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NEW TECHNOLOGIES AND ADJUVANT TREATMENTS FOR THE MANAGEMENT OF TYPE 1 DIABETES

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STETEMENT OF ORIGINALITY

The works reported in this thesis were performed at the University Campus Bio-Medico, Rome, Italy.

The author designed the trials included in this thesis and analysed, described and discussed the results.

I hereby state that this thesis entitled **"New technologies and adjuvant treatments for the management of type 1 diabetes"** has not been submitted for a degree or other qualification at any others universities.

Anna Rita Maurizi

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ABSTRACT

Type 1 diabetes (T1D) is an immune-mediated chronic disease accounting for 5-10% of the total cases of diabetes and its incidence is growing worldwide. As a result of the autoimmune process, inducing the failure of insulin-producing cells, intensive insulin therapy represents the gold standard treatment to maintain blood glucose homeostasis.

In 1993, the effectiveness of intensive insulin therapy in T1D has been widely established in Diabetes Control and Complications Trial (DCCT). Moreover, it was affirmed that optimal glycaemic control is directly related to the reduction of incidence and progression of long-term diabetes complications.

Therefore, tight glycaemic control is overall recommended for T1D patients with near-normalization of blood glucose levels and an HbA1c <7.0% as the treatment goals. However, a great number of T1D patients have a suboptimal glycaemic control and 60% of people with T1D monitor less frequently than American Diabetes Association (ADA) recommendations of ≥3 tests daily.

On the other hand, benefits of intensive insulin treatment observed in the DCCT came with a three times higher risk of severe hypoglycaemia compared to the conventional therapy with one or two daily insulin injections and the fear of hypoglycaemia keeps many patients away from intensive treatment and achievement of recommended glycaemic goals. Furthermore, treatment goals should be achieved safely and

effectively by maintaining daily flexibility to fit individual lifestyle and without major variations in eating behaviours and physical activity promoting patients' compliance and their overall quality of life.

Therefore, to reduce these main limiting factors supporting long-term diabetes management the newer technologies and adjuvant treatments represent areas of investigation with important clinical applications.

The overall aim of my PhD was to find out if new technologies and adjuvant treatments may improve glycaemic control and patients compliance, reducing glucose variability in T1D patients.

This thesis includes two sections: the first one section is focused on the effectiveness of a bolus advisor calculator on glycaemic control in T1D patients undergoing intensive insulin therapy; the second section evaluates the hypothesis that D-Chiro-Inositol (DCI) plus Folic Acid oral supplementation can act as adjuvant treatments to insulin therapy in overweight or obese T1D patients, reducing insulin resistance.

1st section: in order to achieve an optimal glycaemic control in insulin-treated T1D patients the rationale of this part of the thesis has been the higher frequency of errors with empirical calculations of mealtime insulin doses. In fact, 42% diabetic patients administer an uncorrected dose of prandial insulin and the few studies carried-out on bolus calculator systems have been published with controversial results. At the same time, we tested effectiveness and feasibility of a wirelessly meter integrated with a bolus calculator working with a telemedicine system. Furthermore, we investigated the efficacy of this automated bolus calculator (ABC) and wirelessly

communicated blood glucose measurement on glucose variability, by the use of a continuous glucose monitoring system (CGMS).

The rationale is clearly established from the DCCT results suggesting that glycaemic variability may be an additional risk factor for the long- term diabetes complications and the new treatment strategies are increasingly focusing on reducing post-prandial glycaemic excursions. The results of this section have demonstrated that an ABC system is a friendly wirelessly meter that helps to improve glycaemic control and patients compliance to SMBG. Moreover, patients using an ABC showed a significant reduction of time spent in hypoglycaemic range compared to the control subjects.

2nd section: The rationale of this section of the thesis was based on the worldwide rise of obesity in childhood and adolescence with the consequent increase of insulin resistance also in young patients with T1D. In fact suboptimal glucose control is often due to the higher insulin doses causing an increased risk of hypoglycaemic events, weight gain and poor glycaemic control.

On this basis, the second part of this thesis was focused on a research project evaluating the hypothesis that D-Chiro-Inositol (DCI) plus Folic Acid oral supplementation may improve glucose control reducing insulin resistance in overweight or obese T1D patients. In fact, D-Chiro-Inositol (DCI), as putative mediator of intracellular insulin action can accelerate glucose disposal and act as insulin sensitizer. Moreover, folic acid administration seems to improve

glycaemic control reducing insulin resistance in other insulin resistant conditions.

Therefore, we carried-out a 24 weeks, prospective, randomized, control trial in 26 overweight or obese T1D patients, undergoing intensive insulin therapy.

The results of this trial demonstrated for the first time that the DCI plus Folic Acid oral supplementation can improve glycaemic control in overweight T1D patients, as showed by the significant reduction of HbA1c at the end of the study period.

The thesis is based on the following works:

1) *A novel insulin unit calculator for the management of type 1 diabetes*. **Maurizi AR**, Lauria A, Maggi D, Palermo A, Fioriti E, Manfrini S, Pozzilli P. Diabetes Technol Ther. 2011 Apr;13(4):425-8. doi: 10.1089/dia.2010.0190.
2) Abstract "*Use of an automated bolus calculator for the management of insulin therapy in Type 1 diabetes patients*" **Anna Rita Maurizi**, Anda Naciu, Rossella Del Toro, Angelo Lauria, Elvira Fioriti, Silvia Manfrini, Paolo Pozzilli, 76 th session: American Diabetes Association New Orleans, Louisiana 10-14 June 2016.
3) Abstract "*An automated bolus calculator for the control of glucose variability in Type 1 diabetes*" **Anna Rita Maurizi**, Ernesto Maddaloni, Rossella Del Toro, Silvia Pieralice, Anda Naciu, Angelo Lauria, Elvira Fioriti, Silvia Manfrini, Paolo

Pozzilli. 75 th session: American Diabetes Association San Diego, CA 9-13 June 2017.

4) *A pilot study of D-chiro-inositol plus folic acid in overweight patients with type 1 diabetes*. **Maurizi AR**, Menduni M, Del Toro R, Kyanvash S, Maggi D, Guglielmi C, Pantano AL, Defeudis G, Fioriti E, Manfrini S, Pozzilli P. Acta Diabetol. 2017 Apr;54(4):361-365. doi: 10.1007/s00592-016-0954-x.

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ABBREVIATIONS

T1D: Type 1 diabetes

DCI: D-Chiro-Inositol

ABC: Automated Bolus Calculator

SMBG: Self-Monitoring if Blood Glucose

CGMS: Continuous Glucose Monitoring System

IDDM: Insulin-Dependent Diabetes Mellitus

CSII: Continuous Subcutaneous Insulin Infusion

DKA: Diabetic Ketoacidosis

ICA: anti-insular antibodies

IAA: anti-insulin antibodies

GADA: glutamic acid anti-decarboxylase antibodies

IA-2: anti-tyrosine-phosphatase insulin antibodies

ADA: American Diabetes Association

MDI: Multiple Daily Injections

RT-CGM: Real-Time Continuous Glucose Monitoring

CHO: carbohydrate

PPG: postprandial glycaemia

FPG: Fasting Plasma Glucose

GV: glycaemic variability

ISF: Insulin Sensitivity Factor

IOB: Insulin On Board

IR: Insulin Requirement

BMI: Body Mass Index

mHealth app: mobile Health application

ROS: Reactive Oxygen Species

CVD: cardiovascular disease

MAGE: Mean Amplitude Glucose Excursions

MODD: Mean of daily differences

CONGA: Continuous overall net glycaemic action

BG: Blood Glucose

ADRR: Average daily risk range

LBGI: Low blood glucose index

HBGI: High blood glucose index

AUC PP: Post prandial Area under the curve

MARDs: Mean Absolute Relative Differences

FFAs: Free Fatty Acids

IMCLs: Intramyocellular Lipids

DD: Double Diabetes

eGDR: Glucose Disposal Rate estimation

FIRST SESSION

CHAPTER 1:

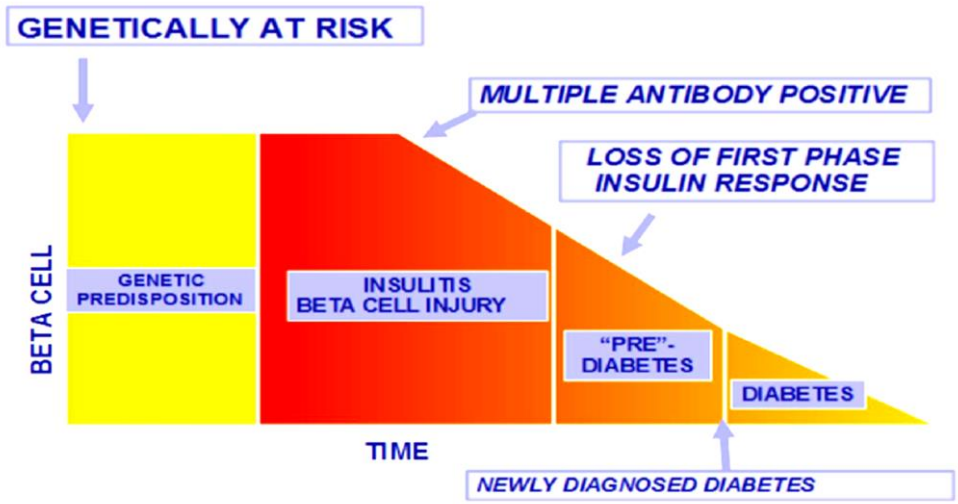
GENERAL BACKGROUND ON TYPE 1 DIABETES

1.1 Introduction

T1D is an immune-mediated chronic disease resulting of a progressive failure of pancreatic β-cells. At the onset of the disease, there is an increase of blood glucose levels due to insufficient insulin secretion, as a result of the loss of β-cells function. Specifically, at the beginning of the autoimmune process, glucose tolerance, depending on the damage of β-cells, is variably conserved.

With the progress of the reduction of β-cells function there is an early loss of natural insulin secretion, causing an impaired glucose tolerance or an impaired fasting glucose, with the appearance of a characteristic clinical symptoms [1] (**FIG. 1**).

FIG. 1 *Natural History of Type 1 Diabetes*



1.2 Epidemiology

T1D, also formerly named insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes, represents one of the most common chronic diseases in children and adolescents.

T1D has a prevalence of 0.2% and an incidence of approximately 10 new cases/100,000 in the age group less than 15 years. Generally, 50% of the new T1D cases are diagnosed ≤ 15 years, 25% between 15-20 years of age, while 15-20% patients are diagnosed over 30 years of age. T1D is rising by 3% every year and approximately 78,000 children under 15 years are estimated to develop T1D with a great worldwide difference.

The incidence rate of T1D has been widely evaluated and a large geographical variability has been found. China shows the lowest incidence rate (0.57/100,000 in T1D patients < 18 years) while countries as Finland or regions as Sardinia present an incidence almost 100 times higher (40-50/100,000 in patients < 18years) (**FIG. 2**).

The incidence rate of T1D in the white population of the USA is higher than those recorded for countries of Northern Europe but significantly lower than those in Sweden and Finland. The incidence of T1D in African Americans was lower than in white Americans with a higher rate among Hispanic compared with non-Hispanic white youth [2].

In our country, the number of people with diabetes is calculated to be around of 3.500,000 and T1D accounts for 350.000 of them [3].

In order to evaluate T1D heterogeneity and to coordinate the registered data on the incidence of the disease, the Registry for Type 1 Diabetes Mellitus in Italy (RIDI) was established in 1997.

The regional registries were pooled in three geographic macro-areas: north, central-south, and insular.

The highest incidence was observed in Sardinia, followed by the Northern and Central-Southern of Italy.

An increasing temporal trend in different areas was reported and the overall incidence during the 10-year study period increased by 3.6% and 3.7% per year in peninsular Italy and Sardinia, respectively [4, 5]

In 2010 the "CSII Diabetes Group in Lazio region, including the capital Rome, in a 6-Year Prospective Study confirmed an increase rate up to 15,68 new cases per 100,000 per year vs. 7,9 and 8,8 described during the 1989–1993 and 1990–1999 years, respectively [6, 7].

These heterogeneous epidemiological data support the role of genetic and environmental factors in the aetiology of the disease.

The growing incidence of T1D suggests a crucial involvement of environmental factors in the pathogenesis of the disease as demonstrated when people relocate from a region of low incidence to regions with higher incidence.

Similarly, it was found that also seasons have a role in the onset of T1D.

However, wide variations in incidence occur between neighbouring areas with similar latitude, suggesting the presence of other contributing risk factors and demonstrating the complexity of the disease' pathogenesis [8].

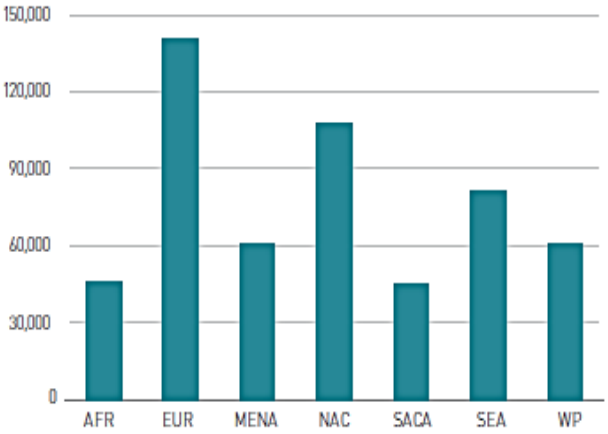
FIG. 2 *Epidemiologic data on T1D [2]*

Global estimates of type 1 diabetes in children (<15 years) for 2015

Child population (< 15 years)	1.9 billion
Type 1 diabetes in children (< 15 years)	
Number of children with type 1 diabetes	542,000
Number of new type 1 diabetes cases per year	86,000
Annual increase in incidence	3%*

* Estimate from the *Diabetes Mondiale study (DIAMOND)*[24], the *Europe and Diabetes study (EURODIAB)*[25].

Estimated number of children (< 15 years) with type 1 diabetes by IDF region, 2015



Top ten countries/territories for number of children with type 1 diabetes (< 15 years), 2015

Rank	Country/territory	Number of children with type 1 diabetes
1	United States of America	84,100
2	India	70,200
3	Brazil	30,900
4	China	30,500
5	United Kingdom	19,800
6	Russian Federation	18,500
7	Saudi Arabia	16,200
8	Germany	15,800
9	Nigeria	14,400
10	Mexico	13,500

Top ten countries/territories for number of new cases of type 1 diabetes (< 15 years) per 100,000 children per year, 2015

Rank	Country/territory	New cases per 100,000 population per year
1	Finland	62.3
2	Sweden	43.2
3	Kuwait	37.1
4	Norway	32.5
5	Saudi Arabia	31.4
6	United Kingdom	28.2
7	Ireland	26.8
8	Canada	25.9
9	Denmark	25.1
10	United States of America	23.7

1.3 Pathogenesis

T1D is a multifactorial chronic disease and different factors are involved in its aetiopathogenesis.

Genetic Factors:

It is well know that there is a genetic susceptibility in T1D, confirmed by the percentage of concordance in homozygous twins. For identical twins, if one twin is affected by T1D, the other one has about 30–50% concordance rate for the disease. The risk of a child to develop T1D is about 5% if the father has it, about 8% if a sibling has it, and about 3% if the mother is affected by the disease.

The genetic susceptibility is strongly related to the HLA system. 95% of individuals with T1D have an HLA DR3 and/or DR4 haplotype (Odds ratio: 7.5-8).

The relative risk for the disease is 7 times greater by DR3, 9 times greater by DR4 and more than 14 times greater if both are present.

However, in the general population, about 20% of subjects presents the haplotype DR3-DR4 but not all develop diabetes.

Therefore, other factors related to environmental agents and to the different age of the disease onset lead patients to the development of clinical T1D [9].

Environmental factors

- Viral infections: for a long time viruses have been suggested as potential environmental triggers for the disease.

However, the relationship between viral infections and the beginning of autoimmune process is not yet fully known.

The most important association between viral infections and T1D involves enterovirus families, of which some strains are able to promote the onset of the disease in the experimental models. Several hypothesis have been considered to explain the mechanism involved in the association between viruses and β-cells damage.

It seems that they can trigger autoimmune mechanisms through a "molecular mimicry" in which a viral epitope that mimics a self-agent may be able to accelerate T1D development.

Enteroviruses, particularly coxsackie virus B4, together with that of rubella, cytomegalovirus and rotavirus, are widely investigated in the studies on T1D aetiopathogenesis.

- Food Factors: Several studies have highlighted the contribution of cow's milk during weaning to the development of T1D.

It was demonstrated the role of β-casein in pathogenesis of the disease. Specifically, B-casein cows' milk protein differs from the human one by 60% of the amino acid sequence, displaying in position 62-66 a sequence of amino acids in common with GLUT-2.

On this basis, the early introduction of cow's milk (during the first three months of life), when the immune system is not yet mature, seems to induce an immune response to β-casein and a cross-reaction with the amino acid sequence of GLUT-2 expressed on β-cells.

Finally, other food factors, as nitrites and N-nitrose compounds are potentially involved in the aetiopathogenesis of the disease [10].

Immunological Factors

The lymphocytic infiltration of the pancreatic isles, called insulitis, is the basis of T1D pathogenesis.

The beta cells inflammation is due to the activation of autoimmune response, with an expansion of autoreactive cells of CD4 + T and CD8 + T cells, B cells producing autoantibodies.

Regard to the autoantibodies, it has been demonstrated that they can predict the progression to the clinical disease.

However, not all subjects with positive autoantibodies show clinical signs of the disease, but the risk for T1D development rises with the number of antibodies. Subjects with three or four antibodies show a risk of progression to T1D of 60–100%. Dosage of islet cell autoantibodies, insulin autoantibodies, autoantibodies targeting the 65-kDa isoform of glutamic acid decarboxylase (GAD), autoantibodies targeting the phosphatase-related IA-2 molecule, and zinc transporter autoantibodies (ZnT8) represents the main approach to identify subjects with genetic susceptibility to T1D.

Most recently, it has been found a novel antibody to post-translationally modified insulin (oxPTM-insulin (oxPTM-INS-Ab). Specifically, being oxPTM-INS-Ab present before the clinical onset of T1D, it could be considered a novel biomarker for prediction of T1D in children [11].

1.4 Clinical presentation

T1D clinical manifestation occurs generally when about 80% of β-cells are destroyed. The failure of beta cells mass leads to the appearance of characteristic clinical symptoms.

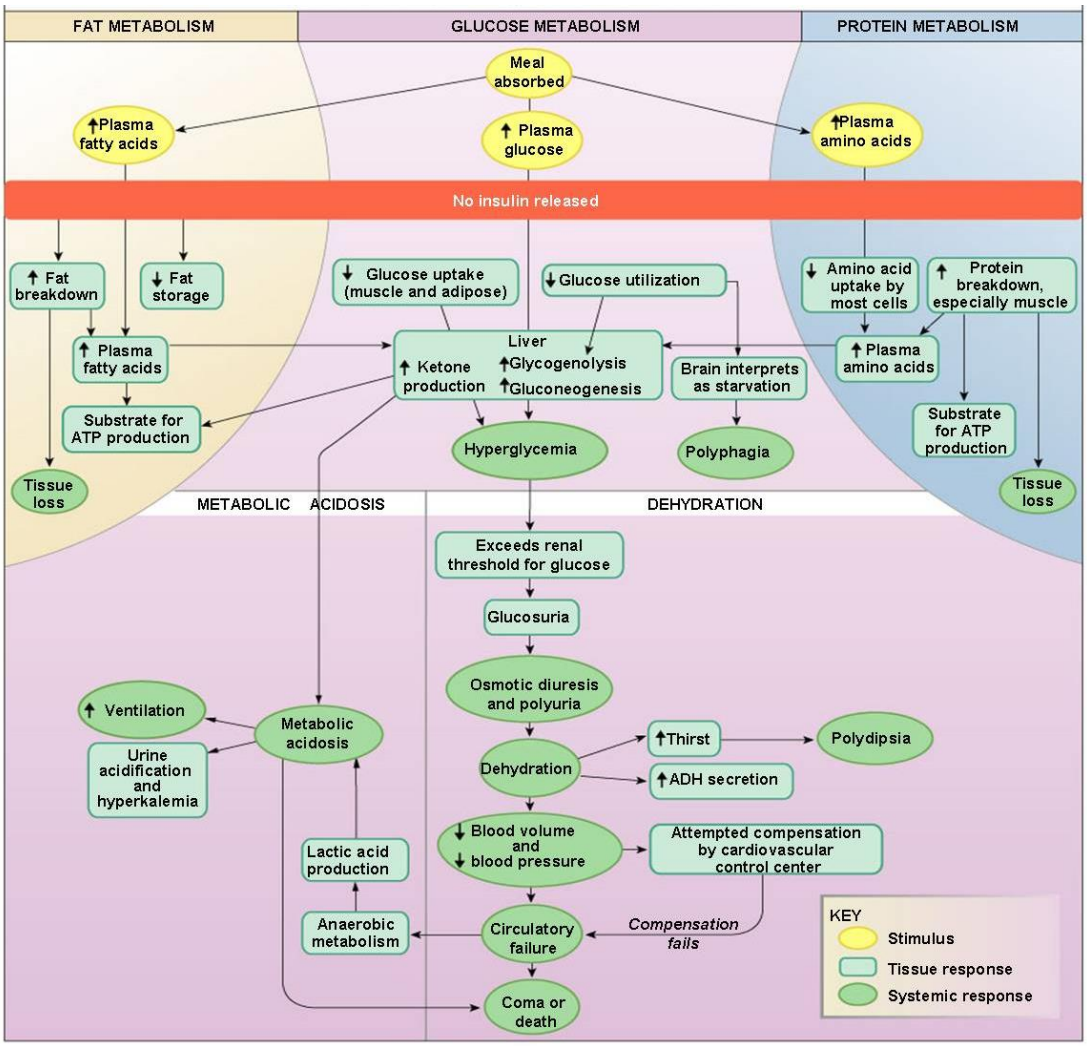
The specific clinical presentation of the disease is represented by classical triad symptoms: polyuria, polydipsia and polyphagia.

The symptoms may develop quite rapidly (weeks or months) in T1D, particularly in younger children. When the glucose concentration in the blood is increased beyond the renal threshold, reabsorption of glucose in the proximal renal tubuli is incomplete and part of the glucose remains in the urine (glycosuria).

This mechanism rises the osmotic pressure of the urine and inhibits the reabsorption of water by the kidney, resulting in increased urine production (polyuria) and enhanced fluid loss. Lost blood volume will be replaced osmotically from water held in body cells, causing dehydration and increased thirst.

T1D patients may also present with diabetic ketoacidosis (DKA), an extreme state of metabolic dysregulation characterized by the smell of acetone on the patient's breath; a rapid, deep breathing known as Kussmaul breathing; polyuria; nausea; vomiting and abdominal pain; and any of many altered states of consciousness or arousal. In severe DKA, coma may follow, progressing to death (**FIG. 3**)

FIG. 3 *Acute pathophysiology of T1D*



1.5 Diagnosis

For a timely diagnosis it is appropriate to consider specific clinical and laboratory parameters.

- Clinical Parameters:

Age, body weight (T1D patients are typically normal or underweight unlike T2D patients), genetic susceptibility or other risk factors.

- Laboratory parameters:

- Glycemia: T1D is characterized by fasting hyperglycaemia with a variable postprandial hyperglycaemia;
- HbA1c: The values are very variable, and depend on the evolution of the pathological process, a value > 7% is considered pathological;
- Chetonuria;
- C-peptide: it is a good marker to estimate the residual β-cells function because it is released into the bloodstream in equimolar quantities to the endogenous insulin.

To preserve β-cells function is useful for an earlier diagnosis the detection of the different types of autoantibodies:

- ICA (anti-insular antibodies)
- IAA (anti-insulin antibodies)
- GADA (glutamic acid anti-decarboxylase antibodies)
- IA-2 (anti-tyrosine-phosphatase insulin antibodies).

1.6 T1D management

Insulin therapy is the cornerstone for the management of T1D as β-cells failure progressively leads to endogenous insulin deficiency. Although insulin administration is crucial to maintain glucose homeostasis, its dosage is a thin balance: over-insulinization can lead to severe hypoglycaemia, on the other hand, the under-insulinization rises the risk of diabetic ketoacidosis and the exposure to chronic hyperglycaemia is directly related to the onset and progression of long-term diabetic complications.

The effectiveness of intensive insulin therapy, based on multiple daily insulin injections (MDI) or on subcutaneous insulin infusion (CSII), has been well established in the Diabetes Control and Complications Trial (DCCT), showing that optimal glycaemic control is able to delay the incidence and progression of long-term diabetes complications [12]. Moreover, it was found that intensive insulin treatment and strictly glycaemic control could also reduce the risk of cardiovascular disease [13].

On this basis, tight glycaemic control is overall recommended for T1D patients with near-normalization of blood glucose levels and an HbA1c <7.0% as treatment target. However, a great number of T1D patients have a suboptimal glycaemic control and 60% of people with T1D monitor less frequently than American Diabetes Association (ADA) recommendations of ≥3 blood glucose tests daily [14].

On the other hand, benefits of intensive insulin treatment showed in the DCCT came with a three times higher risk of severe hypoglycaemia compared to the conventional therapy with one or two daily insulin injections and the fear of hypoglycaemia keeps many patients away from intensive treatment and achievement of recommended glycaemic goals [15]. In order to achieve safely and effectively treatment goals, maintaining daily flexibility and promoting patients compliance, blood glucose monitoring is recommended as a core component of diabetic patient's management. Finally, the management of psychological issues of T1D patients represents a crucial aspect of the overall T1D care.

1.6.1 Insulin administration

As previously stated, intensive insulin therapy is administered by MDI or CSII.

1.6.1.1 MDI

To replace the physiological pancreatic insulin secretion, in MDI treatment are generally required 3 or 4 daily insulin injections; one of a basal insulin to control the hepatic nocturnal and diurnal gluconeogenesis and 3 or more of a rapid-acting insulin for each meal or snacks.

Currently, the most common method of insulin administration is represented by the use of a prandial insulin analogue at meal time (i.e. aspart, lispro, glulisine) and a long-acting basal analogue usually at bedtime (i.e. glargine, detemir or degludec).

Compare to the previous human insulins, the newer short and long-acting insulin analogues have demonstrated an improvement of overall glycaemic control with a reduction of hypoglycaemic events, mainly during the night-time [16].

1.6.1.2 Continuous Subcutaneous Insulin Infusion (CSII)

CSII is well established as a most physiological method of insulin administration currently available. Such as intensive insulin treatment, it offers the opportunity to achieve the tight glycaemic control without increasing the risk of hypoglycaemia and reducing the risk for long-term

diabetic complications in insulin treated diabetes patients [17, 18]. Even though the use of pumps is widely spread, the different diabetic populations with highest likelihood of benefit are still unclear. Moreover, despite several studies have showed better glycaemic control with CSII, its effectiveness on the other outcomes remains controversial. In this regard, there are many discrepancies between the impact on quality of life and the satisfaction reported by pumps users and the insufficient or unconvincing data on quality of life benefits reported in the clinical trials. [19] At present, the CSII management is certainly more challenging than the MDI and it requires knowledge and motivation both in patients and in the healthcare team.

The use of CSII in Italy

Over the years, as diabetes incidence increased, the CSII therapy became a more frequent treatment option for T1D management.

The last national survey on the CSII use published in Italy by Bruttomesso et al. showed that approximately over 10.000 patients were treated with insulin pumps at present [3].

The Italian legislation established that the National Health System refunds therapy with CSII recognizing this device as a valuable tool for the disease management, especially in selected patient groups (e.g. patients with poor glycaemic control or diabetic women during pregnancy). Recently, in order to ensure consistency and appropriateness of insulin pump use the "CSII in Diabetes Study Group" in Lazio region has drafted the "Lazio region Consensus for

CSII". In Lazio, as well as in all other Italian regions, the supply of insulin pumps and its related consumables are under the responsibility of the local Healthcare Institution. During an initial trial period the patient is provided with a specific model of CSII, chosen by the healthcare team. At the end of the trial period, if patient succeeds in its training, the prescribing centre makes the request for a free supply by the local Healthcare Institution. Finally, after the validation by the referring diabetologist, proposed by the local Healthcare Institution, the insulin pump and its related consumables are freely delivered to the patient. Considering the remarkable increase in the insulin pump use, we believe that the provision of specific indications on the clinical management of CSII in diabetic patients is highly recommended [6].

Diabetes management of CSII

In order to provide the knowledge and abilities required to insulin pump users and to support and encourage their self-management through the new technologies, we believe that the modern diabetes management requires a comprehensive approach by a multidisciplinary team of healthcare professionals. The multidisciplinary team provides, in turn, a proper training through a tailored educational program.

The educational process must provide:

a. Pre-pump education session: review of the basic skills and the education criteria before starting pump therapy: accurate carbohydrate counting in a correct diet regimen, insulin/carbohydrate ratio, insulin

correction factor, management of emergencies (e.g. hyper- o hypoglycaemic events).

1st session: Overview of operating modes of insulin pump and learning of the basic functions (general settings, priming and self-insertion of cannula, changing of infusion set, basal rate setting, basal temporary rate, suspension).

b. 2nd session: prandial bolus administration (rapid bolus, square-wave, dual-wave).

c. 3rd session: learning assessment. Management of hyper/hypoglycaemia. Starting insulin pump therapy.

d. 4th - 5th sessions: nutritional reinforcement sessions on carbohydrate counting and healthy eating habits. Interactive group workshops on carbohydrate counting and bolus administration could be introduced as a part of the educational program, including physical activity.

At the beginning of insulin pump therapy, an alternative treatment with multiple subcutaneous insulin injections, which should be used in case of device's failure, must be provided to all patients. In order to avoid possible ketoacidosis, patients must also be trained with behavioural recommendations to follow in case of unexplained hyperglycaemia (e.g. control of insulin infusion set and insertion site).

After the initiation of CSII therapy, the first visit is scheduled after approximately 3–5 days; the second after 10–15 days. Subsequent visits are scheduled every 45 days, with the acquisition of advanced functions (e.g. alternative patterns). After this stage of advanced training, follow-

up visits can be scheduled three to four times a year or according to the needs of each patient. Moreover, once pump therapy has been initiated, the patient's glycaemic records can be emailed weekly or communicated via telephone to the diabetes center, if necessary.

Failure of the educational process can affect both the outcome of CSII therapy and patient safety. Therefore, is important underscore the importance of periodic patient re-evaluation and assessment to determine whether patients need additional training on the technical and educational features of the therapy in order to maximize therapy success and maintain patient safety. To improve adherence to CSII therapy, a behavioural contract that provides patients with specific metabolic goals, including a commitment on the part of the patient to go to the diabetes center for follow-up visits, with the frequency of visits preestablished, and agreement from each patient to fulfil a detailed list of responsibilities is recommended.

Finally, to ensure proper adherence to CSII therapy, if the optimal glycaemic control is not achieved (e.g. HbA1c levels remain stable, >8.5%), discontinuation of the pump therapy can be evaluated [6]. In addition to this, to facilitate follow-up visits, healthcare teams should perform data management using online registries. Generally, the downloading process can be easily performed from the pumps and by the patients themselves. The software currently available gives the healthcare team a retrospective review of all of the uploaded data, which provide, through personalized reports, an overall assessment of glycaemic control. This would allow the healthcare team to adjust each

patient's insulin therapy regimen without the need to use a traditional logbook [20].

1.6.2 The three pillars in modern diabetes glucose monitoring

1.6.2.1 HbA1c

In the last 30 years, HbA1c has been considered the gold standard for assessing glycaemic control in diabetic subjects. Although HbA1c is a good indicator to the risk of developing long-term diabetic complications, as widely showed in observational and interventional studies, it has some limitations. Being an average, HbA1c measures long-term blood glucose exposure but it does not reflect the complexities of glycaemic control and it is unable to provide detailed information on intraday and interday blood glucose excursions, misleading in the optimal diabetes management. Therefore, patients with similar HbA1c levels may have quite different daily blood glucose profile and they can show wide blood glucose fluctuations with high glucose variability, or severe hypoglycaemic and hyperglycaemic events, despite a reasonable HbA1c value [21].

1.6.2.2 SMBG

A fundamental part of diabetes management is SMBG using blood glucose monitoring devices. The role of SMBG is widely appreciated and it is recommended as a fundamental part of diabetes management in

insulin-treated T1D and T2D patients and a desirable component in non-insulin-treated T2D patients [22].

To date, a great number of innovative systems for the SMBG, data download and transmission are available. The so-called "smart" glucometers are equipped with a bolus advisor calculator system that recommend the insulin dose to be administer at each meal taking into account blood glucose levels, insulin/carbohydrate ratio and the carbs intake. Moreover, the newest models are able to wirelessly share a retrospective analysis to the diabetes centre, via computer or smartphones.

Despite, the effectiveness of SMBG on glycaemic control in patients with T1D has been widely demonstrated during the DCCT trial, over 60% of T1D patients monitor less frequently than American Diabetes Association (ADA) recommendations of ≥3 blood glucose tests daily and less of 30% of T1D achieve glycaemic targets. Moreover, it has been widely highlighted the importance of frequency of SMBG that it was found to be directly associated with lower HbA1c levels [23].

Lastly, so that can be successful SMBG should be performed in a structured format to make timely and effective therapeutic choices [24].

Despite these evidences, SMBG has some limitations. In fact, to achieve glycaemic targets the optimization of glucose variability is crucial.

However, SMBG offers limited data on glycaemic excursions and few indications on glucose trends, providing only intermittent snapshots of blood glucose levels and often missing sustained hyperglycaemic and hypoglycaemic fluctuations. In addition to this disadvantage fingerstick

testing remains invasive, painful and time-consuming, thus limiting the frequency of measures per day [6].

1.6.2.3 CGM

Technology of CGM

CGM is a novel technology developed in the last 20 years and available in the market since 1999. It works with an invasive and non-invasive techniques both approved and used in the clinical practice. A typical system consists of a subcutaneous glucose sensor that measures glucose level of interstitial fluid osmotically diffused from the peri-capillary tissue, using enzymatic (glucose oxidase) or microdialysis technique. Each sensor placed under the skin for 6 or 14 days, can continuously measure glucose levels providing up to 288 glucose values in a 24-hour period, depending on the different models.

The limitation of this technique is that glucose level in interstitial fluid lag temporally behind blood glucose value, delaying up to 20 minutes when glycaemic value change rapidly. Moreover, the available systems require 2 or 4 calibration per day (depending on the device), based on capillary blood glucose testing. The CGM showed an absolute difference from 2 to 16% (25-30 mg/dL) compared to capillary blood glucose values.

The detection of hypoglycaemia (<70 mg/dL) showed a sensitivity of 60–70% and specificity of 90% and the detection of hyperglycaemia (>250 mg/dl) displayed a sensitivity and specificity of 63% and 97% respectively [25]. To date, the continuous monitoring can be done in

two ways, Blinded and Real-Time CGM (RT-CGM). The first one records the glucose levels, providing retrospectively information of the overall glycaemic profile, without a real-time display of glycaemic value. This kind of CGM is useful as a diagnostic tool in clinical practice. In fact it allows to evaluate the glycaemic profile in diabetic patients poorly controlled, detecting and preventing unrecognized hypoglycaemic events, mostly in the nocturnal period [26, 27] and to adjust insulin dosage, accordingly to physical activity and diet.

RT-CGM displays data on a monitor or pump screen, and shows the glucose values, the glucose trend graphs, with a waves indicating the direction and rate of change of glucose levels directly to the patient, thus representing an education tool for diabetic subjects.

The systems are equipped with alarms/alerts indicating the risk of hypo- and hyper-glycaemia when the glucose pre-set targets are reached.

Since false negative and false positive alarms are frequent, patients must to detect their glucose level also through the SMBG [28].

The newest generation of glucose monitoring is represented by a flash glucose monitoring system with a small sensor, placed at the back of the arm, providing glucose readings by scanning the sensor also through clothing by a handheld device.

Effectiveness of CGM

The effectiveness of CGM in the management of T1D patients is well known, as recommended in the clinical practice guidelines of the

Endocrinology Society [28]. In fact, CGM allows T1D patients to monitor their glucose levels without the need of repeated fingersticks testing. Moreover, CGM devices providing real-time readings to the wearer, allows for an immediate therapeutic choice, such as the administration of a supplemental dose of insulin. Several studies have demonstrated the improvement of glycaemic control in patients with T1D even when CGM is added to insulin pump therapy [29].

Although the CGM reliability still needs improvement as well as the user-friendliness of the devices and studies on the economic feasibility, CGM represents a reliable monitoring tool to minimize glycaemic variability and to achieve glycaemic targets in T1D without increasing the risk of hypoglycaemia.

Even if it is too early to reach a general conclusion on the specific clinical indications, the RT-CGM can be used as a powerful motivational device to change patients' lifestyle.

Furthermore, the short-term retrospective CGM use may be beneficial in certain clinical situations such as to detect nocturnal or unawareness hypoglycaemia and when significant therapeutic changes must be performed [28].

In fact, the availability of continuous glucose data for T1D patients with low and high glucose alerts may detect unrecognized hypoglycaemia and hyperglycaemic excursions, leading to therapeutic changes as well as a variation of diet and physical activity, modifying diabetes care outcomes and their associated economic costs [21].

CHAPTER 2

SPECIFIC BACKGROUND: Use of automated bolus calculators in T1D

2.1 Introduction

The achievement of treatment goals in T1D patients should be obtained by maintaining flexibility to fit individual lifestyle and without major variations in eating behaviours and physical activity. As well know, diet, physical activity and insulin dosage play a key role in the management of insulin treatment in T1D patients.

Diet, in particular carbohydrate (CHO) meal content, is the main determinant of a rise in postprandial glycaemia (PPG). On the other hand, the contribution of post-prandial excursions to glycaemic variability and on the overall glycaemic control is well known.

Therefore, to achieve post-prandial glycaemic targets the establishment of insulin dose at meal times must be made before each injection, while considering certain parameters, such as target range, insulin sensitivity, insulin/carbohydrate ratio, duration and intensity of post injection physical exercise and so on [30].

Accordingly, an appropriate teaching and training program on CHO counting and specific instructions for the administration of insulin doses need to be implemented for each diabetic patient.

In fact, CHO counting as a meal planning approach offers the flexibility of food choices and allows the achievement of post-prandial glycaemic goals [31, 32].

Although there are several evidences in support of carbohydrates counting effectiveness, empirical calculation remains not easy and time-consuming for most patients, thus reducing their ability to achieve an optimal glycaemic control [33].

In fact, as previously stated, less of 30% of T1D patients achieve the glycaemic target of HbA1c < 7.5% and most patients are not able to calculate correctly pre-meal insulin doses as well reported from several clinical studies [14].

In this regard, already in 2004 Glaser et al. demonstrated a higher frequency of errors with empirical calculations (53-67% incorrect calculations) than with insulin dosage calculation device (25-32% incorrect) [34].

Therefore, to encourage T1D patient's self-management also promoting pre meal insulin doses correct adjustments, in the last two decades many Automated Bolus Calculators (ABCs) are been developed [35].

The first devices are been built in insulin pumps while only in the last few years, since ABCs are integrated in different glucose meters, they have also become available for diabetic patients on MDI [36-38].

2.2 Principles of ABCs

As previously reported, SMBG is essential for T1D self-management, but in order to be effective it could be performed in structured format and, most importantly, each patients must be instructed to interpret different glycaemic patterns appropriately, making the correct therapeutic choices [24].

To facilitate the process, different software products are now available in the "smart" glucose meters; all of them work with a generic formula taking into account the current blood glucose level, target glucose level, CHO meals content (grams), insulin/CHO ratio, insulin sensitivity factor and insulin on board (IOB, the residual active insulin from the previous injection) (Table 1).

Table 1: Parameters of standard ABCs

Parameters evaluated	Parameters not evaluated
▪ Current glycaemic levels	▪ Glycaemic index of foods
▪ Target glycaemic levels	▪ Action of fats and proteins on glycaemic levels
▪ Content of CHOs	▪ Effect of gastric emptying
▪ I/CHO ratio	▪ Effect of insulin absorption
▪ ISF	▪ Health conditions that can impact on post prandial glucose levels
▪ IOB	

The I/C ratio, defined as the amount of CHO (gr) "covered" by 1 unit of insulin, is generally determined in patients using short-acting insulin analogues by the "500 rule": 500/Total Daily Insulin. The ISF, defined as the estimated drop in blood glucose (in mg/dL or mmol/L) expected from the administration of 1 unit of insulin, is calculated by the "1,800 rule": 1800/ Total Daily Insulin.

These parameters based on individual blood glucose measurements, should be periodically evaluated and personalized for each diabetic patients by health care providers (diabetologist/dietician). Finally,

some ABCs consider others parameters such as physical activity or others health conditions affecting blood glucose levels (i.e. fever, menses) and overall data are recorded on an electronic logbook [39].

The standard ABCs work with the following formula [40]:

Insulin dose (IU):

$$\frac{\text{Current glucose levels - Target glucose levels}}{\text{ISF}} + \frac{\text{CHO meals content}}{\text{CHO}} - \text{IOB}$$

2.3 Limits of current ABCs available

As reported in table 1, the ABCs currently available have some limitations.

Therefore, to facilitate the achievement of successful outcomes on glycaemic control, quality of life, patients' compliance and satisfaction, they must have numerous skills and knowledge.

First of all, to promote the proper therapeutic decisions process, patients using an ABC must be confident on SMBG and able to make CHO counting estimation accurately [41]. Moreover, an optimal use of ABC system requires an accurate evaluation of I/C ratio and ISF and a proper knowledge about the glycaemic index and on glycaemic load of the others macronutrients contained in the foods, such as fats and proteins [42-45].

These parameters remain laborious and very difficult to evaluate for both patients and health care providers, thus the newer bolus calculators can aid subjects with poorly numeracy skills.

Nevertheless, the modern ABCs systems do not consider the contribution on the glucose levels derived from fats and proteins intake, which affect postprandial glycaemia, reducing glucose hepatic release, delaying gastric emptying and increasing peripheral insulin resistance [42, 45, 46].

Furthermore, regard to the effect on glycaemic control by dietary fats or proteins the clinical evidences and the ADA recommendation showed that these two dietary compounds should be considered when an insulin bolus is administered [47].

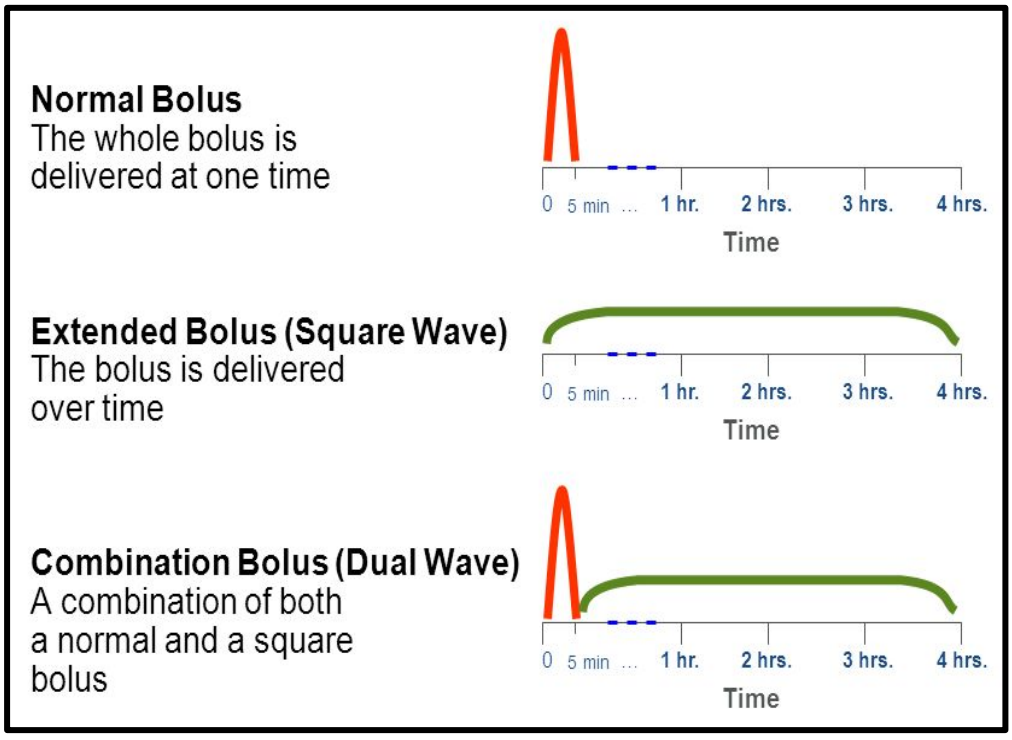
Moreover, the current devices do not take into account the rate of absorption of insulin that varies with individual factors such as gastric emptying, temperature, site and mode of insulin administration.

Not all ABCs consider some factors related to the insulin adjustments, such as physical activity or others health conditions affecting blood glucose levels (i.e. fever, menses).

Finally, these tools are not able to quantify the contribution of long-acting insulin and do not provide any indication on the units of basal insulin to be administer because they not consider fasting glycaemic levels. In this regards, it would be enough recorder the fasting blood glucose trends for a few days to suggest an increase or reduction of the dosage as an automatic titration.

To partially cope with these limitations, patients must be able to choose the most appropriate type of bolus (standard or single wave, dual wave and square wave) (**Fig. 4**) taking into account the content of the meal and its own rate of absorption [42].

Figure 4: *Variable Wave Bolus*



The effective use of the ABCs systems with these limitations need proper skills of diabetic patients to self-manage their insulin therapy as well as of health care providers themselves, in order to promote the correct therapeutic choices.

In fact, as previously stated, diabetic patients have to receive a proper education program on CHO counting and hands-on sessions on the use of the ABC system and mainly they must be able to interpret and

translate in action recorded data, taking into account all factors that could affecting insulin adjustments.

On the other hand, health care provider may also affect the ABCs system outcomes.

In this regards, diabetologist and dietician have to be familiar with these tools as well as they must be trained on the adjustments of device settings since the bolus insulin parameters display continuous intra- and inter- individual variations [48].

Unfortunately, all ABCs have standardized rules and software and frequently the over standardization can minimize the innovations. Therefore, innovative ABCs should be performed with predetermined settings but should allow some modifications of settings for each patient, promoting a personalized use of them [35].

2.4 Clinical evidences of ABCs efficacy on glycaemic control and treatment satisfaction

Following the successful outcomes from the studies evaluating the use of bolus advisor systems integrated into insulin pumps in patients CSII-treated, some Randomized Control Trials (RCTs) were carried-out to evaluate the effectiveness of ABCs in patients on MDIs but the results on glycaemic control and treatment satisfaction are still controversial and unconvincing (**Table 2**).

Table 2: *Effect of the use of an ABC on glycaemic control and treatment satisfaction*

References	Year	Study design	Study duration	Number / Age of patients	Results
Shmidt S. et al.	2012	RCT, open–label three parallel arms	16 weeks	51 T1D patients/ 18–65 years	▪ Significant reduction in HbA1c [<0.8% vs. 0.6% (P<0.0001)][49] ▪ Significant improvement in treatment satisfaction in CarbCount ABC.
Barnard K. et al	2012	Survey	-	488 T1D patients/ 1-70 years	▪ Significant reduction of fear of hypoglycaemia in 13% of patients using ABC ▪ Significant attitude in the estimation of insulin boluses, in 28.0% of subjects
Zucchini S. et al.	2011	Observational study	30 days	30 T1D patients/ Mean age: 13,5 ± 4,5 13,2 ± 4,9 years	▪ Significant reduction of HbA1c [<0.5% vs. 0.3% (p < 0.05)]

Sussman A. et al.	2012	Survey	-	205 T1D patients/ 13-83 years	▪ A major confidence in ABC calculated insulin doses than the manually (83% of cases) ▪ Reduction of errors doses estimation
Ziegler R. et al.	2013	RCT	26 weeks	202 T1D patients and 16 TD2 patients/ > 18 years	▪ Significant reduction of HbA1c in ABC groups compared controls (56.0% vs. 34.4%; P < 0.01) ▪ Significant improvement in treatment satisfaction [11.4 ± 6.0 vs. 9.0 ± 6.3; P < 0.01)]
Vallejo Mora MDR et al.	2017	Extension of CBMDI study	4 months after extension	82 T1D patients/ -	▪ Not significant improvement of HbA1c (7.61 – 0.58 vs. 7.73 – 0.65, P 0.209); ▪ Significant improvement in treatment satisfaction (12.03 – 4.26 vs. 13.71 – 3.75, P 0.007) ▪ Significant decrease in fear of hypoglycaemia (28.24 – 8.18 basal vs. 25.66 – 8.02, P 0.026)

Shmidt S. et al.	2017	Extension of StenoABC	1 year after extension	128 T1D patients/ -	▪ Not significant improvement of HbA1c ▪ Significant improvement in treatment satisfaction (74% of follow-up study respondents were using the bolus calculator)

The BolusCal Study was a 16 weeks RCT carried out on 51 adults T1D patient on MDI with poor glycaemic control (HbA1c 8-10.5%).

T1D patients were randomized into three parallel arms: Control (n = 8), CarbCount (n = 21), or CarbCountABC (n = 22). At 16 weeks, CarbCountABC arm a significant reduction in HbA1c (−0.8% [95% CI −1.4 to −0.1]) and the CarbCount arm had a reduction of−0.6% [−1.2 to 0.1]). Nevertheless, the difference in change in HbA1c between CarbCount and CarbCountABC was insignificant. Furthermore, it was found an improvement of Diabetes Treatment Satisfaction Questionnaire in all study arms, but the improvement was significantly greater in CarbCountABC [50].

In order to evaluate the effectiveness of an ABC on fear of hypoglycaemia by T1D patients on MDIs and to assess patients attitudes and behaviours regarding to its use, in 2011 Barnard et al. performed in UK and Republic of Ireland a survey involving 1,412 T1D patients from

270 hospitals. 588 ABC users for at least 4 weeks on MDIs responded to the survey. Specifically, 76.7% of respondents affirmed to use a bolus calculator advisor system to estimate insulin dosage for meals and snacks always or quite often with a reduction of fear of hypoglycaemia in 39% of cases, that it was significantly reduced in 13% of cases.

Furthermore, 78.8% reported an improvement in attitude in the estimation of insulin boluses, that it was significantly improved in 28.0% of subjects. For 89.9% of respondents the insulin adjustments through the bolus advisor system was easier than the manual calculation, increasing their confidence in the accuracy of their bolus doses.

Zucchini et al., in a 30-day observational study showed that a bolus calculator could be useful to improve glycaemic control and to reduce the calculation errors in insulin boluses in children with T1D, evaluating the efficacy on the percentage of within-target glucose values two hours after meals. Patients using the bolus calculator had a significantly higher frequency of within-target glucose values compared to the control group (67% vs 25%, $p = 0.036$), with similar number of correction boluses and hypoglycaemic events between the two groups. After one month, HbA1c was 0.5% lower than baseline in the group using bolus calculator and 0.3% in the control group ($p < 0.05$).

A major confidence in the meter-calculated insulin doses than the manually was also confirmed by 83% of T1D patients evaluated in a survey performed by Sussman et al. Moreover, this study confirmed that the use of the automated bolus calculator integrated in a meter is helpful

to minimize errors in insulin dose adjustments and more than 80% of patients felt more confident to continue to use the meter with built-in ABC, preferring it to manual calculation [51].

These findings were also confirmed in the ABACUS trial performed in adults with T1D and T2D, poorly controlled. The study showed a major significant reduction of HbA1c in patients using a meter with built-in ABC compared to patients not users (56.0% vs. 34.4%; $P < 0.01$), with a significant improvement in treatment satisfaction (Diabetes Treatment Satisfaction Questionnaire scale) (11.4 [SD, 6.0] vs. 9.0 [SD, 6.3]; $P < 0.01$) and without significant differences in hypoglycaemia (<50 mg/dL) between the two groups [52].

Notwithstanding the results from the mainly studies on bolus advisor systems sustain their effectiveness on glycaemic control and treatment satisfaction, to confirm successful outcomes and adherence to their use in the real life situations wider clinical trials with long-term follow-up are needed.

To date, only two studies have evaluated the long-term efficacy of ABC use in T1D on MDIs. Similarly, the both studies showed a greater treatment satisfaction in ABC users; even though HbA1c was not further significantly improve at the end of the study period [53, 54].

Project Research 1: ***A novel insulin unit calculator for the management of type 1 diabetes***

CHAPTER 3

3.1 Introduction

Intensive insulin therapy, based on multiple daily insulin injections (MDI) or on continuous subcutaneous insulin infusion (CSII), is well established as the gold standard treatment for T1D patient's management. The rationale of intensive insulin therapy is to provide an adequate amount of basal insulin and short-acting insulin to reduce glycaemic excursions. The effectiveness of intensive insulin therapy has been reported in the Diabetes Control and Complications Trial, showing that optimal glycaemic control is directly related to the reduction of incidence and progression of long-term diabetes complications [12].

Achieving treatment goals should be obtained by maintaining flexibility to fit individual lifestyle and without major variations in eating behaviours and physical activity. Diet, physical activity, and insulin dosage play a key role in the management of insulin treatment in T1D patients. Diet, in particular carbohydrate (CHO) meal content, is the main determinant of a rise in postprandial blood glucose. Accordingly, an appropriate teaching and training program on CHO counting and specific instructions for the administration of insulin doses need to be implemented in patients with diabetes.

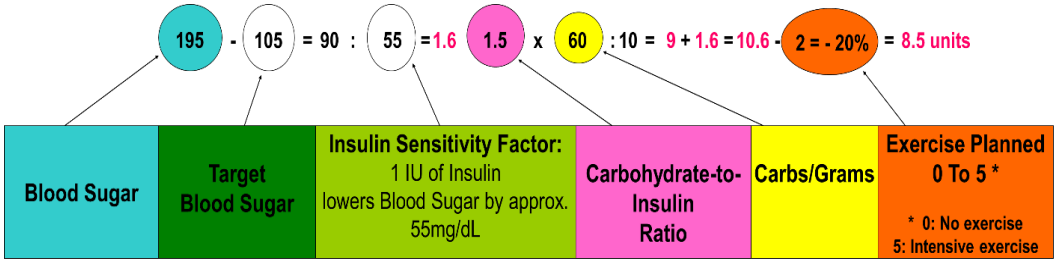
In fact, CHO counting as a meal planning approach offers the flexibility of food choices and allows achieving treatment goals [31, 32].

It has also been demonstrated that a sedentary lifestyle and lack of physical exercise are associated with poor blood glucose control [55]. Regardless of insulin therapy, physical activity helps decrease blood glucose levels and increase insulin sensitivity and peripheral glucose utilization, in addition to reducing cardiovascular risk factors and preventing obesity [13].

To achieve treatment goals, the establishment of insulin dose at mealtimes must be made before each injection, while considering certain parameters, such as blood glucose levels, insulin/CHO ratio, CHO intake, and the intensity of physical exercise after injection.

Calsulin, a simple tool recently developed by Thorpe Products Ltd. (Cambridge, UK) (see www.thorpe-products.com) (**Fig. 5**) calculates the premeal bolus that takes into account all of the above parameters in a matter of seconds, displaying the insulin units to be injected.

FIG. 5: *The insulin units calculator Calsulin (Thorpe Products Ltd.)*



3.2 Aim of the study

The aim of the present study was to demonstrate the efficacy of Calsulin in improving glycaemic control, as assessed by HbA1c in T1D patients using intensive insulin therapy.

3.3 Materials and methods

T1D patients 18–65 years old attending the diabetes outpatient clinic at the University Campus Bio-Medico in Rome, Italy were asked to participate in the study. T1D was defined according to the American Diabetes Association classification, and all patients gave their consent to participate.

Exclusion criteria were learning disabilities or the presence of chronic conditions potentially able to influence daily activities (visual or auditory disability, motor impairment for neurological or orthopaedic problems).

T1D patients affected by severe diabetes complications were also excluded.

In total, 40 consecutive T1D patients who agreed to participate in the study (26 men, 14 women) were randomized 1:1 to Calsulin or standard

education for insulin treatment (control group). All subjects were followed up with visits every 3 months and telephone consultations according to standard procedures operating in our clinic.

At enrolment, all subjects were provided with a logbook and instructed to self-monitor glucose levels, to estimate meal CHO content, and to perform regular exercise, consisting of at least 2 h of physical exercising three times weekly. The target blood glucose, insulin/CHO ratio (I/C ratio), and insulin sensitivity factor (ISF) were determined for all patients, individually, by the physicians.

The I/C ratio, defined as the amount of CHO (g) "covered" by 1 unit of insulin, was determined by the "500 rule." The ISF, defined as the estimated drop in blood glucose (in mg/dL) expected from the administration of 1 unit of insulin, was calculated by the "1,800 rule."

During each clinical visit the haemoglobin A1c (HbA1c) level and frequency of hypoglycaemic episodes were recorded for each patient, and adherence to the use of CHO counting was verified. Patients in the Calsulin group were trained on the use of Calsulin to administer the insulin dosage.

Calsulin setup requires patients to enter four parameters: premeal blood glucose value, I/C ratio, g of CHO contained in the meal, and post-injection exercise.

The potential physical activity, performed after injection, is quantified by a number from 0 to 5 on the numeric keypad, and each number, corresponding to the amount of physical exercise, represents the percentage that will be reduced from the total insulin dose calculated.

Then, the instrument displays the pre-meal insulin units to be injected. HbA1c (%), daily insulin requirement (IR), and body mass index (BMI) were evaluated at entry into the study and at 3- and 6-month follow-ups. The mean daily insulin requirement was expressed as units/kg/day, and BMI was calculated as the ratio of weight (in kg)/(height [in m])2.

Sample size and statistical evaluation

Setting a (probability of type I error) equal to 0.05 and β (probability of type 2 error) equal to 80%, for a difference of HbA1c of 1% with 1% SD at the end of the study period, the required sample size was 34 patients for a two-sided test. To ensure the appropriate sample size, 40 patients were recruited to allow six dropouts.

Paired t test (two tailed) and analysis of variance were used to evaluate differences in HbA1c, IR, and BMI between the two groups.

Data were expressed as ± SD values. A *P* value of <0.05 was considered significant.

3.4 Results

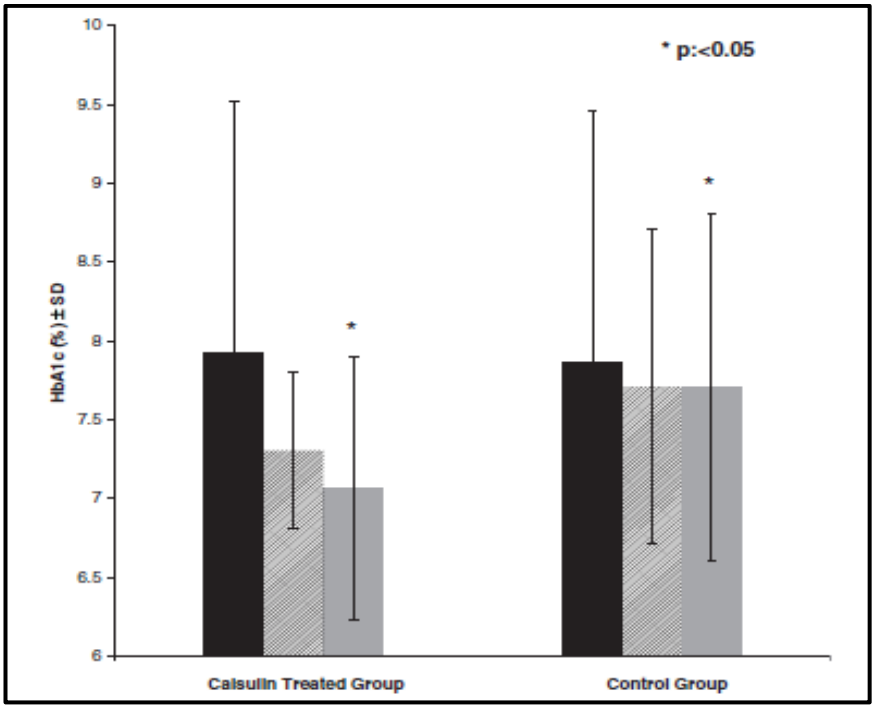
The results of this study are shown in Table 3 and Figure 5

Table 3: *Demographic and Clinical Features of Type 1 Diabetes Patients Participating in the Study*

	Calsulin-treated	*Control patients*	P *value*
Number	20	20	
Male:female	13:7	13:7	
Age range (years)	34.5 ± 15	39.3 ± 13	
Duration of disease (years)	14.4 ± 10.8	13.4 ± 7	NS
BMI at baseline (kg/m^2)	23.7 ± 3.6	24.7 ± 6.1	NS
HbA1c (%)	7.9 ± 1.0	7.8 ± 1.6	NS
Daily insulin requirement at baseline (IU/kg/day)	0.64 ± 0.22	0.57 ± 0.21	NS

Data are mean ± SD values. BMI, body mass index; HbA1c, hemoglobin A1c; NS, not significant.

Figure 5: *Hemoglobin A1c (HbA1c) values at entry (time 0) and after 3- and 6-month follow-ups in the two groups of treated patients*



The comparison of baseline demographic and clinical features indicates that the two groups of patients did not differ in clinical and metabolic parameters.

Level of physical activity was similar between the two groups, and no major differences were reported in socioeconomic status or level of education between the groups.

During the initial 3 months, there was a trend toward a decrease in HbA1c in the Calsulin-treated group versus the control group (7.3±0.5% vs. 7.7±1.0%, respectively; *P* not significant).

At the 6-month follow-up, a significant reduction in HbA1c levels was observed in the Calsulin-treated group versus the control group (±0.85% vs. ±0.07% difference, respectively; P<0.05) (Fig. 2).

There were no significant differences in IR (0.64±0.24 vs. 0.58±0.19, respectively; *P* not significant), BMI (23.9±4.6 vs. 25.1±6.1, respectively; *P* not significant), and frequency of hypoglycaemic events between the two groups.

3.5 Discussion

To achieve treatment goals in diabetes, one crucial aspect is represented by the reduction of postprandial glycaemic excursions.

The metabolic control and the onset and progression of diabetes complications are related to both fasting and postprandial glycemia,8 which results from the CHO content in each meal and of blood glucose levels before meals.

Frequently, it is difficult to calculate the insulin dosage accurately using conventional bolus calculation methods.

As reported in the study by Glaser et al. [34] there is high frequency of error (50–64%) in calculating premeal insulin doses using conventional methods. Often, difficulties in performing dose calculations induce patients to administer fixed prandial insulin doses or to maintain an established amount of CHO in their meals [56].

However, a positive effect of correct premeal insulin doses has been also reported in the Diabetes Control and Complications Trial with better control for patients using CHO counting in the intensively treated group, showing a supplementary 0.56% reduction in HbA1c compared with those not using CHO counting [57].

To be effective, diabetes education must be implemented, not only on CHO counting but also on other features of the disease management, mainly specific algorithms to adjust insulin doses to achieve treatment goals.

Therefore specific algorithms for insulin administration that take into account premeal blood glucose value and g of CHO contained in the meal should be used to reduce postprandial glycaemic excursions and, moreover, to increase flexibility in meal planning, food choices, and physical activity.

In the current study, we demonstrated that this simple pocket instrument, the same size of a small calculator, is an acceptable and practical tool to make the process of calculating the premeal insulin dosage very simple.

Most importantly, it helps improve glycaemic control, as shown by a significant reduction of HbA1c levels compared with standard methods used for calculating the required insulin doses.

Finally, because Calsulin actively involves patients in decision-making for treating their own disease, it may improve to adherence and satisfaction with insulin therapy.

RESEARCH PROJECT 2: *The use of an automated bolus calculator and wirelessly communicated blood glucose measurement can improve patients' compliance to insulin therapy in T1D patients*

CHAPTER 4

SPECIFIC BACKGROUND: Telemedicine and mobile applications for T1D management

4.1 Introduction

The wide use of smartphones such as the growing availability of mobile health care applications with the revolution in the data management has radically changed the communication in the medical field. More than 40% of users collects information on medical therapies through social media, 19% stated that they had healthcare apps, 54% of patients talks about their healthcare condition on the social networks or on the online communities. Although several European research groups have carried-out a great number of clinical research in the field of diabetes technologies, the use of diabetes devices remains currently limited in many European countries, much more than in the United States. Indeed, the scenario for the reimbursement of new and innovative diabetes devices in Europe is very heterogeneous as well shown by the penetration of insulin pump therapy among people with diabetes that varies from 5 to 15% in Europe, while in US it is now close to 40% [3, 21, 30, 58].

The main reason is the late approval of cost coverage by most national healthcare insurance systems, which is still lacking in some countries, causing a great heterogeneity on prescriptive rules and on delivery mode of pumps and other diabetes devices. Partly related with this delay, the number of trained physicians to pump therapy is low in many countries, as well as diabetes educators do not exist as an acknowledged entity in many European countries, and pump manufacturers are excluded from the education process of patients in most of cases.

The economic burden on healthcare insurance systems does not facilitate wider use of the newer diabetes tools, but a significant expansion of them is expected thanks to the growing knowledge of physicians in technological devices and to the increasing patients demand to use technology [59].

As stated, the recent technologies are aimed to diabetes care and especially to the improvement of patients' quality of life, sustaining people with diabetes in their daily activities and increasing their adherence to care pathways. Accordingly, the proper application of the latest technologies helps to obtain successful outcomes, increasing patients' copying and empowerment, as well demonstrated by the data from the literature [21, 30]. As well known, the patient-physician communication has been completely changed by the introduction of telemedicine systems also in diabetes field.

The use of telemedicine offers the opportunity of an easier and immediate communication between diabetologist and patient, optimizing the use of data collected by SMBG and facilitating

educational interventions. Glucose monitoring through telemedicine systems seems to improve patients' quality of life as well as the relationship with the specialist. In this regard, the cornerstone of a "diabetes technological management" is the institution of specialized diabetes centers with proper technical and educational support and highest levels of expertise providing equal access for diabetic patients to the diabetes devices prescription and reimbursement.

Therefore, Diabetes Technology Centers offering not only a technological support should aim to the joint planning of the therapeutic process, allowing to manage diabetes more systematically, accurately and effectively [60].

Thus, diabetes devices are becoming effective to reach the clinical goals and to improve the therapeutic education of digitalized patients, making them more aware and informed. In this way, from telemedicine to the "cloud", from children to adults, the diabetes management is progressively moving across a digital infrastructure.

4.2 Use of telemedicine in Diabetes

To date, the use of new technologies for the management of diabetic patients represents an area of investigation with important clinical application. In fact, in the past decades, the use of diabetes tools is widely grown and it seems to have partly changed the diabetes management [61].

Nevertheless, to evaluate the real benefits of telemedicine in diabetes management it should not only highlight its superiority compared to the

conventional clinical practice but rather it should be investigate how it can be effective in the current clinical practice.

Moreover, the assessment should clearly distinguish between the different data management evaluated and the various technologies applied (**Table 4**).

Table 4: *Remote management of clinical data and different technologies applied*

- Teleconsult: Remote data sharing only between health care providers
- Clinical Folders via the Web (personal health record)
- Remote monitoring of vital parameters in patients with chronic diseases (e.g. in chronic heart failure) for earlier detection of exacerbations
- Telenursing, Call-centers: remote therapeutic and educational programs
- Data transmission from patients to health care providers associated with different kind of feedback (automated messages generated by pre-set software, voice messages, sms, fax by health care providers)
- Telemedicine systems as support for the therapeutic patients self-management
- Data sharing through the use of mobile applications

Finally, the outcomes of the evaluation studies could be dependent on the different goals considered (**Table 5**).

Table 5: *Potential objectives of telemedicine*

- Improvement of clinical outcomes
- Optimization of resources
- Improvement of patients' quality of life and treatment satisfaction

On this basis, diabetic patients and mainly their health care providers should be willing to steadily investigate new technologies to reach proper skills with effective outcomes. Therefore, to ensure achievement of glycaemic targets safely and effectively also improving daily flexibility and the overall diabetic patients' quality of life, a multidisciplinary approach and the definition of general criteria of the diabetes technologies management are crucial [6].

Multidisciplinary approach

In order to provide the knowledge and abilities required to diabetes devices users and to support and encourage their self-management through the new technologies, the diabetes management requires a comprehensive approach by a multidisciplinary team of healthcare professionals [60]. Multidisciplinary clinic team should consist of a

diabetologist/endocrinologist, a diabetes specialist nurse, a certified diabetes educator or counsellor and a diabetes specialist dietitian. Consequently, diabetes care provided by multidisciplinary team is person-centered improving patient's adherence to his treatment. In general, according to their skills, each professional of the healthcare team provide education, information and support to diabetic patient. The trained specialist team is also essential to identify patients, who are suitable candidates for the different diabetes tools. To guarantee the achievement of proper expertise, each team's member provides individual outpatient visits or educational group courses to diabetic patients, ensuring them an appropriate support for the resolution of the different problems related to their condition through the active and effective use of the latest technologies. Therefore, the role of the multidisciplinary team is crucial to taking charge of diabetic patients in different settings [6].

4.3 Evidences of telemedicine in T1D

As well know, the most important area where telemedicine would have some potential benefits in diabetes is the management of intensive insulin therapy.

The results of DCCT have widely demonstrated that the benefits of short and long-term intensive insulin therapy is obtain only with an effective self-management by diabetic patients which requires frequent contacts between patients and health care professionals. In fact, study design included telephone contacts to insulin regimens adjustments daily for

the first week and then weekly thereafter as well as increased frequency of outpatients' visits and intensive SMBG [12].

However, some critical issues are relevant:

- ✓ Often patients have troubles to interpret information obtained from the self-monitoring blood glucose independently;
- ✓ Frequent contacts between specialist and diabetic patients meet often logistical, time and resources obstacles.

In this context, a telemedicine system can offer an easy and immediate medical/patient communication that allows to increase the frequency of contacts and timely therapeutic interventions.

Already in 2005 has been demonstrated that Web-based care management may be a useful tool to manage patients with poorly controlled diabetes [62].

Although, some clinical studies have confirmed that telemedicine has been useful to improve glycaemic control in young diabetic patients poorly controlled, the achievement of metabolic outcomes is strictly related to the continuous and timely medical/patient contact that overcoming the barriers due to geographical distance, to work commitments (younger patients) or to mobility restrictions (elderly patients, acute events, pregnancy) [63-65].

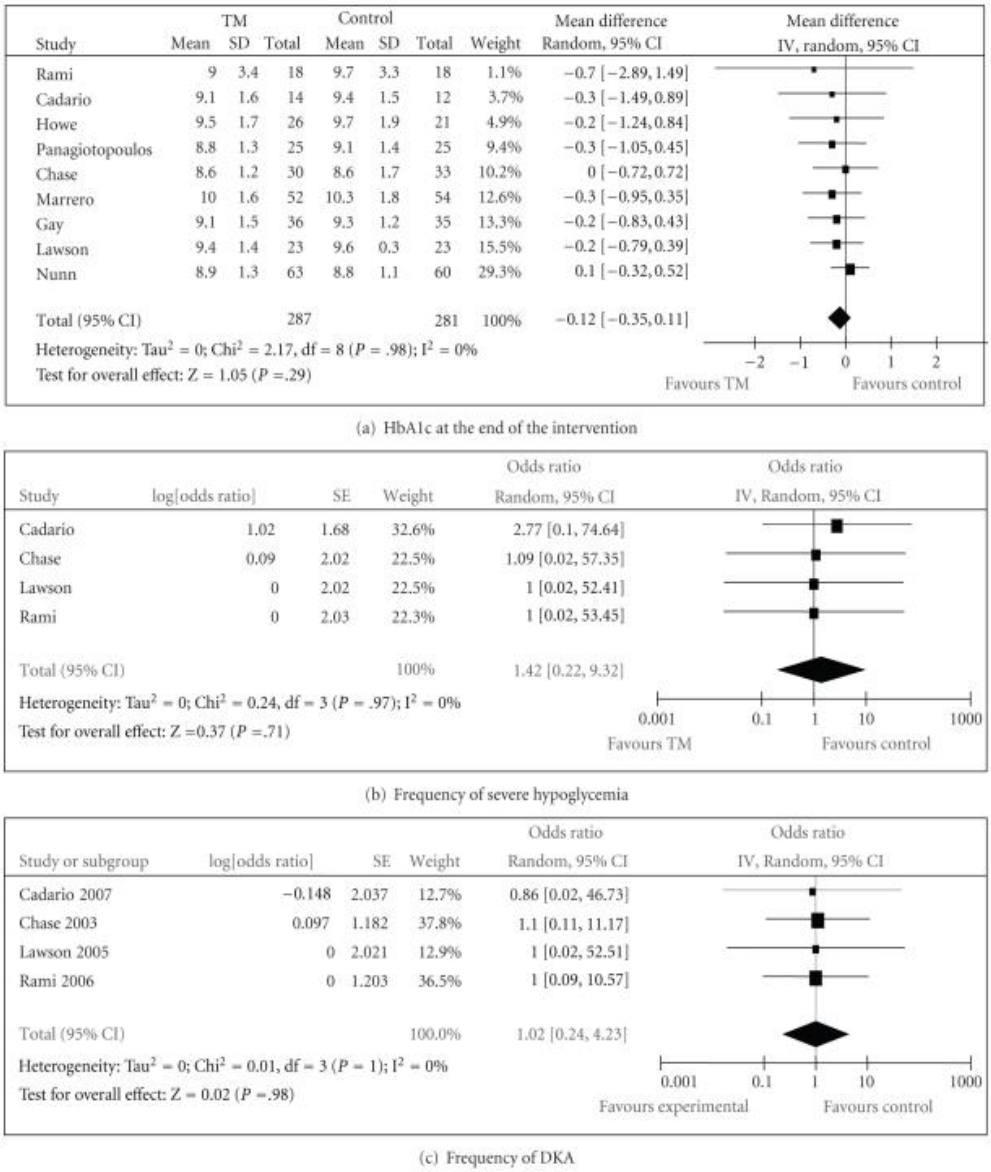
Moreover, this may also result in greater patients' compliance with an active involvement in their therapeutic process.

Nevertheless, in 2010 Shulman et al. in a systematic meta-analysis observed a small effect on glycaemic control of telemedicine compared

to standard care. Their pooled analysis showed an effect of the intervention on HbA1c, likely less than 0.5%.

On the other hand, the study demonstrated the safety of the use of telemedicine as shown by no differences of the risk of hypoglycemia and ketoacidosis (**FIG. 6**) [66, 67].

Fig 6. *Meta-analysis of the effect of Telemedicine. Modified from Ref.* [66]



However, only one study included in the meta-analysis investigated patients' satisfaction showing no significant difference between groups [68].

Similarly, only three studies reported diabetes-related quality of life (QoL) [69-71], and none found significant differences between groups.

Finally, only two studies [68, 72] investigated the cost of a clinic visit; in one of these, the group using telemedicine did not attend the three-month visit with a cost savings of $USD 142.00. Indirect costs such as missed work and school days were considered but not converted into dollar value nor incorporated in the overall cost calculation [72].

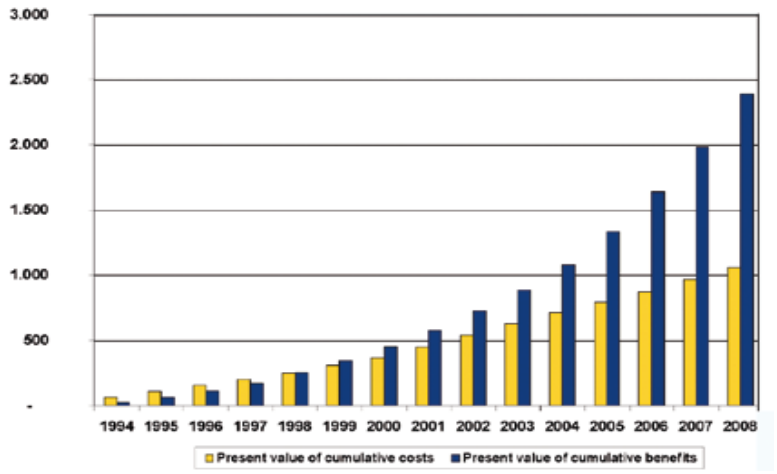
In this regard, a frequent objection to the use of telemedicine is the cost considered to be higher compared to the standard care.

In this regard, a study of European Community showed that the initial expenditures for the implementation of telemedicine systems in healthcare is overcome by economic benefits, over the years (**FIG. 7**).

Lastly, the European Commission has recognized the benefits of telemedicine in the management of chronic diseases, particularly in diabetes, in terms of quality of life, improvement of care, optimization of health resources, recommending the European countries to develop and use telemedicine in clinical practice [72].

FIG. 7: *Expenditures for the implementation of telemedicine systems*

€ mill.



4.4 Mobile applications for diabetes care and self-management

As stated, in the last decade the availability of mobile health devices (mHealth) has widely increased [61].

The Institute of Healthcare Information of Informatics have reported that in 2013 the number of mHealth apps available were more 165,000 and over 1100 diabetes-related apps are now available. Accordingly, the mHealth industry aspect to reach $20 billion by 2018 [73].

Regarding to diabetes, the most relevant mHealth apps are addressed to support diabetic patients in specific areas of their disease management as healthy eating, medication adherence, insulin doses administration, physical activity and SMBG with availability of an electronic logbook where blood glucose measures are wirelessly recorded and emailed to health care professionals [61].

Contrarily to the growing number of mHealth apps on market, in a recent review evaluating mobile apps currently available, it has been clearly demonstrated that only 14 have data published in peer reviewed articles in last five years, satisfying the minimum criteria **(Fig.8)** required to receive approval by US Food and Drug Administration (FDA) or the Conformity European (CE) mark in Europe [74].

FIG. 8: *Criteria considered for mobile apps evaluation Adapt. from [74]*

Box 1 **Criteria used to evaluate mobile apps**
Platform
Smartphone
Meter
Insulin pump
Computer: Web-based software
Other
Function/description
Insulin dose calculator
Basal bolus: pattern adjustment
Activity diary
Education
End user
T1DM
T2DM
Data collection: data source and mode of collection
Glucose
Carbohydrate/nutrition
Activity
Manual versus automatic
Connectivity:
Cloud
Web
Electronic medical record
Other: SMS (short message service)
Availability/regulatory
US Government or EU
FDA cleared or CE marked
Clinical evidence
Study design
Outcomes: safety, efficacy, and QOL

As reported in **Table 6** the 14 mobile apps are been categorized in two major groups:

A) Smartphone-based apps

B) Glucose-meter based mobile apps

Table 6: *Summary of commercially available mobile medical apps for DM management (N: 14). Adapt. from [74]*

Name	Platform	Function/Description	End User	Data Collection	Connectivity: Cloud Web EMR Communication	Availability/ Regulatory	Clinical Evidence/ Reference
Blue Star by WellDoc	App (Android and iOS) or Web based	• Real-time feedback • Touch point messages • Video education • Education library • Longitudinal reporting • The Easy Carb Education library • The Easy Carb Estimator and Restaurant Helper to support healthy eating	T2DM	Manual data entry into app Automatic BG entry with Bluetooth adapter	Cloud: yes Web: yes EMR: no Can send reports to provider	FDA cleared in United States Needs MD Rx	A1C reduction[9] Improvement in self-efficacy[8]
Dexcom Share	App (iOS) for Share (upload to cloud)	Share real-time CGM data with followers	T1DM; T2DM	Dexcom G4 Platinum CGM uploads data to cloud via Bluetooth-enabled receiver	Cloud: yes Web: yes EMR: no	FDA cleared in United States	None
Diabeo	App (Android and iOS)	• Bolus calculator • Adjusts for exercise • Basal bolus pattern recognition • Real-time feedback	T1DM; T2DM on insulin	Manual data entry into app	Cloud: no Web: yes for MD EMR: no Patient can communicate with provider for real-time assistance	Developed in France CE marked in EU	A1C reduction[10–12]

Diabetes Diary	App (Android and iOS)	• Bolus calculator • Tracks BG level, insulin, food, and activity • Provides historical data to facilitate decision making	T1DM	Manual data entry Automatic BG entry with Bluetooth adapter	Cloud: no Web: no EMR: no	Developed in Norway CE marked in EU	A1C reduction[13]
Diabetes Interactive Diary (DID) (Il Diario Interattivo per il Diabete)	App (iOS)	• Logbook for blood sugar, insulin dosing, and events • Nutritional database for counting carbohydrates • Food exchange data • Insulin dose calculator • Physical activity diary • Annual screening reminder • SMS to diabetes provider	T1DM	Manual data entry • BG • CHO selection • Physical activity	Cloud: no Web: no EMR: no Other: SMS sent to diabetes provider	Developed in Italy CE marked in EU	No A1C reduction[14,15] Improved QOL[14] Reduction in hypoglycemia[15]
Glooko	App (Android and iOS)	• Downloads diabetes data from 40+ meters, insulin pumps and CGMs • Integrates health and fitness apps • Nutrition database for CHO counting • Data sharing with providers • Analytics data on clinic population for providers • Hypoglycemia prediction algorithms • Reminders	T1DM/ T2DM	BG upload data via cellular network and MeterSync Blue cable Dexcom CGM data obtained via Apple HealthKit Obtains data from fitness tracking devices	Cloud: yes Web: yes EMR: yes Can email, print, or fax standardized reports to provider	FDA cleared in United States	None

(continued on next page)

(*continued*)

Name	Platform	Function/Description	End User	Data Collection	Connectivity: Cloud Web EMR Communication	Availability/ Regulatory	Clinical Evidence/ Reference
Accu-Chek Aviva Expert	Glucose meter	• Bolus calculator embedded in the meter • Accounts for CHO and insulin on board • Minimizes insulin stacking	T1DM; T2DM on insulin	Glucose data automatic Hand enter CHO and insulin dose	Cloud: no Web: yes Accu-Chek 360 diabetes management software for MD and patient EMR: no	FDA cleared in United States Needs MD Rx	ABACUS 1 RCT clinical trial with A1C reduction[17] Survey results[16]
Accu-Chek Connect (Roche)	Glucose meter and app (Android and iOS or Web based)	• Integrated meter, app, and online portal • Meter automatically transmits data to app • App incorporates bolus calculator • Data shared with health care provider	T1DM; T2DM	Glucose data automatic Hand enter CHO and insulin dose Meal photographs can be attached to BG	Cloud: no Web: yes Uses Accu-Chek 360 View tool for 3-d profile EMR: no	FDA cleared in United States Needs MD Rx	Bolus calculator studied in ABACUS 1 trial[17]
Dario	Glucose meter app (Android and iOS)	• Downloads BG when connected to a smartphone • App contains insulin calculator • Can chart CHO intake, insulin doses, notes • Share results with family, provider	T1DM; T2DM	Glucose data automatic Nutrition, activity, insulin doses manual	Cloud: yes Web: yes	FDA cleared in United States CE marked in EU	None
Diabetes Insulin Guidance System	Glucose meter	• Bolus calculator • Adjusts insulin dosing plan based on historical data • Uses time of day to suggest CHO bolus	T1DM; T2DM on insulin	Glucose data automatic Initial insulin dosing entered manually	Cloud: no Web: no EMR: no Other: connect device to computer to download data	CE marked in EU	A1C reduction, decreased hypoglycemia[18]
FreeStyle InsuLinx	Glucose meter	• Bolus calculator embedded in the meter • Easy mode for fixed CHO meals • Advanced mode for CHO counting • Real-time feedback • Trending reports	T1DM; T2DM on insulin	Glucose data automatic Hand enter CHO	Cloud: no Web: yes FreeStyle Auto-Assist diabetes management software for MD and patient EMR: no	CE marked in EU Needs MD Rx	More accurate meal bolus[19] Confidence in bolus calculation[20]
Gmate	Glucose meter App (Android and iOS)	Downloads BG when connected to a smartphone BG	T1DM; T2DM	Glucose automatic nutrition, activity manual	Cloud: yes Web: yes EMR: yes Data sharing with family, provider via texts, email	FDA cleared in United States	None
Livongo	Glucose meter (In Touch) App (Android and iOS)	• Cellular-enabled glucose meter with touch screen • Tags meals, exercise, medications • Real-time scripted feedback about BG • Displays logbook and patterns • Share results with family, provider, and coach via touch screen	T1DM; T2DM	Glucose and activity data automatically uploaded via cellular network	Cloud: yes Web: yes EMR: yes Patient can communicate with CDE for real-time assistance	FDA cleared in United States	None

(*continued on next page*)

(*continued*)

Name	Platform	Function/Description	End User	Data Collection	Connectivity: Cloud Web EMR Communication	Availability/ Regulatory	Clinical Evidence/ Reference
Telcare	Glucose meter App (Android and iOS) Web portal	• Cellular-enabled glucose meter • Uploads BG automatically to cloud and from there to Web or smartphone • Real-time contextual feedback via text messages • Share data with diabetes care provider • Two-way text messaging between patient and health care professional	T1DM; T2DM; GDM	Glucose data automatic	Cloud: yes Web: yes EMR: yes	FDA cleared in United States	Potential for cost savings when used with disease management program[21] A1c reduction when used with FDA-cleared glucose management software Glucommander[24]

Abbreviations: ABACUS, Automated Bolus Advisor Control and Usability Study; CDE, certified diabetes educators; CGM, continuous glucose monitoring; CHO, carbohydrate; EMR, electronic medical record; GDM, gestational DM; iOS, iPhone operating system; MD, Doctor of Medicine; Rx, therapy; SMS, short message service.

Despite the potential role of health-related apps on improvement of metabolic outcomes such as HbA1c levels or control of hypoglycaemic events as well as on the improvement of quality of life and patients self-management, clinical evidences on their cost-effectiveness are still limited and quite controversial [75].

In 2015, a review carried-out by Garabedian et al. demonstrated that only 20 peer-reviewed articles, published since 2010, have a robust evidence of effectiveness of mHealth interventions for diabetes [73].

In fact only few studies have showed a robust impact on glycaemic outcome, represented by HbA1c levels, as shown in **Table 7** .

Table 7: *Impact of mHealth apps on HbA1c. Adapt from ref. [73]*

Article	Absolute percentage point change in HbA1c	Mean HbA1c level at baseline
Noh et al., 2010	−1.04 %** (−1.53±1.42 in treatment group vs. −0.49±1.07 in control group	9 % (intervention group), 8.6 % (control group)
Quinn et al. 2011 *	−1.2 % (p=0.001; −1.9 in treatment group vs. -0.7 in control group, for highest level of intervention)	9.9 % (intervention group), 9.2 % (control group)
Lim et al., 2011	−0.3 %** (−0.4, p<0.001 in treatment group vs. −0.1, p=0.274 in control group)	7.8 % (intervention group), 7.9 % (control group)
Charpentier et al., 2011	−0.83 %** (−0.66 (95 % CI: 0.28–1.04) in treatment vs. +0.17 (95 % CI: −0.19–0.53) in control group, for highest level of intervention)	9.11 % (intervention group), 8.91 % (control group)
Bell et al., 2012	−0.2 %** (p=−.02; −1.2 in treatment group and −1.0 in control group)	9.6 % (intervention group), 9.0 % (control group)
Kirwan et al., 2013	−1.17 %** (−1.10, p<0.001 in treatment group vs. +0.07, p>0.05 in control group)	9.08 % (intervention group), 8.47 % (control group)
Quinn et al. 2014 *	−1.4 % (p=0.001; −1.8 in intervention group vs. −0.3 in control group for older adult cohort; younger adult cohort difference was −1.0, p=0.02)	9.8 % (intervention group), 8.4 % (control group)—for older adult cohort; 9.9 % (intervention group and control group)—for younger adult cohort
Waki et al., 2014	−0.5 % (p=0.15; −0.4 in treatment group vs. +0.1 in control group)	7.1 % (intervention group), 7.0 % (control group)

*Same intervention studied in multiple articles

**Review authors calcuated difference. Study authors did not calculate whether difference between treatment and control group was significant (i.e., a difference-in-difference estimate)

However, these studies having glycemic control as primary endpoint not investigated other endpoints such as the improvement of patient satisfaction, quality of life, patients' empowerment or other outcomes (e.g. incidence of hypoglycaemia, glycaemic variability, weight loss, serum lipids, cost saving) [76]. Beyond the impact on HbA1c widely varying between the selected studies, other relevant limitations were the small sample size and the short follow-up. In conclusion, to define the clinical and economic effectiveness of this kind of intervention, large clinical randomized trials evaluating other issues of patients self-management with proper power and longer duration are needed [77].

Main barriers to the use of mHealth apps in diabetes management

Despite the mHealth apps may be a useful tools for diabetes management, to date their use presents several barriers.

The main barriers to the mHealth apps in diabetes management are represented by:

costs, few clinical evidences, absence of evaluations in specific populations, data protection and data security, regulatory requirements.

1) *Costs*. Most medical applications on market are paid. Moreover, the use of mobile apps requires additional costs for smartphone or other technological devices and internet services. The internet access in some rural areas or in developing countries could be very difficult. Therefore, although the availability of medical apps may facilitate communication, mainly in certain areas, the relative costs are not certain to justify its adoption [61, 78].

2) *Few clinical evidences*. As previously reported, only few studies evaluating the effectiveness of mobile medical apps have demonstrated their clinical significance with a robust evidence on glycaemic control, assessed by HbA1c. In fact, as highlighted most of the studies were underpowered with a short duration and small simple size. Therefore, large clinical randomized trials with proper power and longer follow-up to establish safety, efficacy, and cost-

effectiveness of patients self-management through apps adoption are needed [76].

3) *Absence of evaluations in specific populations*. Educational level, socio-economic status and clinical conditions may influence the effectiveness of medical apps use. In this regard, most of the available apps may not be useful for the elderly, non-English speakers, disabled with visual or auditory impairments, and subjects from a lower socio-economic status [78, 79].

4) *Data protection and security*. Most of the diabetes technological devices work with a wireless communication, which could be intercepted by electromagnetic devices or hacked by cyber attackers, creating potential risks for users. Furthermore, there are many controversial on who is the owner of the data recorded. This rise the question if the owner of data management is the patient using it or the software owner [61, 80].

5) *Regulatory requirements*. FDA or CE certifications and approvals are an important issue for the safely and proper use of diabetes technological tools. In 2015, FDA guidance stated: "When the intended use of a mobile app is for the diagnosis of disease or other conditions, or the cure, mitigation, treatment, or prevention of disease, or is intended to affect the structure or any function of the

body of man, the mobile app is a device". Similarly, the EU have reported the rules for medical devices [61].

Project Research 2: *The use of an automated bolus calculator and wirelessly communicated blood glucose measurement can improve patients' compliance to insulin therapy in T1D patients*

CHAPTER 5

5.1 Introduction

The results of DCCT have widely demonstrated that the benefits of short and long-term intensive insulin therapy in T1D patients can only be obtained with an effective self-management by diabetic patients.

Moreover, as reported in DCTT, carbohydrates counting is essential to achieve treatment goals [12].

Despite there are several evidences to support carbohydrates counting effectiveness, empirical calculation remains not easy and time-consuming for most diabetic patients, thus reducing their ability to achieve an optimal glycaemic control [41, 81, 82].

In fact, as previously stated, less of 30% of T1D patients achieve the glycaemic target of HbA1c <7.5% and most patients are not able to calculate correctly pre-meal insulin doses [83].

Therefore, to encourage T1D patient's self-management promoting pre meal insulin doses adjustments, many Automated Bolus Calculators (ABCs) are been developed in the last years [84].

Otherwise, the DCCT results showed that an effective self-management by diabetic patients and frequent contacts with health care professionals are needed [12].

On this basis, in the two past decades the new technologies has been aimed to diabetes management and especially to the improvement of patients' quality of life, sustaining them in their daily activities and increasing their adherence to healthcare process [35]. Thus, the use of telemedicine systems offers the opportunity of easier and immediate communications between diabetological team and patient, optimizing the use of data collected by SMBG and facilitating educational interventions. As well know, glucose monitoring through telemedicine systems seems to improve patients' quality of life as well as the relationship with the healthcare providers [63, 85, 86].

In fact, to obtain optimal glycaemic control, adjustments of insulin dose at meal times must be made by taking into account several parameters as current blood glucose levels, target glucose levels, the carbohydrate meals content, the insulin/carbohydrate ratio, insulin sensitivity factor and insulin on board [36].

While the newest technologies using Bluetooth and Wireless connectivity allow entry of glucose data from meters into electronic logbooks, promoting the sharing and remote revision process with a health care professional, only 31% of adults routinely download their capillary glucose testing results for review and only 56% of caregivers reported ever downloading data from one or more devices [87].

Accu-Chek® Aviva Connect, a bolus calculator system integrated in a wireless meter, smartphone app and using an online portal (https://www.accu-chek.com/integrated-systems/connect-system) has been developed for the establishment of the insulin dose to be administer, takes into account all parameters listed above (**FIG 9**).

FIG. 9: *The Accu-Chek® Aviva Connect system*

5.2 Aim of the study

Aim of this randomised trial was to evaluate the efficacy and safely of an ABC integrated in a glucose meter working with a wireless communication on glycaemic control assessed by HbA1c and patients

compliance to SMBG, with a telemedicine-diabetes management system.

5.3 Materials and Methods

The study was designed as a 24 weeks, randomized, parallel, two-arms, controlled trial.

T1D patients 18–65 years old, on MDI and with disease duration more than 1 year, attending the diabetes outpatient clinic at the University Campus Bio-Medico in Rome, Italy were asked to participate in the study.

T1D was defined according to the American Diabetes Association classification, and all patients gave their consent to participate. Exclusion criteria were learning disabilities or the presence of chronic conditions potentially able to influence daily activities (visual or auditory disability or other inabilities to use technological devices). T1D patients affected by severe diabetes complications were also excluded.

The study received the approval by the local ethics committee.

T1D patients were evaluated for eligibility during regular outpatients visit.

Accu-Chek® Aviva Connect was given to all participants and its instructions were given.

After 2-week run-in-period patients were randomly assigned to one of two arms: the treated group using this ABC with bolus calculator and data transmission by App on a Smartphone activated or the control

group with bolus advisor turned off and on standard education for insulin management.

A total of 24 adults T1D enrolled in the study were trained to target blood glucose and CHO counting, I/CHO ratio and ISF were determined for all patients, individually, by the physicians.

Moreover, all patients received hands-on session on Accu-Chek® Aviva Connect use. All subjects were followed up with visits every 3 months and with telephone consultations. During each clinical visit adherence to the use of CHO counting was also verified for each patient.

The primary end-points were HbA1c, measured in the local hospital laboratory and patients compliance, assessed as average number of daily measurements and as total measurements.

As secondary end-points the number of hypoglycaemic events and the total results above target range were evaluated at entry into the trial and at 3 and 6 months follow-up.

HbA1c (%), daily insulin requirement (IR), and body mass index (BMI) were evaluated at entry into the study and at 3- and 6-month follow-ups. The mean daily insulin requirement was expressed as units/kg/day, and BMI was calculated as the ratio of weight (in kg)/(height [in m])^2.

Statistics

Setting a (probability of type I error) equal to 0.05 and b (probability of type 2 error) equal to 80%, for a difference of HbA1c of 0.5% with 0.4% SD at the end of the study period, the required sample size was 24 patients for a two-sided test. To ensure the appropriate sample size, 30 patients were recruited to allow six dropouts.

Paired t test (two tailed) and analysis of variance were used to evaluate differences in HbA1c, IR, and BMI between the two groups. Data were expressed as mean ±SD values. A P value of <0.05 was considered statistically significant.

5.4 Results

The comparison of baseline demographic and clinical features indicates that the two groups of patients did not differ in clinical and metabolic parameters (**Table 7**). Level of physical activity was similar between the two groups, and no major differences were reported in socioeconomic status or level of education between the groups.

Table 7: *Demographic and clinical features of T1D patients*

	Carbs Counting (Treated Group)	No Carbs Counting (Control Group)	All Patients	P-value
Age range (years) (mean ± SD)	36.62±9.39	37.55±7.15	37.04±8.27	0.7905
Disease duration (years) (mean ± SD)	16.15±14.26	12.91±9.25	14.67±12.09	0.5243
Gender				
Female	7/13	6/11	13/24	0.9727
Male	6/13	5/11	11/24	
HbA1c (%) (mean ± SD)	7.65±0.87	7.55±0.98	7.6±0.9	0.7766
BMI (Kg/m^2) (mean ± SD)	23.2±3.28	22.98±1.98	23.1±2.71	0.8491
Daily insulin requirement (IU/kg/day) (mean ± SD)	0.45±0.23	0.58±0.17	0.51±0.21	0.1570

HbA1c at entry was 7.65% ±0.87 (SD) in patients using bolus advisor system with bolus calculator and data transmission by App on a Smartphone activated and 7.55% ± 0.98 (SD) in the control group with bolus advisor turned off and on standard education for insulin management (p:NS).

After 3 months follow-up there was a tendency for an improvement in HbA1c levels in the bolus advisor system treated group vs. control group (7.49%±1.04 vs. 7.99%±2.04, respectively, p:NS). Taking into account the 6 months period of observation, a statistically significant reduction in HbA1c levels was observed in the bolus advisor system treated group vs. control subjects (7.32%±0.82 vs. 8.32±1.38 P=0.04) (**FIG. 10**).

A major compliance to SMBG assessed as mean number of daily measurements (P=0.03) and as total of the measurements for each quarter (P=0.02) was observed in bolus advisor system treated group compared to the control group (**Table 8**).

FIG 10: *Hemoglobin A1c (HbA1c) values at entry (time 0) and after 3- and 6-month follow-ups in the two groups*

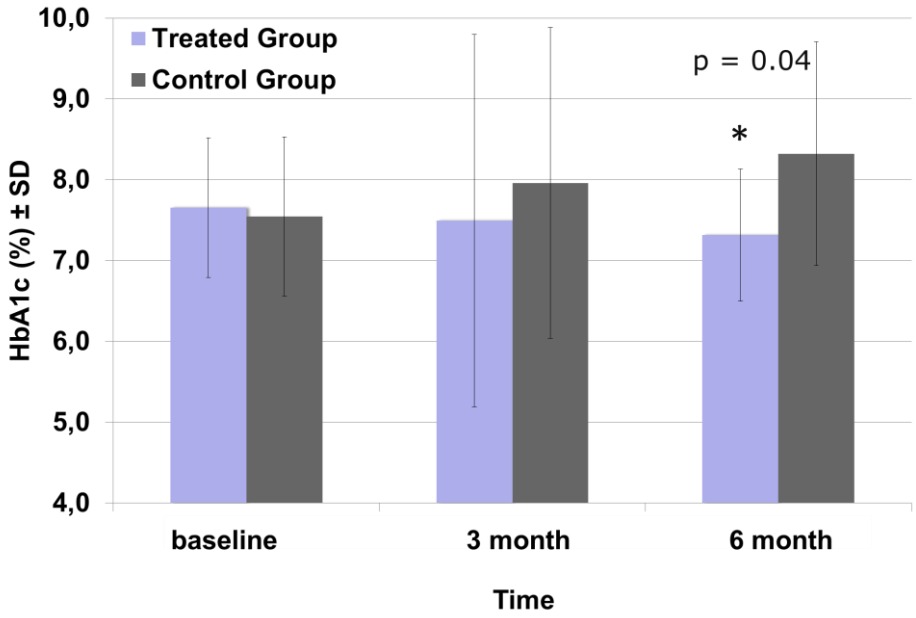


Table 8: *Summary results*

Variable	Visit	Difference	Lower 95% CL	Upper 95% CL	P-Value
HbA1c	0-3 Months	0,527	-0,583	1,637	0,3360
	3-6 Months	1,008	0,258	1,759	**0,04**
BMI	0-3 Months	-0,122	-0,608	0,364	0,6050
	3-6 Months	0,580	-0,242	1,403	0,1564
Insulin Requirement	0-3 Months	0,047	-0,060	0,154	0,3674
	3-6 Months	0,066	-0,034	0,165	0,1823
Average Glucose Values (mg/dL)	0-3 Months	3,224	-32,690	39,139	0,8518
	3-6 Months	-3,005	-25,458	19,447	0,7812
SD Average Glucose Values	0-3 Months	1,520	-11,397	14,436	0,8063
	3-6 Months	0,143	-13,988	14,274	0,9833
Compliance SMBG - Average Number Daily Measurements	0-3 Months	-0,942	-2,125	0,241	0,1116
	3-6 Months	-1,765	-3,428	-0,102	**0,0387**
Total Measurements	0-3 Months	0,853	0,472	1,543	0,5800
	3-6 Months	0,396	0,178	0,880	**0,0253**
Number of Hypoglycaemic Events	0-3 Months	1,413	0,620	3,221	0,3890
	3-6 Months	0,791	0,238	2,622	0,6846
Total Results Above Target Range	0-3 Months	1,232	0,511	2,970	0,6254
	3-6 Months	0,532	0,205	1,381	0,1826

5.5 Discussion

The results of the study demonstrated that this bolus advisor system is a friendly wirelessly meter that helps T1D patients on MDI to improve their glycaemic control, with the achievement of glycaemic targets and the improvement of compliance to SMBG.

These findings are consistent with our previous study showing an improvement of HbA1c in T1D patients using an ABC system [66].

Furthermore, in this study the improvement of glycaemic control was at least partially due to the major compliance to SMBG how showed by the significant increase of daily and total fingerstick measurements in group patients using the telemedicine system.

In fact, patients using the ABC combined to the "real-time" data transmission to the diabetes center by the smartphone app showed a major compliance to SMBG compared to the subjects who empirically estimated their insulin needs and not sharing data [66].

Our results confirm that SMBG patients' compliance is crucial for an effective T1D management as showed by the DCTT trial in which the frequency of SMBG was directly related with lower HbA1c levels [12, 88, 89].

The positive relation between frequency of SMBG and glycaemic control has been also established in the T1D exchange registry showing that patients in the excellent control group (HbA1c <6.5%) performed blood glucose measurement more frequently compared to the poorly control group (≥8.5%) (72% vs. 36% reporting frequency ≥5 times/day) respectively [88].

However, in order to be successful, SMBG should be performed in a structured format, interpreted and then utilized to make appropriate treatment adjustments and timely and effective therapeutic choices [90].

Although patients' satisfaction has not been specifically evaluated in the study, these results on patients compliance to SMBG provide a support indicating that they perceived this tool to be effective and user-friendly.

Despite the application of the new technologies with the use of telemedicine systems and diabetes diary apps can improve T1D self-management, the benefits evaluation of each component is more challenging [91].

For example, although the results of some studies have showed the benefit of CHO counting to the correct mealtime insulin doses administration by the use of mobile apps, its real effectiveness could be likely confounded by other elements, such as the glycaemic index of the meal, the concomitant fat-protein intake and mainly by the patients ability to perform a proper CHO counting [45, 92, 93].

Furthermore, to perform an effective CHO counting, it is important that health care providers and their patients periodically perform comprehensive assessments to ensure the accuracy of insulin parameters and to make adjustments if needed.

On the other hand, the glucose-recording apps have some limitations.

In fact, as documented by Huckvale et colleagues, 67% (n = 31/46) of bolus-calculating apps carried a risk of inappropriate output dose recommendation that either violated basic clinical assumptions (48%, n = 22/46) or did not match a stated formula (14%, n = 3/21) or did not correctly update in response to user input changes (37%, n = 17/46) [94].

Additionally, to achieve successful outcomes, not only diabetic patients have to show knowledge and skills for the correct application of these technologies in their self-management but also health care

professionals must be familiar with these innovative ways for patients treatment [35].

Certainly, in our study the continuous collaborative relationship between patients and health care providers through the sharing of glucose data recorded on an online portal has been an essential component that has facilitated the outcomes achievement.

Nevertheless our study has some potential limitation as the small simple size and the lack of a long-term follow-up of observation.

Although the use of this device may have facilitated review of the data recorded by the health care team, it is not certain whether diabetic patients have in turn reviewed the numbers of their own, accordingly.

Finally, some of the benefits can be simply related to the participation in a clinical trial.

As stated, empirically calculation of insulin meal-time dosage is often argue and time-consuming, and it could provide dosing errors that can limit the achievement of glycaemic targets [95].

Our results suggest that this bolus advisor system is a friendly wirelessly meter that helps T1D patients on MDI to improve glycaemic control with the achievement of treatment targets and the improvement of their compliance to SMBG, by the use of a telemedicine system.

RESEARCH PROJECT 3: *The use of automated bolus calculator and wirelessly communicated blood glucose measurement for the control of glucose variability in T1D patients*

CHAPTER 6

SPECIFIC BACKGROUND:

Glucose Variability and CGM use

6.1 Introduction

The main target of diabetes management should focus on achieving near-normoglycaemic values reducing the risk of hypoglycaemia, thereby minimizing glucose variability (GV). Several studies have showed that glucose variability may be an additional risk factor for long-term diabetes complications [96, 97].

The three clinical parameters related to the long-term diabetic complications are glycated haemoglobin (HbA1c), fasting plasma glucose (FPG) and postprandial glucose (PPG).

In addition to this "glucose triad", the acute glucose fluctuations are involved in the pathophysiological mechanisms of diabetic complications [97, 98].

As clearly demonstrated in DCCT study there is a strong association between glycaemic exposure and the risk of microvascular complication [12].

Similarly, the effect of glycaemic excursions on ROS levels has been widely investigated in T2D [99, 100].

Of note, the study carried out by Monnier et al. in which it was found that glucose excursions are potent activators of oxidative stress, including in T2 diabetic patients not on insulin therapy. It was showed a positive correlation between glucose excursions evaluated by the Mean Amplitude Glucose Excursions (MAGE) and the 24-hour urinary excretion of isoprostanes (8-iso-PGF2α), which is considered a good marker of oxidative stress [101].

Also Ceriello et al. confirmed the importance of acute hyperglycaemic spikes in the physiopathology of diabetic complication, demonstrating that glycaemic spikes are more damaging to the endothelial function, assessed by low-mediated dilatation of the brachial artery (FMD), than sustained chronic hyperglycaemia [102].

Instead, Chang et al. found that both short- or long-term glycaemic excursions could induce oxidative stress and chronic inflammation in T2D [103].

Finally, GV has been considered as a predictor of severe hypoglycaemia because this condition is preceded by glucose disturbances, and several studies reported a decrease of hypoglycaemic events coinciding with lower GV [104-106].

These evidences therefore rise the question of whether we have appropriate tools for GV monitoring and management in clinical practice [107].

SMBG is recommended as a core component of diabetic patient's management but it can provide only intermittent snapshots of blood glucose levels missing often hyperglycaemic or hypoglycaemic excursions [21, 22].

Similarly HbA1c alone is unable to provide detailed diagnostic information and it has several limitations [21, 108].

In the modern diabetes monitoring, CGM could be considered as a third pillar, since it provides information on day-to-day change of blood glucose levels and helps achieving treatment targets without increasing the risk of hypoglycaemia [21].

Therefore the use of CGM may reduce GV, improving glycaemic control and decreasing the risk for long-term diabetes complications [88].

In fact the availability of continuous glucose data for patients with low and high glucose alerts may impact quality of life with short- and long-term effectiveness. Moreover CGM can be used as a powerful motivational device to change T1D patients' lifestyle and to improve their quality of life. Although studies on the economic feasibility to use CGM must be carried out, it can be considered as a powerful motivational device to improve glycaemic control and to control GV [109].

6.2 Pathophysiology of glycaemic variability

As well known, onset and progression of microvascular and macrovascular long-diabetes complications are related to glycaemic excursions and to "dysglycaemia" status.

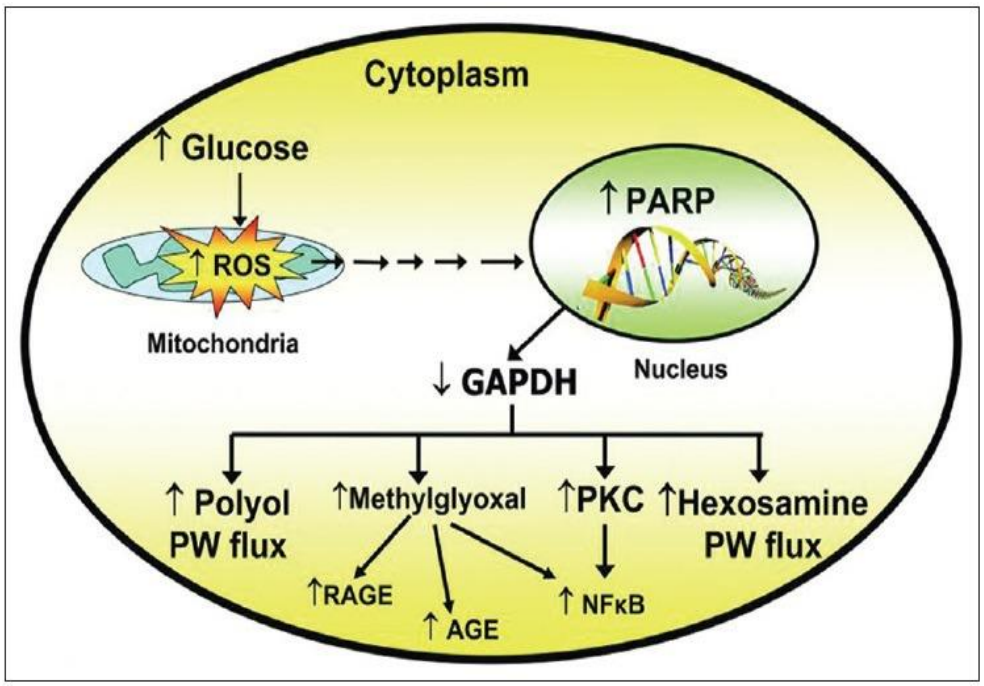
The two primary pathophysiological mechanisms involved in the glycaemic variability are the excessive protein glycation end products and the activation of oxidative stress, which causes vascular damage [110].

Experimental studies widely showed that intermittent glycaemic spikes have more deleterious effects rather than constant exposure to hyperglycaemia [111].

In fact it was found that glucose fluctuations induce oxidative stress and endothelial dysfunction as a result from the overproduction of reactive oxygen species (ROS) by the mitochondrial electron-transport chain [112].

Therefore, this process seems to be the main mechanism of glucose-mediated vascular damage involved in diabetic long-term complications (**FIG. 11**).

FIG 11: *Pathophysiological mechanisms of glucose-mediated vascular damage*



From the overproduction of superoxide by the mitochondrial electron-transfer chain results the activation of the other intracellular pathways as polyol activity, production of advanced glycation end products, activation of protein kinase C (PKC) and nuclear factor- κB and increased of hexosamine pathway flux. All these pathways are able to induce the production of intracellular reactive oxygen species (ROS), causing defective angiogenesis in response to an ischemic damage, activating a number of proinflammatory pathways and promoting long-lasting epigenetic changes that lead to persistent expression of

proinflammatory genes also when normoglycaemia is reached ("hyperglycaemic memory") [110, 113].

Experimental studies in vitro and in animal models have widely showed that these mechanisms, induced by GV and postprandial glucose excursions mainly, are strictly associated with the activation of oxidative stress [101, 114].

Consequently, the hyperglycaemia exposure which induces several endothelial dysfunction by the activation of oxidative stress, is strictly related to cardiovascular disease (CVD) [102, 115, 116].

Furthermore, GV has been considered as a predictor of severe hypoglycaemia because this condition is preceded by glucose disturbances.

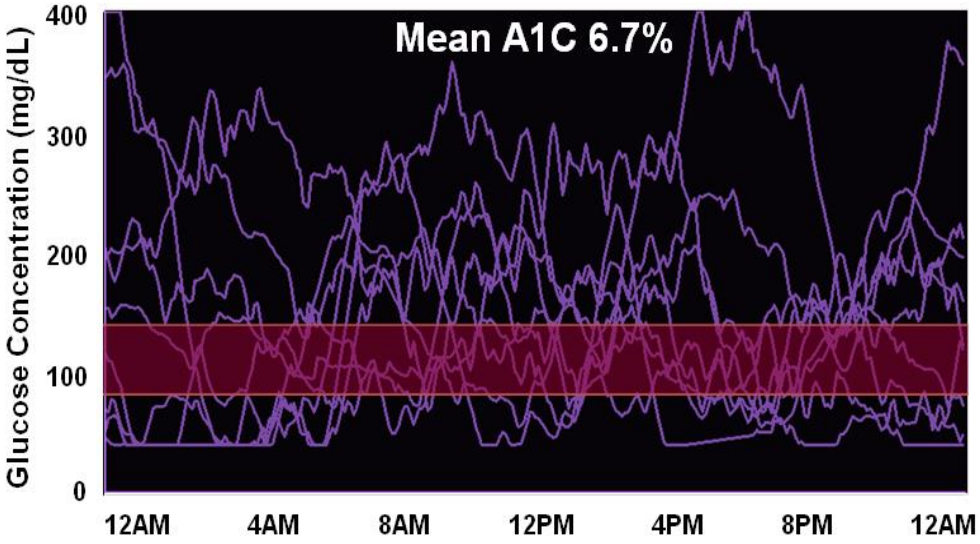
In fact several studies have reported that lower GV was related to the decreased frequency of hypoglycaemic events [117-119].

Moreover, it was found that hypoglycaemia induces in turn an increased platelet and neutrophil activation and the release of inflammatory cytokines, rising the overall vascular damage [120].

6.3 Measurement of glycaemic variability

As previously stated, despite several studies showed that HbA1c levels are directly related to the development and progression of micro- and macrovascular diabetes complications, HbA1c alone incompletely explains GV, mainly if patients show an HbA1c levels in normal range, as reported in **Fig. 11** [98, 121].

FIG. 11: *Glycaemic variability in patients with HbA1c in target range*



In fact, PPG contributing to individual GV in diabetic subjects is also implicated. PPG is the major determinant of overall glycaemic control as expressed by HbA1c in diabetic patients who exhibit HbA1c levels below 7–7.5%, while the contribution of FPG is most important with worsening of glycaemic control [99, 122].

Moreover a growing body of evidence suggests that PPG is an independent risk factor for myocardial infarction, inducing endothelial dysfunction and oxidative stress activation in diabetic subjects [123].

Furthermore, these authors demonstrated that the reduction of hyperglycaemic fluctuations is accompanied to the reduction of some oxidative stress markers [124].

On this basis, a large number of different indices are currently used to assess GV [107].

Despite many glucose variability indices have been proposed, the gold-standard method for the assessment of GV is lacking (**Table 7**) [125].

Table 7: *Main indices for GV evaluation*

Index	*Note*
MAGE	Average size of glycaemic excursions (MAGE-up: from nadir to peaks, indicating hyperglycaemic fluctations; MAGE-down: from peaks to nadir, indicating hypoglycaemic fluctations)
MODD	Evaluates inter-day variability
CONGAn	Intraday glycaemic swings occurring over prederminated intervals (CONGA-1: 1h interval; CONGA-2: 2h interval; CONGA-4: 4h interval)
BG rate	Allows to measure "velocity" of glycemic swings
ADRR	Average Daily Risk Range (based on SMBG data)
LBGI	Index of frequency and severity of hypoglycemias (based on SMBG data)
HBGI	Index of frequency and severity of hyperglycemias (based on SMBG data)
Standard Deviation	Variation or dispersion from the average
Coefficient of Variation	Normalized measure of dispersion from the average
AUC PP	Post prandial AUC calculated from the preprandial glucose levels to the highest peak over 2h period after meal

MAGE- Mean amplitude of glycaemic excursions, MODD- Mean of daily differences, CONGA- Continuous overall net glycaemic action, BG- Blood Glucose, ADRR- Average daily risk range, LBGI- Low blood glucose index, HBGI- High blood glucose index, AUC PP- Post prandial Area under the curve.

However, the most sophisticated GV indices cannot be obtained by SMBG data, thus CGM systems are currently used as adjuvant tool to improve the overall glycaemic control, assessing GV in clinical practice [126].

6.4 Effectiveness of CGM

The effectiveness of CGM in the management of T1D patients is well known, as indicated in the clinical practice guidelines of the Endocrinology Society [28].

In fact CGM allows T1D patients to monitor their glucose levels without the need of repeated fingersticks testing.

Moreover, CGM system can provide real-time readings to the wearer, which allows for an immediate therapeutic choice, such as the administration of a supplemental dose of insulin.

Several studies have demonstrated the improvement of glycaemic control in patients with T1D even when CGM was added to CSII therapy. Similar results were showed by the data from the large studies carried-out on children or and aT1D either on CSII or on MDI [109].

The Juvenile Diabetes Research Foundation (JDRF) in a 26 weeks randomized study involving 322 adults, adolescents, and children with T1D divided in three age groups (8–14 years, 15–24 years, 25 years and older) demonstrated that the use of CGM significantly improved HbA1c by 0.5% in adult patients, without any increase in hypoglycaemia.

Nevertheless, the younger patients (<25 years) did not show significant improvement, which was likely related to <50% adherence in these group [127].

Accordingly, Battelino et al. in a study on 120 well-controlled T1D patients showed a significant reduction of HbA1c in the CGM group

(HbA1c from 6.9% to 6.69%, P=0.008) compared to control subjects after 26 weeks [128].

The GOLD study, a randomized crossover study using Dexcom G4 Platinum, reported a significant decline of HbA1c in T1D patients using CGM compared to patients on SMBG (7.92 [0.8] vs. 8.35 [0.9]) with a great compliance to CGM treatment (the overall meantime of CGM use was 87.8%) [129].

The DIAMOND study was a 24 weeks two-arm randomized controlled trial on T1D patients on MDI, poorly controlled (HbA1c levels between 7.5% and 9.9%) evaluating CGM (Dexcom G4 Platinum) compared to SMBG.

Mean HbA1c decline from baseline was 1.1% at 12 weeks, 1.0% at 24 weeks in the CGM group, 0.5%, and 0.4%, respectively, in the control group (repeated-measures model $P < 0.001$). In the CGM group, full adherence to CGM was 93% in 6 months [130].

Recently, the results of REPLACE-BG study demonstrated that CGM data could be used to make safe treatment decisions, showing no difference at all in those using CGM alone for decision making versus those using a confirmatory SMBG reading. Mean time in 70–180 mg/dL was 63% – 13% at both baseline and 26 weeks in the CGM-only group and 65% – 13% and 65% – 11% in the CGM+SMBG group.

No severe hypoglycaemic events occurred in the CGM-only group, and one occurred in the CGM+SMBG group [131].

In 2016, Bolinder et al. published the results of IMPACT study, a large randomized clinical study, showing that patients who were randomized

to flash CGM for glucose obtained flash glucose monitoring glucose sensor measurements about 15 times per day compared to control subjects who monitored via SMBG on average 5.6 times per day [132].

Other outcomes showed after 6 months a reduction of time spent in hypoglycaemia by 38% dropping by 1.30 h/day from 3.38 h/day at baseline to 2.03 h/day compared with no significant changes in the control group.

Furthermore, it was found an improvement in the GV indices with no significant changes in HbA1 between the two groups [132].

Accordingly, in a study carried-out by Dover et al., mean HbA1c decreased from 8.0% – 0.14% to 7.5% – 0.14% after a 16 week period of flash CGM use in T1D [133].

Ish-Shalom et al. also showed an HbA1c decrease of 1.33% – 0.29% after 8 weeks of flash CGM use in a mixed population of people with T1 and T2D poorly controlled.

McKnight reported a significant change in HbA1c among flash CGM users versus nonusers with T1D in Scotland (-0.2% versus +0.1%, respectively) [134].

Although these clinical evidences, the different CGM systems now available have some limitations as accuracy and adherence. About 25% of CGM users report that accuracy is a major reason for discontinuing its use.

Accuracy is measured using the mean absolute relative differences (MARDs) between CGM and SMBG readings, respectively.

Early CGM devices had quite a high error rate with MARDs of around 20% reported; however, with advances in sensor technology, these rates are now as low as between 9% and 14%. The MARD of the flash glucose monitoring system is 11.4% and that of the latest Dexcom G5 system is just under 10%.

Contributing factors to this inaccuracy, that is sometimes observed, include the physiological lag time, which exists between interstitial and blood glucose. This lag time is between 4 and 10 min and can be longer when glucose concentrations are changing rapidly [135].

In addition, when the descent of glucose levels is rapid on approaching hypoglycaemic levels, sensor glucose can be higher than blood glucose, resulting in false reassurance.

Regarding to the adherence IMPACT, DIAMOND, and Gold studies have all shown that >80% compliance was required to receive optimum benefit with all major available systems.

The cost of CGM also poses a barrier to its use.

Despite the obvious advantages these devices may have, they are not widely available for use by diabetic patients.

Cost restrictions by most national health systems mean that CGM is funded only if a patient meets selected criteria, as reported in **Table 8**.

Table 8: *Minimum criteria for CGM reimbursement*

- Patients with T1D not meeting HbA1c targets or recurrent diabetic ketoacidosis
- Patient with repeated hypoglycemic episodes or hypoglycemia unawareness
- Subjects requiring better glycemic control while avoiding hypoglycemia
- Before or during pregnancy in women with T1DM or T2DM
- Need for improving brittle diabetes

Project Research 3: *The use of automated bolus calculator and wirelessly communicated blood glucose measurement for the control of glucose variability in T1D patients*

CHAPTER 7

7.1 Introduction

Intensive insulin therapy, based on MDI is the gold standard treatment for T1D.

The effectiveness of intensive insulin therapy in T1D has been reported in DCCT, showing that optimal glycaemic control is directly related to the reduction of incidence and severity of diabetes complications [12].

Achieving treatment goals should be obtained by maintaining flexibility to fit individual lifestyle and without major variations in eating behaviours and physical activity [136].

Diet, physical activity and insulin dosage play a key role in the management of insulin treatment in T1D patients.

Diet, in particular CHO meal content, is the main determinant of a rise in PPG [137].

The contribution of post-prandial excursions to GV well known [99].

Several studies have suggested that GV may be an additional risk factor for the long-term diabetes complications [122-124].

Epidemiological evidence from non-diabetic adults has shown that blood glucose level 2-h after a glucose challenge is predictive of both development of cardiovascular disease and mortality [138].

Similarly, in subjects with T2D, there is evidence that PPG is an independent risk factor for myocardial infarction, inducing endothelial dysfunction and oxidative stress generation [123].

The DCCT clearly established a continuous relationship between glycaemic exposure and the risk of microvascular complications [12].

In fact the HbA1c alone is insufficient to explain the onset of complications and PPG also implicated.

It is well-established that the pathophysiology of diabetic complications involves hyperglycaemia-induced oxidative stress and excessive glycation.

In fact, the glycaemic excursions related to PPG lead overproduction of reactive oxygen radicals (ROS) inducing endothelial dysfunction and expression of inflammatory genes [99].

Thus a multitude of parameters to express GV have been described and new treatment strategies are increasingly focusing on reducing post-prandial glycaemic excursions as well as HbA1c levels to reduce the risk for long-term complications [139].

Therefore, to achieve post-prandial glycaemic targets the establishment of insulin dose at meal times must be made before each injection, while considering certain parameters, such as target range, insulin sensitivity, insulin-carbohydrate ratio, duration and insulin on board.

Accordingly an appropriate teaching and training program on CHO counting and specific instructions for the administration of insulin doses need to be implemented in patients with diabetes.

In fact, CHO counting as a meal planning approach offers the flexibility of food choices and allows achieving post-prandial glycaemic goals [32, 140].

On this basis, an ABC (Accu-Chek® Aviva Connect), a bolus calculator system integrated in a wireless meter, working with a smartphone app and online portal (https://www.accu-chek.com/integrated-systems/connect-system) has been developed for the establishment of pre-meal insulin doses, takes into account several parameters as target of blood glucose, current blood glucose level, insulin/carbohydrate ratio, insulin sensitivity factor, carbohydrates intake and insulin on board. In our previous randomized trial, we evaluated 6-months efficacy of this ABC on glycaemic control and patients compliance to SMBG, through a telemedicine system.

In a total of 24 T1D patients we have observed a significant reduction of HbA1c in ABC treated group vs. control group (7.32%±0.82 vs. 8.32±1.38 P=0.04) with major compliance to SMBG assessed as mean number of daily measurements (P=0.03) and as total of the measurements for each quarter (P=0.02) (see **Table 9**).

Table 9: *Previous results from SMBG*

PREVIOUS RESULTS FROM SMBG (24 patients)					
Variable	**Visit**	**Difference**	**Lower 95% CL**	**Upper 95% CL**	**P-Value**
HbA1c	0-3 Months	0,527	-0,583	1,637	0,3360
	3-6 Months	1,008	0,258	1,759	**0,04**
BMI	0-3 Months	-0,122	-0,608	0,364	0,6050
	3-6 Months	0,580	-0,242	1,403	0,1564
Insulin Requirement	0-3 Months	0,047	-0,060	0,154	0,3674
	3-6 Months	0,066	-0,034	0,165	0,1823
Average Glucose Values (mg/dL)	0-3 Months	3,224	-32,690	39,139	0,8518
	3-6 Months	-3,005	-25,458	19,447	0,7812
SD Average Glucose Values	0-3 Months	1,520	-11,397	14,436	0,8063
	3-6 Months	0,143	-13,988	14,274	0,9833
Compliance SMBG - Average Number Daily Measurements	0-3 Months	-0,942	-2,125	0,241	0,1116
	3-6 Months	-1,765	-3,428	-0,102	**0,0387**
Total Measurements	0-3 Months	0,853	0,472	1,543	0,5800
	3-6 Months	0,396	0,178	0,880	**0,0253**
Number of Hypoglycaemic Events	0-3 Months	1,413	0,620	3,221	0,3890
	3-6 Months	0,791	0,238	2,622	0,6846
Total Results Above Target ange	0-3 Months	1,232	0,511	2,970	0,6254
	3-6 Months	0,532	0,205	1,381	0,1826

7.2 Aim of the study

The aim of the study was to investigate the efficacy of this ABC, integrated in a glucose meter and working with a wireless communication, on GV assessed by different indices evaluated by CGM.

7.3 Materials and methods

CGM data for GV assessment have been obtained from a subgroup of 11 T1D patients on MDI recruited in the study previously reported (Chapter 5).

The study was approved by the local ethics committee and all patients gave their informed consent.

Inclusion criteria were T1D for > 1 year, adults 18 years or older, current use of MDI, structured education completed, HbA1c range: 7-9 %.

Exclusion criteria have included any previous or ongoing illness that, in the opinion of the investigator, could have prevented the subjects from completing the study, pregnancy or planned pregnancy for the study duration, learning disabilities or presence of chronic conditions potentially able to influence daily activities (visual or auditory disability, motor impairment for neurological or orthopaedic problems), required continuous use of paracetamol, (paracetamol must not be used in the week before and during the period of CGM-use because it may affect the interpretation of blood glucose reading from Dexcom G4). Subjects with history of allergic reaction to any of CGM materials, those with abnormal skin in the site of sensor attachment (excessive hair, inflammation, infection, rash, tattoo), patients uncomfortable for the CGM use, were also excluded.

Changes in glucose profile were evaluated using masked CGM data collected over 7-day periods at baseline and 6 months after

randomization in 11 diabetic patients (6 for ABC and 5 for control group, respectively).

Patients recruited in the treated group used this ABC with bolus calculator and data transmission by App on a Smartphone activated while patients in the control group performed their SMBG with the bolus advisor turned off and with standard education for insulin dosage management.

All patients received proper education on diabetic self-management, basic skills for insulin adjustments and they were also educated to Dexcom G4 system use.

Glucose variability was assessed from CGM data (Dexcom G4 PLATINUM, Dexcom Inc, San Diego, CA) with the measurement of Average glucose (AG), Standard Deviation (SD), MAGE, CONGA-1 -2 -4, time spent in hypo- hyper-glycaemia and in the normal range at baseline and after 6 months.

Patients compliance was evaluated as average number of daily measurements at entry into the trial and at 6 months follow-up.

Statistics

Differences in outcomes were evaluated by using Kruskal Wallis Test (non parametric ANOVA).

Continuous variables were compared using unpaired T test and analysis of variance was also used to evaluate differences in HbA1c and in the glucose variability indices between the two groups. Data were expressed as mean ±SD values.

No normally distributed variables were evaluated using Mann-Whitney U Test.

A simple regression analysis was performed to assess independent predictors of HbA1c and glucose variability indicators.

A Pearson's correlation analysis was performed to identify any significant correlation between glucose parameters (AG, SD, % of time spent in hypo- hyper- and normal range, MAGE, CONGA-n, HbA1c and the average number of daily measurements).

A P value of <0.05 was considered statistically significant.

Being a pilot study for the assessment of glucose variability in a subgroup of T1D patients the power calculation was not performed.

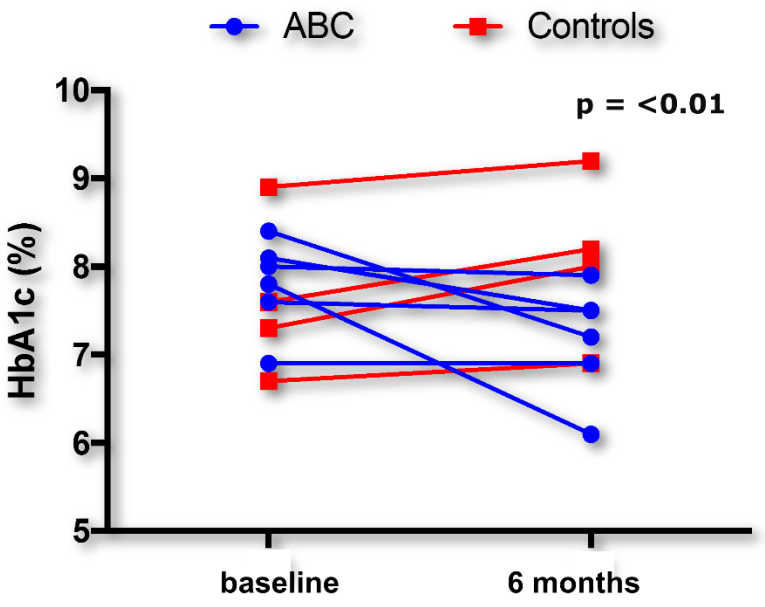
7.4 Results

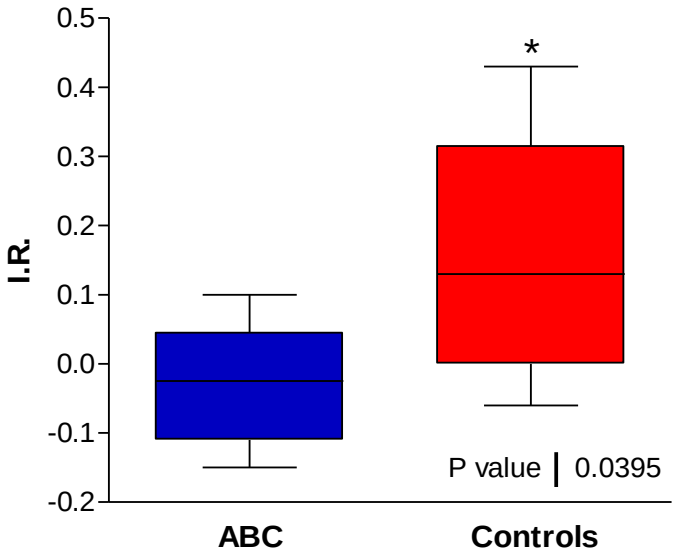
The comparison at baseline indicates that the two groups of patients did not differ in clinical and glycaemic parameters.

After 6 months HbA1c decreased from 7.8± 0.51% to 7.18±0.62% in patients using this bolus advisor system with bolus calculator and data transmission by App on a Smartphone activated and 7.55% ± 0.98 (SD) in the control group with bolus advisor turned off and on standard education for insulin management and increased from 7.6±0.92% to 8.0±0.94% in control group (P <0.01 and P=0.52, respectively), with significant difference between the two groups (P <0.01) (**FIG. 12**).

Moreover, it was found a significant reduction on insulin requirement (I.R.) between the two groups.

FIG. 12: *Results on HbA1c and I.R.*





Regarding to glycaemic parameters derived from CGM evaluation, data for one subject in control group are missing from the analysis due to CGM failure.

Specifically to glycaemic variability indices, no significant differences on AG, SD, % of time spent in hyperglycaemia and normal range, MAGE, CONGA-1 , -2, -4 were found (**Fig. 13, Fig. 14**)

Fig. 13: *Results on glycaemic variability indices (1)*

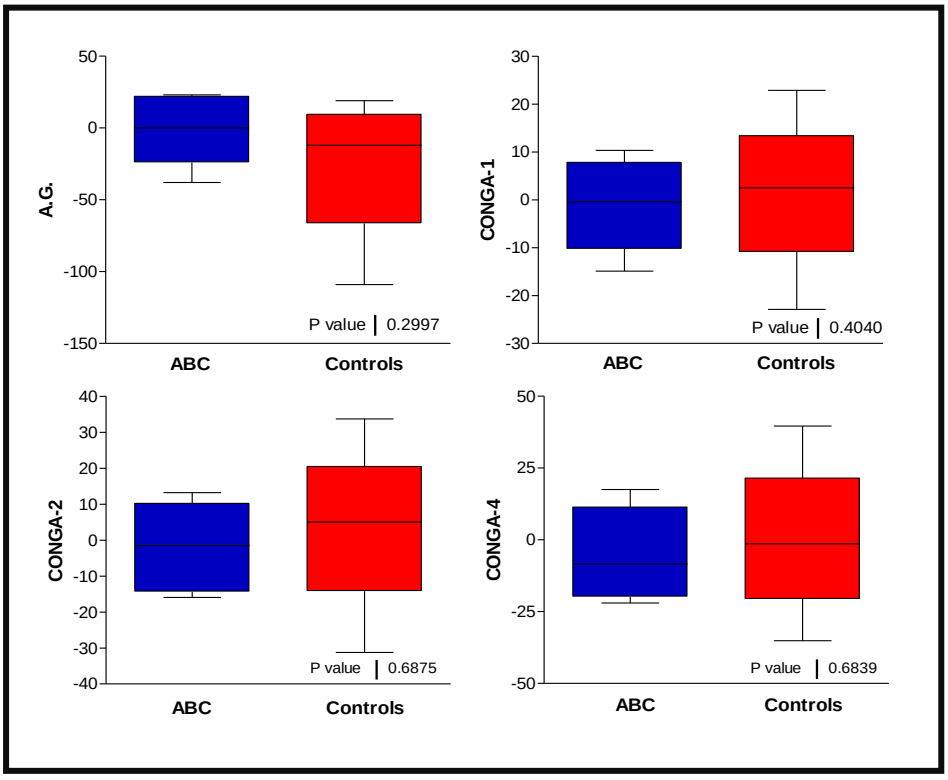
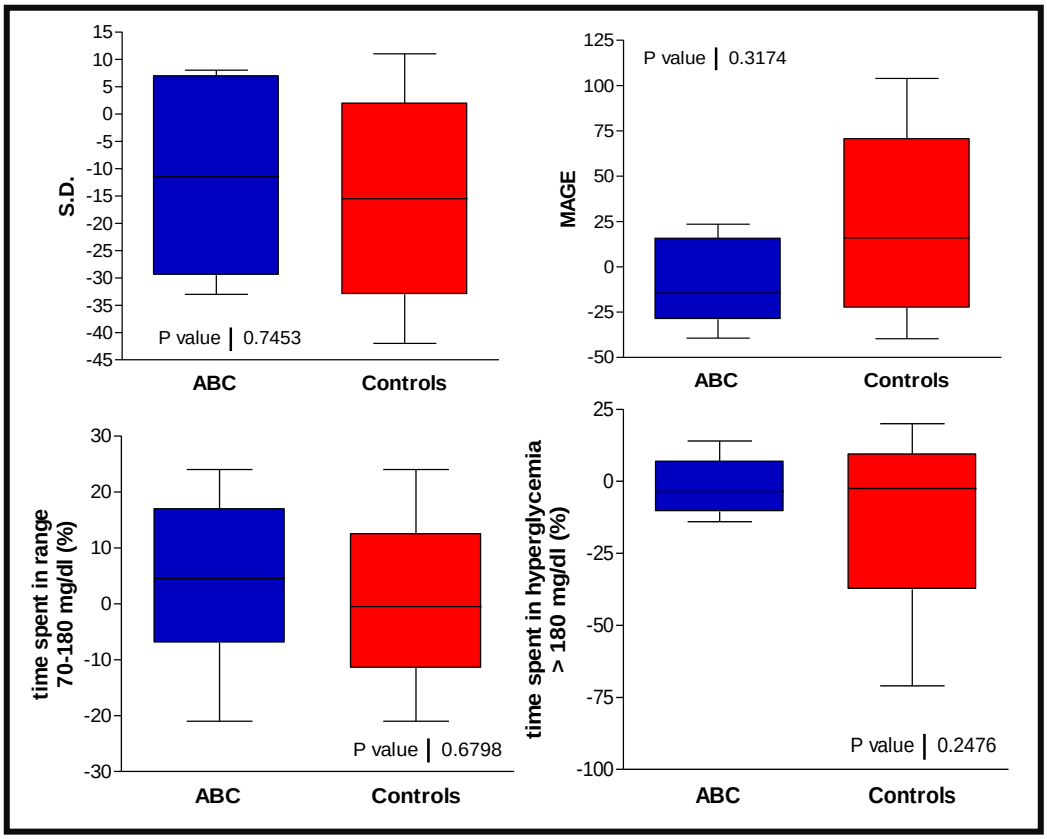
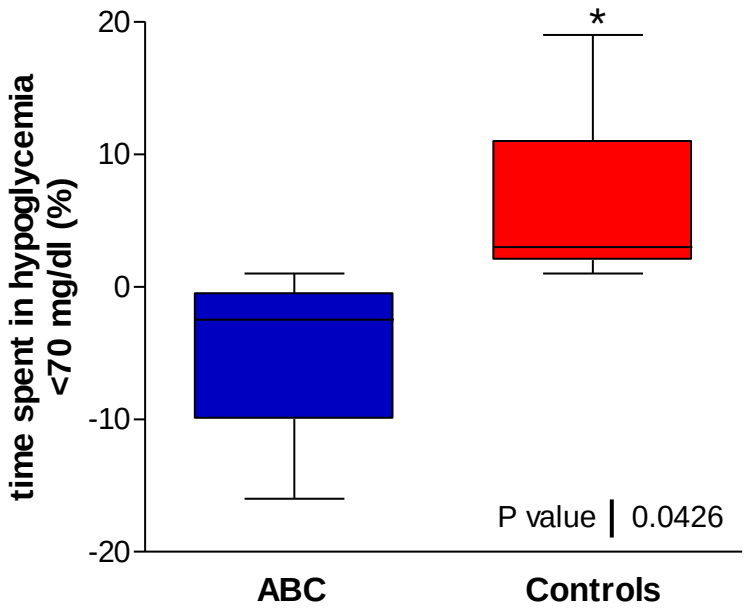


Fig. 14: *Results on glycaemic variability indices (2)*



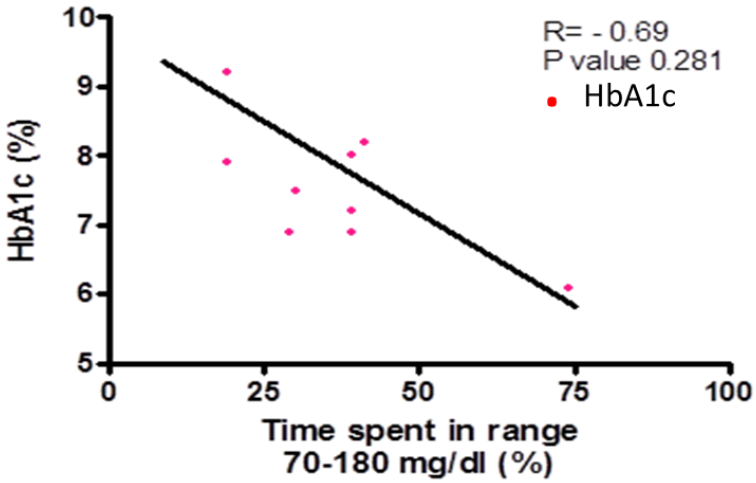
Therefore, ABC group showed a significant reduction of the time spent in hypoglycaemic range (P = 0.04) compared to the control group (**Fig. 15**).

FIG 15: *Reduction of time spent in hypoglycaemic range in ABC treated group vs. control group*



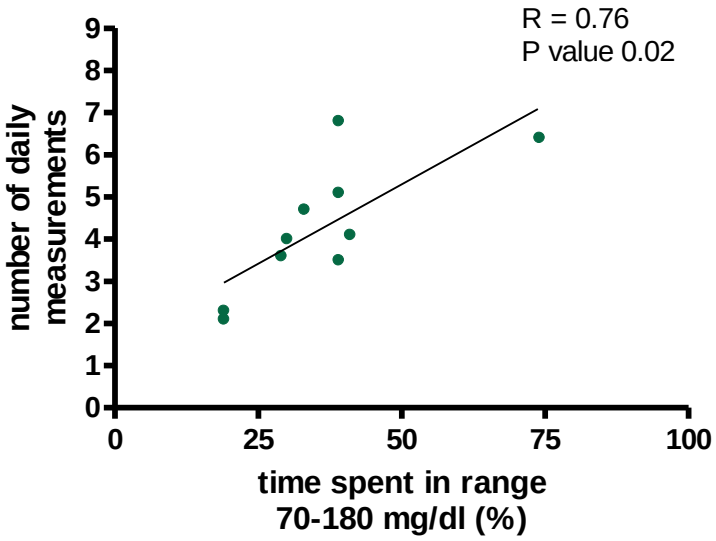
Finally, evaluating the whole population in the linear regression analysis we have found a negative correlation between the time spent in normal range and HbA1c levels. (P = 0.2, r: -0.69), (**FIG. 16)**

FIG 16: *Correlation between time spent in normal range and HbA1c levels*



Moreover, we have found a positive correlation between the number of daily measurements and time spent in normal range (P = 0.02, r: 0.76).

FIG. 17: *Correlation between the number of daily measurements and time spent in normal range*



7.5 Discussion

The results of this study demonstrated that this bolus advisor system is a friendly wirelessly meter that helps T1D patients on MDI to improve glycaemic control increasing patients compliance to SMBG and reducing the risk of hypoglycaemia.

These findings are consistent with our previous study showing an improvement of HbA1c and major compliance to SMBG in T1D patients using this bolus advisor system.

Furthermore, the significant reduction of I.R. in ABC group compared to control subjects suggests that the improvement of glycaemic control could be related with a more appropriate dosage of insulin therapy in patients ABC users.

These findings are also consistent with the results showing a positive relation between the major compliance to SMBG, assessed by the number of daily measurement, and the time spent in glycaemic normal range in group-treated patients using this ABC with the telemedicine system compared to control group.

These observations confirm the evidences from the literature showing that the use of an ABC system with a "real-time" data sharing with the diabetes center by the mobile app can improve the achievement of glycaemic targets, facilitating patients compliance to SMBG and their overall self-management [60, 61].

Specifically to the GV evaluation from the CGM data, we didn't found any significant improvement of GV indices as SD, AG, Conga -1, -2 -4, MAGE nor in time spent in hyperglycaemic or in the normal range.

Probably these results are due to the small population size evaluated by the CGM analysis.

On the other hand, we have found a significant reduction of the time spent in hypoglycaemic range suggesting that the use of this device is able to reduce the incidence of hypoglycaemic events, improving the overall glycaemic control assessed by HbA1c.

As well known, fear of hypoglycaemia is a crucial limiting factor for the achievement of glycaemic targets, negatively influencing insulin adjustments [141, 142].

According to other authors highlighting the beneficial effect of the bolus calculators use on the risk of hypoglycaemia in T1D patients on MDI we can confirm that the use of this advisor system taking into account the amount of the active insulin in prandial and correction boluses is very useful to prevent hypoglycaemic events [142, 143].

As previously stated, the major limitation of this subgroup analysis is the small population size evaluated by the CGM study.

Moreover, we did not investigate patient satisfaction for this tool through specific questionnaires usually used for the evaluation of patients' treatment satisfaction.

Although patients' satisfaction has not been specifically evaluated in this study, the results from patients' adherence to SMBG provide a clear

support indicating that they perceived this tool to be effective and user-friendly.

In conclusion, our results suggest that this bolus advisor system is able to improve glycemic control increasing patients' compliance to SMBG and reducing hypoglycaemia in T1D patients on MDI.

SECOND SESSION

RESEARCH PROJECT 4: *D-Chiro-Inositol plus Folic Acid improve metabolic control in overweight T1D patients*

CHAPTER 8

SPECIFIC BACKGROUND:

Insulin resistance in T1D and potential role of D-Chiro inositol as adjuvant treatment

8.1 Insulin resistance in T1D

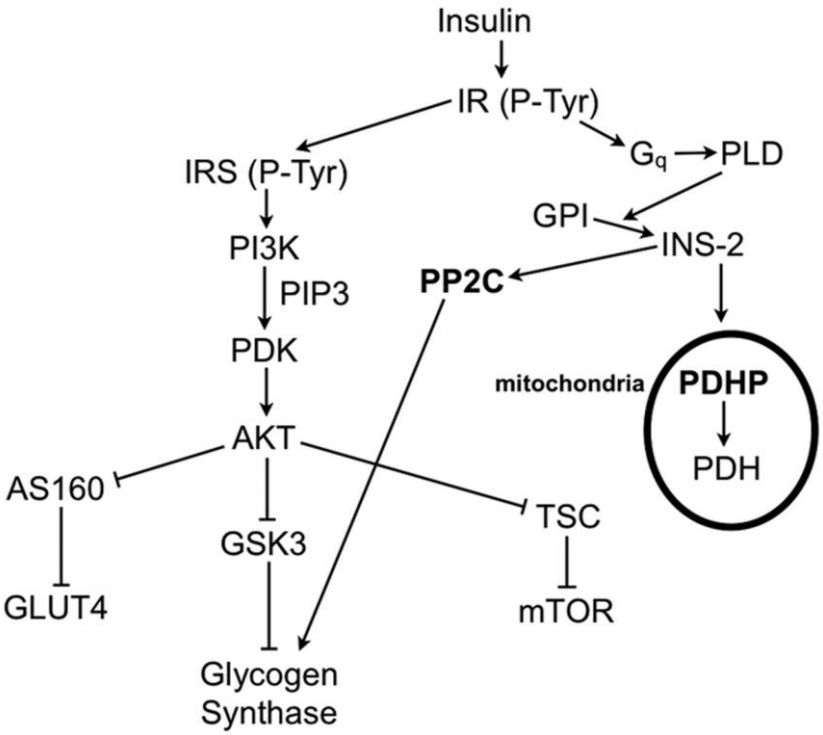
With the rising obesity in childhood and adolescence, insulin resistance is now occurring more frequently also in young patients with T1D [144]. Although the mechanisms involved in insulin resistance in T1D are not clearly defined than in T2D, the impairment of insulin signalling seems to play a key role [145].

In fact, already in 1982 DeFronzo et al. reported that skeletal muscle insulin resistance is a feature of T1D, demonstrating a reduction of glucose transportation into myocytes due to a defective insulin-stimulated regulation of GLUT4 mRNA [146, 147].

The insulin receptor is a transmembrane tyrosine kinase receptor that induces a cascade of reactions including tyrosine phosphorylation of the insulin receptor substrate 1 (IRS-1) and the association with phosphatidylinositol 3-kinase, after activation by insulin binding.

In skeletal muscle and adipose tissue, this process promote the translocation of existing GLUT4 on the cell membrane surface, as well as production of *de novo* GLUT4 protein [148] **(FIG. 18).**

FIG. 18 *Pathways of insulin signalling [149].*



Many abnormalities and dysregulation may occur in the different sites of activation of the insulin-signalling pathway.

Similarly to T2D subjects, in obese patients with T1D lower levels of GLUT4 protein in skeletal muscle were found, without any correlation between GLUT4 and HbA1c, blood glucose levels, insulin doses or disease duration [150].

In T1D animal model, the presence of higher insulin doses induce a decreased GLUT4 translocation. Moreover, insulin administration promote serine rather than tyrosine phosphorylation of IRS-1 with a consequent decreased intracellular glucose transportation [151, 152].

In the insulin resistance in T1D, plasma lipids levels are also involved.

In fact, the intracellular glucose transportation into myocytes through GLUT4 translocation seems to be inhibited by the activation of serine kinase on IRS-1, in place to the correct phosphorylation of tyrosine sites.

The activation of serine kinase sites seems to be also mediated by increased serum free fatty acids (FFAs) levels and intramyocellular lipids (IMCLs).

Similarly, to T2D patients, T1D subjects showed increased IMCLs in skeletal muscle, which are associated with IR, obesity, and defective insulin signalling [153, 154].

Finally, hepatic insulin resistance has also well investigated in T1D.

The liver plays a key role in the regulation of glucose homeostasis: balance is achieved through the regulation of gluconeogenesis and glycogenolysis in the fasting state and glycogen storage in the fed state [147].

In T1D animal model, it was demonstrated that higher doses of insulin promote hepatic gluconeogenesis and glycogenolysis, inhibiting glycogen synthesis.

Furthermore, higher doses of insulin result in impaired insulin-induced tyrosine phosphorylation of the hepatic insulin receptor thus reducing glycogen storage [151].

8.1.1 Double diabetes and long-term diabetes complications

Although insulin resistance is an essential mechanism for T2D development, as previously stated, it can also be a feature of T1D patients.

In fact the rising obesity in youth seems to have induce a change of T1D phenotype, defining a hybrid form of diabetes, known as "double diabetes" (DD) [155].

In DD, patients with autoimmune diabetes show the presence of insulin resistance and the metabolic syndrome features that are well-known risk markers for macrovascular disease [156, 157].

Beyond the euglycemic-hyperinsulinemic clamp is the gold standard method for insulin resistance evaluation, in clinical practice the estimation of insulin resistance in T1D is difficult because simpler tools such as the homeostasis model (HOMA-IR index) are not applicable for kkthis kind of patients [158]. In fact, insulin resistance in T1D patients is often indirectly recognized by their larger insulin requirement or by the presence of typical features of metabolic syndrome. In this regard, more recently a validated method for glucose disposal rate estimation (eGDR) has been developed [159].

The eGDR has been recognized as a surrogate method for the estimation of insulin resistance in the largest epidemiological studies on T1D, mainly for the prediction of the onset of long-term diabetes complication.

Specifically, in the DCCT/EDIC (Epidemiology of Diabetes Intervention and Complications) study it was found that eGDR is a predictor of CVD events and higher eGDR, as a measurement of major insulin sensitivity, was independently associated to a lower risk for CV diseases [160].

Moreover, eGDR was also directly related with a lower risk for microvascular disease as retinopathy and nephropathy in T1D [161].

These data has been also confirmed by the results from Pittsburgh Epidemiology of Diabetes Complications Study [162] and EURODIAB study [163].

8.1.2 Adjuvant therapies for treatment of insulin resistance in T1D

On this basis, to treat insulin resistance in T1D patients improving their insulin sensitivity and preventing long term diabetes complication, some oral and injective agents commonly used for T2D management have been widely evaluated as adjuvant treatments to insulin therapy also in T1D [164].

The potential effectiveness of the various agents by their different mechanisms of action has been widely investigated in several clinical trials, demonstrating their efficacy on improvement of insulin sensitivity and on the overall metabolic control also in T1D patients, as reported in **Table 10**.

The major class includes insulin sensitizer drugs such as metformin and thiazolidinediones acting on insulin resistance, by either inhibiting

hepatic glucose release or improving peripheral glucose sensitivity, mainly in the skeletal muscles [165].

Other agents such as glucagon-like peptide-1 (GLP-1) agonists and dipeptidyl peptidase-4 (DDP-4) inhibitors aim to inhibit hepatic gluconeogenesis and promote the reduction of post-prandial hyperglycaemia, delaying gastric emptying [166].

Finally, sodium glucose cotransporter 2 (SGLT2) inhibitors are newer agents that inhibit glucose reabsorption in the proximal renal tubule [167].

Table 10: *Non-insulin therapies for T1D. Adapt. from [164]*

Class	Mechanism of action	Proposed benefits	Side effects
Insulin sensitizers			
Biguanides: metformin (68, 69)	Inhibit hepatic glucose production Improve peripheral insulin sensitivity	Improved fasting and post-prandial glucose levels Reduction in total insulin dosing Questionable benefits: weight loss, improvement in lipid panel, improvement in HbA1c	Gastrointestinal (diarrhea, flatulence) Hypoglycemia Lactic acidosis (theoretical)
Thiazolidinediones: pioglitazone, rosiglitazone (97, 100, 101)	Reduce insulin resistance in adipose tissue, muscle and liver	Improved glycemic control Positive effect on circulating lipids	Weight gain Hypoglycemia Edema Anemia
Nutrient absorption modification			
Amylin analogues: pramlintide (107, 108, 111, 113, 115)	Reduce gastric emptying and food intake Suppress glucagon secretion and hepatic glucose production	Improved glycemic control Improved post-prandial glucose Reduction of weight	Gastrointestinal Hypoglycemia
Alpha glucosidase inhibitors: acarbose, miglitol (103, 105)	Inhibit digestion into monosaccharides, offsetting post-prandial glucose rise	Insulin sparing Improved glycemic control Reduction of HbA1c Improved post-prandial glucose	Gastrointestinal
Other			
GLP-1 agonists: exenatide, liraglutide, abliglutide, lixisenatide (112, 119, 129)	Regulate post-prandial glucagon release, delay gastric emptying, central effect on satiety	Insulin sparing Improved post-prandial glucose control Weight reduction	Gastrointestinal Pancreatitis
DDP-4 inhibitors: sitagliptin, saxagliptin, linagliptin, alogliptin and vildagliptin (only in Europe) (112, 119, 133)	Inhibit GLP-1 breakdown	Insulin sparing Improved glycemic control	Gastrointestinal Pancreatitis
SGLT2 inhibitors: canagliflozin, dapagliflozin (135)	Selectively inhibit SGLT2, increasing urinary glucose excretion	Improved glycemic control Reduction of body weight and blood pressure	UTI, genital infections Hypovolemia DKA

DDP-4, dipeptidyl peptidase-4; DKA, diabetic ketoacidosis; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; SGLT2, sodium glucose co-transporter 2; UTI, urinary track infection.

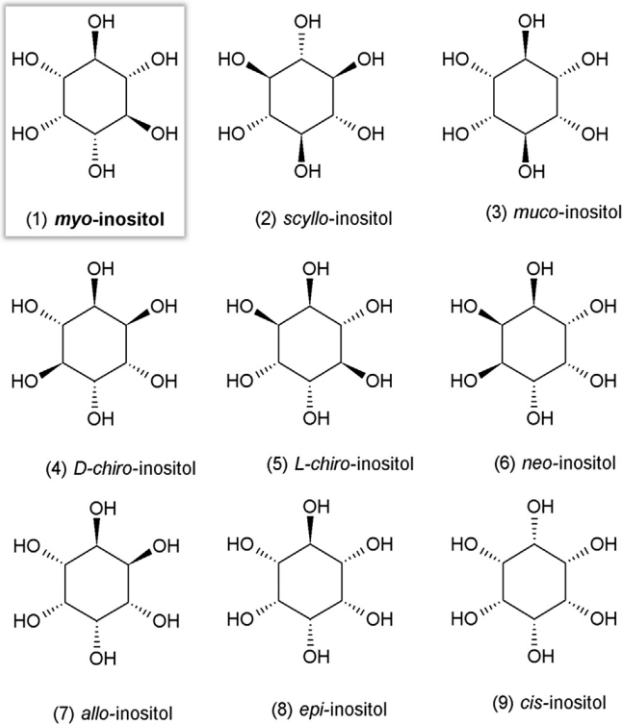
8.2 The potential role of inositol in insulin resistance

In the last years, to reduce insulin resistance in obese or overweight T1D patients various non-pharmacological approaches are also recognized as potential adjuvant treatments.

Among these, the inositol family plays an important role in insulin signaling transduction, improving insulin sensitivity [168].

Inositol is a polyol widespread in foods and cells eukaryotic. In particular, we find it well represented in fresh fruit, vegetables, beans and nuts. In nature, there are nine isomers of inositol that differ in their position of hydroxyl groups [169] (**FIG. 19**).

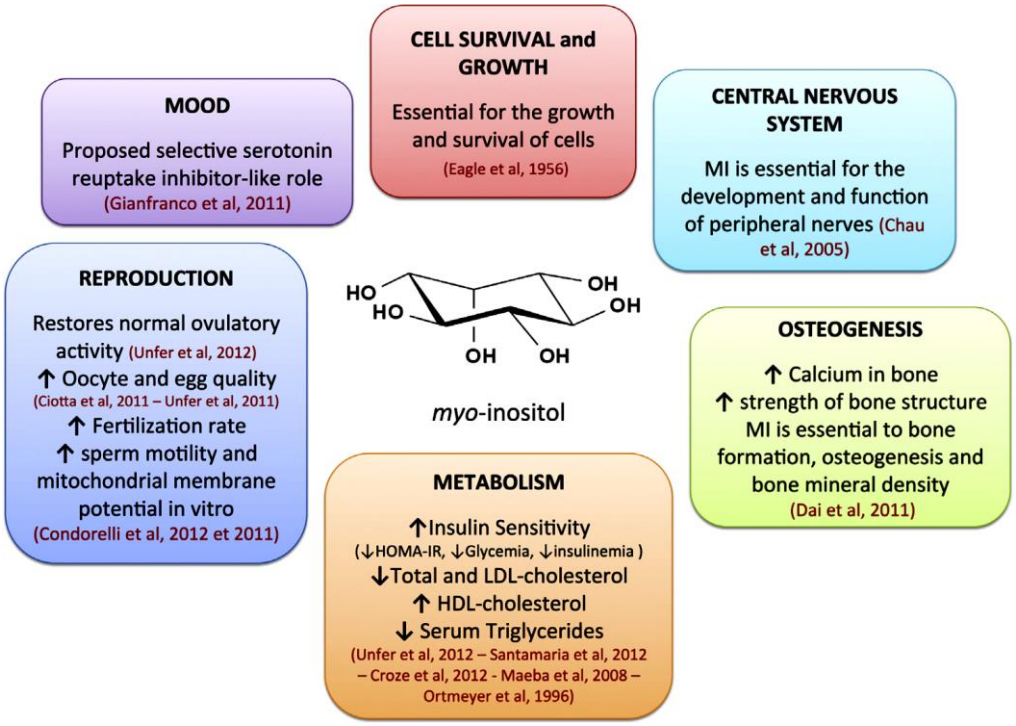
FIG. 19: *Structures of the nine stereoisomers of inositol. Adapt from* [169]



The most common isomer of inositol is myo-Inositol widely present in plant and animal tissues.

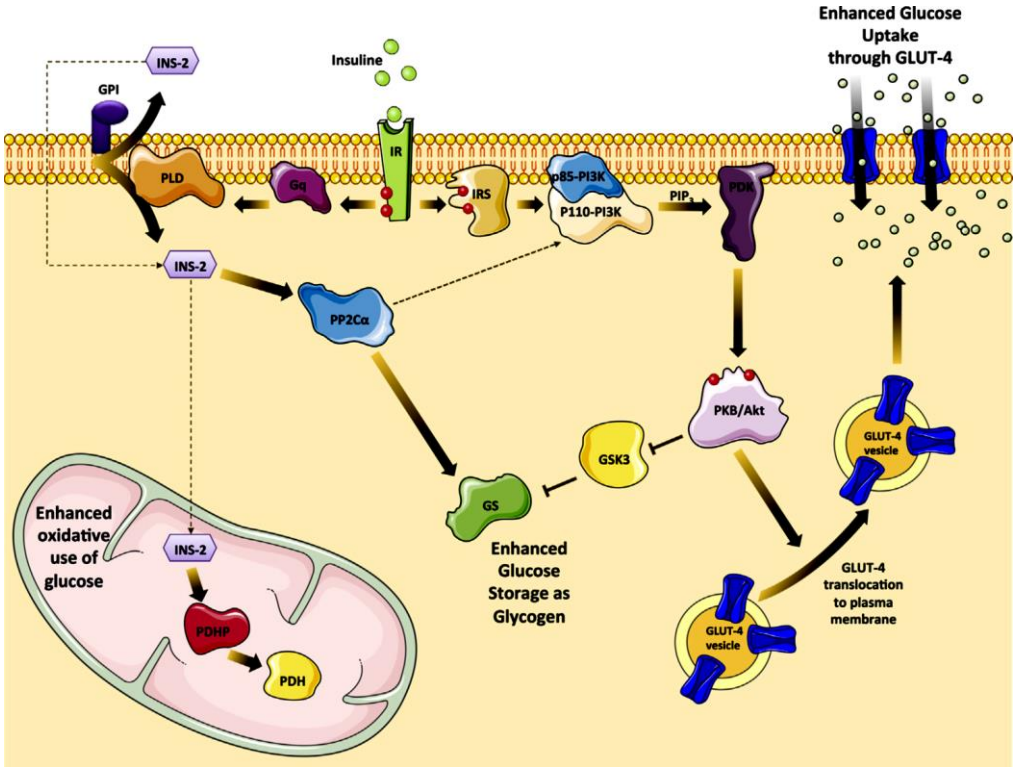
As secondary messenger, Myo-Inositol is involved in numerous cellular processes, as the cell growth and survival, peripheral nerves function, osteogenesis and ovarian function **(FIG. 20)** [169].

FIG 20: *Functions and benefits of a myo-inositol diet supplement for human health. Adapt from* [169]



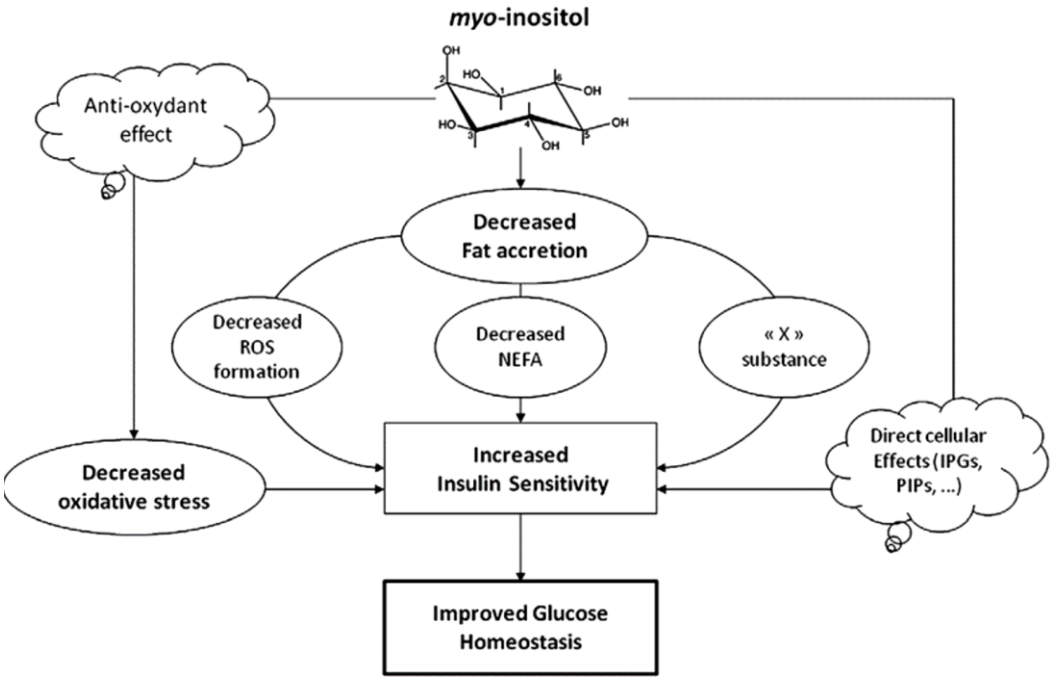
Several studies in vitro and in vivo on Myo-Inositol have demonstrated its ability to mimic a great number of the metabolic insulin actions, thus it is considered as putative mediator of intracellular insulin action [170]. Regarding to insulin signaling, Myo-inositol, containing inositol glycans, as putative second messenger of insulin (INS-2), induces GLUT-4 translocation with a consequent enhanced glucose storage, stimulating P13K and glycogen synthase activity and inhibiting GSK-3 activity, as reported in **FIG. 21** [169].

FIG. 21 *Inositol glycans as putative second messengers (INS-2) of insulin. Adapt from [169]*



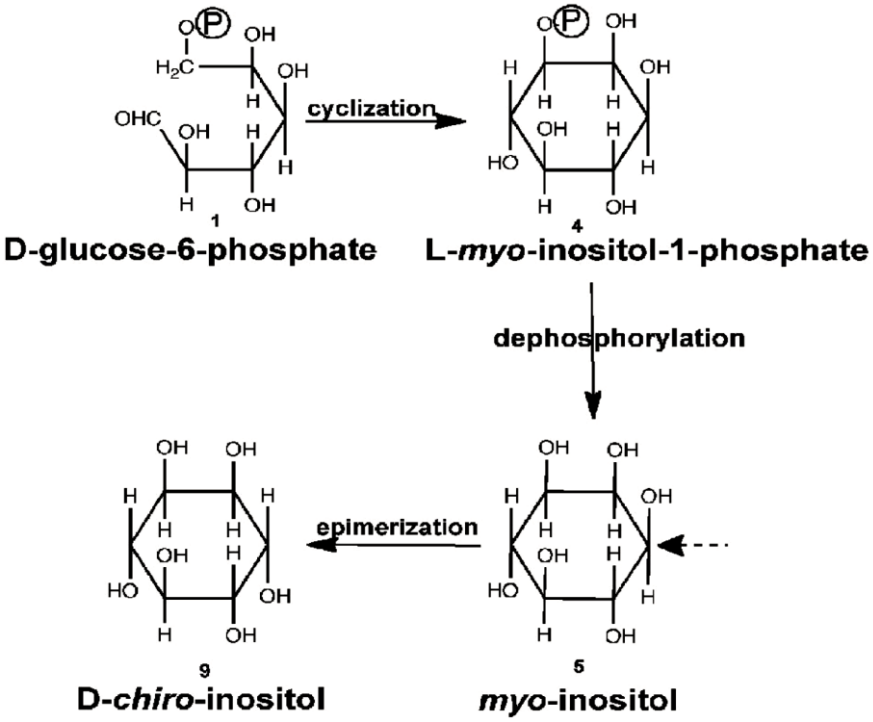
On this basis, myo-Inositol with its insulin-mimetic properties is recognized as an insulin sensitizer molecule stimulating lipogenesis, improving insulin sensitivity and enhancing oxidative glucose disposal, mediated by mitochondrial pyruvate dehydrogenase (PDH) (**FIG. 22**) [169].

FIG. 22: *Effects of chronic treatment with myo-inositol on insulin sensitivity in mice. Adapt from [169]*



D-chiro-inositol (DCI) is the active form of myo-Inositol resulting from the epimerization by oxido-reductive inversion of hydroxyl 3 of myo-Inositol (**FIG. 23**) [149].

FIG. 23: *Conversion of myo-Inositol to DCI. Adapt from [149].*



In vitro, DCI has showed insulin mimetic effects accelerating the dephosphorilation of glycogen synthase and pyruvate dehydrogenase involved in the regulation of non-oxidative and oxidative glucose disposal and in glycogen storage [171].

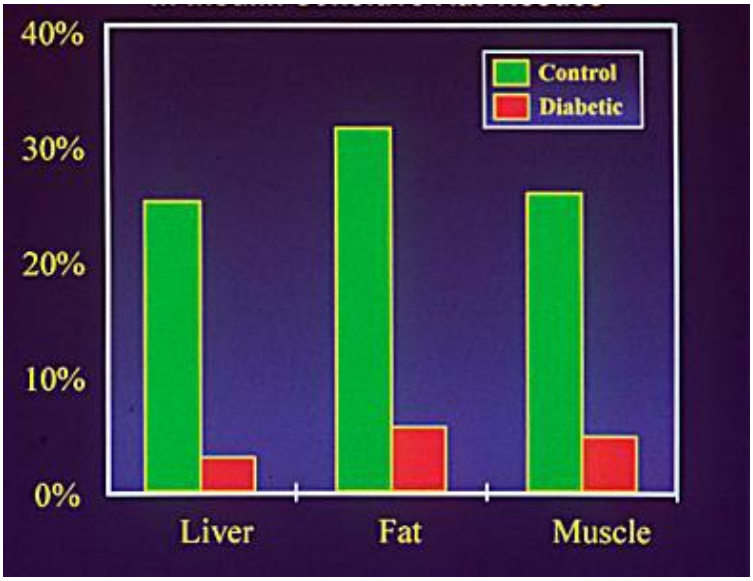
8.2.1 The inositol imbalance

In some insulin resistance conditions, a defective myo-Inositol epimerization to DCI was observed. This condition results in an "inositol imbalance" that is characterized by higher myo-Inositol and lowest DCI levels in insulin-sensitive tissues. **FIG. 24** [171, 172].

It has been hypothesized that DCI may act to by-pass a defective normal epimerization of myo-Inositol to DCI, which is related to insulin

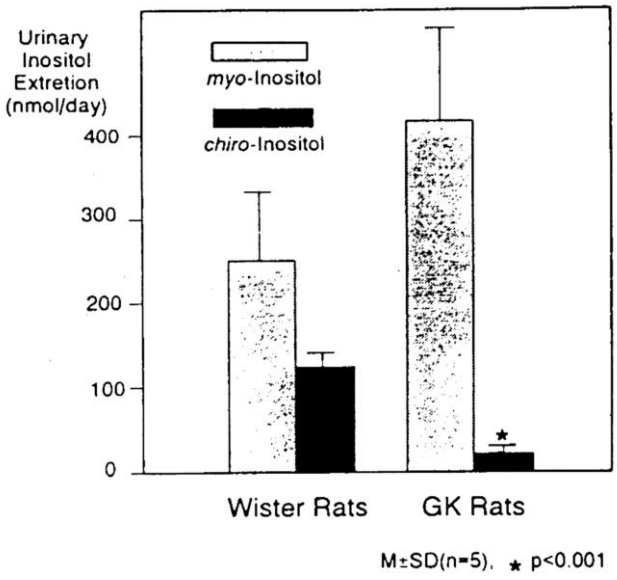
resistance and it can act to at least partially restore insulin sensitivity and glucose disposal [168, 171].

FIG 24: *Defective conversion of Myo-inositol in DCI in insulin-sensitive rat tissues. Adapt from [149]*



Accordingly, in animal models and in T2D patients, a reduction of DCI plasma levels with a decreased DCI urinary excretion related to the different degree of insulin resistance was reported (**FIG. 25**) [172, 173]

FIG 25: *24 hr urinary excretion of myo- and chiro-inositols in G/K rats. Adapt from [171]*



On this basis, inositol oral administration could play an important role in insulin resistance pathogenesis, avoiding the faulty epimerase and improving peripheral insulin sensitivity as shown in vivo and in the experimental studies [174, 175].

Consequently, in some insulin resistance conditions such as polycystic ovary syndrome (PCOS), the efficacy of DCI oral supplementation has been widely demonstrated [176]. In insulin-resistant women with PCOS, DCI is effective in improving ovulatory function, metabolic parameters and serum androgen concentration [176-180].

Similarly, Gluck et al. have demonstrated the DCI putative effect on glucose homeostasis and insulin sensitivity in streptozotocin (STZ)-induced diabetic rats [149].

Accordingly, clinical studies showed an improvement of glycaemic control with a reduction in fasting blood glucose and decreased HbA1c levels in T2D patients.

Project Research 4: *A pilot study of D-chiro-inositol plus folic acid in overweight patients with type 1 diabetes*

CHAPTER 9

9.1 Introduction

Intensive insulin therapy, based on multiple daily injections (MDI), is the gold standard treatment in T1D. Rationale for intensive insulin therapy is to provide an adequate amount of basal insulin and short-acting insulin in order to achieve optimal glycaemic control in these patients.

The effectiveness of tight glucose control through intensive insulin treatment has been reported in DCCT trial that showed as optimal glycaemic control is related to the reduction in incidence and severity of microvascular diabetes complications [12]. However, to achieve and maintain good glucose control through the standard therapy, insulin doses are often increased causing a higher risk of hypoglycaemic events, weight gain and poor glycaemic control.

Furthermore, with the worldwide increase of obesity in childhood and adolescence, insulin resistance is now occurring more frequently also in young patients with T1D.

Consequently, the rising obesity in youth seems to induce a change of T1D phenotype, defining a hybrid form of diabetes, known as "double diabetes" (DD) [156].

On this basis, to improve glycaemic control and peripheral insulin sensitivity, some insulin-sensitizing drugs generally used in Type 2 Diabetes (T2D), such as metformin, are more frequently used as adjuvant treatment also in patients with DD [49, 156]. Moreover, as recently reported, metformin is able to enhance the acute insulin-sensitizing effects of a single bout of exercise in insulin-resistant patients [181].

Despite the effect on glycaemic control reducing body weight and IR in overweight or obese T1D patients, several limitations are related to metformin.

In this regard, metformin may have many gastrointestinal side effects reducing patients' adherence to treatment and consequently its own effectiveness [182].

Similarly to metformin, but without its adverse effects, studies in vitro and in animal models have shown that D chiro-inositol (DCI) is an important insulin-sensitizing compound by acting as intracellular second messenger of insulin signalling.

Specifically, DCI through its insulin-mimetic activity seems to promote intracellular glucose disposal and glycogen storage in the insulin-sensitive peripheral tissues accelerating the glycogen synthase and mitochondrial pyruvate dehydrogenase dephosphorylation [149].

Moreover, in some insulin resistance conditions, a defect of myo-inositol

epimerization to DCI was observed, resulting to an "inositol imbalance" with higher myo-inositol and lowest DCI levels in insulin-sensitive tissues compared to healthy subjects [171, 172].

Accordingly, in animal models and in T2D patients, a reduction in DCI plasma levels with a decreased DCI urinary excretion related to the different degree of insulin resistance was reported [173].

On this basis, inositol oral administration could play an important role in insulin resistance pathogenesis, avoiding the faulty epimerase and improving peripheral insulin sensitivity as shown in vivo and in the experimental studies [175, 183].

Similarly to metformin and DCI, folic acid oral administration seems to improve glycaemic control with a reduction in insulin resistance in insulin-resistant T2D patients. Previous studies suggested that folic acid oral administration with its homocysteine-lowering effect may improve insulin sensitivity and reduce insulin levels in patients with metabolic syndrome [184]. Furthermore, its administration may be of benefit to endothelial dysfunction, decreasing oxidative stress and asymmetric dimethylarginine levels by preventing NO synthase dysfunction [185].

Therefore, when administered in addition to myo-inositol, folic acid has reduced the risk of developing gestational diabetes in pregnant women at high risk for diabetes [186].

9.2 Aim of the study

On this basis, we hypothesize that the administration of the two compounds may have a synergistic effect by acting on their specific cellular pathways in overweight T1D patients.

The purpose of this pilot study was to evaluate the effect of DCI oral supplementation in addiction to folic acid compared to folic acid alone on glycaemic control as assessed by HbA1c in overweight or obese T1D patients undergoing intensive insulin therapy.

9.3 Materials and methods

A 24-week, prospective, randomized control trial was carried out in T1D patients, aged 17–50 years (13 males, 13 females), with disease duration >1 year and BMI >25, attending as outpatients the Endocrinology and Diabetes Unit of University Campus Bio-Medico in Rome.

The clinical features of the patients population are showed in **Table 11**.

Table 11 *Demographic and clinical features of T1D patients (data are mean ± SD)*

	Treated group (DCI + folic acid)	Control group (folic acid)	*p* value
No.	15	11	NS
Male/female	7/8	6/5	NS
Age range (years)	35.5 ± 10.0	37.0 ± 11.3	NS
Disease duration (years)	20.4 ± 10.5	21.3 ± 11.5	NS
BMI (kg/m^2)	26.2 ± 2.1	27.1 ± 1.2	NS
HbA1c (%)	8.0 ± 1.1	7.5 ± 1.1	NS
Daily insulin requirement (IU/kg/day)	0.56 ± 0.26	0.55 ± 0.16	NS

Exclusion criteria were learning disabilities or presence of chronic conditions potentially able to influence daily activities (visual or auditory disability, motor impairment for neurological or orthopaedic problems). T1D patients affected from diabetic complications were also excluded.

Specifically, in order to detect the potential insulin-sensitizing additive effect of DCI compared to folic acid alone, we designed a two-arm randomized controlled trial in which the active group was represented by insulin-treated T1D patients receiving DCI plus folic acid, while in the control group insulin-treated T1D patients receiving folic acid alone were enrolled. All patients were randomly assigned to 1 g DCI plus 400 mcg folic acid once daily (treated group) or to 400 mcg folic acid only once daily (control group) (see Flow Diagram of the study).

HbA1c (%), IR and body mass index (BMI) were evaluated at entry into the trial at 3- and 6-month follow-up.

Mean daily IR was expressed as IU/kg/day, and BMI was calculated as the ratio: weight in kg/(height in m)2.

To detect the potential insulin-sensitizing additive effect of DCI compared to the folic acid alone, all patients were equipped with the same algorithm for self-titration of insulin doses based on SMBG, insulin/carbohydrate ratio and carbohydrate intake at each meal; they also received the same dietary indications and educational programme, thus ensuring the homogeneity between the two groups and similar intervention in both groups.

The sample size for the study has been calculated taking into account 80% power and a difference of HbA1c of 0.3% at the end of the study period. Paired t test (two tailed) and analysis of variance were used to evaluate differences in HbA1c, IR and BMI between two groups at different time points.

The protocol was consistent with the principles of the Declaration of Helsinki, it was approved by the Ethic Committee at University Campus Bio-Medico, and all participants gave written informed consent. Data were expressed as means ± standard deviation (SD). A p value <0.05 was considered significant.

9.4 Results

Comparison of baseline demographic and clinical features showed that two groups were appropriately randomized (Table 11).

After 3-month follow-up, a significant reduction in HbA1c levels was observed in DCI-treated group versus control group [7.5% (58 mmol/mol) ± 1.1 vs. 8.1% (65 mmol/mol) ± 1.9, respectively, p\0.05]. At the end of the study period (6 months), HbA1c reduction in DCI treated group vs. control group was statistically confirmed [7.5% (58 mmol/mol) ± 0.9 vs. 7.9% (63 mmol/-mol) ± 1.7, respectively, p\0.05] (Fig. 26: HbA1c in the two groups).

Otherwise, no statistically significant differences in BMI and IR between the two groups were observed [(BMI 25.7 ± 2.8 vs. 26.7 ± 1.0, respectively, p NS, **In the control group only 7 patients had recorded*

BMI at T2 and the analysis was performed accordingly); (IR 0.52 ± 0.26 vs. 0.52 ± 0.19, respectively, p NS)] (Figs. 2, 3: BMI and I.R. in the two groups).

Moreover, the differences in each group, from baseline to the end of the study, were also not significant as showed by the all p values for HbA1c, BMI and I.R. in DCI-treated group and in the control group from T0 to T2, respectively [HbA1c T0/T2: DCI-treated group p = 0.14 control group p = 0.92; BMI T0/T2: DCI-treated group p = 0.49 control group p = 0.53; I.R. T0/T2: DCI-treated group p = 0.82 control group p = 0.87]. Finally, there were no significant differences in the frequency of hypoglycaemic events between the two groups and no relevant adverse effects occurred during the period of observation.

Fig. 26: *HbA1c in the two groups. T0: baseline, T1: 3 month, T2: 6 month*

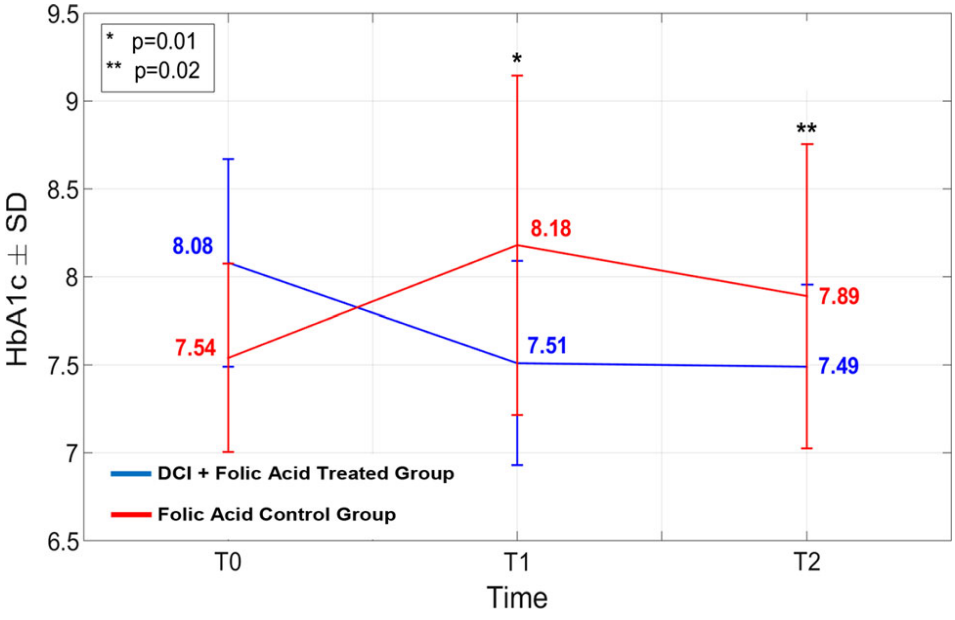


Fig. 27: *BMI in the two groups. T0: baseline, T1: 3 month, T2: 6 month*

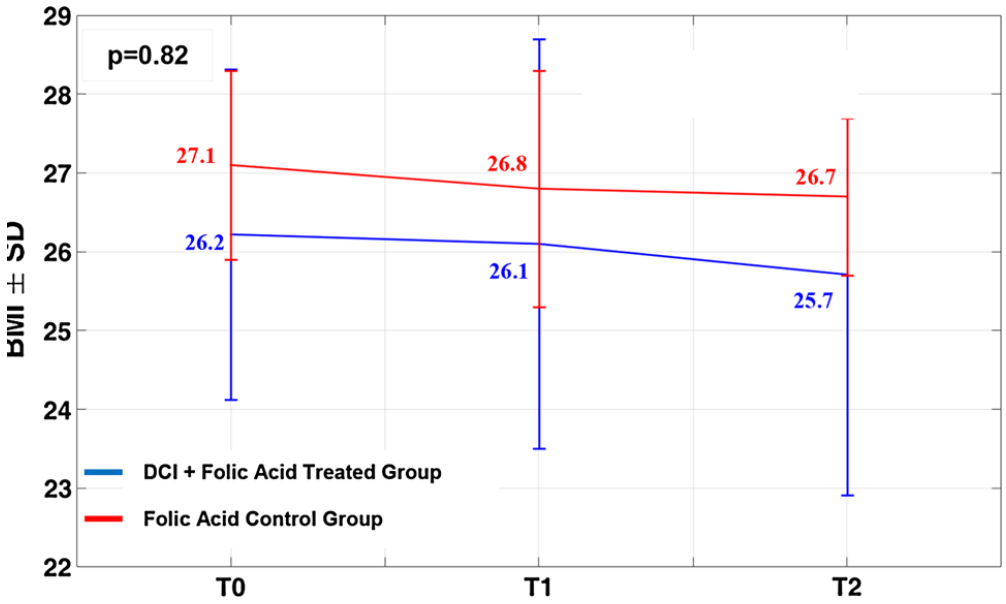
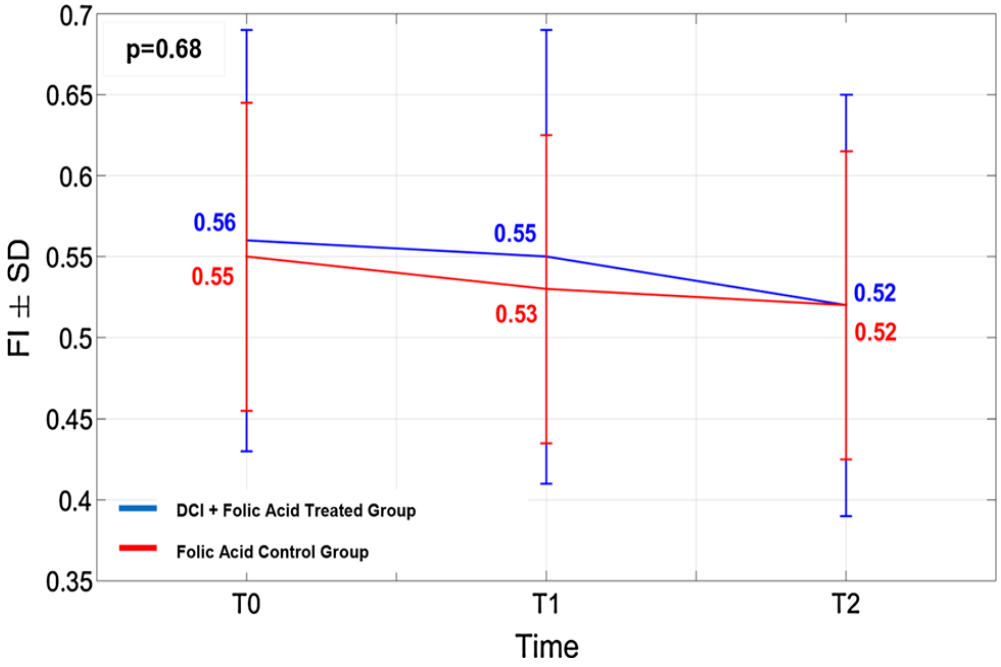


FIG. 28: *I.R. in the two groups. T0: baseline, T1: 3 month, T2: 6 month*



9.5 Discussion

The results of this study showed for the first time that the DCI and folic acid oral supplementation may represent an adjuvant treatment in overweight or obese insulin-treated T1D patients.

Although several studies have supported the hypothesis that inositol is involved in glucose control mechanism, to date, no studies have evaluated its use as an adjuvant treatment in T1D.

As previously stated, DCI oral supplementation may improve glucose metabolism bypassing the defective epimerization of myo-inositol to DCI, thus partially restoring peripheral insulin sensitivity [171].

Consequently, in some insulin resistance conditions such as polycystic ovary syndrome (PCOS), the efficacy of DCI oral supplementation has been widely demonstrated [176]. In insulin-resistant women with PCOS, DCI is effective in improving ovulatory function, metabolic parameters and serum androgen concentration [176-180].

Similarly, Gluck et al. have demonstrated the DCI putative effect on glucose homeostasis and insulin sensitivity in streptozotocin (STZ)-induced diabetic rats [149].

Accordingly, clinical studies showed an improvement of glycaemic control with a reduction in fasting blood glucose and decreased HbA1c levels in T2D patients [187, 188].

Likewise to DCI, folic acid with its anti-inflammatory activity seems to have a potential therapeutic role in some insulin-resistant conditions. Acting through an amelioration of insulin sensitivity, it improves

insulin resistance and endothelial dysfunction, decreasing the homocysteine and inflammatory mediators plasma levels [184, 185].

Although T1D and T2D pathogenesis are significantly different, with the fast increase of childhood obesity, a great number of evidences support the hypothesis that insulin resistance plays a crucial role also in T1D pathogenesis [157]. Consequently, the therapeutic approach in these patients has considerably changed leading to a widespread use of insulin-sensitizing drugs as adjuvant treatment of insulin therapy. On this basis, we have designed this pilot study to test the efficacy of DCI oral supplementation in addiction to folic acid on glycaemic control in T1D patients undergoing intensive insulin therapy.

Specifically, we have evaluated the effects on glycaemic control, assessed by HbA1c, of DCI plus folic acid daily administration compared to folic acid daily supplementation (control subjects) in overweight or obese T1D patients. At 3-month follow-up, we found an improvement of metabolic control with a significant reduction in HbA1c levels in the DCI-treated group compared to folic acid treated group with this difference confirmed at 6 months of treatment.

Differently, there was only a tendency for a reduction in BMI and IR in DCI compared to folic acid group. According to the rationale of this study, we have demonstrated significant HbA1c reduction in the DCI treated group compared to the control subjects despite a similar reduction in daily insulin dosage, expressed by IR, between the two groups.

Therefore, it is likely that these results are due to the influence of DCI on insulin sensitivity, by means of its additive insulin-sensitizing effect when administered in addition to the conventional insulin treatment. We also found a slight reduction in the waist circumference in DCI-treated group vs. folic acid group (90 ± 13.6 vs. 92.5 ± 13.3 cm, respectively, p NS). These results suggest that the effect of DCI supplementation on metabolic control may be independent of body weight reduction.

Indeed HbA1c reduction could be related to DCI insulin-like activity promoting GLUT-4 membrane translocation, glycogen synthase activity with intracellular glucose disposal and glycogen storage [189].

Nevertheless, our study has several limitations as the small sample size, the short follow-up such as the lack of eGDR (estimated glucose disposal rate) evaluation before and after intervention in each group.

9.6 Conclusions

In conclusion, although intensive insulin therapy remains the standard treatment for patients with T1D, we reported for the first time that the DCI and folic acid oral supplementation may represent an adjuvant treatment in overweight or obese T1D.

Further randomized placebo control trials with wider sample size and longer follow-up are required to confirm the potential role of DCI oral administration on insulin resistance in T1D patients.

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