglycemia may be fatal and, especially if occurring frequently, is associated with permanent mild cognitive impairment (20,40). Inadequately low doses are the reason for high blood glucose concentration (i.e., hyperglycemia) with symptoms like polyuria, polydipsia, blurred vision and tiredness (18,23,66).

Furthermore, severe insulin deficiency, often accompanied by hyperglycemia, leads to ketoacidosis by the production of ketone bodies from unrestrained fatty acid oxidation (65). This ketoacidosis is a potentially life-threatening emergency situation (19,40,65). In some cases, the nonketotic hyperosmolar syndrome may occur due to acute hyperglycemia without severe insulin deficiency (17).

Inadequate or insufficient treatment results in mean blood glucose concentration above the physiologic target range, i.e., chronic hyperglycemia. This chronic hyperglycemia causes so-called long-term complications, classified as macrovascular or microvascular, resulting in damage to peripheral nerves, kidney failure, blindness, cardiac disease, stroke, and others (5,17,66).

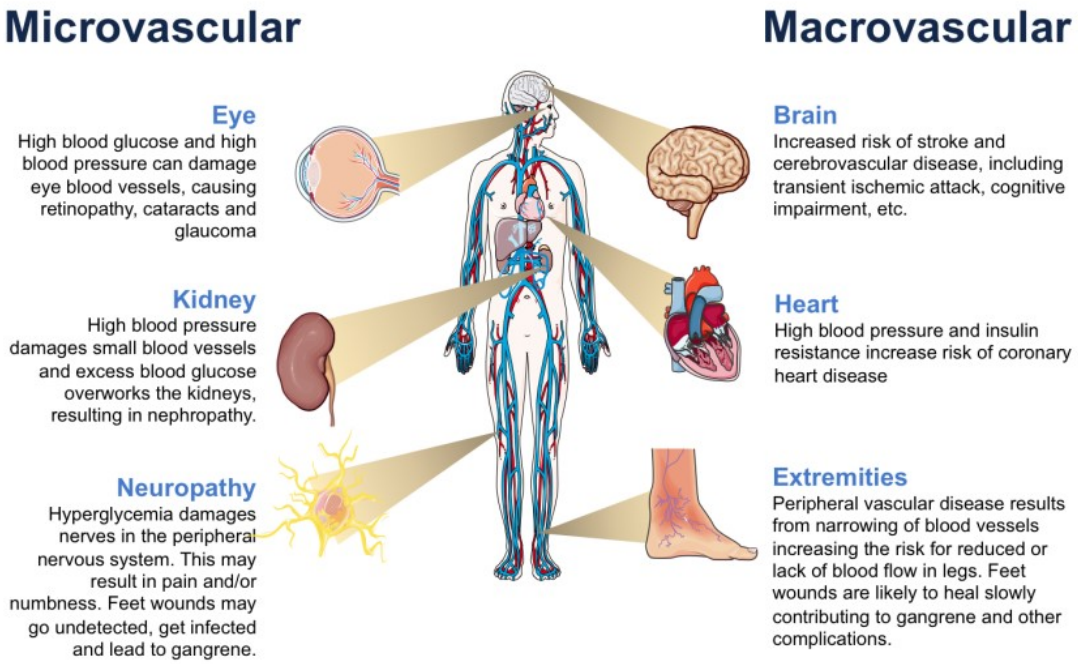
Early intensive therapy of DM reduces the risk of complications and lengthens the complication-free time, but currently cannot prevent those diabetes-related complications completely (20,68,69). Once complications arise, any improvement in the treatment leads to slowing down of the progression rate and may even cause complete remission of some complications (20,70,71).

Tissue trauma due to repetitive insulin injection into the same subcutaneous area is associated with the possible development of lipodystrophy (38). Lipodystrophic areas cause unpredictable changes in insulin absorption, and therefore general advice is to change injection and infusion site regularly (39).

Furthermore, an invasive procedure, e.g., injection or infusion, is a possible source of infection. Consequently, meticulous disinfection and thorough monitoring of the injection and infusion site are recommended (43).

To prevent the occurrence of diabetes complications, further improvement of therapeutic strategies and, thus research, is necessary. With regards to improving CSII, early detection of any problem related to CSII may decrease the risk of prolonged hyperglycemia and its potential complications (57). It would be desirable to determine the longest possible catheter wear-time individually and to inform the patient when to change the infusion site (44).

Figure 8: Major Complications of Diabetes mellitus



Adapted from rcsb.org (67).

1.3 Changes in the Insulin Absorption During Catheter Wear

Several groups have studied the effect of the catheter wear-time on insulin pharmacokinetics and pharmacodynamics. Swan et al. compared pharmacodynamics after insulin bolus (0.2 units/kg body weight) in 17 patients with T1DM on day one and day four and showed significantly earlier peak and shorter duration of action on day four (72). Clausen et al. tested ten healthy subjects on four consecutive days with a 0.1 units/kg body weight bolus and also showed an earlier peak in insulin action (63). Both groups could not find any significant change in the total amount of insulin absorbed. Thus, the extent of insulin absorption is not affected. A third group, Luijf et al., studied the effect of a three-day wear-time on insulin absorption in twenty subjects with T1DM and found lower postprandial glycemic excursions on day three, compared to the first day of catheter wear (73). Liu et al. also conclude that insulin infusion becomes faster after three days of catheter wear (74). Karlin et al. investigated the effect of catheter wear-time on the number of catheter failures in 20 patients with T1DM over a seven-day period. In addition, patients wore a CGM system during this seven-day period. Within the group with a seven-day catheter survival, they found a decrease in mean daily glucose concentration after 24 to 48 hours of catheter wear. Thereafter, mean daily glucose concentration increased steadily as the cannula use continued (75). These results indicate that during the first two days of catheter wear insulin absorption is improved, but it may worsen as the catheter wear continues.

Taken together, there is evidence for improving insulin absorption during the first three days of catheter wear (63,72–75). Furthermore, there is evidence for deteriorating insulin absorption as catheter wear continues (75). However, there is a general recommendation of infusion set manufacturers to change the catheter every two to three days (76). Thus, the question arises: If the catheter wear-time can be safely extended in many patients and the insulin absorption becomes more like the endogenous insulin secretion after three days of catheter wear, why change the catheter and the infusion site while the absorption is best?

1.4 Changes in the Hydraulic Tissue Resistance During Catheter Wear

Depending on the structure and physical properties, tissue opposes the infusion or injection of any fluid to a certain degree (44,46,77). This resistance exerted by the tissue upon the fluid entering the tissue is termed hydraulic tissue resistance (TR). It can be determined by measuring the pressure needed to counteract this opposition to inject or infuse the fluid into the subcutaneous tissue, see Figure 10 (44).

Preliminary experiments at the Medical University of Graz have shown a change of the TR in healthy individuals with T1DM over the duration of catheter wear (44). It varies from infusion site to infusion site and appears to decrease during the first two to four days of infusion site use but may exponentially increase as the use of the infusion site continues. Changes in the subcutaneous tissue following the tissue damage due to the catheter insertion (58–60) and/or the aggregation of insulin molecules, i.e., fibrillation, in the extracellular space surrounding the cannula may be the cause of this TR increase (78–80).

1.5 Rationale and Objectives of the Clinical Study Performed within the EU Project "AP@home"

In view of the previous findings that insulin absorption is improving during the first two to three days of catheter wear, whereas TR appears to decrease during this time, and that insulin absorption is decreasing after six days of catheter wear, while TR increases. We were reasoning that the absorption rate of the infused insulin may be inversely dependent on the TR. Thus, a very high TR value observed during infusion site use may indicate that insulin absorption is strongly reduced and that the maximum duration of the infusion site use is reached. Hence, a new infusion site should be established in order to achieve appropriate insulin absorption rates again. Furthermore, since TR can be easily determined by measuring the counter pressure during insulin delivery, we were reasoning that quantitative information on the absorption properties of insulin at the infusion site could be easily obtained by monitoring the TR during infusion site use.

Therefore, as a first step, a study was designed to determine the relationship between the TR and insulin absorption during prolonged use of the infusion site. The aims of this study, which was conducted within the EU project “AP@home”, were:

- Determining changes in the TR over a prolonged catheter wear-time.
- Determining changes in the insulin absorption over a prolonged catheter wear-time.
- Determining the relationship or correlation between the TR and the insulin absorption.

1.6 Aims of this Diploma Thesis

The aim of this diploma thesis was the collection of pressure and glucose data during this clinical study, the statistical analysis of the collected pressure and glucose data as well as the graphical representation and description of the analysis results.

2 Material and Methods

To determine the changes of the TR over a prolonged catheter wear-time as well as the changes of insulin absorption between the first and last study day of this prolonged catheter wear-time, a study was planned and conducted at the Clinical Research Center (CRC) of the Medical University of Graz with ten patients, diagnosed with T1DM. Patients had to be without residual insulin secretion (C-peptide negative) and treated with CSII. During the study, patients were treated with CSII, using a commercially available insulin pump and infusion catheter. The catheter wear-time was extended. Infusion pressure and tissue resistance were assessed daily. Plasma glucose concentration was assessed during an OGTT performed at the first and the last day of catheter wear. The resulting data of the TR and blood glucose concentration were then analyzed, and graphics were generated. The study was performed in accordance with the moral, ethical and scientific principles governing clinical research as set out in the declaration of Helsinki (81) and the applicable guidelines for good clinical practice (82). The study protocol is described in the section below.

2.1 Study Design for the Determination of Tissue Resistance and Insulin Absorption

The study was a single-center study with ten subjects and a within-subjects (or repeated-measures) design. Subjects were recruited from the CRC's database of patients with T1DM. The subjects came to the CRC on the screening day and each day during the catheter wear-time, which depended on the measured time course of the TR.

The TR was determined on the first study day shortly after the insertion of a new infusion catheter and each consecutive day until the TR reached values exceeding ten times the basal value, see Figure 9.

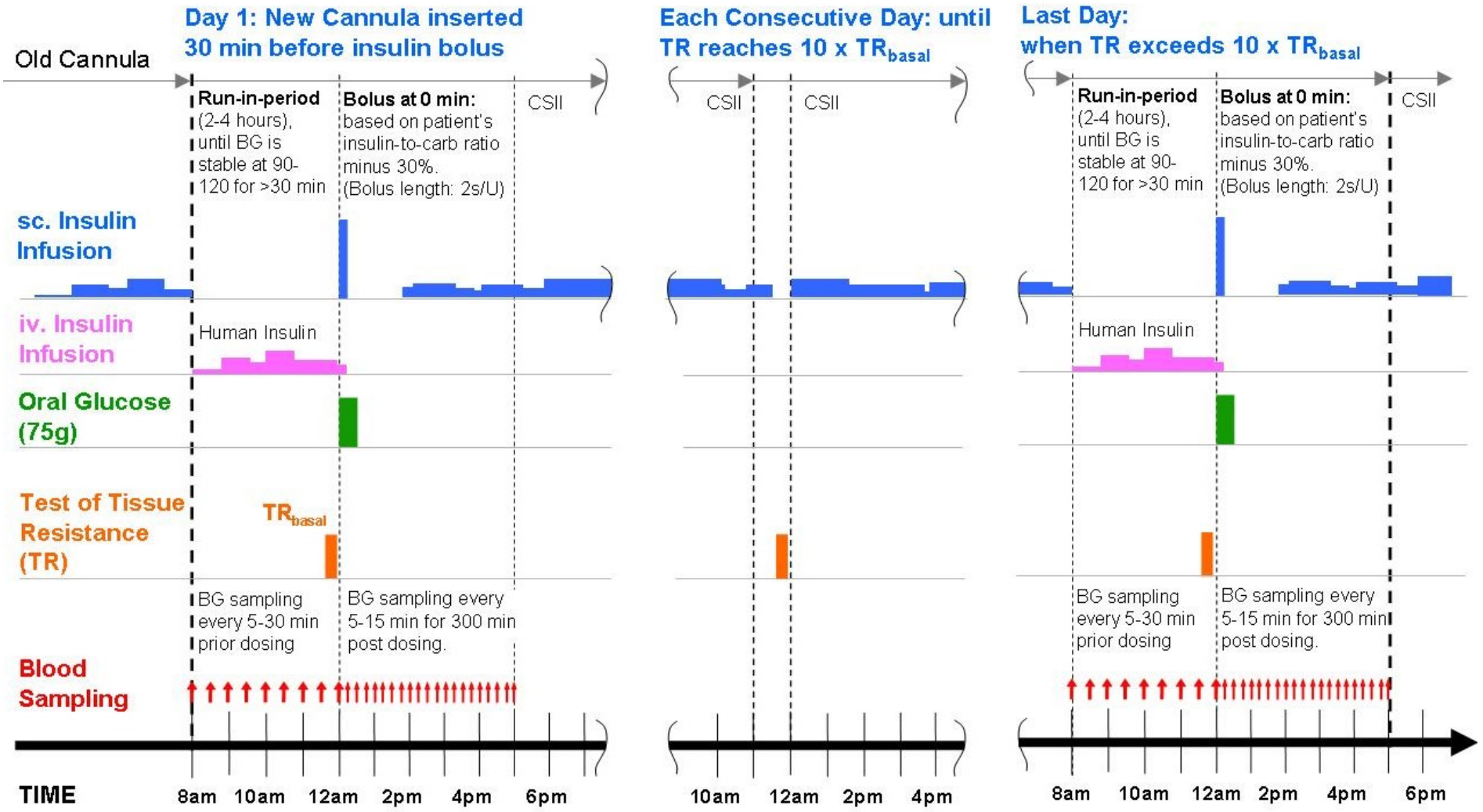
A disposable pressure sensor was placed between the reservoir of an insulin pump (Animas IR 2020) and the female Luer-lock end of an infusion catheter from Medtronic Minimed (Quick-set) to determine the TR.

To assess the relationship between the TR and the insulin absorption within a subject, a 75g OGTT was performed on two study days, one shortly after the

insertion of the new infusion catheter and one on the last day of the infusion catheter use. Subjects fasted overnight before these study days. Twenty minutes before the ingestion of the 75g of glucose, a bolus of rapid-acting insulin (Novorapid; Novo Nordisk) was administered via the infusion catheter using the insulin pump from Animas. The size of the insulin bolus administered amounted to 70% of subject's usual insulin dose (calculated using medical records on the subject's insulin-to-carbohydrate ratio). Following administration of the insulin bolus, rapid-acting insulin was delivered at basal rates via the infusion catheter. During the first OGTT, the basal insulin delivery was periodically adjusted to re-establish near-normal plasma glucose by ~4h after glucose ingestion. However, during the second OGTT, the basal insulin delivery rates used was identical to those rates applied during the first OGTT.

To establish comparable baseline glucose concentrations on the days on which OGTTs were performed and to avoid different accumulations of insulin in the subcutaneous tissue before administration of the boluses at the start of the OGTTs, subcutaneous insulin delivery was stopped ~4 hours before the insulin bolus administration and, instead, an intravenous infusion of regular human insulin (Actrapid, Novo Nordisk) was maintained during the 4-hour period before administration of each subcutaneous insulin bolus (Figure 9). The adjustments in the rate of the intravenous insulin infusion were based on the frequently measured plasma glucose concentration.

Figure 9: HYDRA-01 Study protocol

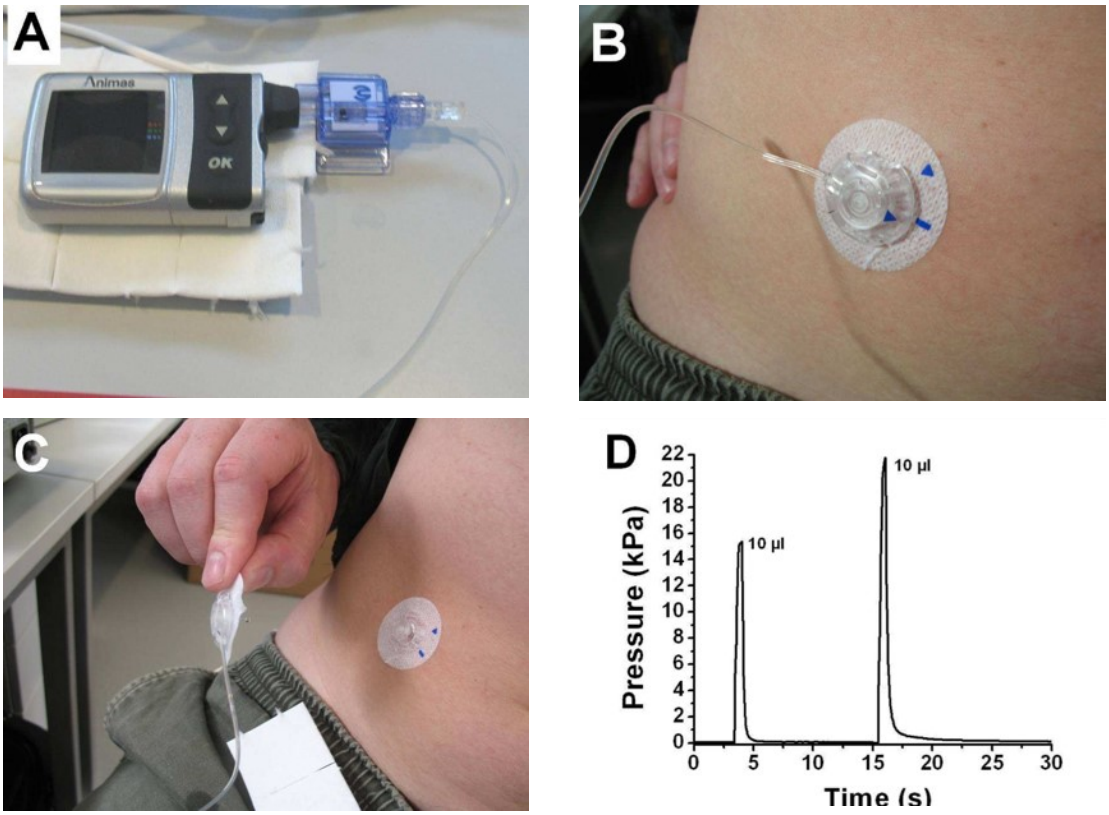


2.1.1 The First Study Day

The subjects admitted themselves to the CRC at ~ 7:30 AM after an overnight fast. Upon admission, vital signs (blood pressure, heart rate, and body temperature) were assessed, and subjects were asked to remove their own infusion set and insulin pumps. At ~ 7:45 AM, an arm vein was cannulated with a 20-gauge intravenous catheter (Vasofix® Safety; B. Braun, Melsungen AG, Germany) to be used for an intravenous insulin infusion during a baseline period preceding the OGTT. A second 20-gauge intravenous catheter (Vasofix® Safety) was then inserted into a vein in the opposite forearm to allow blood withdrawal during the study day. The hand with this catheter was then placed in an electric heating pad (P10; Beurer GmbH, Ulm, Germany) and maintained at 55°C to ensure the arterialization of the venous samples. After insertion of the catheters, an intravenous infusion of human insulin (Actrapid®, 100U/ml; Novo Nordisk A/S, Bagsvaerd, Denmark) was started using a syringe pump (B. Braun Perfusion® Space Syringe Pump; B. Braun, Melsungen AG, Deutschland). During the subsequent baseline period lasting 3-5 hours, the insulin infusion rate was adjusted on the basis of frequent plasma glucose measurements (every 5-20 minutes) to achieve and maintain target glucose levels between 90 and 120 mg/dl for the last hour prior to the subcutaneous insulin bolus administration. Preceding the subcutaneous insulin bolus by forty minutes (t = -40 min), the intravenous insulin delivery was set at a rate which should maintain the plasma glucose concentration within the target glucose range. At t = -10 min, this insulin infusion was tapered (75% from t = -10 to -5 min; 50% from -5 min to 0 min) and discontinued at the time of the subcutaneous bolus administration (at t = 0 min). Forty minutes before the subcutaneous insulin bolus, a subcutaneous insulin infusion cannula (Quick-set; Medtronic Minimed, Dublin, Ireland) was inserted into the periumbilical subcutaneous adipose tissue. Using an infusion set tube (Quick-set; Medtronic Minimed, Dublin, Ireland), the inserted cannula was connected to a reservoir (Animas® Insulin Pump Cartridge; Animas Corp., West Chester, PA, USA) filled with an insulin diluting solution (Insulin diluting medium for NovoLog®; Novo Nordisk A/S, Bagsvaerd, Denmark). For the determination of the hydraulic tissue resistance (TR), a disposable pressure sensor (DPT-100, Utah Medical Products Inc., UT, USA) was placed between the reservoir of the insulin pump and the Luer-lock end of the infusion set tube. The reservoir was then inserted into an insulin

pump (IR2020; Animas Corp., West Chester, PA, USA), and the TR was measured as described in Figure 10. After the measurement of TR, the reservoir and infusion set tube filled with the insulin diluting solution (IDS) were disconnected from the infusion cannula and replaced by a reservoir and infusion set tube filled with rapid-acting insulin (NovoRapid®; Novo Nordisk, Bagsvaerd, Denmark). At t=0 min, a bolus of rapid-acting insulin was then administered via the subcutaneous infusion cannula using the insulin pump. The size of the insulin bolus administered was 70% of the subject's usual insulin dose to cover 75g of glucose (calculated using medical records on the subject's insulin-to-carbohydrate ratio). At t=15 min, the subject ingested 75 g glucose dissolved in 300 ml of water (Glucoral; Unipack, Wr. Neustadt, Austria). Following glucose ingestion, rapid-acting insulin was delivered at basal rates via the subcutaneous infusion cannula. Basal insulin delivery was periodically adjusted on the basis of frequent plasma glucose measurements (every 5-20 minutes) to re-establish euglycaemic plasma glucose by ~4 h after glucose ingestion. If the plasma glucose levels decreased below 3.22 mmol/l (58 mg/dl) during the experiments, the subjects were asked to ingest additional glucose. To determine the plasma insulin concentrations during the OGTT, blood was sampled at t = -15 (predose), 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 105, 120, 150, 180, 240, and 300 min. To determine the TR at the end of the OGTT, the reservoir and infusion set tube filled with rapid-acting insulin were disconnected from the infusion cannula and connected to the reservoir and infusion set tube filled with IDS. After the measurement of TR; the pump, reservoir and infusion set tube filled with IDS were disconnected from the infusion cannula and replaced by the subject's insulin pump, reservoir and infusion set tube. Subjects then received a meal and left the study site after that.

Figure 10: Experimental set up for determining the tissue resistance



(A) *A disposable pressure sensor (DPT-100, Utah Medical Products Inc., UT, USA) is placed between the reservoir of an insulin pump (Animas Corp., West Chester, PA, USA) and the female Luer-lock end of an infusion tubing (60 cm line length and 9 mm cannula length, Quick-set; Medtronic Minimed, Northridge, CA, USA). IDS boli of the size of 10µl (the volumes corresponding to 1U of a 100 U/ml insulin solution) are administered using the pump. During the IDS delivery, the pressure in the infusion set is monitored using the disposable pressure sensor connected to a modular data acquisition system consisting of a 24-bit full-bridge module (NI 9237), a USB chassis (NI cDAQ-9172), a data acquisition program based on Lab VIEW® 7.0 (all from National Instruments Inc., Austin, TX, USA), and a notebook computer.* ***(B)*** *The pressure changes monitored during the infusion are generated by the combined resistance of the infusion line (i.e., hydraulic resistance from pumping the solution through a tube with a small diameter) and the tissue (i.e., hydraulic resistance tissue) to the fluid flow. To separate and quantitate the pressure induced by the infusion line and that induced by the tissue, the pressure generated by pumping the IDS through the infusion line alone is monitored before the IDS infusion into the tissue. To perform this measurement, a cannula identical to the cannula inserted into the tissue is connected to the infusion tube* ***(C)****.* ***(D)*** *Graph showing typical pressure changes generated by pumping the IDS through the infusion line alone (left peak) and pressure changes induced by both the hydraulic tissue resistance and the flow resistance in the infusion line (right peak) (44).*

2.1.2 Consecutive Study Days

Subjects were admitted to the CRC on the next day between 8:00 AM and 12:00 PM. Upon arrival, the subject's insulin pump and infusion set tube were disconnected from the infusion cannula. After a visual inspection of the subcutaneous infusion site, the insulin pump and infusion set tube filled with IDS was connected to the infusion cannula, and the TR was measured as described in Figure 10. After the measurement of TR, the insulin pump and infusion set tube filled with IDS were disconnected from the infusion cannula and replaced by the subject's insulin pump and infusion set tube. Afterward, subjects left the CRC. Subsequently, the determination of the TR was repeated every day until TR reached values exceeding ten times the value observed on the first study day (i.e., TR > 10 x TR_{basal}). On the day the TR value reached this threshold value, the subjects were asked to fast overnight and come to the CRC on the next morning.

2.1.3 The Last Study Day

Subjects were admitted to the CRC at ~07:30 AM. Subsequently, the experimental procedures applied were the same as those used on the first study day, except that (A) the insulin pump and infusion set tube filled with IDS were connected to the infusion cannula when the intravenous insulin infusion was started, (B) the infusion cannula was used for insulin delivery throughout the OGTT (i.e., no new subcutaneous infusion cannula was inserted at t = -40 min), (C) the basal insulin delivery rates used after glucose ingestion were identical to those rates applied during the first OGTT, and (D) the subject's infusion cannula and infusion site were changed at the end of the study visit.

2.2 Parameters

- G_{mean}, the average plasma glucose concentration during the OGTT.
- ΔG_{mean}, the average increase of plasma glucose concentration above basal plasma glucose concentration
- TR, the tissue resistance determined at each day during infusion site use.
- sTR, the specific tissue resistance determined at each day during infusion site use.
- R_{SYS}, the hydraulic resistance exerted by infusion line alone.
- P_{max}, the maximum hydraulic pressure during infusion site use.
- ΔP_{Smax}, the maximum hydraulic pressure during infusion line use alone.
- P_{mean}, the mean hydraulic pressure during infusion site use.
- ΔP_{Smean}, the mean hydraulic pressure during infusion line use alone.

2.3 Study Materials

In this section, the medicinal products and medical devices used in the study are listed.

2.3.1 Pharmaceuticals

The following pharmaceuticals were used in this study:

- During the study, insulin aspart (100 U/ml, NovoRapid®; Novo Nordisk, Bagsvaerd, Denmark) was administered subcutaneously using an insulin pump.
- During the study, an insulin diluting solution (Insulin diluting medium for NovoLog®; Novo Nordisk A/S, Bagsvaerd, Denmark) was subcutaneously infused (NovoLog® is the name under which insulin aspart is marketed in the USA).
- During the OGTT on the first and last study day, the patency of the blood sampling catheter will be maintained by slow infusion of saline (0.9%, Fresenius Kabi, Graz Austria).
- 75 g of glucose for performing the OGTT (Glucoral; Unipack, Wr.- Neustadt, Austria).

- Human insulin solution for the intravenous insulin administration on the OGTT study days [100U/ml] (Actrapid®; Novo Nordisk, Bagsvaerd, Denmark).

2.3.2 Medical Devices

The following medical devices were used in this study:

- For determination of the hydraulic tissue resistance, a disposable pressure sensor (DPT-100, Utah Medical Products Inc., UT, USA) was applied.
- For the subcutaneous infusion of rapid-acting insulin and IDS, insulin pumps (Animas IR2020; Animas Corporation, West Chester, Pennsylvania, USA),
- An insulin infusion set (Quick-set, Medtronic, Dublin, Ireland), and
- Pump reservoirs (Animas Reservoir; Animas Corporation, West Chester, Pennsylvania, USA) were used.
- For frequent plasma glucose measurements, a laboratory glucose meter (Super GL2; Dr. Müller Gerätebau, Freital, Germany) was used.
- Intravenous lines for blood sampling and infusion of insulin or glucose on the OGTT study days (Vasofix® Safety; B. Braun, Melsungen AG, Germany)
- An electric heating pad for blood sample arterialization (P10; Beurer GmbH, Ulm, Germany)
- A syringe pump for intravenous insulin infusion on the OGTT study days (B. Braun Perfusion® Space Syringe Pump; B. Braun, Melsungen AG, Deutschland)

2.4 Data Management and Data Collection

Subject identification details were coded according to professional standards of confidentiality and protected by strict security measures as well as restricted accessibility. The study related data were locally stored. The study was registered in a public registry of clinical trials (EudraCT number 2013-001791-38).

The study Case Report Forms (CRFs) were prepared in advance and used for data collection during the study. The collected data were transferred by hand from the source documents to a computer and submitted for review. A person, other than the

person transferring the data, performed this review. The subjects, as well as any biological material obtained from the subjects, were identified by the subject number and trial identification number. Finally, to protect the identity of the subjects in all presentations and publications, appropriate measures such as data encryption or deletion were enforced according to the local/regional ethics committee rules. In case of screening failures, no data were entered into the database.

2.5 Data Analysis

2.5.1 Endpoints Related to Insulin Absorption

The endpoints (G_{mean}, ΔG_{mean}) related to insulin absorption were derived from the individual time courses of the plasma glucose concentration and the plasma insulin concentration observed during the OGTTs on the first and last study day.

2.5.2 Endpoints Related to Hydraulic Tissue Resistance

The TR, was calculated as TR = $\Delta P_T/FR$, where FR is the flow rate of the liquid in mm^3/s (or µl/s) and ΔP_T is the difference in the pressure values obtained during the infusion of insulin or IDS into the tissue and that obtained during the IDS or insulin infusion through the infusion set alone (in Pa). As the tissue resistance calculated in this way depends on the cross-sectional area of the applied cannula, it is advantageous to first normalize the flow rate of the liquid for the cross-sectional area of the cannula end at the infusion site (A; in mm^2) and then calculate a specific tissue resistance (sTR; in $Pa \cdot s \cdot mm^{-1}$) as sTR = $\Delta P_T/(FR/A)$ = $(\Delta P_T \cdot A)/FR$. Thereby, the specific tissue resistance obtained for a delivery site is independent of the cannula applied and may reflect a property of the tissue purely. The specific tissue resistance can also be calculated from the tissue resistance and the cross-sectional area of the cannula end as sTR = TR·A, see Figure 11.

As the pressure was continuously recorded, the tissue resistance (in $Pa \cdot s \cdot mm^{-3}$) was derived by calculating differences between the area under pressure curve obtained during the infusion of insulin or IDS into the tissue ($AUC_{Tissue+System}$; in Pa·s) and that obtained during the infusion of insulin or IDS through the infusion line alone (AUC_{System}; in Pa·s), and by dividing the obtained difference by the infused amount

of IDS or insulin (Vd; in mm^3). The specific tissue resistance (in Pa·s·mm-1) was then calculated by multiplying the tissue resistance value by the cross-sectional area of the used cannula (i.e., Quick Set: 0.113 mm^2). The system resistance (R_{SYS}; in $Pa \cdot s \cdot mm^{-3}$) was derived by dividing the AUC_{System} (in Pa·s) by the infused amount of IDS or insulin (Vd; in mm^3). The integrated areas under the pressure curves (AUC's) were calculated using the trapezoidal rule. P_{mean} and ΔP_{Smean} were calculated as the means of the pressure values observed during the infusion of insulin or IDS into the tissue, and that observed during the IDS or insulin infusion through the infusion set alone, respectively. P_{max} and ΔP_{Smax} were determined as the highest-pressure value seen during the infusion of insulin or IDS into the tissue and that seen during the IDS or insulin infusion through the infusion set alone, respectively. The recorded pressure time courses and the derived endpoints were represented in graphs and tables for each subject.

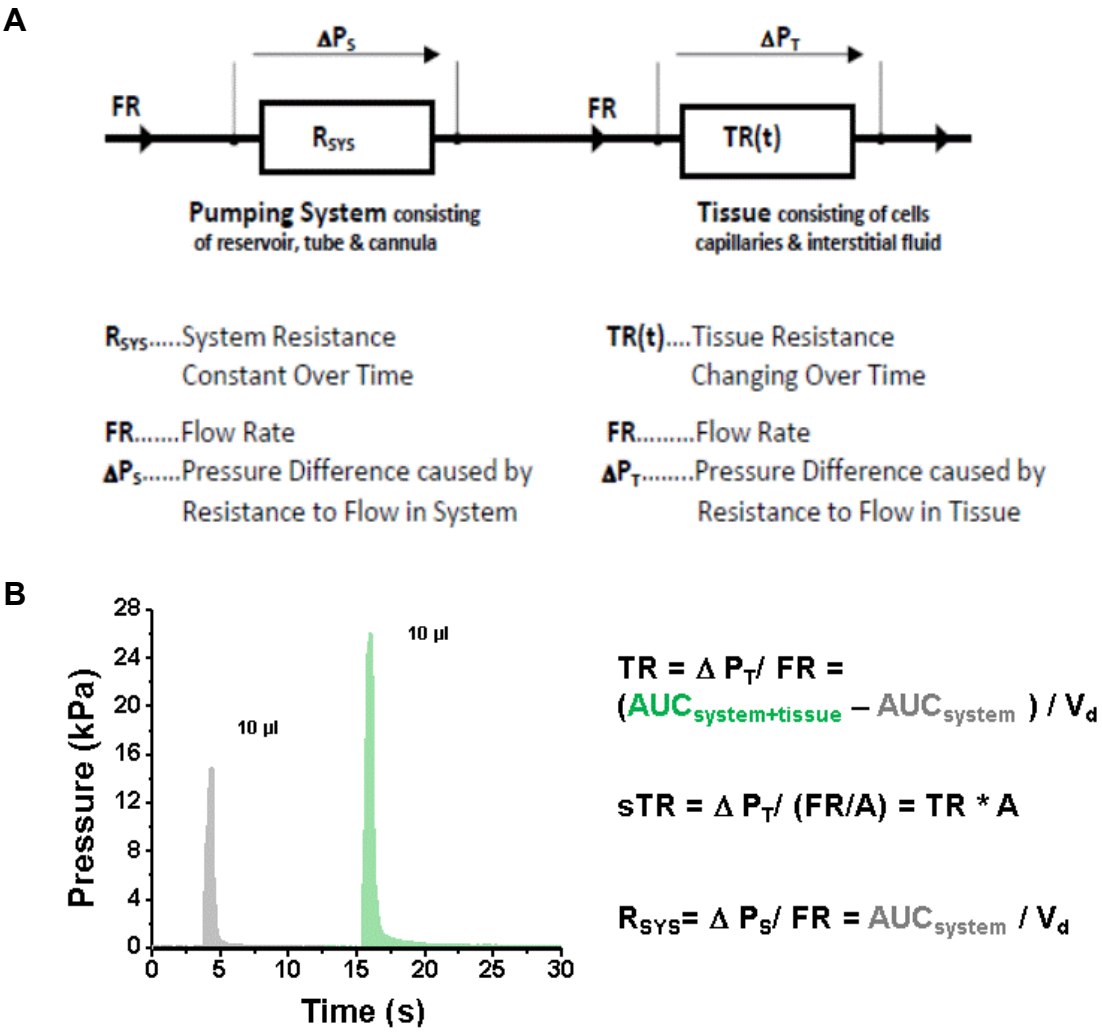
2.6 Statistical Analysis

The statistical analysis of the data was performed using an OriginLab software package (Version 8.5; OriginLab® Corp. Northampton, MA).

2.6.1 Comparison of Endpoint Values Between Study Days

To determine whether the average values of the endpoints changed during the first seven study days (day six was the last day with all 10 subjects enrolled), the endpoint values (sTR, P_{max}, P_{mean}, ΔP_{Smax}, ΔP_{Smean}, R_{SYS}, TR, G_{mean}) observed on the study days 1-7 were examined by the non-parametric Friedman's test.
The average values of the endpoints were furthermore compared between day 0 and every other study day with n≥5, using Wilcoxon Signed Rank Test.
P values <0.05 were considered significant.

Figure 11: Calculation of the hydraulic tissue resistance



(A) The pressure changes monitored during the IDS or insulin infusion are generated by the combined resistance of the infusion line (i.e., hydraulic resistance from pumping the solution through a tube with a small diameter, R_{SYS}) and the tissue (i.e., hydraulic tissue resistance, TR) to the fluid flow.
(B) Graph showing typical pressure changes generated by pumping the IDS or insulin through the infusion line alone (grey peak) and pressure changes induced by both the hydraulic tissue resistance and the flow resistance in the infusion line (green peak). Also shown are the equations used to calculate the hydraulic resistances.

3 Results

3.1 Subject Characteristics

14 patients with T1DM and CSII treatment were invited to take part in the study. All of them met the inclusion criteria. During the study, four subjects met study day exclusion criteria: two subjects withdrew consent for further study participation, one subject accidentally removed the infusion cannula, and one subject had a skin irritation from the tissue adhesive. These four subjects were replaced, so in total ten subjects completed the study. No severe adverse event occurred in the study.
A summary of the demographic characteristics of the ten subjects who completed the study is given in Table 2. The individual data can be found in the case report forms of each subject.

Table 2: Subject characteristics

n (female / male)	10 (2 / 8)
Age (years)	31.09 ± 7.7 (21.1 – 44.2)
Weight (kg)	83.8 ± 8.6 (73 – 98)
Height (cm)	177.4 ± 10.2 (158 – 191)
BMI (kg / m²)	26.7 ± 2.8 (23.5 – 30.9)
HbA1c (%)‡	7.7 ± 0.5 (6.8 – 8.6)
Diabetes duration (years)	20.1 ± 10.4 (6.8 – 35.7)
Time in study (days)	10.4 ± 2.5 (6 – 13)

Data are means ± SD (range); ‡ Normal range 4.3 – 5.9%

3.2 Infusion Set Function

The ten subjects participating in the study wore the insulin infusion catheters for an average 10.4 (6 to 13) days. The insertion site of the catheters was the periumbilical abdomen. The insertion of the catheter was performed manually by the study personnel. In one subject, very high infusion pressure values (i.e., occlusion) were found directly after catheter insertion. The catheter was withdrawn from the tissue and replaced at the contralateral periumbilical abdomen. Additionally, there were

three instances in which the adhesive tape of the cannula housing became loose, and thereby causing the fluid to leak out around the infusion catheter (i.e., due to backflow from the cannula tip along the cannula shaft towards the skin surface). To prevent further fluid leakage at these infusion sites, the adhesive tapes of the cannula housings were re-secured to the subject's skin using additional adhesive strips and liquid tissue adhesive.

3.3 Hydraulic Pressure Readings and Derived Study Endpoints

The hydraulic pressure measurements performed in the subjects were described and documented in the study case report forms. The hydraulic pressure time courses were recorded as tabulator separated text format files and graphically represented. A typical individual pressure time course is displayed in the appendix, see 6.1. From the individual hydraulic pressure time courses, the study endpoints were calculated for each subject as described in 2.5.2.

3.3.1 Normality Tests

Normality tests performed using normal probability plots, and Kolmogorov-Smirnov tests indicated that the derived endpoint data are log-normally distributed. A typical result of the normality tests, using sTR data that were observed on days 6 to 13, is shown in Figure 12 and Figure 13. One can see that the untransformed sTR data do not fall onto the reference line (Figure 12), whereas the $\log_{10}$-transformed sTR data coincide with the reference line (Figure 13). Also, the results of the Kolmogorov-Smirnov test indicated that, in contrast to the untransformed sTR data ($p<0.01$), the log-transformed sTR data are normally distributed ($p=1$).
Therefore, to adequately describe the frequency distributions of the endpoints, the geometric mean (GeoMean), the geometric standard deviation (GeoSD), the median (x), as well as the first and third quartile (Q_1 and Q_3) were calculated for each endpoint.

Figure 12: Representative normal probability plot of untransformed sTR data

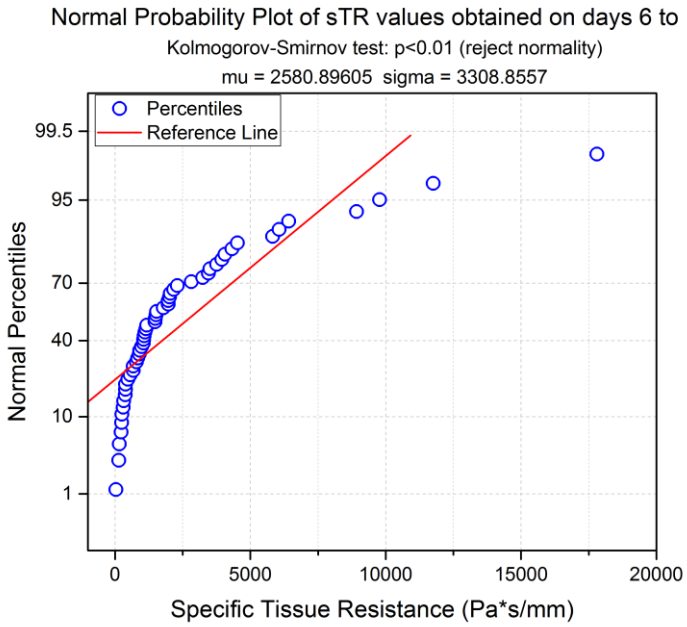
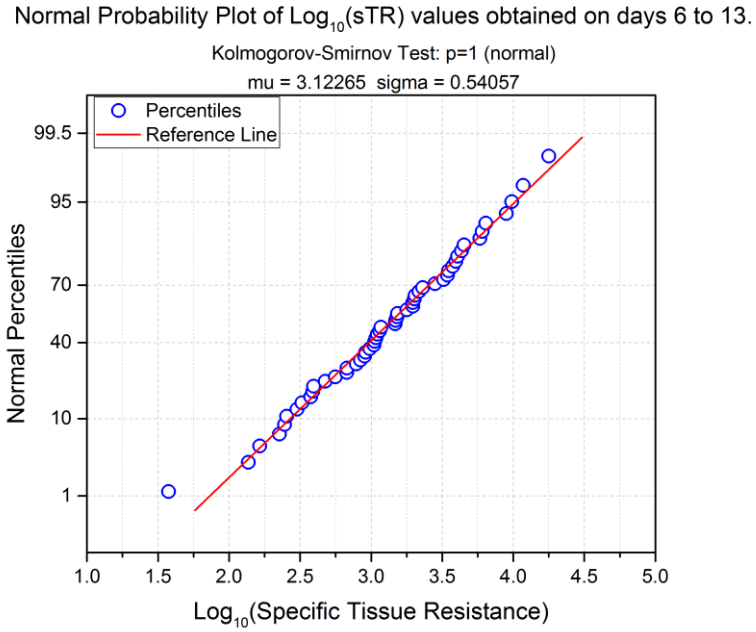


Figure 13: Representative normal probability plot of log_{10}-transformed sTR data

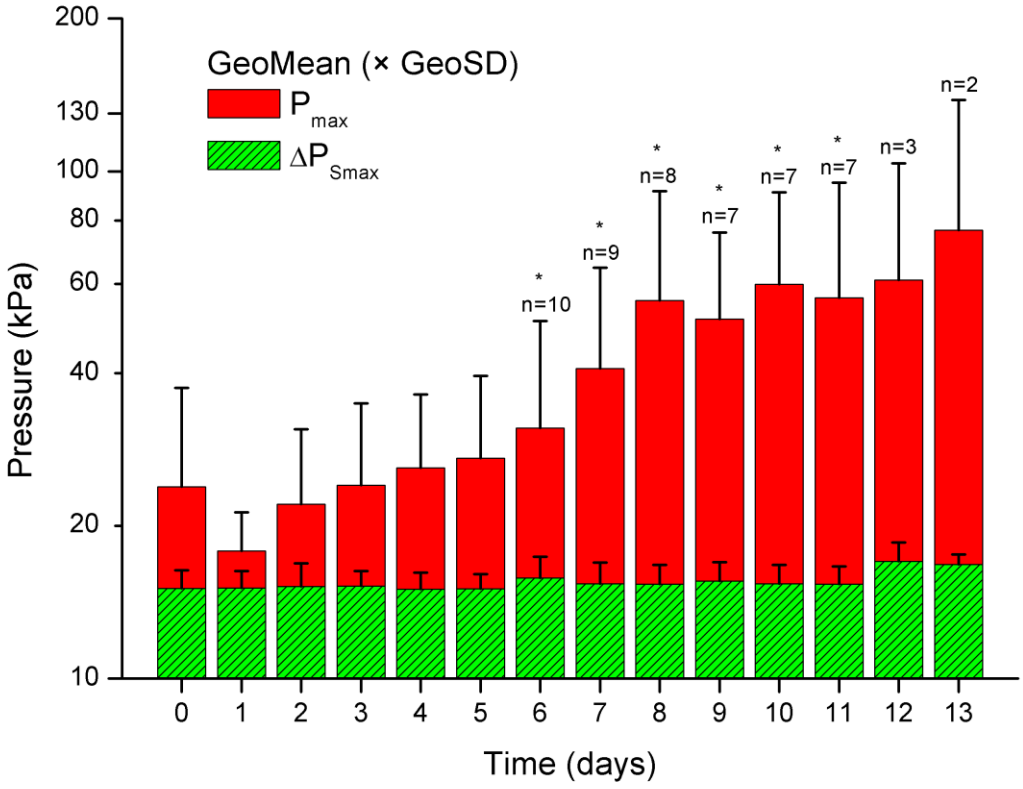


3.3.2 Maximum Pressure (P_{max}, ΔP_{Smax}) Values

Average values of the maximum pressure observed during bolus delivery at the infusion site (P_{max}), as well as the average values of the maximum pressure generated by pumping IDS through the infusion line alone (ΔP_{Smax}, i.e., detached from the tissue cannula and attached to an identical cannula without body contact), are shown in Figure 14 and given in Table 3.

It can be seen that the P_{max} values during bolus delivery changed over the catheter wear-time of seven days (Friedman ANOVA, n=10, $p<0.01$), while ΔP_{Smax} remained constant during this time period (Friedman ANOVA, n=10, $p=0.18$). A day-to-day comparison, comparing day 0 with every other day (i.e., days with more than four study subjects, $n \geq 5$), showed that the pressure values observed from day six onward were significantly different to those values observed on day 0. Average values of P_{max} observed on day one of catheter wear had a tendency to be lower (GeoMean 17.8 kPa) than P_{max} values observed directly after catheter insertion on day 0 (GeoMean 23.9 kPa). However, this decrease was not significant (Wilcoxon Signed Rank test, $p=0.11$). Following this initial decrease, P_{max} progressively increased over the catheter wear-time. The highest increase in P_{max} values was observed at the infusion site of subject 12. In this subject, P_{max} increased from a value of 18.6 kPa on the first study day to a value of 132.0 kPa on the last study day.

Figure 14: Average pressure values (P_{max}, ΔP_{Smax}) observed each day



* p value<0.05 using Wilcoxon Signed Rank test to compare with day 0.

Table 3: Average pressure (P_{max}, ΔP_{Smax}) values observed each day

Day No.	N (Subjects)	P_{max} (kPa)		ΔP_{Smax} (kPa)	
		Median (1st & 3rd Quartile)	GeoMean (geoSD)	Median (1st & 3rd Quartile)	GeoMean (geoSD)
0	10	19.4 (18.6 – 25.7)	23.9 (1.6)	15.1 (14.6 – 15.6)	15.1 (1.1)
1	10	17.9 (16.0 – 20.7)	17.8 (1.2)	15.3 (14.6 – 15.8)	15.1 (1.1)
2	10	20.8 (19.1 – 25.4)	22.1 (1.4)	15.7 (14.9 – 16.3)	15.2 (1.1)
3	10	24.0 (20.7 – 27.9)	24.0 (1.5)	15.5 (14.9 – 15.9)	15.2 (1.1)
4	10	26.9 (20.3 – 34.8)	26.0 (1.4)	15.4 (14.7 – 15.8)	15.0 (1.1)
5	10	25.4 (22.2 – 37.5)	27.2 (1.5)	15.3 (14.5 – 15.8)	15.0 (1.1)
6	10	28.2 (24.5 – 32.8)	31.2 (1.6)	15.9 (15.3 – 16.5)	15.8 (1.1)
7	9	35.7 (31.0 – 53.4)	40.8 (1.6)	15.4 (14.9 – 16.4)	15.4 (1.1)
8	8	54.5 (39.6 – 75.4)	55.6 (1.6)	15.4 (15.2 – 15.6)	15.4 (1.1)
9	7	50.8 (38.7 – 61.6)	51.1 (1.5)	15.2 (15.1 – 17.0)	15.6 (1.1)
10	7	52.0 (43.9 – 74.8)	59.9 (1.5)	15.7 (14.2 – 16.2)	15.4 (1.1)
11	7	42.3 (36.3 – 100.8)	56.3 (1.7)	15.8 (14.4 – 15.9)	15.3 (1.1)
12	3	46.7 (43.3 – 112.4)	61.0 (1.7)	17.8 (15.3 – 18.2)	17.0 (1.1)
13	2	83.3 (50.4 – 116.2)	76.5 (1.8)	16.8 (16.2 – 17.4)	16.8 (1.0)
P-value#		<0.01#		0.18#	

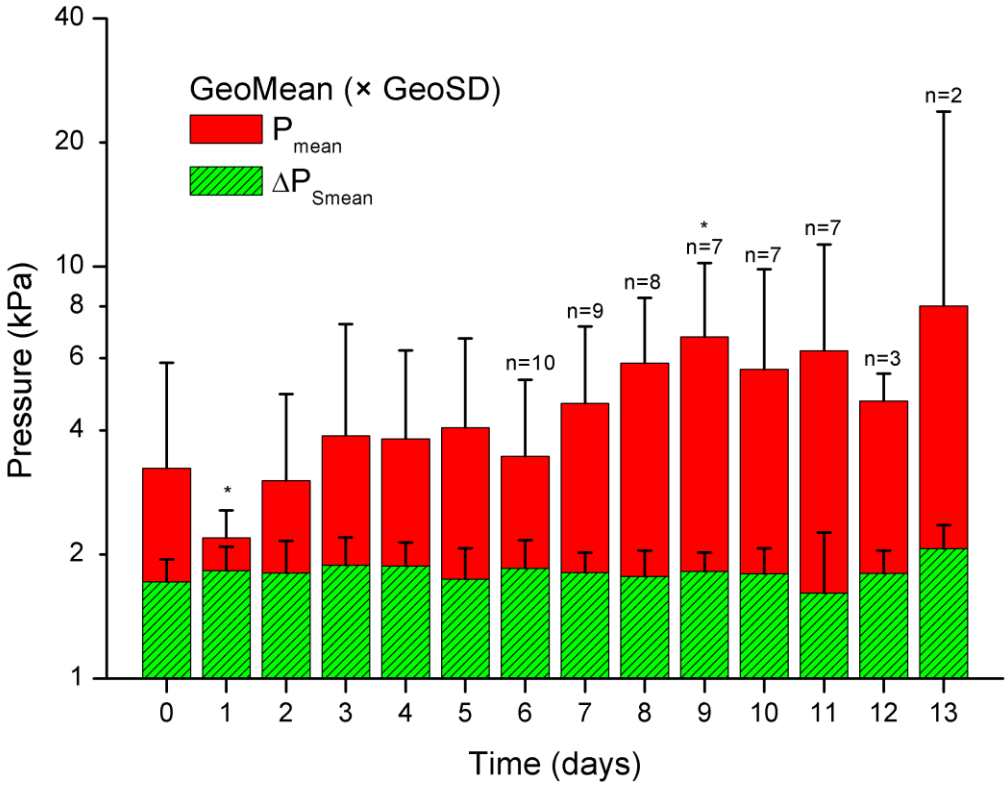
#Comparison with Friedman's test
P-values for the data range from day 1 to day 7
(day 7 was the last day with all ten subjects enrolled)

3.3.3 Mean Pressure (P_{mean}, ΔP_{Smean}) Values

Average values of the mean pressure observed during bolus delivery at the infusion site (P_{mean}) as well as the average values of the mean pressure generated by administering the bolus the infusion line alone (ΔP_{Smean}, i.e., detached from the tissue cannula and attached to an identical cannula without body contact) are shown in Figure 15 and given in Table 4.

Comparing figures 14 and 15, it can be seen that the obtained average time course of the parameter P_{mean} is similar to that of P_{max}. P_{mean} values during bolus delivery changed over the catheter wear-time of seven days (Friedman ANOVA, n=10, $p<0.01$). A day-to-day comparison, comparing day 0 with every other day (i.e., days with $n \geq 5$), showed a significant decrease of P_{mean} on day 1 (Wilcoxon Signed Rank test, $p=0.04$). On every other day, compared to day 0, a tendency to increase was found in P_{mean} values. However, a significant increase in P_{mean} values was only found when comparing day 9 to day 0 (Wilcoxon Signed Rank test, n=7, $p=0.03$).

Figure 15: Average pressure (P_{mean}, ΔP_{Smean}) values observed each day



* P value<0.05 using Wilcoxon Signed Rank test to compare with day 0.

Table 4: Average pressure (P_{mean}, ΔP_{Smean}) values observed each day

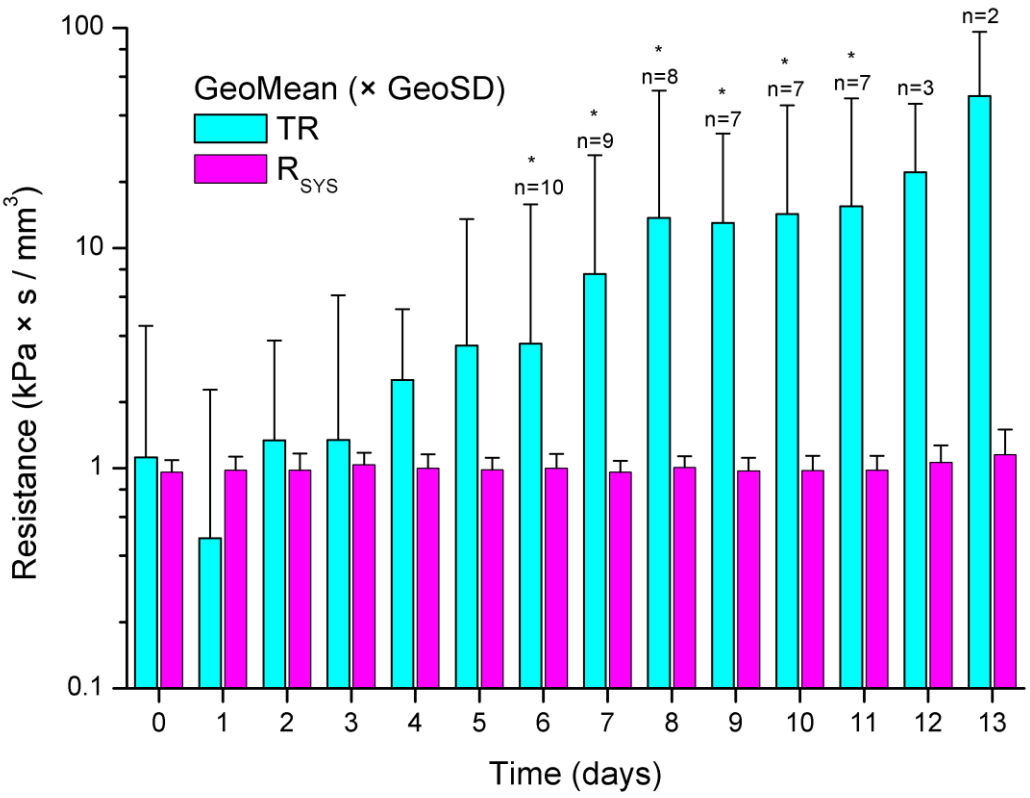
Day No.	N (Subjects)	P_{mean} (kPa)		ΔP_{Smean} (kPa)	
		Median (1st & 3rd Quartile)	GeoMean (geoSD)	Median (1st & 3rd Quartile)	GeoMean (geoSD)
0	10	2.7 (2.4 – 3.5)	3.2 (1.8)	1.7 (1.5 – 2.0)	1.7 (1.2)
1	10	2.2 (2.0 – 2.5)	2.2 (1.2)	1.9 (1.8 – 1.9)	1.8 (1.1)
2	10	2.8 (2.1 – 3.8)	3.0 (1.6)	1.8 (1.5 – 2.0)	1.8 (1.2)
3	10	4.1 (2.1 – 6.6)	3.9 (1.9)	1.9 (1.9 – 2.0)	1.9 (1.2)
4	10	3.5 (2.7 – 6.2)	3.8 (1.6)	1.9 (1.8 – 2.2)	1.9 (1.1)
5	10	4.6 (2.7 – 6.1)	4.1 (1.6)	1.8 (1.5 – 1.9)	1.7 (1.2)
6	10	3.6 (2.7 – 4.4)	3.5 (1.5)	1.8 (1.7 – 2.2)	1.9 (1.2)
7	9	4.7 (3.6 – 5.4)	4.7 (1.5)	1.8 (1.7 – 1.9)	1.8 (1.1)
8	8	6.1 (4.4 – 7.8)	5.8 (1.4)	1.8 (1.6 – 2.0)	1.8 (1.2)
9	7	5.5 (5.0 – 10.7)	6.7 (1.5)	1.8 (1.7 – 1.9)	1.8 (1.1)
10	7	4.1 (3.7 – 9.3)	5.6 (1.8)	1.9 (1.5 – 2.1)	1.8 (1.2)
11	7	6.9 (3.7 – 11.4)	6.2 (1.8)	1.7 (1.5 – 2.0)	1.6 (1.4)
12	3	4.3 (4.3 – 5.6)	4.7 (1.2)	1.7 (1.7 – 2.1)	1.8 (1.1)
13	2	10.5 (3.7 – 17.3)	8.0 (3.0)	2.1 (1.9 – 2.3)	2.1 (1.1)
P-value[#]		<0.01		0.19[#]	

[#]Comparison with Friedman's test
P-values for the data range from day 1 to day 7
(day 7 was the last day with all 10 subjects enrolled)

3.3.4 Tissue Resistance (TR) and System Resistance (R_{SYS}) Observed

Average values of the TR and R_{SYS} measured at the infusion site are shown in Figure 16 and given in Table 5. It can be seen that the TR values during bolus delivery changed over the catheter wear-time of seven days (Friedman ANOVA, n=10, p<0.01). A day-to-day comparison, comparing day 0 with every other day (on days with n≥5), showed that the TR values observed from day six onward were significantly different to those values observed on day 0. As was the case with the parameters P_{max} and sTR, the average TR values obtained at the infusion site showed a tendency to decrease, but significance was not obtained (p=0.23).
As was with ΔP_{Smax} and ΔP_{Smean} values, the R_{SYS} values, observed during the seven-day period, remained constant (Friedman ANOVA, n=10, p=0.07).

Figure 16: Average resistance values (TR, R_{SYS}) observed each day



*P value<0.05 using Wilcoxon Signed Rank Test to compare with day 0

Table 5: Average resistance (TR, R_{SYS}) values observed each day

Day No.	N (Subjects)	TR (Pa × s / mm^3) Median (1st & 3rd Quartile)	TR (Pa × s / mm^3) GeoMean (geoSD)	R_{SYS} (Pa × s / mm^3) Median (1st & 3rd Quartile)	R_{SYS} (Pa × s / mm^3) GeoMean (geoSD)
0	10	0.8 (0.3 – 3.2)	1.1 (4.0)	1.0 (0.9 – 1.0)	1.0 (1.1)
1	10	0.4 (0.2 – 1.1)	0.5 (4.7)	1.0 (0.9 – 1.0)	1.0 (1.2)
2	10	1.2 (0.5 – 2.7)	1.3 (2.8)	1.0 (0.9 – 1.1)	1.0 (1.2)
3	10	2.0 (1.2 – 3.2)	1.3 (4.5)	1.0 (1.0 – 1.1)	1.0 (1.1)
4	10	2.8 (1.9 – 4.4)	2.5 (2.1)	1.0 (0.9 – 1.1)	1.0 (1.2)
5	10	4.3 (2.8 – 9.4)	3.6 (3.7)	1.0 (0.9 – 1.0)	1.0 (1.1)
6	10	3.6 (1.5 – 9.4)	3.7 (4.3)	1.0 (1.0 – 1.1)	1.0 (1.2)
7	9	5.9 (3.5 – 17.8)	7.6 (3.5)	1.0 (0.9 – 1.0)	1.0 (1.1)
8	8	11.7 (5.8 – 33.5)	13.7 (3.8)	1.0 (1.0 – 1.0)	1.0 (1.1)
9	7	17.3 (7.9 – 19.2)	13.0 (2.5)	1.0 (0.9 – 1.0)	1.0 (1.1)
10	7	15.7 (8.7 – 28.6)	14.3 (3.1)	1.0 (0.9 – 1.0)	1.0 (1.2)
11	7	13.4 (7.0 – 34.8)	15.5 (3.1)	1.0 (0.9 – 1.0)	1.0 (1.2)
12	3	31.0 (9.8 – 35.8)	22.1 (2.0)	1.0 (0.9 – 1.3)	1.1 (1.2)
13	2	54.6 (30.5 – 78.2)	49.0 (2.0)	1.2 (1.0 – 1.4)	1.2 (1.3)
P-value[#]		<0.01[#]		0.07[#]	

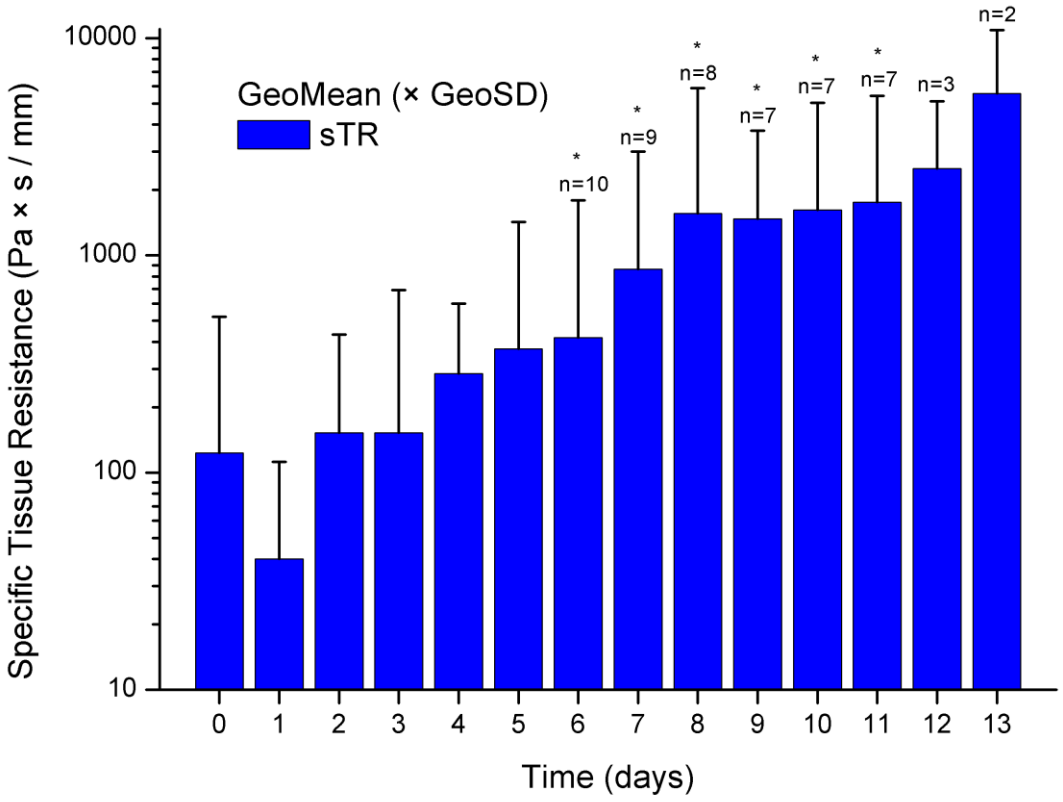
[#]Comparison with Friedman's test
P-values for the data range from day 1 to day 7
(day 7 was the last day with all 10 subjects enrolled)

3.3.5 Specific Tissue Resistance (sTR) Values

Average values of the specific tissue resistance (sTR) measured at the infusion site are shown in Figure 17 and given in Table 6.

As was the case with the parameters P_{max} and TR, the average sTR values increased over the catheter wear-time of seven days ($p<0.01$). A day-to-day comparison, comparing day 0 with every other day (i.e., days with $n \geq 5$), showed that the pressure values observed from day six onward were significantly higher than those values observed on day 0. The average sTR values obtained on day one showed a tendency to decrease, but significance was not obtained (Wilcoxon Signed Rank test, n=10, p=0.06).

Figure 17: Average sTR values obtained each day



*P value<0.05 using Wilcoxon Signed Rank test to compare with day 0.

Table 6: Average specific tissue resistance (sTR) values observed each day

Day No.		sTR (Pa × s / mm)	
	N (Subjects)	Median (1st & 3rd Quartile)	GeoMean (geoSD)
0	10	94.2 (51.4 – 367.1)	123.1 (4.2)
1	10	49.1 (23.1 – 84.9)	40.0 (2.8)
2	10	135.3 (61.6 – 307.6)	151.9 (2.8)
3	10	227.3 (134.9 – 365.1)	152.1 (4.5)
4	10	318.5 (210.5 – 500.9)	285.1 (2.1)
5	10	431.2 (187.4 – 1062.2)	371.8 (3.8)
6	10	404.3 (164.9 – 1061.0)	417.6 (4.3)
7	9	674.0 (394.0 – 2016.0)	863.9 (3.5)
8	8	1321.1 (658.4 – 3798.6)	1556.0 (3.8)
9	7	1966.0 (899.2 – 2172.9)	1472.8 (2.5)
10	7	1783.8 (981.2 – 3244.7)	1618.5 (3.1)
11	7	1523.2 (788.5 – 3948.8)	1752.3 (3.1)
12	3	3512.2 (1105.9 – 4065.0)	2508.7 (2.0)
13	2	6190.1 (3453.4 – 8926.9)	5552.3 (2.0)
P-value#		<0.01#	

#Comparison with Friedman's test
P-values for the data range from day 1 to day 7 (day 7 was the last day with all 10 subjects enrolled)

3.3.6 Pressure (P_{max}, ΔP_{Smax}, P_{mean}, ΔP_{Smean}) and Resistance (TR, R_{SYS}, sTR) Values Observed at the First and Last Study Day

Average pressure (P_{max}, ΔP_{Smax}) values observed at the first and last day of catheter wear (days of the OGTTs) are shown in Figure 18 and given in Table 7. A comparison between the P_{max} values showed a significant change between first and last day (Wilcoxon Signed Rank test, $p<0.01$). As can be seen, average P_{max} values were significantly higher on the last day than those obtained on the first day (Wilcoxon Signed Rank test, $p<0.01$). Furthermore, average ΔP_{Smax} values did not change.

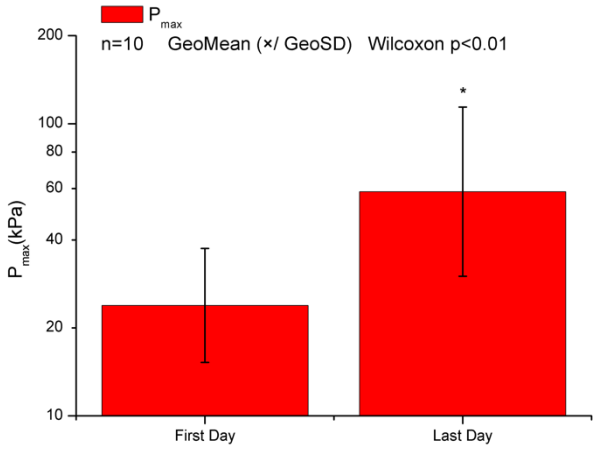
Average pressure (P_{mean}, ΔP_{Smean}) values at the first and last day of catheter wear are shown in Figure 19 and given in Table 7. Average P_{mean} values showed a tendency to increase. However this increase was not significant (Wilcoxon Signed Rank test, $p=0.32$). Furthermore, average ΔP_{Smean} values did not change (Wilcoxon Signed Rank test, $p=0.7$).

Average resistance (TR, R_{SYS}) values at the first and last day of catheter wear are shown in Figure 20 and given in Table 7.

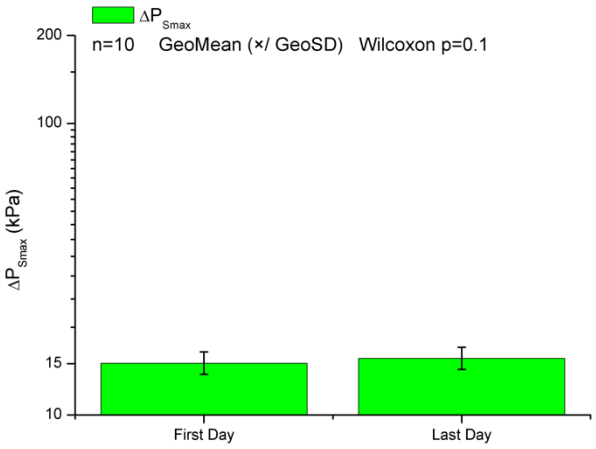
A comparison between the TR values showed a significant increase between first and last day (Wilcoxon Signed Rank test, $p<0.01$). As can be seen, R_{SYS} values did not change ($p=0.16$).

Figure 18: Average pressure (P_{max}, ΔP_{Smax}) values observed at the first and last study day

A



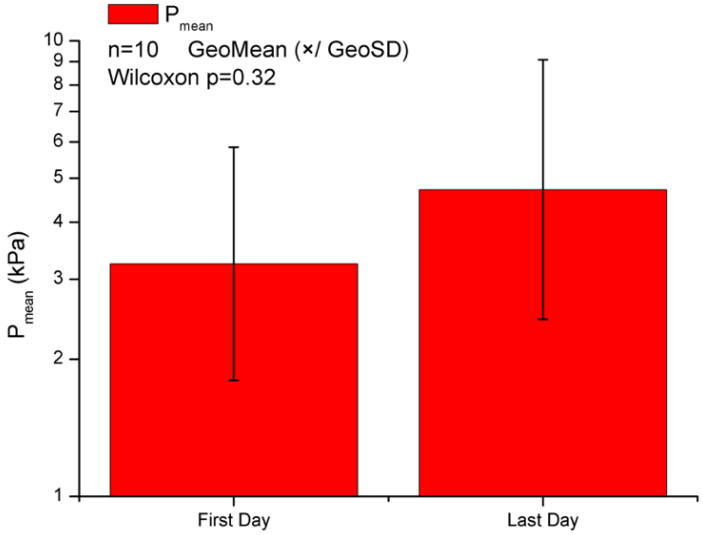
B



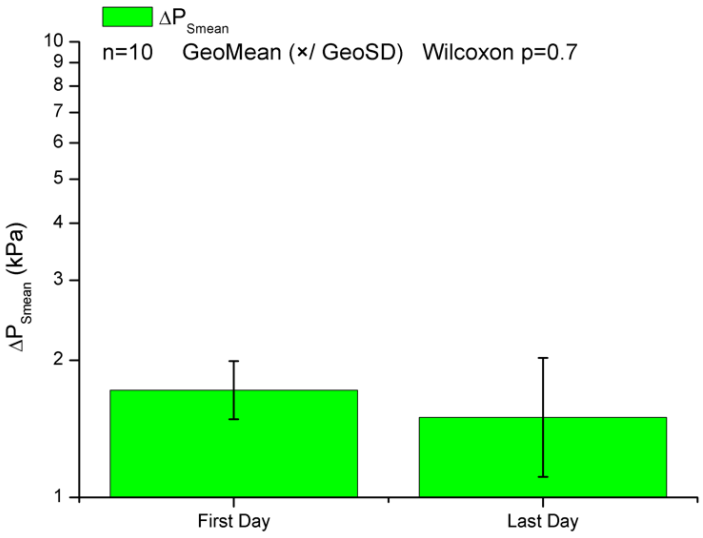
*P value<0.05 using Wilcoxon Signed Rank Test to compare with day 0

Figure 19: Average pressure (P_{mean}, ΔP_{Smean}) values observed at the first and last study days

A



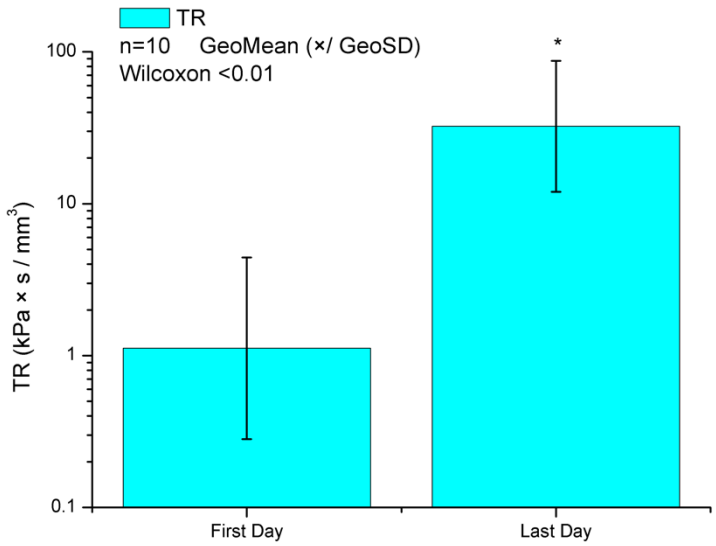
B



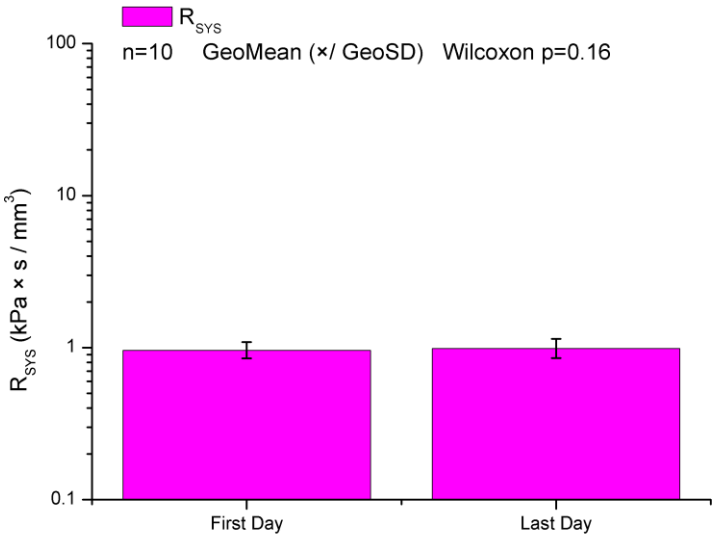
*P value<0.05 using Wilcoxon Signed Rank Test to compare with day 0

Figure 20: Average resistance (TR, R_{SYS}) values observed at the first and the last study day

A



B



*P value<0.05 using Wilcoxon Signed Rank Test to compare with day 0

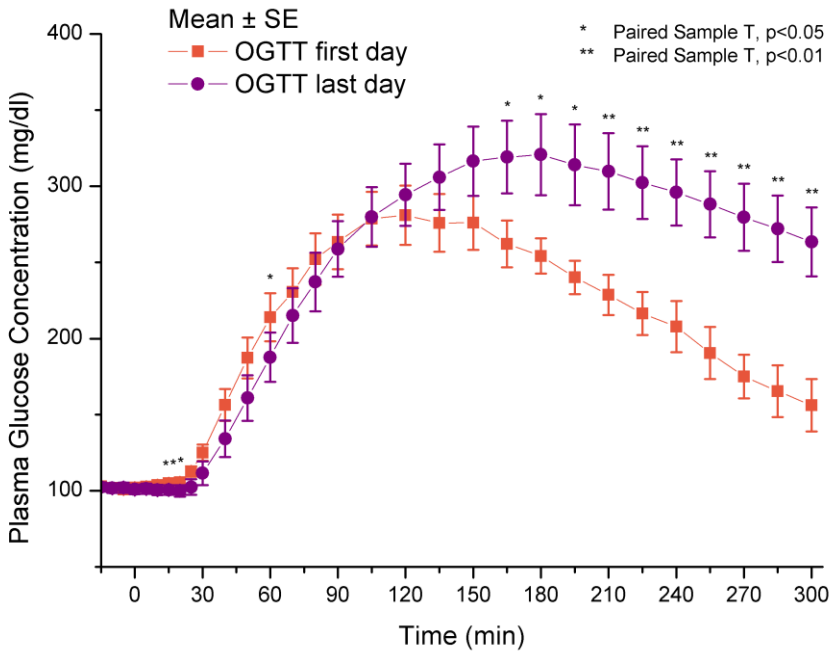
Table 7: Average pressure (P_{max}, ΔP_{Smax}, P_{mean}, ΔP_{Smean}) and resistance (R_{SYS}, TR, and sTR) values observed at the first and last study days

Parameter	Median (1st & 3rd Quartile) and GeoMean (GeoSD)				P-value†
	First Day		Last Day		
P_{max} (kPa)	19.4 (18.6 – 25.7)	23.9 (1.6)	46.9 (35.9 – 116.2)	58.5 (1.9)	<0.01
ΔP_{Smax} (kPa)	15.1 (14.6 – 15.6)	15.1 (1.1)	15.5 (14.5 – 16.4)	15.6 (1.1)	0.11
P_{mean} (kPa)	2.7 (2.4 – 3.5)	3.2 (1.8)	4.0 (2.7 – 5.6)	4.7 (1.9)	0.32
ΔP_{Smean} (kPa)	1.7 (1.5 – 2.0)	1.7 (1.2)	1.6 (1.5 – 1.7)	1.5 (1.4)	0.70
sTR (Pa×s/mm)	94.2 (51.4 – 367.1)	123.1 (4.2)	3482.8 (2016.0 – 8926.9)	3669.2 (2.7)	<0.01
TR (Pa×s/mm ^{3})	0.8 (0.3 – 3.2)	1.1 (4.0)	30.7 (17.8 – 78.7)	32.4 (2.7)	<0.01
R_{Sys} (Pa×s/mm ^{3})	1.0 (0.9 – 1.0)	1.0 (1.1)	1.0 (0.9 – 1.0)	1.0 (1.2)	0.16

3.4 Plasma Glucose (G_{mean}, ΔG_{mean}) During the OGTTs at the First and the Last Study Day

During the study, blood glucose concentrations observed during the OGTTs were recorded in a study CRF for each subject. From the recorded glucose concentrations, the study parameters were calculated as described in chapter 2.5.1. Average time courses of the blood glucose concentrations for the OGTTs performed on the first and the last study day are plotted in Figure 21 and given in Table 9. As can be seen, no significant difference between both OGTT was found during the first 150 minutes of the OGTT. However, from time point 165 minutes onward, average glucose concentrations observed during the OGTT performed on the first day of catheter wear were significantly lower than those observed during the OGTT on the last day of catheter wear. This observed temporal pattern of change in plasma glucose was also reflected in the derived study parameters, where the average ΔG_{mean} and G_{mean} (Table 8) obtained for the OGTT at the first day were significantly lower than those obtained for the OGTT at the last day of catheter wear.

Figure 21: Average plasma glucose concentrations observed during the first and the last study day



Average time courses (n=10, mean ± SE) of the plasma glucose concentrations from the OGTTs performed on the first and the last study day.

Table 8: Plasma glucose during the OGTT

Parameter	Mean (SE)		P-value†
	OGTT first day	OGTT last day	
G_{mean} (mg/dl)	215.1 (7.0)	256.4 (16.7)	< 0.05
ΔG_{mean} (mg/dl)	112.9 (5.9)	154.6 (54.7)	< 0.05

Data are means ± SD (range).

Table 9: Average plasma glucose concentration observed during the first and the last study day

	Plasma Glucose Concentration (mg/dl)				
	1. OGTT	2. OGTT		1. OGTT	2. OGTT
Time (min)	Mean (± SE)	Mean (± SE)	Time (min)	Mean (± SE)	Mean (± SE)
-15	103.0 (2.5)	102.5 (3.0)	90	263.5 (17.9)	258.9 (18.2)
-10	101.8 (2.0)	101.8 (3.2)	105	278.8 (17.6)	279.9 (19.6)
-5	100.8 (1.8)	102.0 (3.3)	120	281.0 (19.5)	294.4 (20.4)
0	102.1 (1.9)	101.0 (3.2)	135	276.0 (18.9)	306.0 (21.5)
5	102.6 (2.2)	101.3 (3.4)	150	276.1 (17.7)	316.6 (22.7)
10	103.7 (2.2)	100.5 (3.4)	165*	262.2 (15.4)	319.2 (23.9)
15*	104.9 (2.3)	100.6 (3.2)	180*	254.2 (11.6)	320.8 (26.7)
20*	105.7 (2.6)	100.0 (3.8)	195*	240.2 (11.0)	314.1 (26.5)
25*	112.7 (3.4)	102.5 (5.1)	210**	228.8 (13.2)	309.8 (25.2)
30	125.1 (5.3)	111.6 (7.8)	225**	216.5 (14.2)	302.5 (23.9)
40	156.4 (10.4)	134.1 (12.0)	240**	207.9 (16.9)	296.0 (21.7)
50	187.3 (13.5)	161.0 (15.0)	255**	190.5 (17.2)	288.2 (21.6)
60*	214.0 (15.7)	187.7 (16.3)	270**	175.1 (14.3)	279.6 (22.0)
70	230.6 (15.5)	215.2 (17.9)	285**	165.4 (17.0)	272.1 (21.8)
80	252.2 (16.9)	237.2 (19.3)	300**	156.1 (17.2)	263.5 (22.6)
P-value[#]				* p<0.05	** p<0.01

[#]Comparison with Paired Sample T-test

4 Discussion

The primary goal of the study was to monitor the hydraulic tissue resistance at the site of a subcutaneous insulin infusion over a prolonged catheter wear-time and to determine and compare the insulin absorption at the first and the last day of catheter wear in ten subjects with T1DM who were treated with insulin pump therapy. On each study day, hydraulic tissue resistance was determined by administering a pre-determined bolus of insulin diluting solution. In order to assess the insulin absorption, an OGTT was performed on the first and last study day.

We found that the infusion pressure and tissue resistance values increased significantly during the catheter wear (average wear-time 10.4 days). On the last study day, the TR was approximately 30-fold higher than on the first day of catheter wear. Furthermore, the average increase in the plasma glucose concentration during the OGTT on the last day was higher than that during the OGTT at the first day of catheter wear ($p<0.05$), thus indicating a significant reduction in the insulin absorption at the end of catheter use.

Therefore, these data suggest that the observed increase in the TR at the end of the catheter wear may be associated with a decrease in the efficiency of insulin absorption from the infusion site.

4.1 Log_{10} Normality Distribution of the Pressure and Resistance Data

In the present study, the average specific tissue resistance (sTR) obtained at the insulin infusion site initially decreased from day 0 to day 1, and then progressively increased over the wear-time (Figure 17). This increase in the hydraulic tissue resistance at the infusion site induced a strong increase in the maximum and mean hydraulic pressure (P_{max}, P_{mean}) in the used infusion set.

Statistical analysis indicated that the obtained tissue resistance and hydraulic pressure data may be log_{10}-normally distributed (Figure 13) and may, therefore, be adequately described by using the geometric mean (GeoMean) and geometric standard deviation (GeoSD). In Tables Table 3Table 7, the observed GeoMean and GeoSD values are given alongside with the Median, the first and the third Quartile for the parameters P_{max}, P_{mean}, TR, R_{SYS}, and sTR.

The pressure time courses are mainly influenced by the infusion set used, the flow rate applied and the tissue at the infusion site. By distinguishing R_{SYS} from TR, by their determination and normalizing both to a standard length and diameter, infusion pressure can be predicted for any infusion set. The information on the distribution of the resistance and pressure values may be very useful in designing future insulin pumps. For example, after seven days of infusion site use, P_{max} increased to a GeoMean value of 31.2, with a corresponding GeoSD value of 1.6. By taking these values, it can be estimated that when applying an Animas Vibe insulin pump and using the infusion site for seven days, 67% and 95% of the arising P_{max} values will be smaller than 49.9 and 97,8 kPa, respectively.

4.2 Effects of the Catheter Wear-Time on the Infusion Pressure and the Tissue Resistance

We monitored the pressure during bolus delivery in ten patients over an average wear-time of 10.4 days. The catheters were used by the patients to administer insulin as required for their regular treatment throughout the study. We found decreasing pressure and TR values during the first 24 to 48 hours of catheter wear. As the wear-time continued, these parameters increased steadily. Towards the end of catheter wear, TR values increased 30-fold, maximum pressure values increased 2.45-fold. As system pressure and resistance remained largely unchanged, the results suggest a change of the hydraulic properties in the tissue at the site of a continuous subcutaneous insulin infusion.

To the author's knowledge, changes in infusion pressure have only been monitored in a few studies over a short catheter wear-time (2 to 4 days).

Patte et al. examined tissue resistance pressure during a saline infusion on four consecutive days and found no significant changes in tissue resistance over the catheter wear-time (77).

Another group, Højbjerre et al. (61), infused saline as well and monitored infusion pressure over 48 hours. They observed a small (1.46-fold; from 4.87 to 7.12 kPa), but significant increase in maximum infusion pressure after 48 hours catheter wear. In contrast to the increased infusion pressure values found by Højbjerre et al., we

found infusion pressure values decreasing over the same period of 48 hours catheter wear.

A possible explanation for the disparity between our results and those of Højbjerre (58; pressure increase) and Patte (73; no change) is likely to be related to the different infusion solutions (saline vs. insulin solution) and infusion profiles (constant basal rate vs. basal and bolus insulin administration) used during the catheter wear. Our study was not designed to determine the cause of the changes at the infusion site. However, it is tempting to speculate that mechanisms of wound repair following the tissue damage due to the catheter insertion as well as fibrillation of insulin may play a role in these changes. Following an injury, e.g., catheter insertion, wound healing is a four-step process, comprising of (1) immediate coagulation and inflammation, (2) clot digestion and wound contraction, (3) fibroplasia, matrix formation and remodeling and (4) angiogenesis and epithelialization (60). Especially during the first three phases the aggregation of cells, e.g., macrophages and fibroblasts, and proteins in the tissue surrounding the cannula, may result in higher tissue density (58–60). Thus, it is conceivable, that after catheter insertion these changes result in the increase of TR which was observed from day four of catheter wear onwards.

In addition to wound healing processes, the aggregation of insulin molecules, i.e., fibrillation, in the extracellular space surrounding the cannula may further hinder fluid transport (78–80). Thus, during prolonged use of the infusion catheter, size and number of insulin aggregates may increase in the vicinity of the catheter and thereby induce strongly increased TR.

Further investigation of the effects of different infusion solutions on infusion pressure and tissue resistance is required.

4.3 The Possibility to Detect Catheter Malfunction by the Monitoring of Infusion Pressure Time Courses

Catheter malfunction, e.g., leakage or insertion failure, may result in reduced glycemic control and possible complications, e.g., ketoacidosis. Thus, early detection of catheter malfunction and replacement of the infusion catheter would improve the safety and glycemic control of patients treated with CSII. Our proposed

method for measuring infusion pressure and determining tissue as well as system resistance could be used to detect patterns during insulin delivery, which are characteristic for specific types of catheter malfunction.

For example, in one lean subject, very high hydraulic pressure values were measured during bolus delivery via a newly inserted catheter. The most likely reason for the elevated hydraulic pressure during bolus delivery via this cannula may have been that the tip of the cannula passed right through the subcutaneous layer and reached a more compact tissue layer, like muscle fascia. Following catheter replacement with a shorter infusion cannula, pressure values during bolus delivery returned to average levels.

In three cases a decrease in infusion pressure was observed from one bolus to another. In these three cases (subjects 10, 13 and 14) visual inspection of the infusion site showed leakage at the infusion site. The loosening of the catheter may have been the cause of the leakage, possibly due to backflow from the cannula tip along the cannula shaft towards the skin surface. Following the re-securing of the catheter with a liquid tissue adhesive as well as additional adhesive tape, infusion pressure returned to values similar to those before the leakage and decrease in infusion pressure. No further leakage was detectable at the re-secured infusion sites.

Thus, our proposed method of monitoring the infusion pressure could be implemented into an insulin pump. Characteristic changes in bolus infusion pressure could then be used to detect possible catheter malfunction, e.g., leakage or insertion failure. An additionally implemented algorithm could then notify the patient immediately that imminent action is required. Thus, possibly dangerous situations could be averted.

4.4 The Inverse Relationship between Tissue Resistance and Insulin Absorption

The results of the present study suggest an association between increased (30-fold) tissue resistance and decreased insulin absorption. This study was not designed to determine the cause of changes in the insulin absorption. However, one may speculate that a possible reason for the reduced insulin absorption observed at the

end of the catheter wear may be increased action of proteolytic enzymes in the subcutaneous tissue surrounding the catheter (83). Another reason for the reduced insulin absorption at the end of catheter wear may be the reduced bioavailability of subcutaneously administered insulin due to fibrillation of insulin (79). Further studies are necessary to identify the cause of the reduced insulin absorption at the end of prolonged catheter wear.

We found reduced TR values during the first 24 to 48 hours of catheter wear. However, we did not assess insulin absorption at these time points. Insulin absorption after these 2 to 3 days of catheter wear has been investigated in other studies. Luijf et al. (73), Clausen et al. (63), Swan et al. (72) as well as Liu et al. (74) found increased insulin absorption rates after two to three days of catheter wear-time but did not determine TR. Thus, taken together, decreased TR may be associated with increased insulin absorption.

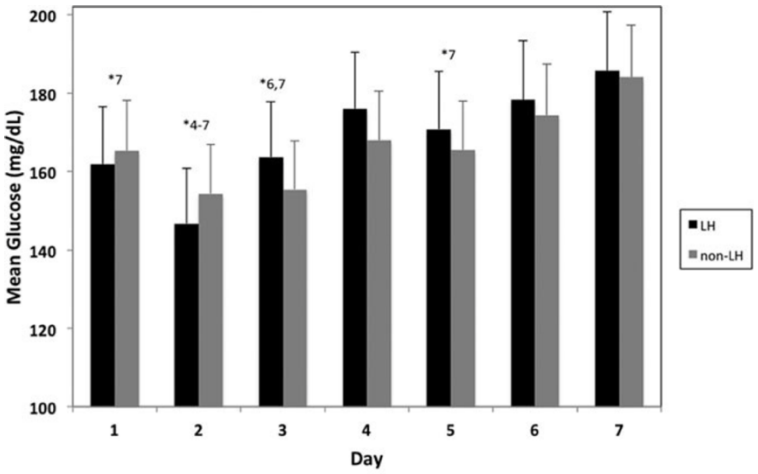
In a further study, Karlin et al. investigated the effect of catheter wear-time on the number of catheter failure in 20 patients with T1DM over a seven-day period. In addition, patients wore a CGM system during this seven-day period. They found a decrease in mean daily glucose after 24 to 48 hours of catheter wear. Thereafter, mean daily glucose increased steadily as the infusion site use continued (75).

It is conceivable that a change in mean glucose concentration may be the result of changed insulin absorption (increased glucose values reflecting reduced insulin absorption and vice versa). We extracted the mean glucose readings of the CGM from their publication (Figure 22 A) and correlated them with our log10-transformed sTR values (Figure 22 B). A high correlation coefficient (r value) was found (r=0.89), indicating a strong inverse correlation between tissue resistance and glucose concentration, see Figure 22 C.

Overall, these results strongly suggest, that the rate of insulin absorption may be inversely dependent on TR. Further studies are necessary to investigate the association between TR and insulin absorption. If this inverse relationship between TR and extent of insulin absorption is confirmed in further investigations, TR could be used as an indicator of the efficiency of insulin absorption and applied in future insulin pumps for the determination of the optimal wear-time of an infusion catheter.

Figure 22: Comparison of the average time courses of sTR and CGM-measured glucose concentration

A



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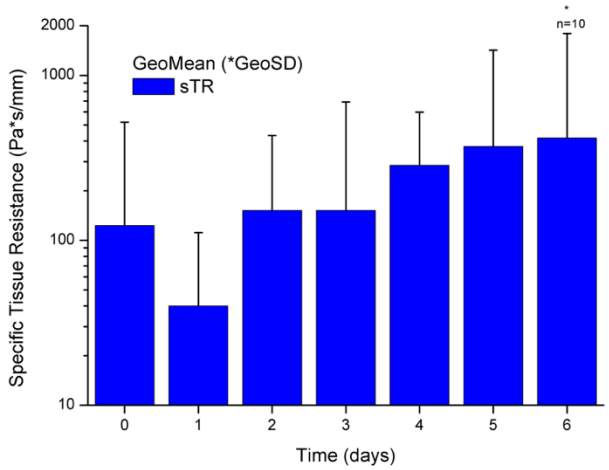
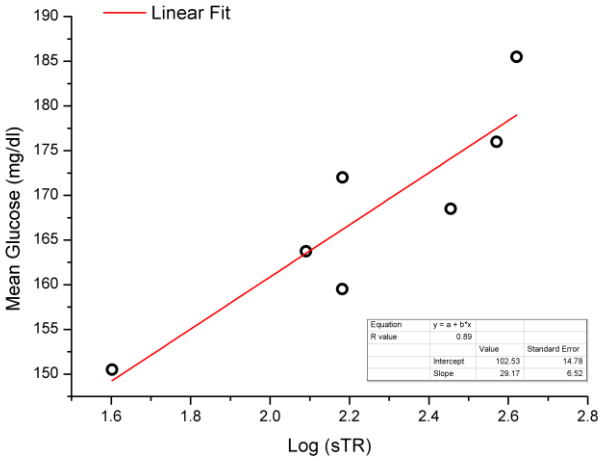


C



(A) Average CGM-measured glucose values during a seven-day catheter wear-time (75)*. (B) Average sTR values during a seven-day catheter wear-time. (C) A linear fit of CGM-measured glucose values, extracted from* (75) *and* Log_{10}*-transformed sTR values. A correlation coefficient (R-value) of 0.89 was found.*

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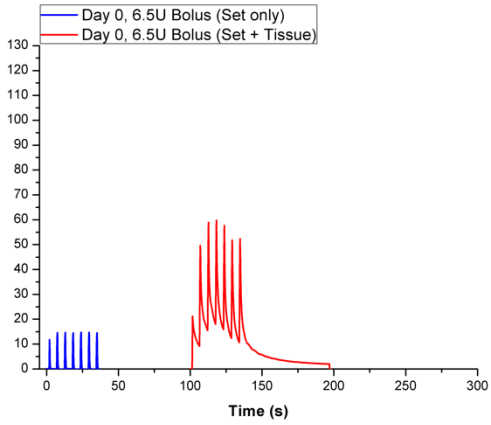
6 Appendix

6.1 Typical Pressure Time Course

Day 0



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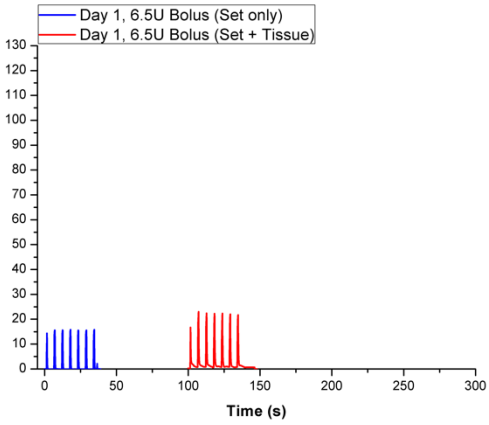

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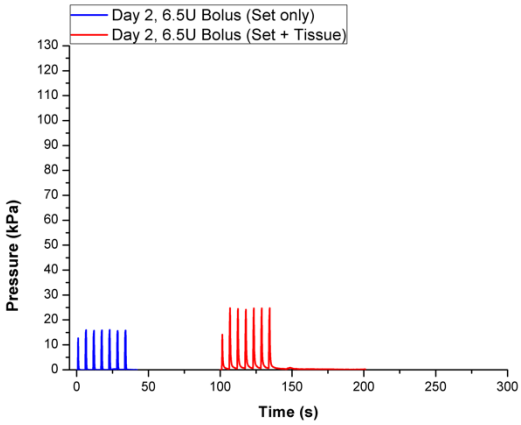

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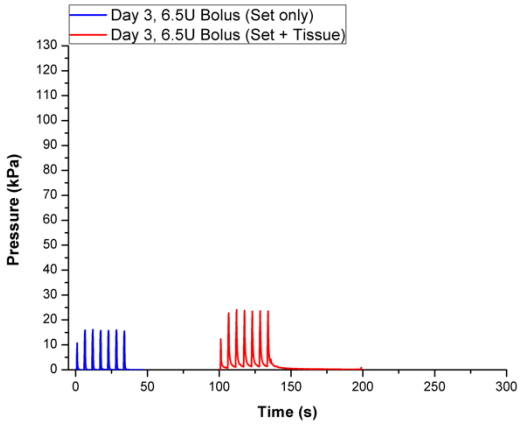
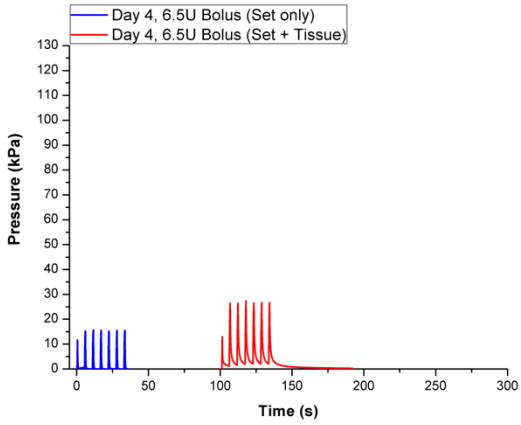
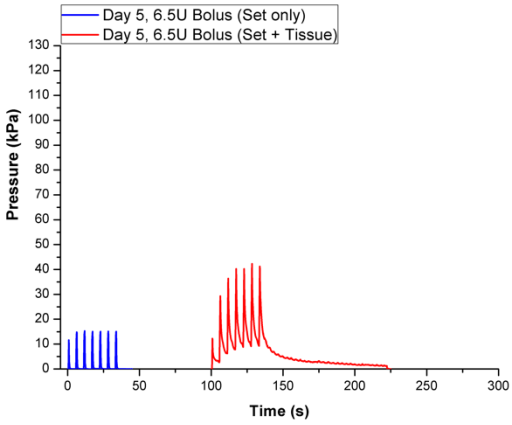


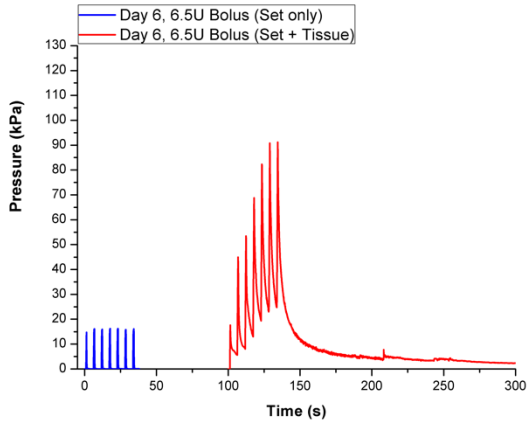
Day 4



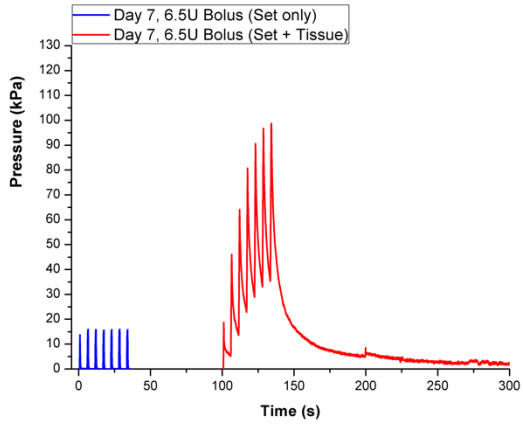
Day 5



Day 6



Day 7



Day 8