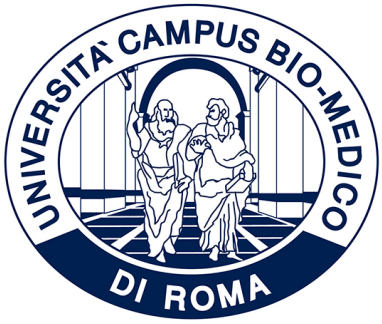

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THE BLIND SIDES OF GLUCOSE CONTROL IN TYPE 1 DIABETES: FROM PATHOGENESIS TO CLINICAL IMPLICATIONS

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Abstract

Introduction

Type 1 diabetes (T1D) can be acquired at any age and accounts for about 5% to 10% of all diabetes mellitus cases. It is a metabolic disease caused by a cellular-mediated autoimmune destruction of pancreatic β cells which results in a deficiency of insulin secretion. What causes the pathological autoimmune response is not yet fully understood but includes genetic susceptibility in combination with an environmental trigger. Insulin deficiency causes hyperglycaemia which is the main characteristic of T1D. In clinical practise, achieving a good glucose control represents the most important target. HbA1c and continuous glucose monitoring (CGM) parameters (Time in Range, Time Above the range and Time below the range) are currently used as glucose control indicators. HbA1c is a particular form of hemoglobin modified by glucose which determines the three-month average blood glucose level. It can be used both as a diagnostic test for diabetes and to assess glycaemic control. Time in range, time above the range and time below the range represent the amount of time a person spends in, above and below the target range (generally 70-180 mg/dl). Time in range should reach at least a value of 70%. Patients with long history of T1D can develop chronic complications, including ischemic cardiopathy, stroke and diabetic retinopatht, nephropathy and neuropathy. With the increasing use of CGM, TIR is expected to become a core indicator for short-term blood glucose assessment and for the risk of diabetic complications According to 2019 ADA guidelines a 5% increase in TIR is associated with significant clinical benefit in patients with T1DM. However, the relationship between TIR and diabetic complications has not been fully studied, and whether TIR value resulting from the extensive fingertip glucose monitoring and non-GCM is equally meaningful remains to be investigated.

Study 1

Poor glucose control has been associated with markedly increased mortality in COVID-19 patients with Type 1 Diabetes (T1D), however, the impact of glucose control on immunogenicity to SARS-CoV2 vaccines is not clear. The aim of the present study was to assess the effect of glucose control on antibody response to SARS-CoV2 vaccination in T1D. 26 patients (14 males, mean age 39.3 ± 11, mean disease duration 21.4 ± 10.1), scheduled to receive two doses of the SARS-CoV2 mRNA vaccine BNT162b2, were enrolled in our single-centre six-months cohort study. Patients underwent blood samples at 5 time-points T0-T4

(baseline within three days before the first vaccine dose; T1 just before the second vaccine dose; T2 two weeks after the second dose; T3 three months from baseline and T4 six months from baseline). The main outcomes were IgG antibodies to Spike glycoprotein by ELISA, HbA1c and CGM parameters. Longitudinal IgG response to spike reached a peak at T2, followed by a progressive decline across later timepoints ($P<0.001$). Peak IgG at T2 was not significantly correlated with baseline HbA1c, but strongly correlated with baseline glucose time in range (TIR) and glucose time above range (TAR) in patients wearing a CGM device for at least 10 days during the two weeks before baseline. Our findings indicate a strong relationship between glucose control and antibody response following SARS-CoV2 vaccine, highlighting the importance of achieving well-controlled blood glucose control.

Study 2

According to Sims et al. (Diabetes Care 2018), 96% long-standing T1D had detectable serum proinsulin (>3.1 pmol/L) despite low or absent C-peptide (a marker of insulin secretion). The Proinsulin to C-peptide ratio is a marker of beta-cell stress, indicating the inability of Beta-cell to convert proinsulin to insulin and C-peptide. Residual Beta-cell function has been associated with lower risk of chronic complications in T1D. We hypothesized that the Beta-cell stress marker Proinsulin/C-peptide is higher in patients with complications. The aims of the present study were to evaluate whether proinsulin and the proinsulin/C-peptide ratio are associated with chronic complications and glucose control in patients with long standing T1D. 100 T1D patients (64 males, 36 females) were enrolled in our single-centre cross-sectional cohort study. Patients were divided in two groups: without complications (74 subjects, mean age 42.3 ± 15.8, mean disease duration 12.7 ± 7) and with complications (26 subjects, mean age 42.16 ± 8.58, mean disease duration 24.5 ± 8.89). Chronic complications assessment were performed to screen diabetic neuropathy, nephropathy and retinopathy according to international guidelines. The main outcomes were proinsulin, C-peptide, proinsulin to C-peptide ratio, HbA1c and CGM parameters. No significant correlation was observed between C-peptide and proinsulin. C peptide, proinsulin and the Proinsulin to C-peptide ratio (PI:CP) resulted unrelated to chronic complications and glucose control. Beta cell stress is present in most T1D patients, however, proinsulin/C-peptide ratio is not associated with T1D complications and glucose control.

Study 3

Carbohydrate (CHO) counting is often performed inaccurately by patients with T1D. We hypothesized that mobile App “Dietrometro”, that estimates CHO content of food figures,

would ameliorate glucose control. Fifty-four T1D subjects (26 males), on multiple daily injections (n=23) or continuous subcutaneous insulin infusion (n=31), were randomly assigned to three groups: no counting (group 1; n=19, mean age 44,37 ± 15,79), "self- managed" counting (group 2; n=19, mean age 42,21 ± 15,09) and App-assisted counting (group 3; n=16, mean age 38,31 ± 13,69). Outcomes were one- and three months follow-up CGM parameters, estimated by flash or continuous glucose monitoring, and HbA1c. At the baseline TIR were similar between groups, while HbA1c was lower in group 3 compared to group 1 (6.9±1.06 vs. 7.8±0.85%; $p<0.05$). At one-month follow-up, TIR was higher in group 2 and 3 compared to group 1 (63.58 ± 11.55 vs. 52.32 ± 13.22%; $p = 0.014$, and 71.25 ± 9.75 vs. 52.32 ± 13. 22%, respectively; $p<0.001$). TAR at one-month follow-up was significantly lower in group 3 (31.25 ± 19.18 vs. 22.31 ± 10.89%; $p<0.001$), while no differences were observed in TBR . At three-months follow-up, groups 2 and 3 had a lower HbA1c than group 1 (7.16 ± 0.647 vs. 6.56 ± 1.91 vs. 7.96 ± 1.0%; $p<0.05$). App-assisted CHO counting might improve short-term glucose control. Patient's counseling to increase compliance should be part of disease management to achieve a better long term glucose control

Study 4

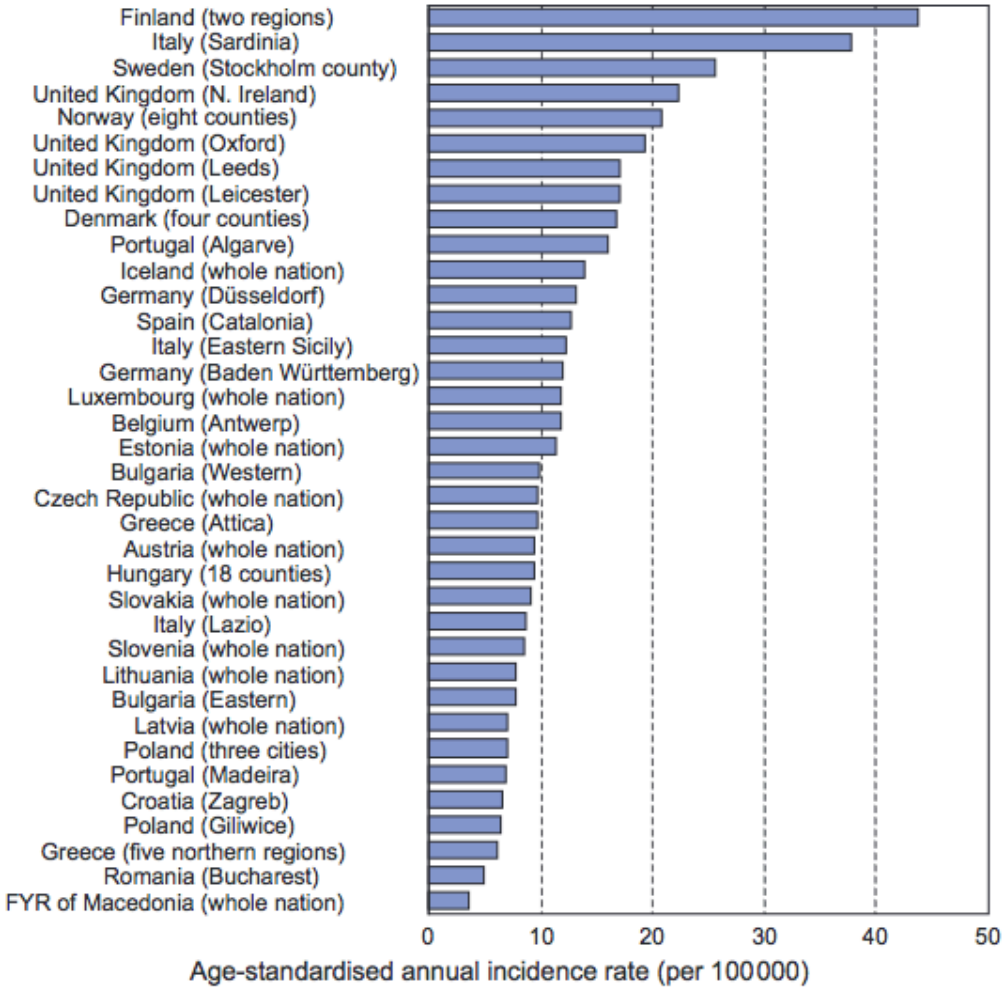
Technological advances in glucose monitoring and continuous subcutaneous insulin infusion (CSII) should aim to improve glucose control and quality of life in type 1 diabetes (T1D). The primary aim of study 4 was to test the overall effect of new technologies in the treatment of type 1 diabetes in terms of quality of life. The exploratory aim was to compare the different devices (both sensors and insulin pumps) on patients' quality of life.. Sixty-nine T1D patients (31 males, mean age 39 ± 12) were recruited. 36 were on multiple daily insulin injections (MDI), 33 on CSII devices including Medtronic Minimed 640G and 670G, Theras Omnipod, Roche Insight and Movy Tandem. Glucose monitoring was performed with Dexcom-G6, Guardian sensor and Flash Freestyle Libre. The Diabetes Treatment Satisfaction Questionnaire (DTSQ), the Diabetes Specific Quality Of Life Scale (DSQOLS) and The Short Form (36) Health Survey (SF-36) were administered to test quality of life. The main outcomes were HbA1c and CGM parameters. Patients belonging to CSII group had higher treatment-related satisfaction (84.8% vs 52.8%, $p = 0.004$), and better disease acceptance (84.8% vs 52.8%, $p = 0.012$) compared with patients on MDI, despite similar age (MDI mean age 38 ± 12.5, CSII 41 ± 11.6). No differences were observed among devices (p = ns). TIR resulted higher in the CSII group than in the MDI group ($p=0.001$). Technological devices improve quality of life and glucose control, but not patient's self perception of disease.

Chapter 1: autoimmune diabetes

1.1 Epidemiology

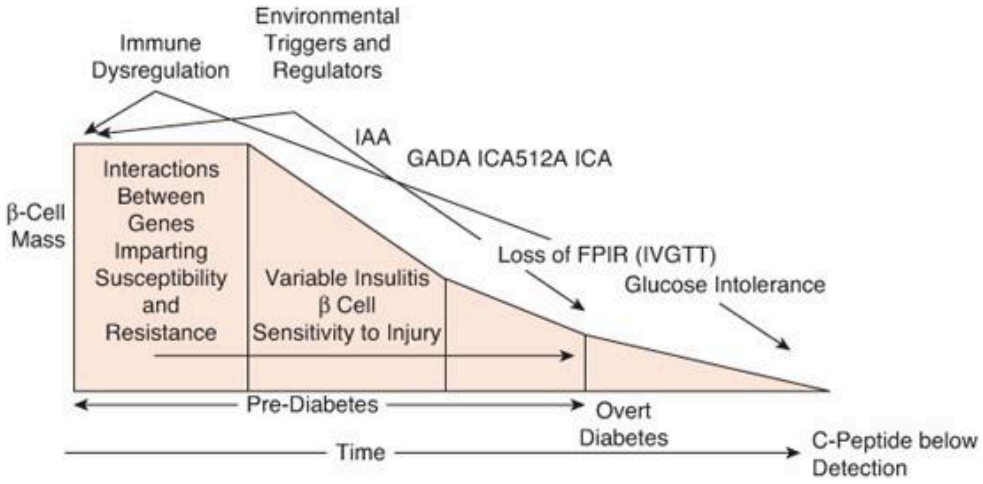
Autoimmune or Type 1 diabetes (T1D) is characterized by chronic insulin deficiency and hyperglycemia due to extensive destruction of insulin producing β-cells. At diagnosis, just 15-20% of insulin producing β-cells can still secrete insulin [1]. The incidence of Type 1 diabetes is progressively increasing world-wide and it is influenced by ethnicity, gender, familiar history, body mass index (BMI) and geographyc area of origin and growth. The highest incidence of type 1 diabetes is observed between 9 months and 12-14 years [2].

Figure 1: T1D incidence ncidence rates in 1989–1998 for 36 EURODIAB centers (Pediatric Diabetes, 2007)



T1D is the result of a complex interaction between genetic background and environment, so that it is possible to define T1D a multifactorial autoimmune disease. The autoimmune nature of this process is supported by the presence of a pool of auto-antibodies against β-cell antigens as well as the association with genes controlling immune homeostasis. Genetic, epidemiologic and immunologic studies led to distinguish different phases in its natural history, summed-up in the below figure [3,4].

Figure 2: T1D natural history (Biology, Medicine, November 2012)



1.2 Genetics

T1D is a polygenic disorder, with nearly 40 loci known to influence disease susceptibility [5]. The greatest susceptibility to T1D is determined by genes involved in immune response. In particular, the HLA complex gene region (short arm of chromosome 6) determines about 40% of the familiar clustering of the disease. Other genes (insulin VNTR, PTPN22, CTLA4, IL2RA) are involved in the determination of genetic susceptibility but they play a minor role. The *HLA* complex region containins alleles that encode for molecules involved in recognition and presentation of peptide antigens to T-cells. This risk is conferred by a combination of alleles of the *HLA* class II super-types *DRB1*03-DQB1*0201* and *DRB1*04-DQB1*0302* [5]. *DQB1*0302-A1*0301 (DQ8*) haplotype defines the greatest risk for the disease, with an increased diabetogenic effect if the *DRB1*0401* allele is inherited as part of the haplotype. A predisposing effect to T1D has been also found for other *DRB1*04* alleles, **0402*, **0404* [6] and **0405* [7]. Conversely, *DRB1*1501* and *DQA1*0102-DQB1*0602* provide disease resistance. The HLA complex locus is the most polymorphic gene region. The HLA-encoded risk of diabetes is determined by the HLA genotype (HLA haplotypes of both chromosomes) and there is a spectrum of risk: the highest risk is associated with heterozygous DR3/4 genotype, which is found in over one third of patients, but only in 2-3% of healthy individuals. Other genotypes are classified as moderate and low risk

HLA haplotypes. Among the non –HLA genes, only those for the insulin VNTR, *PTPN22, CTLA4*, and *IL2RA* are associated with odds ratios greater than 1.1 [8]. The insulin gene encode for the preproinsulin, a peptide of 110 amino acids which is processed to proinsulin and then to insulin by removal of C-peptide within the islet β-cells. Insulin gene comprises three exons and two introns. T1D susceptibility is associated with a variable number tandem repeats (VNTR) located about 0.5kb upstream the transcription site. Three different classes of alleles have been identified at this locus that are named short class I (26-63 repeats), intermediate class II repeates and larger class III repeats (140-210), respectively [9]. Class I homozygosity confers increased risk being found in 80% of T1D subjects compared with 60% of healthy individuals. Conversely, longer class III VNTR is rare conferring protection[10]. Evidence from animal studies seem to suggest that increased insulin production within the thymus in presence of the protective insulin VNTR III may favor positive and negative selection of T-cells. In contrast, lower expression of insulin associated with VNTR I may lead to less effective selection of insulin by T-cells [11]. Genetic contribution in individuals diagnosed with T1D has changed over the last five decades. The incidence of childhood-onset of T1D has been increasing progressively over the last half century and it is accounted for by individuals with lower-risk HLA genotype who, in the past, would not have developed diabetes in childhood [12]. As demonstration of the major role of HLA genotype in disease development, a relationship between the HLA-encoded risk and presence and/or titers of beta cell autoantibodies exists.

Figure 3: HLA – Complex (National Cancer Institute, 2012)

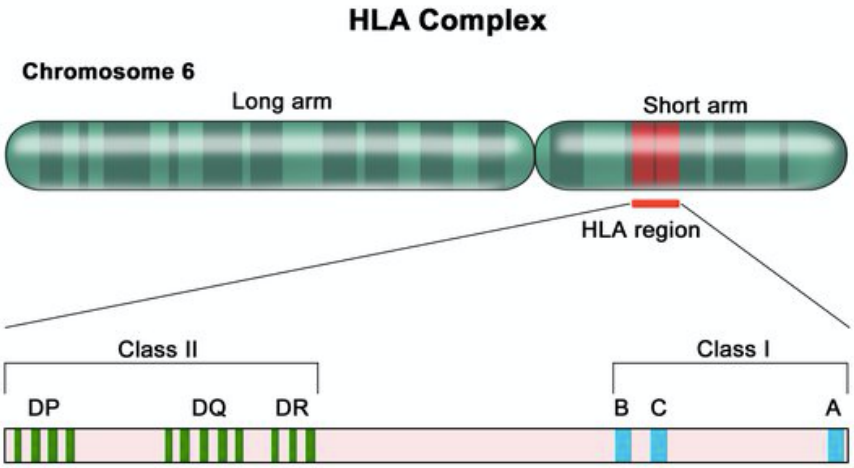


Table 1: HLA – Complex and risk of T1D

Individual risk	0.3%
Individual risk in presence of DQB1*0201/0302	1.7%
Risk of a first degress relative (no mention of HLA alleles)	3-6%
Risk of a first degress relative (no common HLA alleles)	≤ 1%
Risk of a first degress relative (one HLA allele in common)	6%
Risk of a first degress relative (two HLA allele in common)	16%
Monozygotic twins no DR3/DR4	35%
Monozygotic twins withDR3/DR4	70%

Table 2: Association between DRB1/DQB1 aplotypes and T1D

DR	DQ	57th amino acid DQ chain	Association
DR2	0602	Asp	Negative
DR3	0201	Ala	Positive
DR4	0301	Asp	Neutral
DR4	0302	Ala	Positive
DR5	0301	Asp	Negative
DR6	0603	Asp	Neutral
DR7	0201	Asp	Neutral
DR8	0401	Asp	Neutral
DR9	0303	Asp	Neutral

1.3 T1D autoimmunity

1.3.1 Diabetes autoantibodies

Islet cell autoantibodies (ICA) were the first autoantibodies showed to correlate with the development of T1D [13]. Other autoantibodes to insulin (IAA), glytamic acid decarboxylase (GADA), protein tyrosin phosphatase (IA-2A or ICA512) have been subsequently discovered. Diabetes specific autoantibodies can anticipate diabetes diagnosis by years and the presence of persistently positive and multiple antibodies can be predictive of T1D.

Islet cell autoantibodies. The first clue for autoimmune etiology of T1D was provided by the association between T1D and Addison's disease, an autoimmune disease

involving adrenal cortex. The histological analysis of islets of patients with new-onset type 1 diabetes showed the presence of lymphocytic processes within the islets, termed "insulitis" [14]. Then the identification of cytoplasmic ICA was made [13]. Detection of ICA results from the reaction of sera with human pancreatic tissues and then staining for these autoantibodies. Patients with Addison disease may present increased levels of ICA, which binds to certain islet ell surface and cytoplasmic antigens. ICA quantification has been standardized to Juvenile Diabetes Foundation (JDF) units [15] and high JDF levels associated with the development of T1D in relatives of patients with the disease [16]. Their presence before clinical onset provided evidence for the long disease prodrome [17] and allowed their use as predictive marker of T1D. Moreover, ICA positivity also detect a subgroup of patients that progressed more quickly to insulin treatment [18].

Insulin autoantibodies. Insulin and the precursor proinsulin are the only known specific beta cell antigen[15]. It was well recognized that treatment with exogenous insulin induced generation of insulin antibodies, suggesting that insulin preparations purified from other species were able to trigger insulin autoimmune reactions [19]. However, in 1983 Palmer discovered the presence of insulin antibodies in patients with new-onset T1D before administration of exogenous insulin therapies [20]. As shown by a number of subsequent studies, insulin auto-antibodies (IAA) anticipated diabetes diagnosis by years and correlated inversely with age [15]. Unlike other putative autoantigens, proinsulin is expressed almost exclusively by beta cell, which is consistent with the specific targeting of the autoimmune response mediated by T-cell toward pancreatic islets. IAA are the first marker of beta cell autoimmunity to appear in young subjects with type 1 diabetes and are also found in the non-obese diabetic (NOD) mouse, an experimental model of autoimmune diabetes, supporting the idea that the disease might be driven primarily by a loss of tolerance toward this molecule. IAA may appear as early as at 6 months of age in children genetically at risk for type 1 diabetes. Data from the BABYDIAB and DIPP birth cohorts have shown that the highest incidence of IAA is between the first and second year of age [21]. Both prevalence and levels of IAA at diagnosis are inversed correlated with age [22]. Indeed, in first-degreee relatives of patients with type 1 diabetes, more than 90 percent of children below 5 years of age are positive to IAA, compared with only half of young adults aged 15 to 21. Although no

gender difference have been shown in term of prevalence or levels of IAA, peack incidence in BABYDIAB was at nine months in boys and two years in girls. Both prevalence and levels of IAA showed some association with HLA-DRB1*04 in patients and their relatives [23]. In relatives, an association have also been described for HLA-DQA1 alleles, with *DQA1-*0101, *0102, *0103*, *0201*, or *0301* associated with higher IAA levels in comparison to *DQA1*0401, *0501, *0601*. Interestinly, a Swedish study found that insulin autoimmunity was related with the diabetes susceptibility class I allele of VNTR-INS [24], although this results have not been replicated in other populations [25]. It is believed that VNTR affect insulin expression in the thymus and therefore it is believed to influence tolerance toward insulin and possibly the development of IAA response.

Glutamic acid decarboxylase auto-antibodies. The enzyme glutamic acid decarboxylase (GAD) catalyzses the decarboxylation of glutamate to γ-amino butirric acid (GABA) within the nervous system and islet cells. In humans, two main isoforms of GAD have been identified encoded by two different genes –GAD1 and GAD2, encoding for the isoforms GAD67 and GAD65, with molecular weights of 67 kDa and 65 kDa, respectively. GAD1 is expressed in the brain and pancreas, while the GAD2 isoform is expressed only in the pancreatic alpha and beta cells [21]. Antibodies against GAD (GADA) were first shown in subjects with the stiff-man syndrome, a neurological disorder characterized by progressive rigidity and stiffness, and were subsequently found in patients with new-onset T1D as well as individuals with autoimmune polyendocrinopathy syndrome type 2 (ASP2) 108. GADA develop in over 70 percent of individual with T1D independently of age at onset and are a main marker for autoimmune diabetes in adults. In the early phase of GAD65 autoimmunity, GADA predominantly bind to an epitope located in the middle region of the molecule, although subsequently the epitope extend to the N-terminus [15]

Protein tyrosine phosphatase IA-2 antibodies. ICA512, also called IA-2A, have also been detected in patients with type 1 diabetes and related to the development of the disease. These autoantibodies bind to the protein tyrosine phosphatase IA-2 and its cognate IA-2β also named *phogrin*. This antigen is expressed primarly in neuroendocrine celles such as the central nervous system and the pancreatif islets. There, its anchored in the membrane of insulin granules [15]. The main target of these

antibodies appear to be the intracellular domain AA 601-979, which is only exposed in case of cell damage. Prevalence of IA-2 ranges between 60 to 80 percent in recent onset type 1 diabetes and decreased to about 45% in individuals developing the disease after the age of 20 [26]. Compared with GADA, these autoantibodies are less common in patients diagnosed older than 30 years and tend to disappear earlier after the diagnosis [26]. The positivity is highest in individuals positive to *HLA-DRB1*0401* and presenting the *DQA1*0301-DQB1*0302* haplotype. At least 90 per cent of children are positive to either IA-2A or GADA at diabetes onset. IA-2A tends to appear later compared with GADA and therefore are associated with a quicker progression to the disease [27].

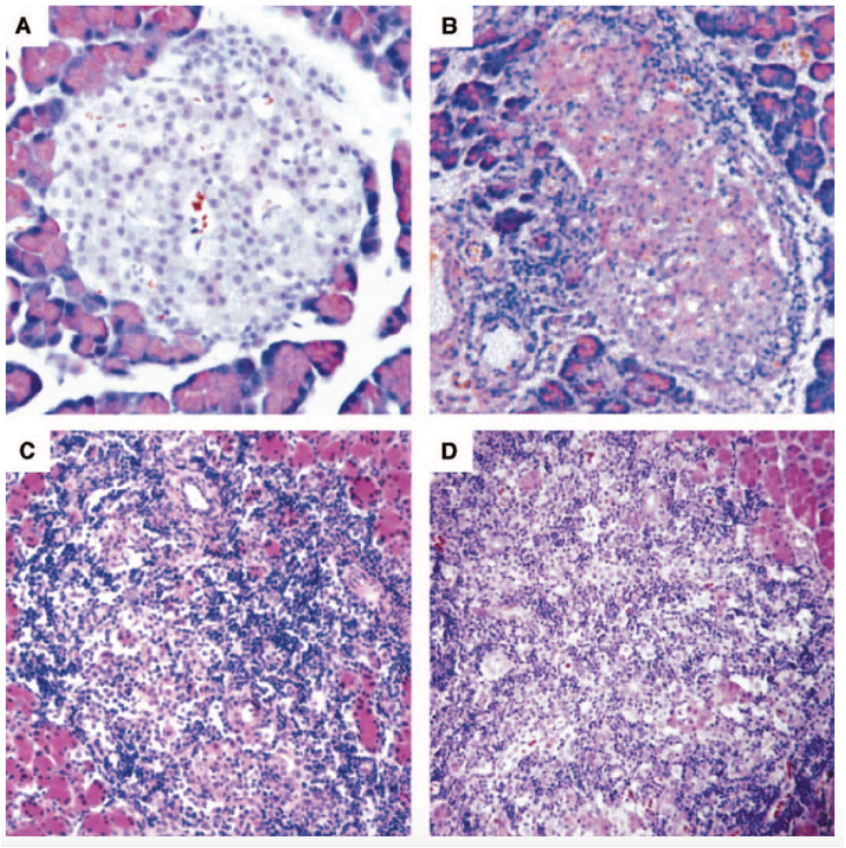
Zinc transporter-8 antibodies. More recently, the zinc transporter-8 (ZnT8) has been described as a major islet autoantigen [28]. ZnT8 is a zinc transporter associated with the membrane of islet cell granules modulating the zinc flux and therefore formation of complexes with insulin in storage crystals. Reactivity against ZnT8 is evident in 70 percent of patients and is of value for T1D prediction. Polymorphysms involving the ZnT8-encoding gene SLC30A8 may influence diabetes risk with the presence of a homozygous SNP at position 325 for either arginine or tryptophan leading to the greatest risk of disease progression [29].

Association of autoantibodies with HLA. Markers of beta-cell autoimmunity such as glutamic acid decarboxylase auto-antibodies (GADA), insulinoma-associated antigen-2 auto-antibodies (IA-2A) and insulin auto-antibodies (IAA) are strongly associated with *HLA-DR4* and/or *HLA-DR3* not only in type 1 diabetes mellitus, but also in first-degree relatives of type 1 diabetes mellitus, as well as in the general population [27]

The HLA confers the greatest genetic risk for type 1 diabetes and has proven to be a significant predictor of the disease in addition to diabetes specific autoantibodies. To date no specific association between HLA alleles and autoimmunity to islet autoantigens have been demostrated but some correlation has came up from some studies indicating that HLA genotype may influence response to specific autoantigens and autoantibodies production. Indeed, autoantibody positivity to GAD65 have been correlated with *DRB1*03* and/or *DQB1*0201* haplotypes [24]. Presence of IA-2 antibodies was increased in patients carrying the *DQ8* [30] and/or *DRB1*04* positivity [31] but reduced in those carrying the DR3/DQB1*0201 [24]. On the other hand, IAA and ICA

develop more commonly in subjects positive for *DRB1*04* (105) and *DQ8* [24]. The *DRB1*04/DQ8* haplotype confers the highest risk for T1D while the *DRB1*03/DQ2* alleles are more associated with a broad-based autoimmune risk for other autoimmune diseases. Therefore, it is believed that *DRB1*03* associated antibody responses (such as GAD65A) are marker of general autoimmunity, while *DRB1*04* associated IA-2A define a more specific marker of islet autoimmunity [31]. IAA have been associated with polymorphisms within the *VNTR-INS* gene but this has not been replicated by subsequent studies.

Figure 4: Progression of insulitis during autoimmune diabetes development in rat (Diabetes, 2005)



Non infiltrated pancreatic islet of a nondiabetici, control animale representing stage 0 (A). In the early infiltration stage (stage 1), the infiltrate was restricted to the islet periphery of a normoglycaemic T1D rat at day 50 (B). In stage 2, in normoglycaemic animala t day 55, the whole islet was infiltrated to a low degress (C), stage 3 represented a severely infiltrated islet of a diabetic animal at day 59 (D)

1.3.2 Diabetes and ROS

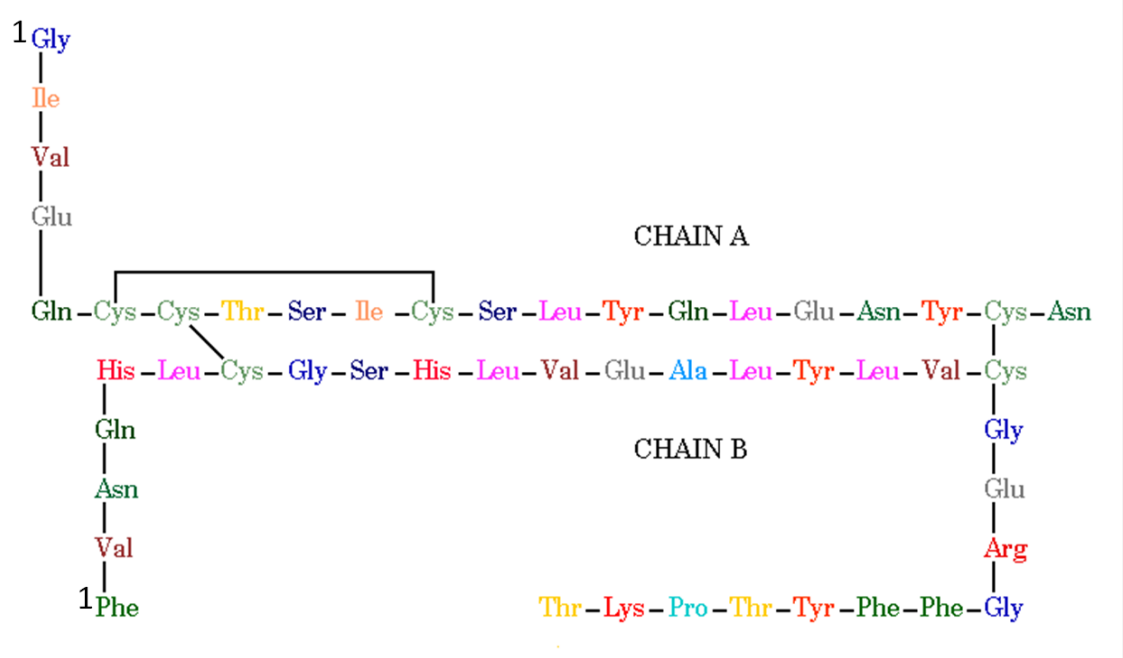
Hyperglycemia is the hallmark of the disease and is a central player in the pathogenesis of chronic complications. Elevations of blood glucose induce oxidative stress and

changes in the cellular redox state. NADPH oxidase has been responsible for formation of high levels of ROS in response to high glucose [32]. A second source of ROS production is the excessive production of advanced glycation end products (AGE). Two AGE, namely carboxymethyl-lysine and pentosidine are related to severity of diabetic nephropathy and are addressed as 'carbonyl stress' [33]. The main toxic effect of both ROS and AGE is the induction of abnormal post-translational modifications of self-antigens and generation of neo-antigens, thus by-passing immune tolerance and contributing to the development of autoimmune responses [34]. The involvement of oxidative stress in type 1 diabetes mellitus has been implied by the presence of auto-antibodies against oxidized-GAD [35]. In addition, experimental diabetes can be induced in rats by feeding with alloxan and streptozotocin, two substances which work by generating ROS and inducing a selective damage of beta cells [36].

1.3.3 Insulin structure

Insulin is a small globular protein of about 5.8 kDa consisting of two chains, the A-chain of 21 amino acids and the B-chain of 30 amino acids, linked by three disulfide bonds, one intrachain bond, A6-A11 and two interchain bonds, A7-B7 and A20-B19). Insulin is processed by its precursors preproinsulin (PPI) and proinsulin (PI). PPI contains an N-terminal signal peptide of 24 amino acids, which is cleaved to generate PI into the endoplasmic reticulum. In the endoplasmic reticulum [37] PI is reduced and unfolded, then oxidized and folded to generate a polypeptide consisting of A-chain and B-chain linked by a 35-amino acid connecting peptide (C-peptide). PI moves then to the Golgi where it is cleaved to Insulin by removing the C-peptide in a reaction involving the protease carboxypeptidase E. Insulin aggregate in hexamer and zinc ions participate in structure stabilization within the post-Golgi and sorted in secretory granules [38]. Two crystal forms exist with either two or four zinc atoms per six insulin molecules. Each beta-cell contain over 10,000 secretory granules and insulin represent more than 50% of total beta cell mRNA. Upon secretion into the portal circulation, insulin hexamers dissociate into bioactive insulin monomers.

Figure 5: Insulin sequence and primary structure



1.4 Environmental factors

Just a few subjects with genetic susceptibility to T1D develop the disease, so that it is reasonable to recognize to environmental factors a key role in the autoimmune pathogenetic process [3].

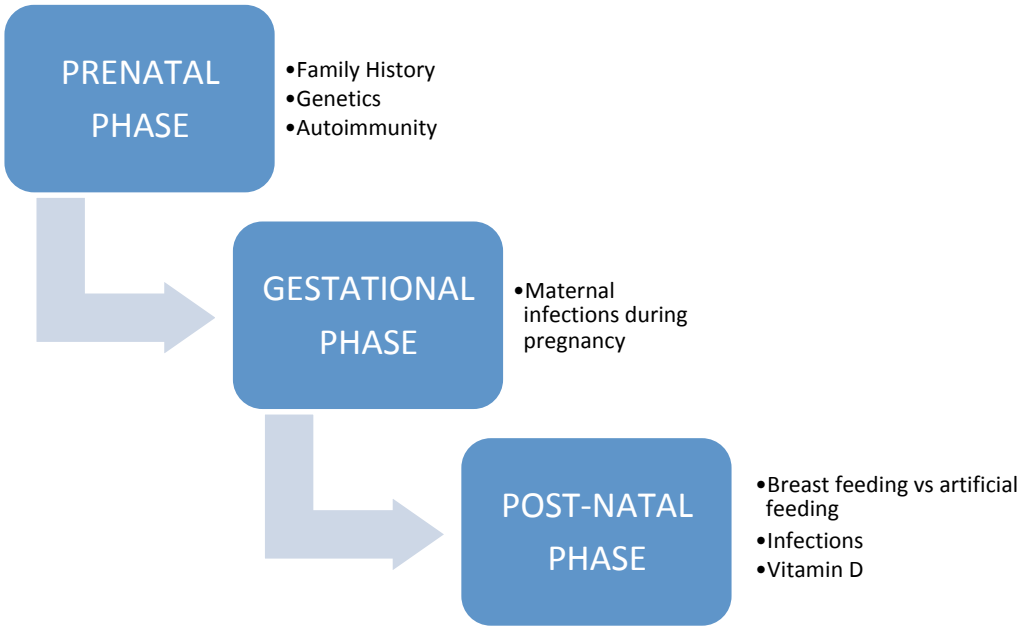
- Immune system**:** enterovirus, in particular coxsackie B virus, seem to be related to T1D onset. A recent systematic review showed a strong correlation between coxsackie B infection and T1D [39]. This correlation resulted even higher in subjects with high-risk HLA-DQB1 aplotype. Viruses seem to infect β-cells directly, leading to cytolysis and exposure of β-cell autoantigens to immune-cells. Some studies suggest that infections developed by pregnant women could affect fetal pancreas, increasing the risk of developing T1D during childhood[40]. Regulatory-T cells up-regulation and T helper-1 dysfunction could be responsible of the autoimmune impairment that lead to β-cell distruction [4,41,42].

Finally, vitamin D deficiency, which contribute to immune response, could increase or accelerate the development of T1D because of the consequent imbalance between pro- and anti-inflammatory factors [43]

- Breast-feeding and cereals: artificial breast-feeding with bovine milk is one of the most studied environmental factors associated with T1D pathogenesis. One milk protein seem to trigger autoimmune activation against β-cells. Many T1D patients present antibodies anti- bovine milk proteins. Some studies suggest an inverse correlation between duration of breast-feeding and T1D onset: in particular children breast-fed for less than 3 months seem to be more prone to develop T1D [44]. Weaning and feeding during early childhood with solid foods have been widely studied in T1D pathogenesis, especially in subjects genetically prone. The DAISY study confirmed that the introduction of cereals (both with or without gluten) during weaning increased the risk of development of T1D autoimmunity [45]. In this same field, the BABYDIET study evaluated if gluten exclusion for the first 6 months of life of newborns with a first degress relative diagnosed T1D could reduce the risk of T1D onset, however no significant benefits were obtained [46,47].

- Body Mass Index (BMI): BMI can condition C-peptide decline and T1D progression in children. Barker et al. designed a study involving more than 3.000 subjects aged between 0 to 18 years and diagnosed T1D within one years to establish if BMI at the diagnosis could become an independent predicor of C-peptide reduction during the first months of disease. In subjectes diagnosed T1D between 0-5 years, 5-10 yerars and after 18 years, no relevant correlation between BMI and C-peptide was observed. Between 10-18 years, otherwise, to higher BMI values corresponded a stronger C-peptide reduction [48].

Figure 6: Environmental factors and T1D



1.5 T1D complications

1.5.1 Macrovascular complications

The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body [49]. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries. Angiotensin II may promote the oxidation of such particles. Monocytes then infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes. T-lymphocytes, in turn, induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the process is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of this lesion leads to acute vascular infarction or stroke [50].

In addition to atheroma formation, there is strong evidence of increased platelet adhesion and hypercoagulability in case of hyperglycaemia. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation. Elevated levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis in patients with diabetes [51].

Cardiovascular disease (CVD) is the primary cause of death in people with T1D. In fact. Among macrovascular diabetes complications, coronary heart disease has been associated with diabetes in numerous studies beginning with the Framingham study [52]. More recent studies have shown that the risk of myocardial infarction (MI) in people with diabetes is equivalent to the risk in nondiabetic patients with a history of previous MI [53]. These discoveries have lead to new recommendations by the ADA and American Heart Association that diabetes be considered a coronary artery disease risk equivalent rather than a risk factor [54]. Diabetes is also a strong independent predictor of risk of stroke and cerebrovascular disease, as in coronary artery disease. Risk of stroke-related dementia and recurrence, as well as stroke-related mortality, is elevated in patients with diabetes [55]. Patients with type 1 diabetes also bear a disproportionate burden of coronary heart disease. Studies of have shown that these patients have a higher mortality from ischemic heart disease at all ages compared to the general population. In individuals > 40 years of age, women experience a higher mortality from ischemic heart disease than men. Observational studies have shown that the cerebrovascular mortality rate is elevated at all ages in patients with T1D [56]. The increased risk of CVD has led to more aggressive treatment of these conditions to achieve primary or secondary prevention of coronary heart disease before it occurs. Studies in T1D have shown that intensive diabetes control is associated with a lower resting heart rate and that patients with higher degrees of hyperglycemia tend to have a higher heart rate, which is associated with higher risk of CVD [57]. Even more conclusively, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study demonstrated that during 17 years of prospective analysis, intensive treatment of type 1 diabetes, including lower A1C, is associated with a 42% risk reduction in all cardiovascular events and a 57% reduction in the risk of nonfatal MI, stroke, or death from CVD. There is additional benefit to lowering blood pressure with ACE inhibitors or ARBs. Blockade of the renin-angiotensin system using either an ACE inhibitor or an ARB reduced cardiovascular endpoints more than other antihypertensive agents [51,58]. It

should be noted that use of ACE inhibitors and ARBs also may help slow progression of diabetic microvascular kidney disease. Multiple drug therapy, however, is generally required to control hypertension.

Another target of therapy is blood lipid concentration. Numerous studies have shown decreased risk in macrovascular disease in patients with diabetes who are treated with lipid-lowering agents, especially statins. These drugs are effective for both primary and secondary prevention of CVD, but patients with diabetes and preexisting CVD may receive the highest benefit from treatment. In addition to statin therapy, fibric acid derivates have beneficial effects. They raise HDL levels and lower triglyceride concentrations and have been shown to decrease the risk of MI in patients with diabetes in the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial [51,54,59–62].

1.5.2 Microvascular complications

1.5.2a Retinopathy

Retinopathy occurs in all forms of diabetes . Several high-quality studies have defined the natural history of retinopathy in T1D using stereoscopic fundus photography. As with all diabetes-specific complications, the development of retinopathy depends on the duration of the disease [63]. The first and most common visible lesions are small (diameter, <100 micrometers) microaneurysms arising from the terminal capillaries of the retina. Dot and blot hemorrhages appear when erythrocytes escape from the microaneurysms. The retinal vessels are abnormally permeable and leak serous fluid, leading to the formation of hard exudates. Microaneurysms, dot and blot hemorrhages, and hard exudates are described as “background” retinopathy (because of their common occurrence in diabetes) or, preferably, nonproliferative retinopathy. Unless nonproliferative retinopathy occurs near the maculae and causes macular edema, it does not lead to the loss of vision. Macular edema occurs when leakage of fluid from abnormal vessels near the maculae disrupts the light path to the maculae and results in the loss of visual acuity. With increasingly severe retinopathy, the abnormal vessels can become occluded, leading to retinal ischemia with infarctions in the nerve layer of the retina, seen as soft, or “cotton wool,” exudates (preproliferative retinopathy). In response to ischemia, new vessels develop (neovascularization). The new vessels proliferate out of the retinal surface and into the vitreous cavity (proliferative

retinopathy). They are attenuated and fragile and tend to bleed into the vitreous. The vitreous hemorrhages can obscure vision, but they are usually reabsorbed in one to three months. Subsequent fibroproliferative changes result in retinal traction and detachment and the loss of vision. Proliferative retinopathy is clinically divided into neovascularization of the disk (which occurs within one disk diameter of the disk) and neovascularization elsewhere, on the basis of the differential risks for loss of vision associated with these lesions [64]. The natural history of diabetic retinopathy has been defined best in T1D, in which the date of onset of clinical diabetes can be accurately ascertained. In general, nonproliferative retinopathy does not appear until after three to five years, and may not appear at all before puberty. After seven years, approximately 50 percent of patients with T1D have some degree of retinopathy detectable by stereoscopic fundus photography [65]. Direct ophthalmoscopy is less sensitive, especially if there are few lesions. The prevalence of any retinopathy reaches more than 90 percent after 20 years. The development of macular edema and proliferative retinopathy is also duration-dependent, although those complications are less frequent than nonproliferative retinopathy. Nonproliferative retinopathy and proliferative retinopathy are the characteristic lesions of diabetic retinopathy. Patients with diabetes are also at higher risk for other ophthalmic disease, such as cataracts [66].

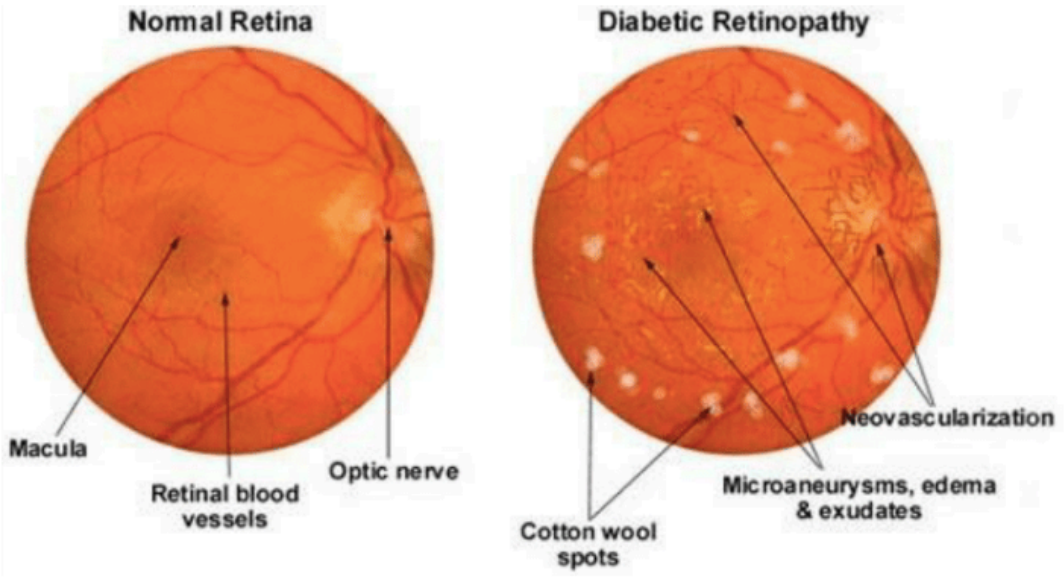


Figure 7: Retinophaty (ResearchGate, January 2013)

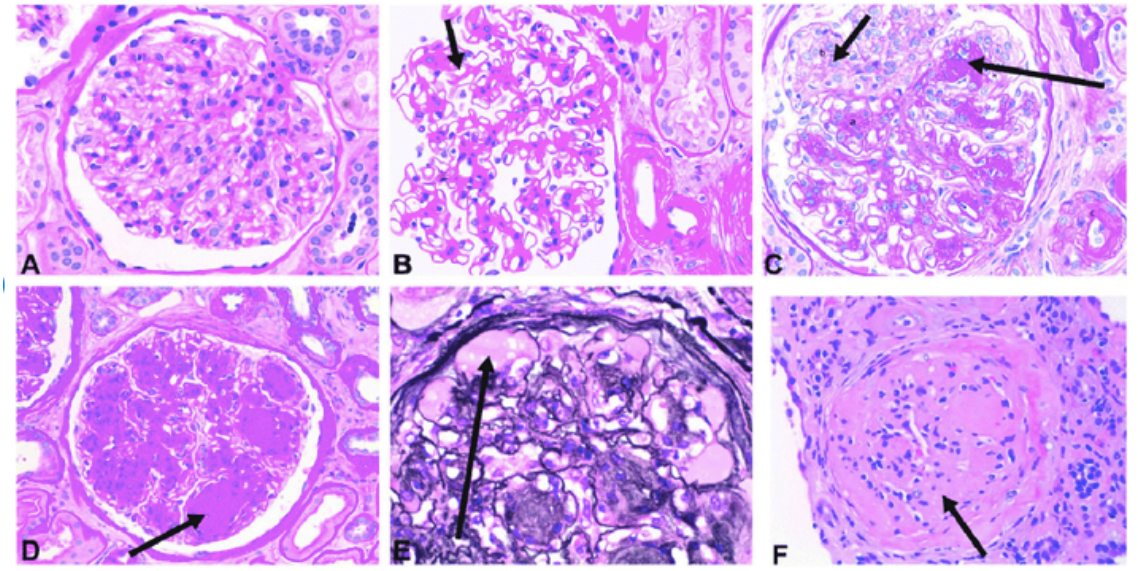
1.5.2b Nephropathy

Nephropathy is the diabetes-specific complication associated with the greatest mortality [67]. Although the vast majority of diabetic patients have some degree of retinopathy, nephropathy develops in only 35 to 45% percent of patients with T1D [68]. The natural history of clinically detectable diabetic nephropathy begins with the development of microalbuminuria (30 to 300 mg of albumin per 24 hours), which may occur as early as five years after the onset of diabetes**.** This stage of incipient nephropathy may be more likely in patients with glomerular hyperfiltration (i.e., a glomerular filtration rate >150 ml per minute) [69]**.** After another 5 to 10 years of diabetes, overt proteinuria (>500 mg of protein per liter, equivalent to >300 mg of albumin per 24 hours) develops in patients destined to have end-stage renal disease. Hypertension invariably develops during this period. In the next 5 to 10 years, the nephrotic syndrome develops and the glomerular filtration rate falls, resulting in end-stage renal disease. The mean durations of T1D before the development of overt proteinuria and end-stage renal disease are 17 years and 23 years, respectively [70]**.** Although a small fraction of patients with T1D who have nephropathy may die of uremia, the majority die of concurrent cardiovascular disease, the risk of which is 30 to 40 times that in patients with IDDM who do not have nephropathy[68].

Unlike the prevalence of retinopathy, the prevalence of nephropathy does not rise continuously with the increasing duration of diabetes [71]. If overt proteinuria, the most reliable indicator of diabetic nephropathy, has not developed after 25 to 30 years of disease, the risk of nephropathy begins to decrease [72]. Although histopathological changes do not always mirror clinical severity, they too follow a stereotypical course [73]. Initially, there is renal hypertrophy, with expansion of the glomeruli, including the mesangium and glomerular basement membrane, and an increase in kidney size [74]**.** Glomerular composition changes more slowly, leading to characteristic mesangial expansion, thickening of the glomerular basement membrane, and afferent and efferent arteriosclerosis. With more advanced nephropathy (progressive proteinuria), glomerular closure occurs. There is compensatory hypertrophy of the functioning glomeruli during this stage [75]**.** Kimmelstiel-Wilson nodular glomerulosclerosis is a relatively late phenomenon that affects only a minority of patients with nephropathy [76]**.** End-stage

renal disease is characterized by small, atrophic kidneys with diffuse glomerulosclerosis [77]

Figure 8: Nephrophaty (Clinical Journal of the American Society of Nephrology, 2017)



| Diabetic glomerulopathy. Changes in glomerular histology in diabetic glomerulopathy (A) Normal glomerulus. (B) Diffuse mesangial expansion with mesangial cell proliferation. (C) Prominent mesangial expansion with early nodularity and mesangiolysis. (D) Accumulation of mesangial matrix forming Kimmelstiel-Wilson nodules. (E) Dilation of capillaries forming microaneurysms, with subintimal hyaline (plasmatic insudation). (F) Obsolescent glomerulus. A-D and F were stained with period acid-Schiff stain, and E was stained with Jones stain. Original magnification, 3400.

1.5.2c Neuropathy

Clinical manifestations of neuropathy in patients with T1D can be different. A peripheral, symmetric sensorimotor neuropathy is the most common form of diabetic neuropathy, whose other forms include cranial and peripheral motor neuropathies and autonomic neuropathy. Although neuropathy is also more common with a longer duration of diabetes, a relatively severe, early-onset polyneuropathy has been described [78]. Electrophysiologic studies demonstrate subclinical abnormalities, including slowed motor- and sensory-nerve conduction in most patients, after 5 to 10 years of diabetes [79]. Distal symmetric sensorimotor neuropathy detectable on physical examination is only minimally bothersome for most patients. Symptoms, including paresthesia, are characteristically worse at night. Because loss of sensation in the feet and altered foot architecture make foot care problematic, the principal risk posed by peripheral neuropathy is of foot trauma and diabetic ulcers. A minority of patients have painful peripheral neuropathy with lancinating or burning dysesthesia, severe enough in some

to be associated with depression and anorexia [80]. Symptoms often wax and wane. Focal motor (cranial and peripheral) and compression neuropathies and mononeuritis multiplex are less common than the sensorimotor neuropathies. Radiculopathies may also occur, mimicking disk disease. Except for the compression neuropathies, such as carpal tunnel syndrome, which may require surgical decompression, the motor neuropathies usually resolve spontaneously in six weeks to six months. Autonomic neuropathy can affect gastric or intestinal motility, erectile function, bladder function, cardiac function, and vascular tone. Although subclinical changes (e.g., the loss of variation in heart rate with respiratory phase or altered gastrointestinal contractility) can often be detected within 5 to 10 years after the onset of IDDM, clinical autonomic neuropathy is less common. Gastroparesis may not only cause symptoms but also alter the absorption of meals and make glycemic control problematic. Diabetic diarrhea and incontinence are rare but can be disabling. Impotence is the most common clinical manifestation of autonomic neuropathy, affecting more than 50 percent of men with diabetes. Cardiac autonomic neuropathy may result in resting tachycardia and postural hypotension [68].

1.6 Follow-up

Patients with type 1 diabetes of > 5 years' duration should have annual screening for microalbuminuria. All patients with diabetes should have serum creatinine measurement performed annually [81]. Patients with microalbuminuria or macroalbuminuria should be treated with an ACE inhibitor or ARB unless they are pregnant or cannot tolerate the medication [82]. Patients who cannot tolerate one of these medications may be able to tolerate the other [83]. Potassium should be monitored in patients on such therapy. Patients with a GFR < 60 ml/min or with uncontrolled hypertension or hyperkalemia may benefit from referral to a nephrologist [84]. Patients with T1D should receive a comprehensive eye examination and dilation within 3-5 years after the onset of diabetes. Patients should strive for optimal glucose and blood pressure control to decrease the likelihood of developing diabetic retinopathy or experiencing progression of retinopathy [85]. All patients with diabetes should undergo screening for distal symmetric polyneuropathy at the time of diagnosis and yearly thereafter. Atypical features may

prompt electrophysiological testing or testing for other causes of peripheral neuropathy. Patients who experience peripheral neuropathy should begin appropriate foot self-care, including wearing special footwear to decrease their risk of ulceration. They may also require referral for podiatric care [86]. Screening for autonomic neuropathy should take place 5 years after the diagnosis of type 1 diabetes [87]. Medication to control the symptoms of painful peripheral neuropathy may be effective in improving quality of life in patients but do not appear to alter the natural course of the disease. For this reason, patients and physicians should continue to strive for the best possible glycemic control. In light of the above strong evidence linking diabetes and CVD and to control and prevent the microvascular complications of diabetes, the ADA has issued practice recommendations regarding the prevention and management of diabetes complications. Blood pressure should be measured routinely. Goal blood pressure is <130/80 mmHg. Patients with a blood pressure ≥ 140/90 mmHg should be treated with drug therapy in addition to diet and lifestyle modification. Patients with a blood pressure of 130-139/80-89 mmHg may attempt a trial of lifestyle and behavioral therapy for 3 months and then receive pharmacological therapy if their goal blood pressure is not achieved. Initial drug therapy should be with a drug shown to decrease CVD risk, but all patients with diabetes and hypertension should receive an ACE inhibitor or ARB in their antihypertensive regimen [81]. Lipid testing should be performed in patients with diabetes at least annually. Lipid goals for adults with diabetes should be an LDL < 100 mg/dl(or < 70 mg/dl in patients with overt CVD), HDL > 50 mg/dl, and fasting triglycerides < 150 mg/dl. All patients with diabetes should be encouraged to limit consumption of saturated fat, trans fat, and cholesterol. Statin therapy to lower LDL by 30-40% regardless of baseline is recommended to decrease the risk of CVD in patients > 40 years of age. Patients < 40 years of age may also be considered for therapy. In individuals with overt CVD, special attention should be paid to treatment to lower triglycerides or raise HDL [81].

1.7 T1D therapy

The type 1 diabetes therapy concept consists of insulin therapy, nutritional knowledge, training and glucose self-monitoring.

1.7.1 Multiple daily injection therapies (MDI)

The indication for insulin therapy in T1D is permanent and lifelong. A prerequisite for the substitution of lacking insulin is knowledge of the physiological insulin requirement as well as the pharmacokinetic and pharmacodynamic properties of the insulins used for therapy. The individual insulin requirement depends on the physiological insulin secretion. This occurs both without food intake (basal insulin requirement) and after food intake (prandial insulin requirement). When dosing insulin, it must be taken into account that the absolute insulin requirement also depends on the individual insulin sensitivity.

Conventional therapy is characterized by a binding specification of both the insulin dose and the sequence and size of the meals (fixed carbohydrate portions). A blood glucose self-measurement is recommended 3–4 times daily. Fixed insulin mixtures are administered twice a day for breakfast and dinner and, as far as possible, adapted to the eating behaviour of the patients. A simple conventional insulin therapy can only be successful with a fixed diet plan. In contrast to intensified therapy, this form of insulin therapy is a subordinate therapy option for people with type 1 diabetes in the following cases:

- For people who cannot meet the requirements of an intensified therapy (due to cognitive impairment, illness or age),
- For people who decide against intensified therapy after receiving extensive information on the risks and benefits,
- For people with a significant problem adhering to longterm therapies.

Since medium and longterm glycaemic control is crucial for reducing the risk of diabetes-associated complications, conventional insulin therapy can be sufficient if the individual HbA1c target value are reached, hypoglycaemia is avoided, and the quality of life is not restricted by the therapy.

The intensified insulin therapy is defined as the administration of at least three insulin injections per day. Above all, however, it is characterised by substituting the basal insulin requirement with long-acting basal insulin and by substituting prandial insulin requirement with rapid-acting bolus insulin at mealtimes (basal bolus principle). Synonyms of intensified insulin therapy are functional insulin therapy and flexible insulin therapy. This therapy can be performed with insulin syringes, insulin pens or insulin pump pens [81].

1.7.2 Continuos subcutaneous insulin infusion (CSII)

Good control of blood glucose levels is known to be associated with reduced long-term complications of diabetes, however many patients with T1D can't reach this goal. Even if MDI therapy with insulin analogues is the treatment of choice many patients do not achieve the target glycosylated haemoglobin (HbA1c) level of <7%, and about 20% of patients with type 1 diabetes experience episodes of severe hypoglycaemia at a frequency of about 1 per patient per year. Insulin pump therapy or continuous subcutaneous insulin infusion (CSII) was introduced in the 1980. Insulin pumps have been designed to infuse insulin subcutaneously and are able to provide a background or basal insulin infusion in association with bolus doses that can be administered with food or to correct high blood glucose levels (Figure 1). Bolus doses may also be a fraction of a unit allowing for finer dose adjustments. Infusion of rapid-acting insulin into one site should reduce glycaemic variability, compared with multiple injections into different sites. Furthermore, CSII introduces the possibility of varying the background or basal infusion rates according to patient needs, and having as many bolus doses of rapid insulin that may be needed to correct for high readings or for added unscheduled snacks. CSII has developed over the years so that there are a number of different models available with functions such as extended boluses, temporary basal rates, and more compact 'patch' pumps that do not require tubing. These pumps need to be worn 24 hours a day and the cannula site changed every 3 days. Although pumps are worn continuously, there are now accessories that allow for more discreet wearing of pumps under clothing, and advances that allow for less handling of the pump and remote activation. CSII has improved outcomes for patients in terms of hypoglycaemia reduction, HbA1c improvement and quality of life, but there is a limit to the benefits, and some patients continue to struggle to achieve optimal control.1 One of the

limitations of CSII is that subcutaneous insulin administration is peripheral, whereas pancreatic insulin, which involves the portal system, has important effects on hepatic glucose metabolism. Furthermore, subcutaneous insulin absorption is slow, compared with the fast onset and offset of normal beta cell function. Furthermore, the requirement for regular blood glucose testing is no different with CSII, compared with standard MDI [81].

Figure 9: Medtronic 780G (A), ACCU-CHEK Insight (B) Insulin Pumps and Theras Omnipod Patch Pump (C)

1.7.3 Glucose sensors

Subcutaneous CGM has been developed and now has proven benefit in type 1 diabetes. It involves the subcutaneous insertion of a glucose sensor attached to a transmitter that sends signals to either an insulin pump or a hand-held meter. These are worn for 7 days with the sensor inserted into subcutaneous abdominal fat. Most of these devices need regular calibration and blood glucose testing about twice a day. The accuracy of these

devices has been an issue, but they have been improved in recent years. Subcutaneous glucose levels change more slowly than plasma glucose, and this may be an important limitation, particularly if glucose levels are changing rapidly. Subcutaneous glucose levels, therefore, have a short time lag, compared with blood glucose measurements, and measurements may not always match blood glucose. Nevertheless, when worn regularly (changed every 7 days), they improve outcomes in terms of hypoglycaemia and hyperglycaemia. As the devices and associated pumps have advanced, patients can be alerted to hypo – and hyperglycaemia, and take early action to correct blood glucose levels. If worn in association with last generation pumps, a low glucose level identified on CGM will not only alert the patient through an alarm system, but shut the pump off until glucose levels recover. This is known as the 'low glucose suspend' feature.

CGM is also available as a 1-week diagnostic test in which the patient is blinded to glucose levels at the time and the sensor is downloaded at the end of the week. The literature behind this type of retrospective or blinded CGM is not as strong as with the real-time CGM, but it can be used in primary care [81].

Table 3: characteristics of main glucose sensors

	Guardian & Enlite sensor	Dexcom G6	Freestyle Libre
Agency	Medtronic	Theras	Abbott
rt-CGM	Yes	Yes	No
Detected glyacemic range	40-400 mg/dl	40-400 mg/dl	40-500 mg/dl
Paracetamol interference	Yes	No	No
Age	No limits	> 2 years	> 4 years
Pregnancy	Yes	Yes	Yes
Duration	7 days	10 days	14 days
Wearability	High	High	Very hogh
Tendence arrows	Yes	Yes	Yes
Alarms	Yes	Yes	No (FS-1) Yes (FS-2)
Predictive alarms	Yes	Yes	No
Calibrations per day	Yes (Enlite and Guardian sensor 3) No (Guardian sensor 4)	No	No
Need of glucometer check	Yes	No	No
Insulin pumps interface	Yes	Yes	No
Web data platforms	Carelink	Dexcom Clarity	LibreView

Figure 10: Glucose sensors (A: Medtronic Guardian Connect; B: Theras Dexcom G6; C: Abbott Freestyle Libre; D: Ascensia Eversense)

1.7.4 Selection of patients for CSII and CGM

Most patients with type 1 diabetes are adequately maintained on MDI together with insulin adjustment for carbohydrate content, exercise and acute illness. In the majority of cases, pump use commences in childhood, although there are growing numbers of users commencing as adults. Many paediatricians and parents prefer children and adolescents to use pumps because of the flexibility and improved control associated with CSII, particularly in view of the erratic lifestyle and growth issues in these age groups. In adults with established type 1 diabetes, indications may vary and include lifestyle and quality-of-life factors, and regular hypoglycaemia on MDI therapy. Many people with type 1 diabetes have impaired quality of life associated with frequent hypoglycaemia, erratic blood glucose levels and fear of hypoglycaemia. A smaller

percentage of patients experience frequent episodes of severe hypoglycaemia that require assistance or hospitalisation. These are examples of circumstances in which CSII should be considered and discussed with the patient. Some degree of technological capability is required to run a pump, so elderly patients with type 1 are less likely to start using a pump. Patients with T1D preparing for pregnancy may decide to trial CSII before pregnancy in order to achieve and maintain better control during pregnancy. In all cases, patients need to have a high level of compliance with monitoring glucose frequently, as well as having an adequate grasp of carbohydrate counting.

Pump starts require a specialist multidisciplinary team including an endocrinologist/paediatrician, credentialled diabetes nurse educator and a dietician. Pre-pump education includes:

- assessment of the indication and patient expectations
- dietician review and initiation or reinforcement of carbohydrate counting
- discussions regarding pump types and general workings of pumps re-siting, adjusting rates and problem solving.

In the event of rapidly rising blood glucose levels, patients need to have an action plan including the ability to re-site cannulae, as one of the common problems encountered is kinking or blocking. The next few weeks require regular follow-up and adjustments with the team, and after-hours numbers must be available for problem solving in the event of unstable blood glucose levels. Many of the modern pumps have software that patients can use to download their data and send it to the team for assessment and adjustments. Patients need to be aware that pumps may fail at a rate of about 1 in 5 over 4 years, so clinicians must inform patients that they might need to restart MDI in case of an emergency. CGM is a useful tool in pump and non-pump users with T1D. The most commonly used CGM is in combination with CSII, and most modern pumps allow for CGM to be read in real time on the pump. In selected cases CGM can also be used in non-pump users. Many patients are concerned about severe, nocturnal hypoglycaemia, and CGM should be considered for high-risk patients, given the morbidity and possible mortality associated with severe nocturnal hypos. High-risk groups include patients with hypoglycaemia unawareness and particularly ‘frequent flyers’ with regular severe hypos. Another important group is the patient with T1D who is pregnant and aims for

meticulous glucose control. CGM should be considered in patients who have occupations that require warning of hypoglycaemia such as professional drivers, or those working in remote or offshore environments. Unfortunately, CGM is expensive and has government support in selected cases [88–90].

1.7.5 Carbohydrate counting

Carbohydrate counting is one of the dietary approaches that can be used in the management of T1D. It can be used by patients on multiple daily injections (basal bolus regimen) or continuous subcutaneous insulin infusion to manage diabetes. It focuses on carbohydrates as the primary macronutrient affecting postprandial glycaemic response and is used to adjust insulin dose levels according to the carbohydrate content of the meal. With carbohydrate counting, the patient is made aware of the effect of carbohydrate-containing foods on blood glucose levels. Patients are taught to quantify the amount of carbohydrates by visualisation using education tools like plate models or hand portions. They are then taught to give the correct amount of insulin depending on the portion of carbohydrate, to prevent hyper- and hypoglycaemia and maintain normal blood glucose levels. The National Institute for Health and Care Excellence (NICE) guidelines recommend that carbohydrate counting should be offered to all adults with T1D as part of self-management structured education [91]. For those who are not able to gain access to such structured education groups, it is recommended that it be given on a one-on-one basis. Carbohydrate counting has been shown to improve glycaemic control as well as quality of life but must be taught by someone who has clinical expertise in this field, such as an experienced registered dietitian. Carbohydrate counting has been known since the 1920s and was one of the selected nutrition interventions used together with intensive insulin therapy to attain normoglycaemia in subjects who participated in the Diabetes Control and Complications Trial (DCCT) trial [56]. This method was effective in achieving glycaemic control as well as allowing for flexibility with food choices. A meta-analysis of the current literature on the effectiveness of carbohydrate counting in comparison with other diet methods showed that carbohydrate counting resulted in a significant reduction in HbA1c. There are three levels of carbohydrate counting. Level 1 is the basic level of carbohydrate counting that can be taught to patients with T1D. Level 2 is for patients who have mastered level 1 and desire further

skills pertaining to blood glucose patterns and food intake. Level 3 is designed primarily for people with T1DM on intensive insulin regimes who use insulin-to-carbohydrate ratios [92]. Carbohydrate counting is recommended as standard care for the management of T1D in the United States of America (USA) and the United Kingdom (UK). The American Diabetes Association (ADA) recommends that carbohydrate counting should form part of the standard care for patients with T1D [81].

Study 1: The effect of glucose control on immune response to a SARS-COV2 vaccine in T1D

2.1 Background

Metabolic homeostasis is severely compromised in T1D, affecting different organs including liver, adipose tissue, bowel, brain and skeletal muscle [81] Glycosilation and oxidation processes, in particular, are enhanced, resulting in non enzymatic modifications of proteins performing different biological functions [93–96]. Signs and symptoms of SARS-CoV-2 infection can be extremely diversified [97]. Patients can be asymptomatic or mildly symptomatic, experiencing more or less severe symptoms that can involve just the upper respiratory airways (20.86% of cases) or both upper and lower respiratory airways up to Acute Distress Respiratory Syndrome (ARDS), septic shock and Multi Organ Failure (MOF) [98]. People with history of diabetes, cardiovascular events, obesity, and hypertension present an higher risk to develop a more severe COVID-19-related outcome [99–104]. Many studies demonstrated that diabetic patients show a less effective immune response against infective agents [105,106] as a consequence of the hyperglycaemic environment and the chronic inflammatory state [107,108]. For instance, diabetic patients exposed to vaccines against hepatitis or influenza present a weaker antibody response and studies suggest that patients in poor glucose control are more at risk of worse outcome if infected by COVID-19 [109]. Our group has previously demonstrated how glycation can modify significantly antibody response. Glycated insulin, for example, is not recognized by anti-insulin autoantibodies in type 1 diabetes, while, on the contrary, glycated collagen type II enhances autoantibody response both in type 1 diabetes and in rheumatoid arthritis [110,111].

2.2 Aim

The aim of study 1 was to verify if hyperglycaemia could interfere with antibody response in patients with T1D undergone COVID-19 vaccine.

2.3 Matherials and Methods

2.3.1 Study population

26 patients with type 1 diabetes, undergone mRNA Pfizer mRNABNT162b2 (Comirnaty) vaccine, were enrolled in the study. All type 1 diabetes patients were on intensive insulin therapy, respectively on multiple daily insulin injection regimen (MDI) and on continuous subcutaneous insulin infusion (CSII). CSII devices included Medtronic Minimed 640G and 670G (4), Theras Omnipod (4) and Roche Insight (2). Glucose monitoring was performed with Dexcom-G6 (6), Guardian sensor (4) and Flash Freestyle Libre (6). Blood samples were collected during medical consultations in the Day Hospital and the outpatients clinics of the Endocrinology and Metabolic Diseases Unit of Policlinico Campus Bio-Medico of Rome in order to test time-related variations of post-vaccine antibody titer. Analysis and storage of the blood samples was performed in Endocrinology and Metabolic Diseases Laboratory. of Policlinico Campus Bio-Medico of Rome Inclusion criteria were: age > 18 years, previous SARS-COV2 mRNA Pfizer vaccine, signed inform consent and, just for patients with type 2 diabetes, ongoing therapy with at least two anti-diabetic medications. Exclusion criteria were: age <18 years, diagnosis of type 1 or type 2 diabetes for less than three months, pregnancy or breast-feeding, end-stage kidney failure, chornic steroid or immunosoppressive therapy and advanced cancer. Patients were recruited from March 2021 to September 2021. Age, sex, body weigh in Kg, body height in cm, daily insulin dosage and anti-diabetic pharmacological check were recorded at the baseline and at the different timing points. HbA1c, Time In Range (TIR), Time Above the Range (TAR), Time Below the Range (TBR) were recorded as indicators of glucose control, while coefficient of Variation (CV) was recorded as indicator of glycemic variability. The analysis was adjusted for sex, age and duration of the disease.

2.3.2 Biological sample collection, analysis and storage

Blood samples were collected by nurses or physicians during medical consulations and were stored in Endocrinology and Metabolic Diseases of Laboratory of Policlinico Campus Bio-Medico of Rome in dedicated freezers at -80°C, following the time schedule reported below:

T0: day of the first vaccine or no more than 3 days before

T1: day of the second vaccine or no more than 5 days before

T2: 5 weeks from T0

T3: 12 weeks from T0 (+/- 1 week)

T4: 24 weeks from T0 (+/- 1 week)

2.3.3 Spike glicoprotein glycation

Spike glicoprotein was exposed *in vitro* to different concentrations of ribose in order to obtain a non enzymatic glycosylation, following established and validated protocols. Spike protein was replenished in 1xPBS to obtain a 0,4 mg/mL concentration and was stored together with ribose 1 M overnight at 37 ° C. Final concentration was a 0,2 mg/mL blend of spike protein and ribose 0.5 M. SDS-PAGE was performed to confirm the result. A resolving 10% SDS-PAGE gel and a 5% stacking gel were prepared

Table 4:

	10% Resolving gel (10 mL)	**5% Stacking gel (5 mL)**
H_2O (mL)	4.0	3.4
30% acrylamide mix	3.3	0.83
1.5 M Tris (pH 8.8)	2.5	0.63
10% SDS	0.1	0.05
10% ammonium persulfate	0.1	0.05
TEMED	0.1	0.005

Upload of the sample:

- 5 µL di proteina Spike (native)
- 5 µL Laemmli *Sample Buffer*

Upload of the sample:

- 10 µL di proteina Spike (glycated)
- 10 µL Laemmli *Sample Buffer*

Gel was put in the running chamber *BIO-RAD Mini-PROTEAN Tetra System* for 2 hours at 120 Volt.

Staining phase: gel were colored for two hours with Coomassie blu
Destaining phase: gel were treated with 50 mL H_2O, 40 mL methanol, 10 mL acido acetico e water overnight.

Pictures were realized through Bio-rad ChemiDoc MP imaging system.

2.3.4 Evaluation of antibody response in type 1 diabetes after anti-COVID-19 vaccination

Nunc 96-well ELISA plates were covered with 2 µg/mL native SARs-CoV-2 spike protein (10549-CV-MTO, R&D systems) or 0,5 M ribose glycated SARs-CoV-2 spike protein and incubated overnigh at 4 °C. Plates were fixed with 200 µL for blocking pad cockpit (1x PBS / 5% skimmed milk / 0,1% Tween20) for one hour at room temperature. After removing the blocking pad and after whashing plates for three times, different time point sera (from T0 to T4) were diluted 1:1280 in 1% milk in PBST and incubated for one hour at room temperature. Plates were whashed again and incubated with 100uL of anti-human rabbit IgG-HRP at 1:3000 in 0,1% PBST for one hour at room temperature. After the incubation, plates were whashed again and added to 100 uL of TMB substrate diluted 1: 100 in sodium acetate 0.1 M pH 6.0 per cockpit for 3 minutes. The reaction was stopped with 50 µL of 20% sulfuric acid. The optic density of the plates was read at 450 nm. Plates were whashed between each phase four times with 1x PBS/0.05% Tween20.

2.4 Results

Table 5: study population features

	T0	T1	T2	T3
Age (years)	39,3 ± 11			
Disease duration (years)	21,4 ± 10,1			
BMI (Kg/m^2)	24,8 ± 3,5			
HbA1c %	7,3 ± 0,6			
TIR %	66,5 ± 15,1	66,2 ± 14,9	69,8 ± 14,9	68,3 ± 18,9
TAR %	28,86 ± 14,9	29,36 ± 14,7	25,3 ± 14,8	26,1 ± 19,8
TBR %	4,14 ± 4,2	4,2 ± 4	4,2 ± 4,3	5,6 ± 4,8

The primary exposure and outcome measures were baseline TIR and IgG response to the COVID-19 vaccine, respectively. We prospectively enrolled 26 patients with T1D. All patients tested negative to spike IgG at baseline. After vaccination, the IgG response significantly increased and reached a spike at T2, which was followed by a progressive decline across later timepoints (P<0.001; Fig 11). Pre-vaccination HbA1c was inversely related with antibody response to spike glycoprotein at peak antibody response (T2), although the relationship did not reach statistical significance (r= -0.33; P=0.14). However, both pre-vaccination time in range (TIR) and time above range (TAR) strongly predicted the antibody response over the six months timeframe (AUC x time) (TIR: r= 0.75; p= 0.02; TAR: r -0.81; p= 0.008). The strongest relationship was found at the peak antibody response (time 2), which was correlated positively with baseline TIR (r=0.82, P=0.004; Fig. 11) and inversely with baseline TAR (r= -0.73, P=0.016; Fig. 1C), respectively. This was consistent with the association between TIR and the antibody neutralization potency assessed in the cell-based assay (correlation between TIR and the reciprocal neutralising antibody titre at IC50: r=0.49; p=0.042), indicating that longer the percentage of time spent with blood glucose levels in the target range at baseline, greater the antibody neutralization potency. Glucose control along the study timeframe was also associated with IgG response as showed by the correlation between time-dependent mean of TIR and TAR during follow-up and IgG-AUC (TIR: r = 0.93, p

<0.0001; TAR: r = -0.84, p <0.0001). TBR was unrelated with either peak-IgG or IgG-AUC (-0.04 < r < -0.018; p >0.90). Females showed slightly stronger antibody response compared with males (median AUC: 104.5 [IQR 98.9-118.0] vs. 119.8 [109.1-130.7], P=0.03; T2: median IgG OD at T2: 1.088 [1.036-1.187] vs. 1.204 [1.145-1.365], P=0.057). Peak IgG response was unrelated to age (r=0.03; P=0.88), BMI (r= -0.14; P=0.53), or disease duration (r=0.05; P=0.84), while the IgG AUC across time correlated negatively with BMI (r= -0.53; P=0.04) and disease duration (r= -0.53; P=0.03), but not significantly with age (r=0.42; P=0.098)

Figure 11

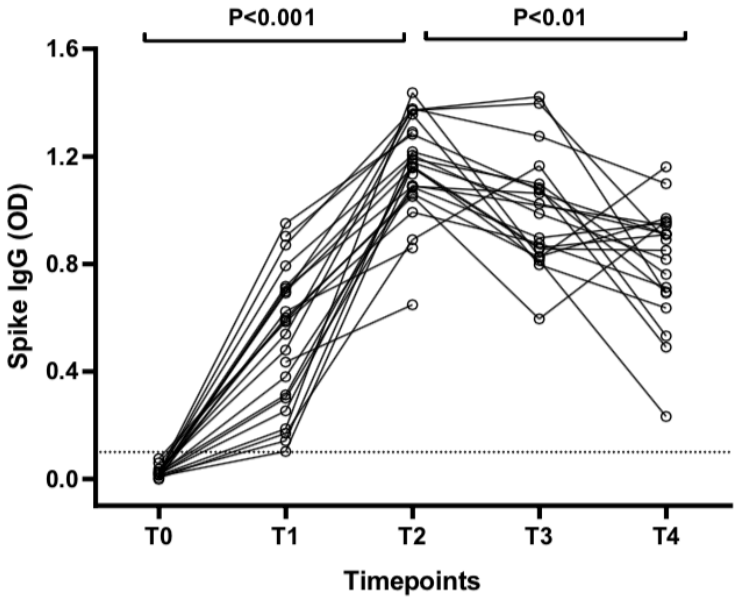
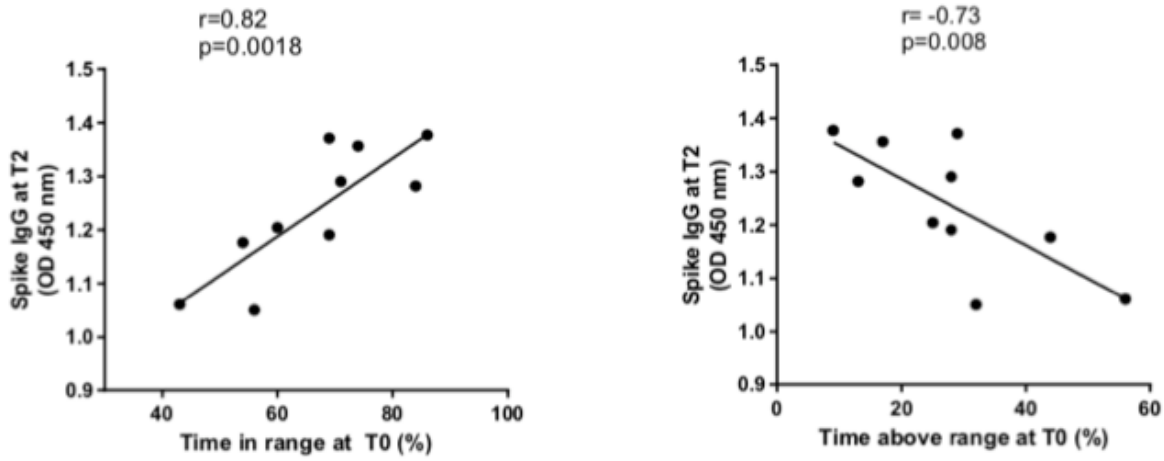


Figure 12



2.5 Discussion

Many studies have widely demonstrated the bad influence of poor glucose control in T1D patients on immune system response, in particular against infective agents [105,106]. The results of the present study confirm that glucose control and protein glycosilation can interfere with antibody response in T1D [93–95]. In detail, a worse glucose control resulted associated to a less effective immune response after SARS-COV2 vaccine Glucose control was estimated through HbA1c, TIR and TAR. HbA1c resulted not significantly associated with immune response strengh. TIR and TAR, otherwise, resulted positively ($p = 0.008$) and inversely ($p = 0.016$) related to immune response. This result suggests that HbA1c cannot be considered a reliable glucose control marker in all clinical cases because of the interference of glucose variabily. Protein structure in condition of hyperglyacemia can change as a consequence of non enzimatic glycosilation processes. This reduced immune response against glycated spike protein in comparison to native spike protein are corresponding to the results of previous studies. In particular Strollo et al. in 2015 demonstrated that insulin glycosilation affects auto-antibodies bond affinity to insulin target in T1D patients. Ribosylation, formylation and other chemical reactions modify the molecular structure of different amino-acids (mainly lysine and phenylalaline) localized in correspondence to immuno-dominant epitopes, reducing native protein autoantibodies bond affinity and exposure to their

main epitopes [110]. Finally, comparing antibodies neutralization potency, it resulted stronger in presence of higher TIR values ($p = 0.042$). This result suggests that T1D patients in poor glucose control could be more at risk of COVID19 infection even after SARS-COV2 vaccine compared to T1D patients in good glucose control. The stronger correlation between IgG response and CGM, compared to HbA1c, may also imply that the timeframe immediately close to vaccination (as close as two weeks), is the most crucial for the achievement of optimal immune response. By contrast, baseline HbA1c, whci covers longer timeframe (e.g. three months before vaccinations), may not fully catch the effect of glucose on immune response following vaccine administration. Furthermore, HbA1c does not take into account glucose variation, and may show some degree of discordance with CGM data in around 40% of T1D patients. It should be also noted that the majority of our patients has a relatively good glucose control (only 20% patients had an HbA1c higher than 7.5%), which makes the population of patients with poor glucose control underrepresented. Further studies are needed to explore if other actors involved in immune response (i.e. T cells activation) could play a crucial role. Several mechanisms have linked hyperglycaemia to reduced vaccination efficiency and increased risk of infection, including impaired antigen recognition, altered cytokine expression, immune-senescence, and antigen glycation. Although a time-dependent decline in antibody levels might increase the risk of breakthrough infections, the antibody cut-off predicting such risk is still unknown, which is a major study limitation. The relationship between glucose control and effectiveness of SARS-CoV2 vaccine in preventing COVID-19 should be assessed in future studies. In conclusion, our findings indicate a strong relationship between glucose control and antidoby response after SARS-CoV2 vaccination, highlighting the importance of achieving well-controlled blood glucose for COVID-19 prevention.

Study 2: The association between markers of Beta-cell stress and chronic complications in T1D

2.1 Preliminary considerations

C-peptide, the 33 amino acid physiological biomarker of insular Beta cell function, is the product resulting from the clivation of pro-insulin (insulin precursor) in the pancreas. Each molecule of insulin match with one molecule of C-peptide with a 1:1 ratio. Range C-peptide values, fasting, are generally 0,26 – 0,62 nmol/L [112]. Reduced C-peptide levels are related with the type of diabetes and the duration of the disease. Values inferior to 0.2 nmol/l are suggestive for type 1 diabetes, while values between 0.2 and 0.6 nmol/l could be found also in type 2 diabetic patients, above all if with long history of diabetes. Reasonably, in case of low levels of C-peptide, insulin secretion is expected to be insufficient as well. As expression of residual Beta cell function, C-peptide is the preferred marker because of its major half-life compared to insulin and because of the lack of evidence of antibody response against it even in course of insulin therapy. Clinical studies support a predictive role of C-peptide in terms of progression of diabetes, development of chronic complications, worse glucose control and risk of hypoglycaemia. Some patients continue to produce relevant quantities of C-peptide even many years after diagnosis of diabetes, generally with a better glucose control in comparison with patients with no evidence of C-peptide secretion [113].

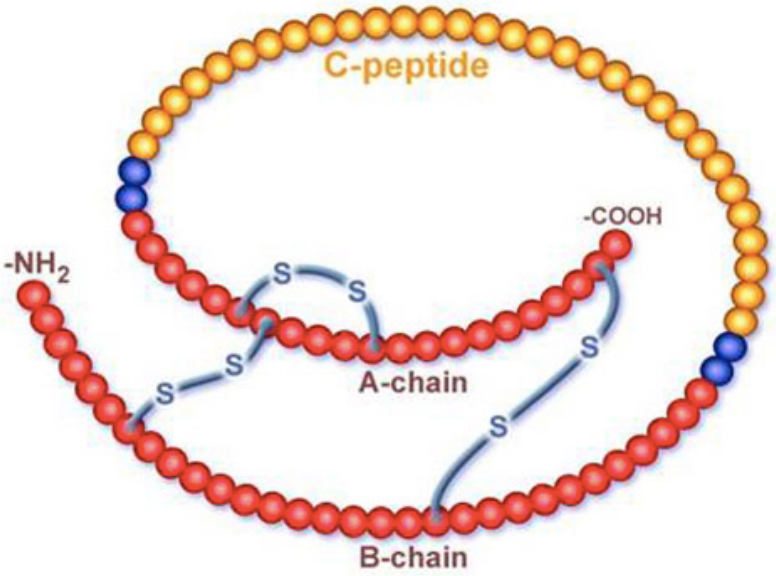


Figure 13: insulin structure

Proinsulin, the 86 amino acids precursor of insulin, is the product of clivation of another precursor, pre-proinsulin. C-peptide is clived only after disulfide bonds between A- and B-chains are built. To secrete hormones, β cell takes a well-functioning endoplasmic reticulum in order to assembly proteins correctly [112]. In oxidative stress conditions, insulin request can overcome endoplasmic reticulm capabity to produce insulin, leading to β cell impairment. Endoplasmic reticulum stress can be quantified through Proinsulin/C-peptide ratio [114].

2.2 Background

Analysis of pancreatic sections of patients diagnosed type 1 diabetes show still functioning insule even many years after the diagnosis of diabetes. Furthermore, some studies confirmed that patients diagnosed type 1 diabetes can produce low, but detectable levels of C-peptide. β cell dysfunction, consequent to the increased oxidative stress which affect the endoplasmic reticulum, lead to the release of not correctly synthesized proinsulin [114,115]. The measurment of just C-peptide could underestimate β cell capability to secrete insulin. Proinsulin has been detected even in sera of patients without detectable levels of C-peptide, suggesting that the lack of insulin, main actor of type 1 diabetes pathogenesis, could be caused by defects in proinsulin clivage more than in proinsulin synthesis [115]. There is still no evidence of a possible correlation between proinsulin levels and glucose control or time of onset and severity of complications

2.3 Aim

The aim of study 2 was to evaluate wether proinsulin and the proinsulin to C-peptide are associated with chronic complications in patients with long standing T1D.

2.4 Matherials and Methods

2.4.1 Study population

100 patients with type 1 diabetes attending the Endocrinology and Metabolic Diseases Unit of Policlinico Campus Bio-Medico of Rome were enrolled in the study. 64 males and 36 females, aged between 18 and 70 years (mean age 43 ± 10), duration of the disease 23.8±8.7 were recruited. 23 patients showed diabetc complications, among them the most common was diabetic retinophaty. All patients were on intensive insulin therapy. Patients attending Endocrinology and Metabolic Diseases Day Hospital or outpatient clinics of Policlinico Campus Bio-Medico of Rome were recruited from January 2020 to Dicember 2020. Inclusion criteria were: age 18-70 years, ongoing intensive insulin therapy, signed inform consent and diagnosis of type 1 diabetes for at least six months. Exclusion criteria were: age <18 years, diagnosis of type 1 diabetes for less than six months, high impairment due to psychic or physical stress or cognitive issues, pregnancy or breast-feeding, end-stage kidney failure, chornic steroid or immunosoppressive therapy, advanced cancer. Age, age at diagnosis, duration of the

disease, sex, body weigh in Kg, body height in cm and daily insulin dosage were recorded. HbA1c, C-peptide expressed in ng/ml, total cholesterol HDL, triglycerides, creatinine, AST, ALT, GTT, microalbuminuria were measured after collecting blood samples during medical consultations in the Day Hospital of the Endocrinology and Metabolic Diseases Unit of Policlinico Campus Bio-Medico of Rome. Analysis and storage of the blood samples was performed in Policlinico Campus Bio-Medico of Rome Laboratory Analys as part of routine patient's follow-up with the exception of proinsulin dosage which was performed in Endocrinology and Metabolic Diseases Laboratory.

2.4.2 Proinsulin measurment

Blood samples were processed to separate serum, stored at -20° C and subsequently defrosted before the analysis. To detect proinsulin was performed an ELISA assay exploiting two monoclonal murine antibodies. Standard proteins, sera from enrolled patients and sera from control were tested. 50µL of Working Strength Protease Inhibitor were used to cover a 96-weel plate, then centrifuged for one hour at 700-900 rpm at room temperature. The plates were whashed four times with 350 µL of Working Strength Wash Buffer. 100 µL di Working Strength Detector Antibody were used to cover the 96-well plate. The plates were centrifuged for one hour at 700-900 rpm at room temperature and then whashed other four times with 350 µL of Working Strength Wash Buffer. 100 µL of HRP (horseradish peroxidase) – conjugate streptavidin (SA) were added to the 96-well plates, next covered with a sealant and centrifuged for 30 minute at 700-900 rpm. at room temperature After the SA-HRP the plates were whashed four times with 350 µL di Working Strength Wash Buffer. 100 µL di Working Strength chemiluminescent substrate were added to the 96-well plates. The optical analysis was performed with 10 minutes after the addition of the substrate with Relative Light Units (RLU) directly proportional to proinsulin contained in the sample.

2.4.3 Diabetes complications

- Cardiovascular diseases: familiar and personal medical history focused on cardiovascular death, heart attack, PTCA, CABG, stroke
- Diabetic retinopathy: fondus oculi
- Diabetic nefropathy: microalbuminuria, glomerular filtration rate (GFR) ml/min
- Diabetic neuropathy: Michigan screening

2.5 Results

Table 6

	No complications	**With complications**	**P**
Age (years)	42.3 ± 15.8	42.16 ± 8.58	0.02
Disease duration (years)	12.7 ± 7	24.5 ± 8.89	0.009
BMI (Kg/m$^{2)}$	26.01 ± 3.27	27 ± 4.89	Ns
C-peptide* ng/ml	0.59 ± 1.85	0.025 ± 0.005	Ns
Proinsulin* pg/ml	5.2 ± 1.49	9.4 ± 11.28	Ns
HbA1c %	7.47 ± 0.49	7.44 ± 1.28	Ns
TIR %	62.3 ± 10.6	39.3 ± 26.3	Ns
TAR %	33.5 ± 10.6	50.5 ± 23.60	Ns
TBR %	5 ± 5.20	10.1 ± 8.3	Ns

*Proinsulin and C-peptide mean value ± SD have been calcutaled only when detectable

Table 7:

	Detectable C-peptide	Undetectable C-peptide
Complications (n = 26)	5	21
No complications (n = 74)	9	65

	Detectable proinsulin	Undetectable proinsulin
Complications (n = 26)	11	15
No complications (n = 74)	34	40

Analyzing sera collected from 100 enrolled patients, proinsulin resulted detectable in 45 and C-peptide in 14 of them (45% and 14%, respectively). Circumscribing the analysis to the 26 subjects with chronic complications of diabetes and to the 74 subjects without chronic complications of diabetes, proinsulin resulted detectable in 11 and 34 patients, respectively. C-peptide, otherwise, resulted detectable in 5 and 9 patients, respectively. C-peptide, in absence of complications, resulted undetectable (<0,03 ng/ml) in 21 subjects (28.3%) with mean value of 0.59 ± 1.85 ng/ml, while proinsulin resulted undetectable in 40 subjects (54%) with a mean values of 5.2 ± 1.49. C-peptide, in presence of complications, resulted undetectable in 5 subjects (19.2%) with mean value of 0.02 ± 0.005, while proinsulin resulted undetectable in 11 subjects (57.69%) with a mean values of 9.4 ± 11.28. No correlation between C-peptide and proinsulin was observed (figure 17). C-peptide and proinsulin did not differ significantly in presence or absence of chronic complications of diabetes (C-peptide 0.025 ± 0.005ng/ml vs 0.59 ± 1.85 ng/ml p value NS and proinsulin 9.4 ± 11.28 pg/ml vs. 5.2 ± 1.49 pg/ml; p value NS, respectively, figure 24). C-peptide, proinsulin and C-peptide/proinsulin ratio were related to different parameters including age, disease duration, age at onset of disease, BMI, HbA1c and time in range. C-peptide resulted significantly related to duration and age ad onset of disease (r = -0.25, P = 0.01; r = 0.2, P = 0.005) while proinsulin and C-

peptide/proinsulin ratio resulted related only to BMI (r = 0.25, P = 0.02; r = 0.24, P = 0.01). No other significantly correlations were observed.

Figure 14:

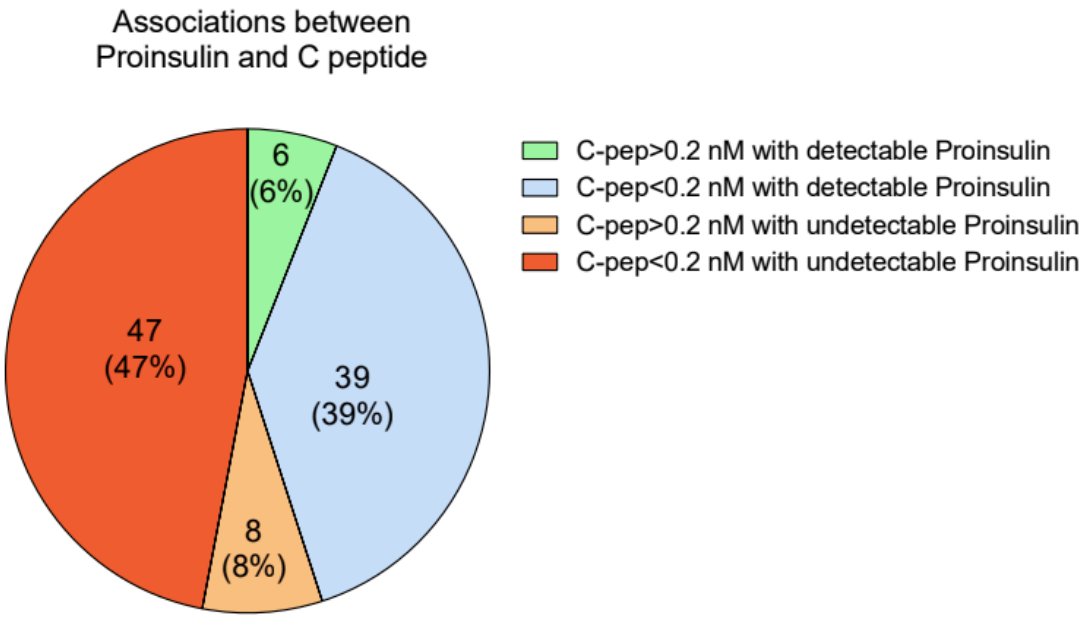


Figure 15:

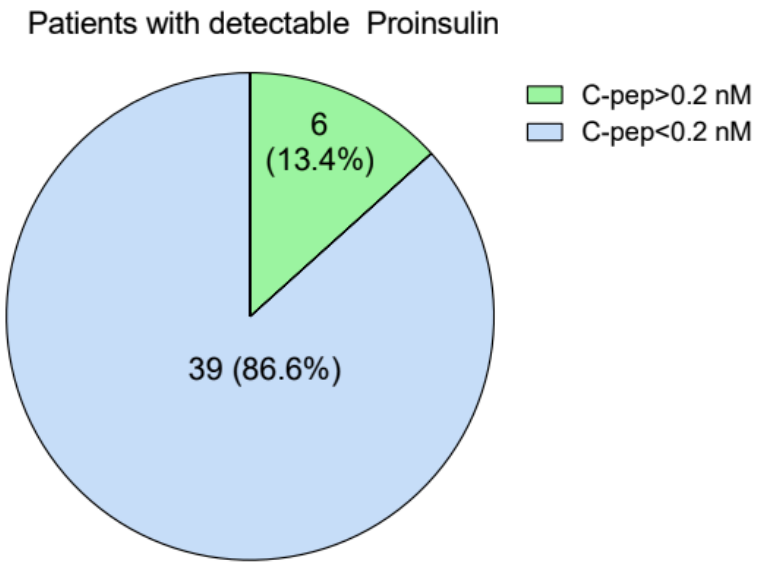


Figure 16:

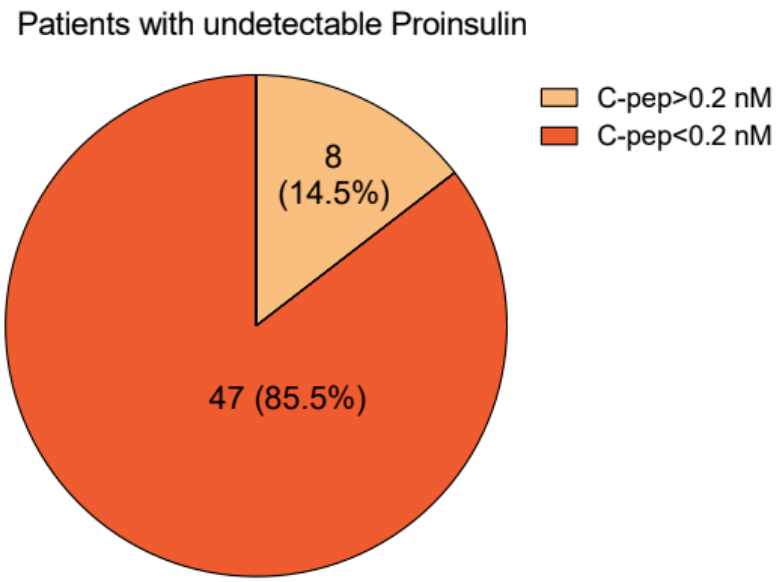


Figure 17:

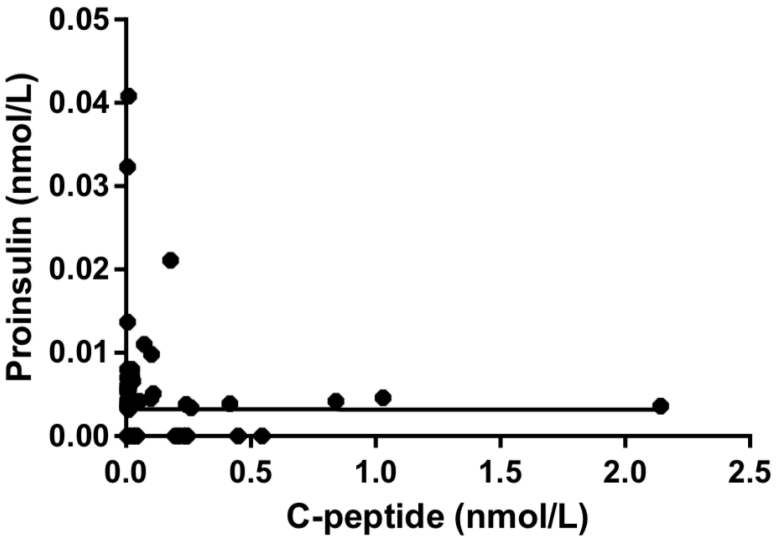


Figure 18

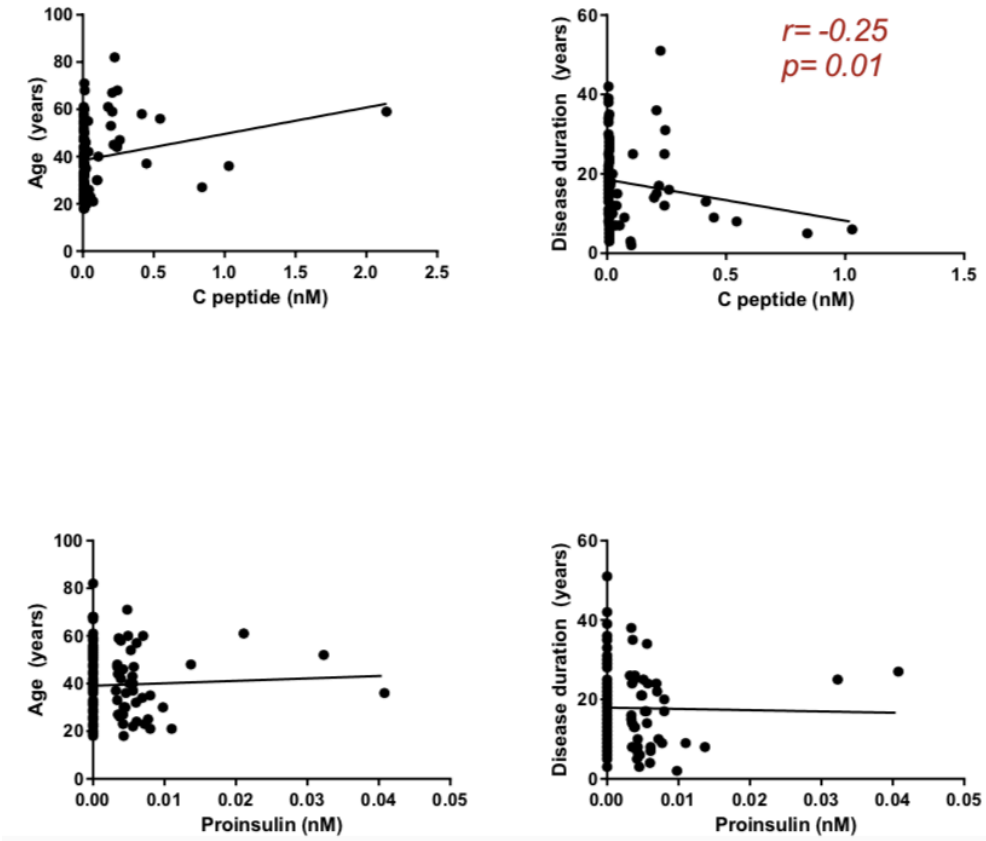

Age (years)
C peptide (nM)
Disease duration (years)
C peptide (nM)
r= -0.25
p= 0.01
Age (years)
Proinsulin (nM)
Disease duration (years)
Proinsulin (nM)

Figure 19:

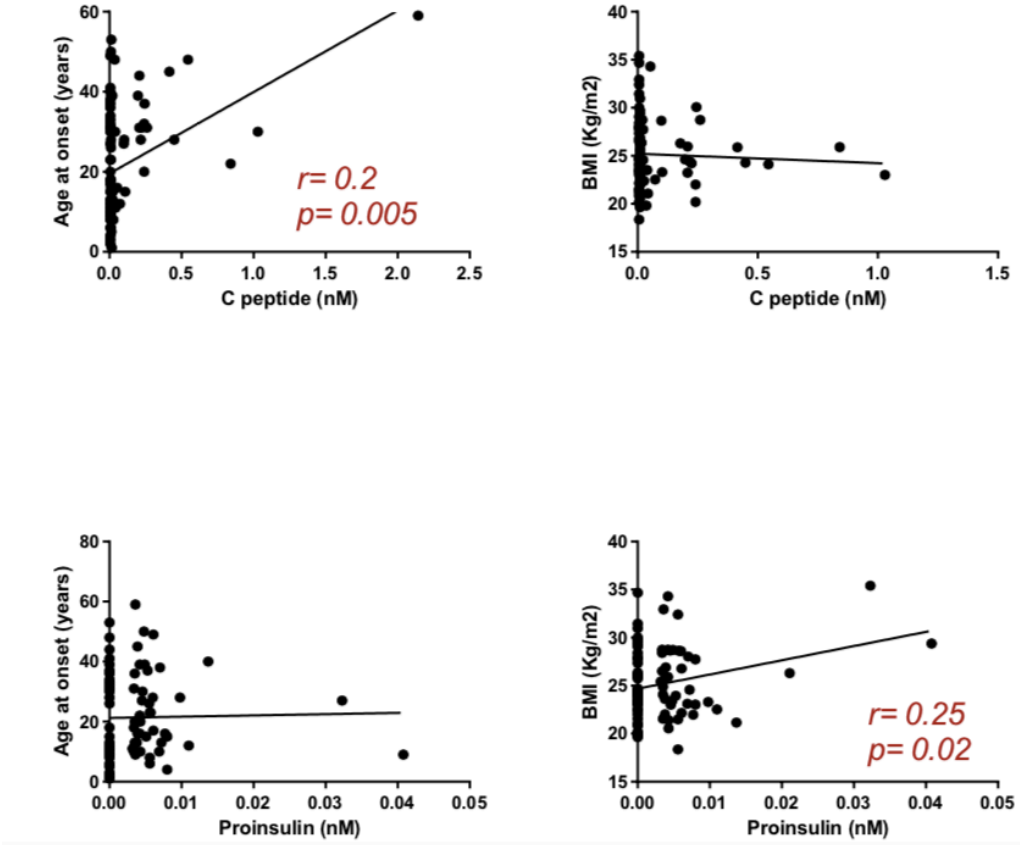
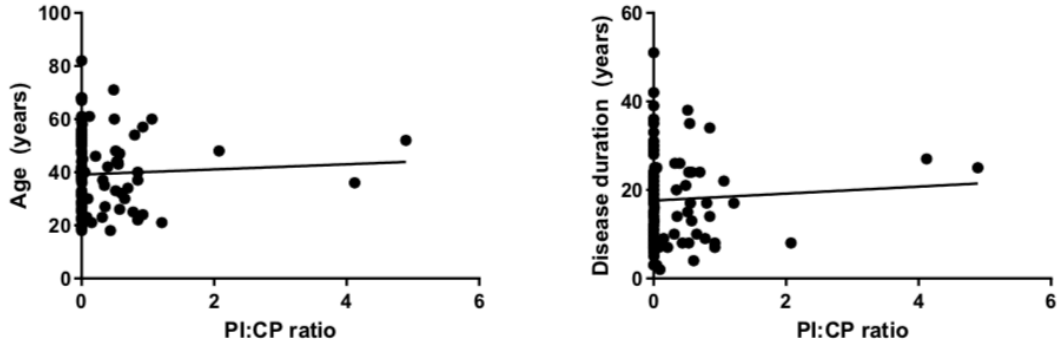


Figure 20:



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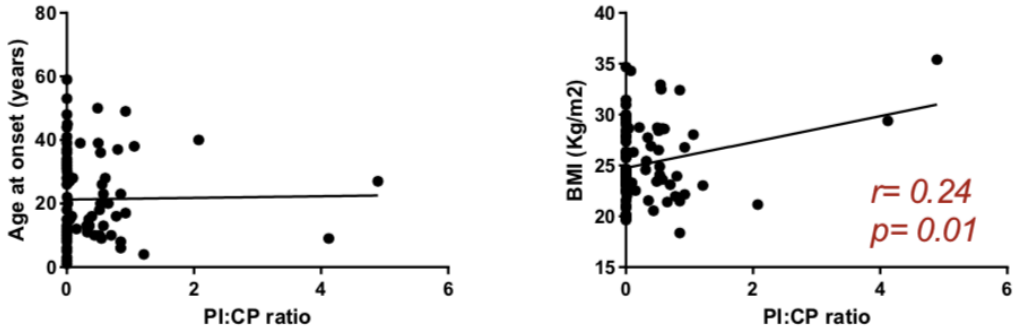


Figure 22:

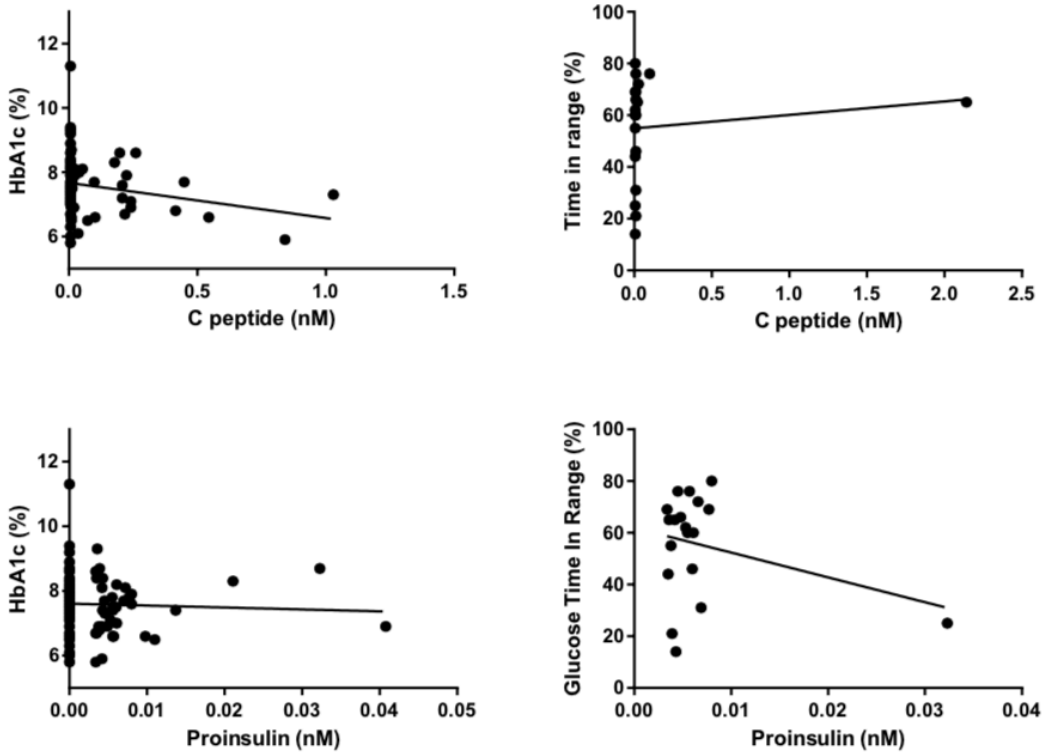


Figure 23:

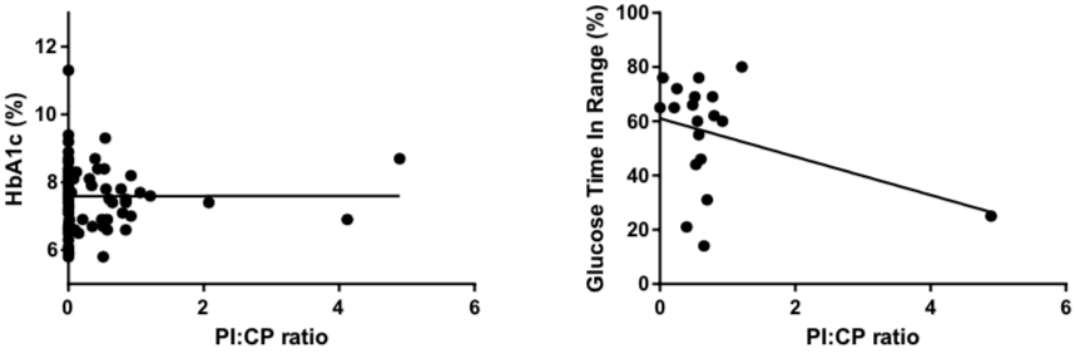


Figure 24:

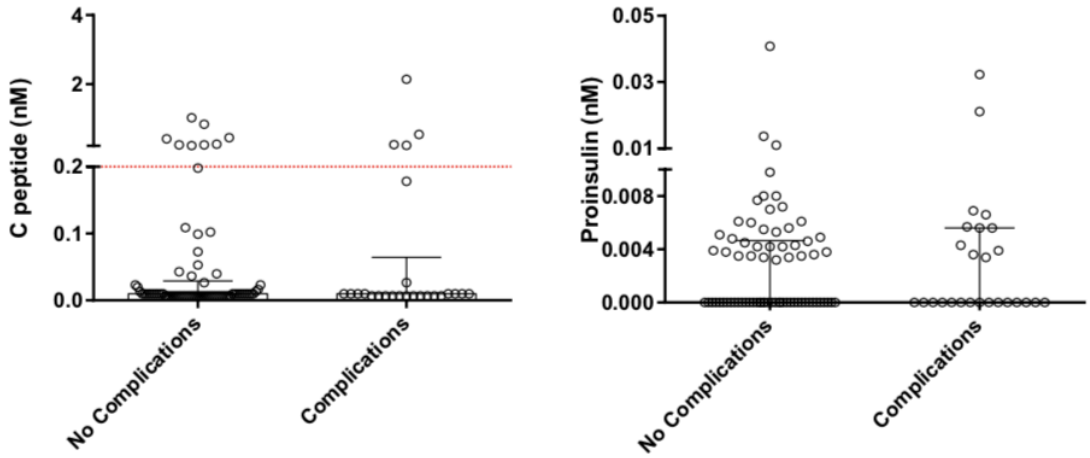
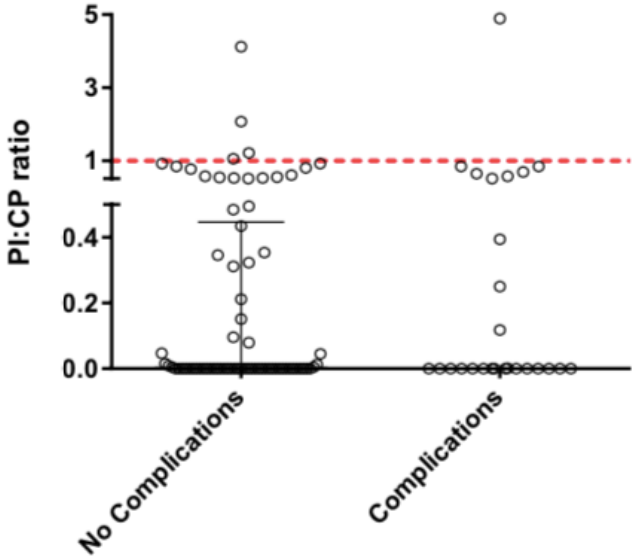


Figure 25:



2.6 Discussion

Other studies have showed that a consistent share of subejcts can secrete proinsulin even many years after T1D diagnosis. Sims et al., analyzing data from a study population composed by patients with a long history of T1D, demonstrated that 95.9% showed detectable proinsulin (>3.1 pmol/L) even if 89.9% had undetectable C-peptide (<0.017 nmol/L). In their sample proinsulin levels appeared substantially unchanged during a follow-up of four years, while C-peptide decreased progressively. In conclusion, after many years of disease, Beta cells seemed to preserve their proinsulin secretive capability, independently from C-peptide [115]. In our study population, in absence of detectable C-peptide, proinsulin resulted detectable and undetectable in 39% and 47% of patients, respectively. C-peptide and proinsulin appeared not related. Finally, no consistent correlation was observed between proinsulin and other parameters, in particular glucose control and presence of complications, the main actors of T1D evolution. These results suggest that proinsulin detectability doesn't change the natural history of T1D. It is likely that the differences observed between proinsulin and C-peptide are due to biochemical mechanisms involving Beta-cell and its capability to secrete hormones in the blood stream in presence of hyperglycaemia. Multiple biochemical pathways and mechanisms of action for glucose toxicity have been

suggested, including glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism, sorbitol formation, and oxidative phosphorylation. There are many potential mechanisms whereby excess glucose metabolites traveling along these pathways might cause beta cell damage. However, all these pathways have in common the formation of reactive oxygen species that, in excess and over time, cause chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis. Further studies are needed to explore more deeply this field [116–118]. A limit of the study is represented by differences in disease duration between the groups. As expected, patients with chronic complications had longer disease duration compared to those without complications. However, when adjusting the statistical analyses for disease duration, we did not find significant differences between proinsulin and C-peptide to proinsulin ratio between the two study groups. Finally, oyr study was focues on microvascular complication, and it is unknown whether proinsulin and the proinsulin C-peptide ratio may associate with macrovascular complications. This should be addressed in future studies.

Study 3: Effect of a novel app-based strategy for carbohydrate counting on glucose control in T1D

3.1 Background

The Amerinca Diabetes Association Standards for the Treatment of Diabetes Mellitus affirm, in the recommendations dealing with nutritional medical therapy, that "The counting of carbohydrates is confirmed, in the context of medical nutritional therapy, an essential component, and represents the most effective strategy for glycemic control in the patient with diabetes under intensive insulin treatment (Level of evidence I, Strength of recommendation B)" [81]. Type 1 diabetes is a chronic disease characterized by a life-long treatment and impairment in quality of life due to specific needs of care [119]. Carbohydrate counting and insulin therapy are the cornstones of T1D management. However, patients with type 1 diabetes face a variety of other ordinary day life troubles including the risk and, above all, the fear of hypoglycemia, the constant control of glycaemic values and nutritional concerns. In this field, patients have to deal every day with carbohydrate counting to estimate the appropriate meal-related insulin dose [120]. This calculation is not only associated to the quantity of carbohydrates to ingest, but also to quality of carbohydrates, to the self established insulin/carbohydrate ratio, to the current glycaemic value, to the glycaemic target, to any residual insulin activity from the last dose administered and to the physical activity performed or intended to carry out [121–123]. Medical nutrition management is recommended for all people with diabetes as an essential part of care and should be managed by a nutrition expert, such as a dietician. Its role in improving glucose control with a consequent reduction in HbA1c and complications associated with diabetes has been amply highlighted by clinical studies that have also recognized its strong impact on reducing health costs. The goals of medical nutritional management are to obtain and maintain the best possible glycaemic and lipidic levels, to ensure adequate caloric intake, to promote normal growth and psycho-physical development, and to prevent, delay or treat diabetic complications with the aim of improving or maintaining the patient's health. A diet based on the intake of fixed carbohydrate quantities is a possible option for diabetic patients on insulin therapy, especially in the elderly, in order to avoid wide variations in insulin dosage. The main advantage of this nutritional approach consists in its simplicity

and reproducibility, guaranteeing a complete diet and restricting variations in insulin administrations to compensate for eventual pre-prandial hyperglycaemias. However, even if theoretically easy, taking a constant quantity of carbohydrates at each meal could become challenging and frustrating, compromising patient's compliance with possible consequent hypo- or hyperglycemias. Carbohydrate counting, therefore, represents a valid alternative. Recent studies demonstrate that a better glycaemic control and a significant impact on HbA1c levels in those patients experiencing carbohydrate counting [124–126]. On the other hand, insulin therapy remains the therapy of choice for the treatment of type 1 diabetes, bacause of the damage of the pancreatic insulin secretive Beta-cells that progressively leads to insulin deficiency and hyperglycaemia. The administration of insulin is essential for maintaining glucose homeostasis, ensuring the achievement of adequate glycemic compensation. It can be achieved by intensive insulin treatment, with multiple daily administrations of exogenous subcutaneous insulin, or by continuous subcutaneous insulin infusion therapy with a pump (Continuous Subcutaneous Insulin Infusion, CSII). The latter is considered the gold standard of intensive insulin therapy, moreover if associated with a continuous glucose monitoring system (real time or intermittent), or in combination with the monitoring of capillary blood glucose (self-blood glucose monitoring) only [127]. Compared to multi-injection therapy, the insulin pump therapy reproduces pancreatic insulin secretion more physiologically [128,129]. Currently, thanks to technology, to better manage decisions regarding insulin therapy and in particoular insulin boluses based on the amount of carbohydrates to ingest, it is possible to use smartphone applications to perform more easily carbohydrate counting [127]. These tools use pictures and tables to identify the precise content of carbohydrates, adjusted for the portion usually consumed by patients. Dietometro is an italian application for smartphones that exploits pictures representing the quantity of carbohydrates of different foods. Each sheet consists of a photographic representation of portions of the most common foods, indicating their weight (both raw and cooked) and the relative carbohydrate content. For some types of food, graphics cards are available with different portions (small, medium and large), in order to facilitate the recognition of the portion closest to that usually consumed by the patient, with the relative grams of carbohydrates contained in it. The application is very intuitive and easy to use. The patient can select a category of food to search for the dish supposed to consume [130,131]. The categories featured on the home page include:

- First courses, including boiled pasta, parboiled rice, rice salad, rice and peas, tortellini, gnocchi, lasagna, spelled or barley etc..
- Legumes, both dried and frozen
- Side dishes, including mixed salad, spinach, courgette or carrots both raw and cooked, potatoes and mashed potatoes;
- Second courses, including raw weighed meat and fish, cold cuts, meat and fish in box, eggs and dairy products
- Baked goods and cereals, in which you can find regular type bread or wholemeal, wraps, dry baked goods such as rusks and biscuits, breakfast cereals and oat flakes
- Sandwiches, flat bread and pizzas, where you can find stuffed sandwiches and various types of flat bread and seasoned pizzas
- Appetizers, including chips, pizzas, pretzels, rustici, canapés and popcorn
- Fruit, where there are both fresh fruit and dried fruit
- Desserts, in which portions of tarts, various types of cakes, but can be recognized also panettone, pandoro, easter cake, or even breakfast sweets like brioches and muffins, various snacks, ice creams, popsicles and jams
- Beverages, including milk, yogurt, cappuccino, hot chocolate, fruit juices, freshly squeezed orange juice, smoothies, wine, beer and sweetened carbonated drinks
- Aperitifs, both non-alcoholic and alcoholic such as Spritz and various types of cocktails
- Condiments, where you can find all the sauces, pesto, vinegar, vinegar glaze balsamic or even grated cheese

Among the categories that can be selected there are also customized foods. Patients can add specific foods by manually entering the values shown on the label of the same. Favorite foods can be selected from the main list to more easily find the ones consumed habitually. The application includes also a "Food Diary" section, which allows each patient to record the foods consumed during the day or the week, to calculate the average of the grams of carbohydrates consumed and therefore to estimate the units of insulin self-administered daily/weekly.

Figure 26: exemplary page from Dietometro

3.2 Aim

The aim of study 3 was to to study the effect of "Dietometro" on carbohydrate ounting and glucose control

3.3 Matherials and Methods

3.3.1 Study population

Fifty-four subjects with type 1 diabetes attending the Endocrinology and Metabolic Diseases Unit of Policlinico Campus Bio-Medico of Rome. 26 males and 28 females, aged between 18 and 70 years (mean age 43.01 ± 14.7), were recruited. All patients were on intensive insulin therapy: 23 on multiple daily insulin injection regimen (MDI) and 31 on continuous subcutaneous insulin infusion (CSII), respectively. CSII devices included: Medtronic Minimed 640G and 670G, Theras Omnipod, Roche Insight and Movy Tandem. Glucose monitoring was performed by Dexcom-G6, Guardian sensor

and Flash Freestyle Libre. Subjects were allocated to three groups. Group 1 include 19 subjects not practicing and unwilling to learn carbohydrate counting in their everydaylife management of the disease. Other recruited subjects were randomly assigned to Group 2 and Group 3. Group 2 include 17 subjects performing "self-managed" counting, in which the patients managed carbohydrate counting without the help of any technological device. Group 3 include 16 patients in which carbohydrate counting was performed with the support of "Dietometro" application. Patients attending Endocrinology and Metabolic Diseases Day Hospital or outpatient clinics of Policlinico Campus Bio-Medico of Rome were recruited from January 2020 to Dicember 2020. HbA1c, Time In Range (TIR), Time Above the Range (TAR), Time Below the Range (TBR) were collected as indicators of glucose control, while coefficient of Variation (CV) was collected as indicator of glycemic variability. The analysis was adjusted for sex, age and duration of the disease. HbA1c was detected at the baseline and after three months of follow-up. Time In Range (TIR), Time Above the Range (TAR) and Time Below the Range (TBR) were recorded at the baseline, after one month of follow-up and after three months of follow-up. Inclusion criteria were: age 18-70 years, ongoing intensive insulin therapy, signed inform consent and diagnosis of type 1 diabetes for at least six months. Exclusion criteria were: age <18 years, diagnosis of type 1 diabetes for less than six months, high impairment due to psychic or physical stress or cognitive issues, pregnancy or breast-feeding, end-stage kidney failure, chornic steroid or immunosoppressive therapy, advanced cancer. Age, sex, BMI and daily insulin dosage were recorded at the baseline and at the different timing points. The study was approved by Università campus Bio-Medico ethical committee.

3.4 Results

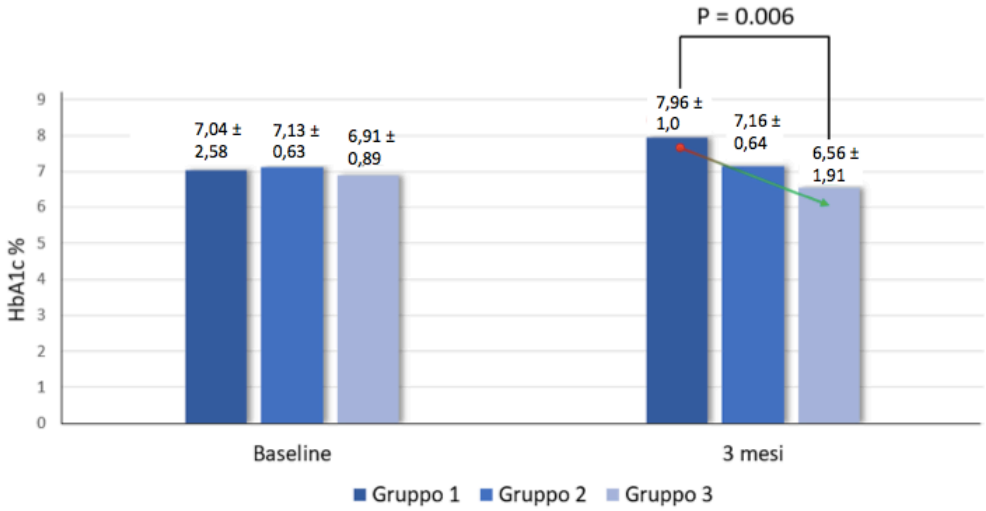
Table 8: study population features

	Group 1	Group 2	Group 3
Age (years)	44,37 ± 15,79	42,21 ± 15,09	38,31 ± 13,69
Disease duration (years)	27,58 ± 12,44	17,00 ± 7,26	21,63 ± 12,65
BMI	28,86 ± 3,13	25,55 ± 3,09	25,38 ± 5,34
HbA1c (baseline) %	7,04 ± 2,58	7,13 ± 0,63	6,91 ± 0,89

3.4.1 HbA1c

At the baseline mean HbA1c was 7,03% ± 1,62%, without significant differences among mean group 1, group 2 and group 3 HbA1c, respectively (7,04 ± 2,58 vs 7,13 ± 0,63 vs 6,91 ± 0,89). After three months, HbA1 changed significantly in group 1 and 3, even if no differences were observed in group 2 (7,13 ± 0,63 vs 7,16 ± 0,647). In detail, group 1 or "no counters" group HbA1c rised up to 7,96 ± 1,0 starting from 7,04 ± 2,58, while group 3 or "Dietometro-App counters" group HbA1c decreased from 6,91 ± 0,89 to 6,56 ± 1,91. Finally, HbA1c resulted significantly lower in group 3 compared to group 1 (6,56 ± 1,91 vs 7,96 ± 1,0; $p < 0,05$).

Figure 27: baseline HbA1c vs 3-months follow-up HbA1c



4.4.2 Time in range (TIR), time above the range (TAR) and time below the range (TBR)

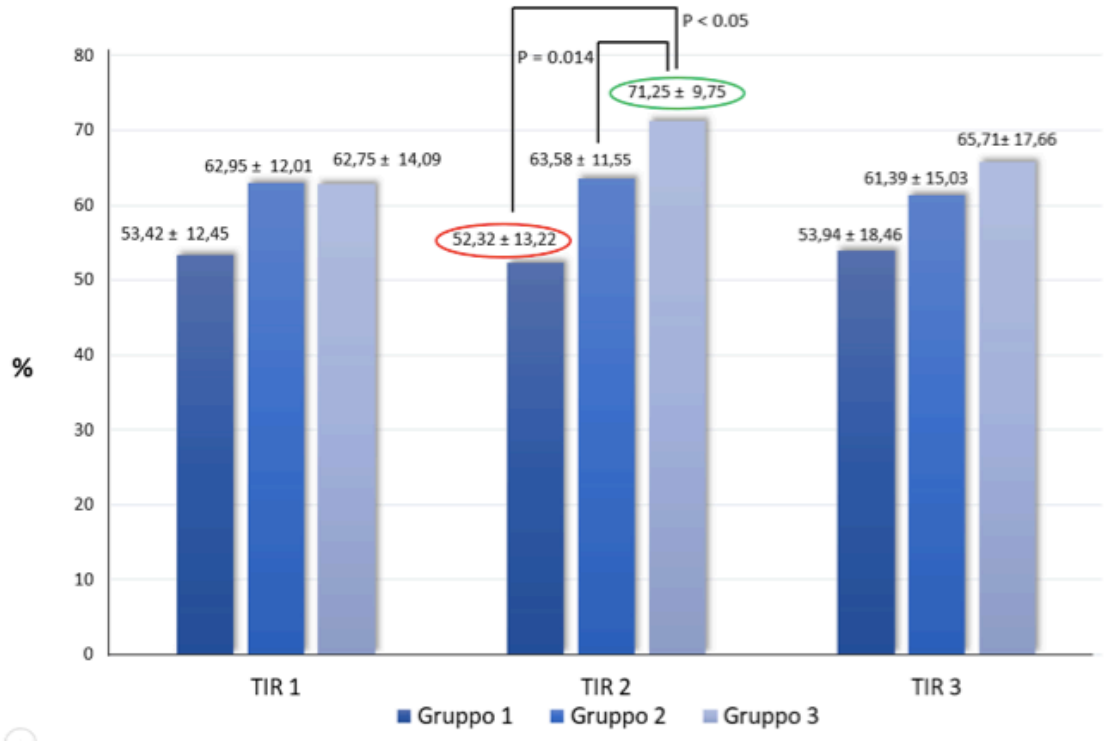
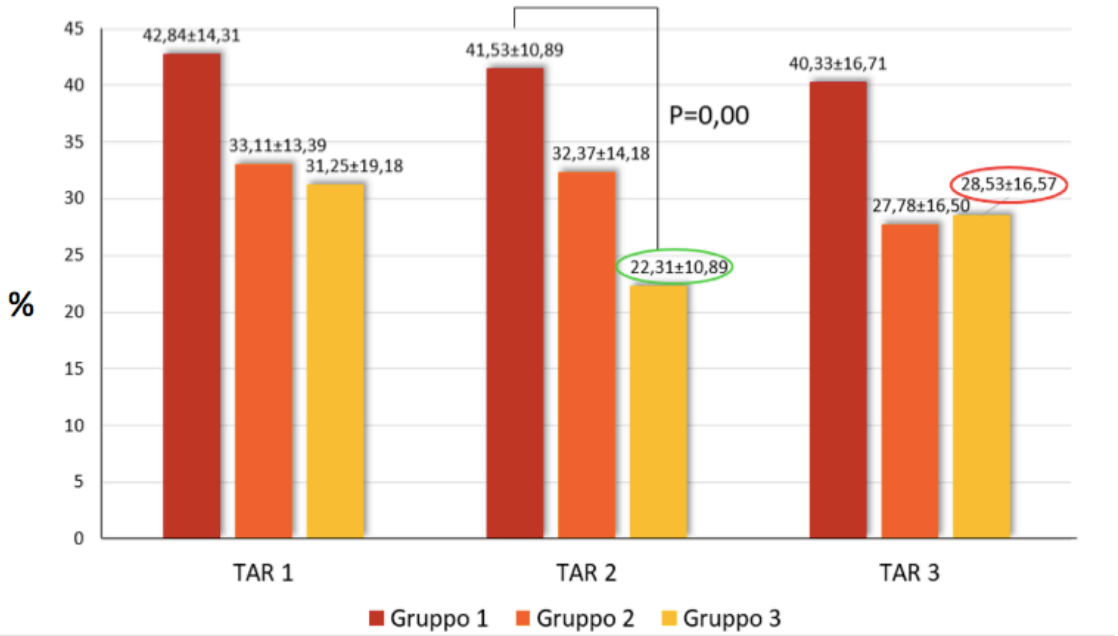


Figure 28: baseline TIR vs 3-months follow-up TIR

3.4.2 CGM

Concerning Time In Range (TIR), at the baseline it resulted similar between group 2 and group 3 (62,95% ± 12,01% vs 62,75% ± 14,09%) and lower in group 1 (53,42% ± 12,45%). After one month, group 1 and group 2 TIR did not change significantly (53,42% ± 12,45% vs 52,32% ± 13,22% and 62,95% ± 12,01% vs 63,58% ± 11,55%, respectively), while group 3 TIR decreased consistently (53,42% ± 12,45% vs 71,25% ± 9,75%). A significant difference was observed between group 1 and group 2 (52,32% ± 13,22% vs 63,58% ± 11,55%; $p < 0,05$) and, moreover, between group 1 and group 3 (52,32% ± 13,22% vs 71,25% ± 9,75%; $p < 0,05$). At the baseline and after one month, TBR resulted similar in the three groups, (5,58% ± 10,34% vs. 4,53% ± 4,63% vs. 6,44% ± 8,89%), while TAR, similar in the three groups at the baseline, resulted significantly lower in group 3 compared to group 1 (22,31% ± 10,89% vs. 31,25% ± 19,18%; p=0,00). After three months, however, this difference disappeared: group 3 TIR decreased from 71,25% ± 9,75% to 65,71% ± 17,66%, almost returning to the starting values (62,75% ± 14,09%). Even group 3 TAR after three months rised up to values similar to the starting ones (TAR3 = 28,53% ± 16,57% vs. TAR1 = 31,25% ± 19,18%; p = 0,051).

Figure 29a: significant difference between group 1 and group 3 TAR after 1 month of follow-up



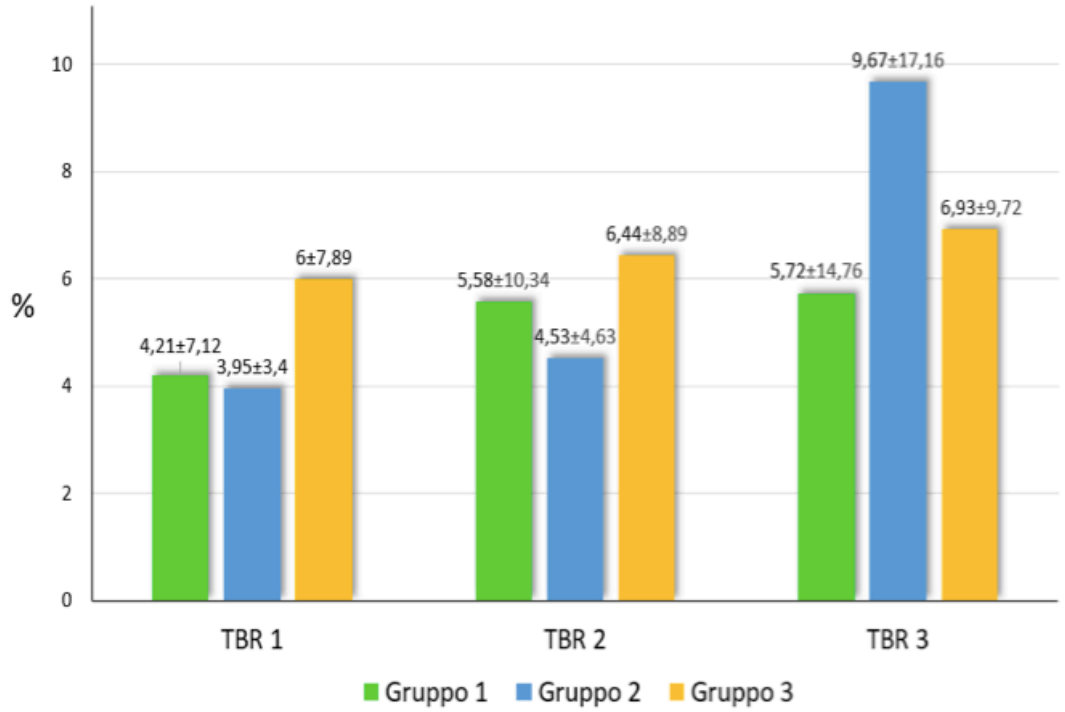


Figure 29b: baseline TBR vs 3-months follow-up TBR

3.5 Discussion

Many clinical trials, among them the DCCT (*Diabetes Control and Complication Trial*) [85] and the DAFNE (*Dose Adjustment For Normal Eating*) [132], have demonstrated that an accurate CHO counting is essential in T1D management to guarantee a good glucose control and to avoid hyperglycaemias and/or hypoglycaemias, with a positive impact on long-term HbA1c values. Other studies, as the BolusCal, have been performed to test if the good impact of CHO counting on glucose control could be observed not only in trial settings, but also in ordinary clinical assistance, showing an improvement of HbA1c values [133]. The results of the present study confirm how CHO counting can consistently influence glucose control. In particular, we observed that patients belonging to group 3 (using App Dietometro to perform CHO counting) showed a significantly lower HbA1c after 3 months of follow-up in comparison to baseline HbA1c and in comparison to group 1 (no counters) HbA1c after 3 months of follow-up. No difference in TIR after one month of follow-up was observed in group 1 and group 2 (conventional counters),

while TIR decreased significantly in group 3. After three months of follow-up, however, this difference disappeared, with a TIR values close to the baseline ones. After interviewing patients enrolled in groups 3 to investigate the possible reasons of the observed loss of effectiveness of App-guided CHO counting on glucose control, a reduction in patient's compliance to the App was identified. Nowadays technologies have an high impact in T1D, thanx to their capability to support patients in many ordinary activities (i.e. use of CSII and CGM devices, bolus calculator function to adapt insulin bolus to each meal) [134,135]. App for mobile phones with different characteristic compared to Dietometro have already been object of clinical studies, suggesting their potential in facilitating T1D patients in performing CHO counting [130,131]. The dowside of techinology in T1D management is represented by the constant necessity to be updated and to use correctly techonological instruments. Patients with T1D have to face calculations life-long to estimate CHO content of food, to estimate the correct insulin dose before the meal, to adapt insulin dose to pre-meal glycaemia, to estimate a correct CHO intake before doing physical activity and to adapt it to pre-activity glycaemia. With this background, it is likely to expect a reduction or loss of compliance if the proposed techonological devices are not intuitive and easy to use.

Study 4: T1D technology and quality of life: glucose control and beyond

4.1 Background

The management of type 1 diabetes mellitus has changed consistently over the past 25 years. Technological advances in glucose monitoring and continuous subcutaneous insulin infusion have improved the ability to optimize glycaemic control [136], however, the healthcare costs for chronic complications of diabetes continue to rise. This may suggest that despite both types of diabetes are life-long, given the generally earlier onset of the disease and the higher proportion of patients not reaching glycaemic goals, patients with type 1 diabetes may reach higher morbidity and higher health care resource consumption and cost to the system. One of the most important components (18%) of this amount is the prescription of drugs to manage only the complications of diabetes [137,138]. A better glycaemic control, thanks to techonology, could be effective in reducing healthcare costs by reducing the incidence and prevalence of chronic complications. However, the first aim should always be to improve quality of life [139]. According to the World Health Organization (WHO), the concept of health-related quality of life (HRQoL) refers to the perceived physical and mental health of an individual. Each patient has different characteristics and experiences the disease in a personal way, often without a real acceptance of the disease, especially by teenagers and young adults, that are the most vulnerable. Diabetes has a heavy psychosocial impact as it leads to changes in multiple areas of personal identity and relationships, increasing the risk of developing mood and behavioral disorders. These disorders can be triggered by "Diabetes Distress", a condition that refers to the negative emotions deriving from living with the disease and the burden of self-management of diabetes. For this reason, the choice of introducing insulin pump or sensor (or both) is a very delicate decision that must be made only after a deep counseling [139–142]. The use of CGM and CSII can improve quality of life, however, CGM satisfaction is not always associated with good glycaemic outcomes.

4.2 Aim

The primary aim of study 4 was to test the overall effect of new technologies in the treatment of type 1 diabetes in terms of quality of life. The exploratory aim was to compare the different devices (both sensors and insulin pumps) on patients' quality of life.

4.3 Matherials and Methods

4.3.1 Study population

69 subjects with type 1 diabetes attending the Endocrinology and Metabolic Diseases Unit of Policlinico Campus Bio-Medico of Rome were enrolled in the study. 31 males and 38 females, aged between 18 and 70 years (mean age 39 ± 12), were recruited. All patients were on intensive insulin therapy, respectively 36 on multiple daily insulin injection regimen (MDI) and 31 on continuous subcutaneous insulin infusion (CSII). CSII devices included Medtronic Minimed 640G and 670G (15), Theras Omnipod (7), Roche Insight (7) and Movy Tandem (3). Glucose monitoring was performed with Dexcom-G6 (15), Guardian sensor (10) and Flash Freestyle Libre (15). Patients attending Endocrinology and Metabolic Diseases Day Hospital or outpatient clinics of Policlinico Campus Bio-Medico of Rome were recruited from January 2020 to Dicember 2020. HbA1c, Time In Range (TIR), Time Above the Range (TAR), Time Below the Range (TBR) were collected as indicators of glucose control, while coefficient of Variation (CV) was collected as indicator of glycemic variability. The analysis was adjusted for sex, age and duration of the disease. HbA1c, Time In Range (TIR), Time Above the Range (TAR) and Time Below the Range (TBR) were recorded during medical consultations as glucose control parameters. Inclusion criteria were: age 18-70 years, ongoing intensive insulin therapy, signed inform consent and diagnosis of type 1 diabetes for at least six months. Exclusion criteria were: age <18 years, diagnosis of type 1 diabetes for less than six months, high impairment due to psychic or physical stress or cognitive issues, pregnancy or breast-feeding, end-stage kidney failure, chornic steroid or immunosoppressive therapy, advanced cancer. Age, sex, BMI and daily insulin dosage were recorded for each patient.

4.3.2 Quality of life assesment

Quality of life was investigated administering three questionnaires during medical consultations: the Diabetes Treatment Satisfaction Questionnaire (DTSQ), the Diabetes Specific Quality Of Life Scale (DSQOLS) and The Short Form (36) Health Survey (SF-36). The DTSQ was developed in the early 1980s and is still widely used, both in clinical trials and in routine clinical monitoring. It was specifically designed to estimate satisfaction concerning diabetes therapy and to compare specific tools of satisfaction in patients on different treatment regimens. The questionnaire investigates the self perception of blood glucose levels (both high or low) and some peculiar characteristics of intensive insulin therapy (i.e. flexibility) for a total of 8 items. The DSQOLS carefully focuses on the differences between insulin regimens in type 1 diabetes mellitus and investigates patient satisfaction in relationship to individual goals. This 64-item questionnairecan detect social inequalities in correlation with therapheutic regimen and can identify eventual motivational deficits and adapting strategies. The SF-36 questionnaire, finally, is a survey to the test patient's self-estimated state of health. It is characterized by brevity (on average it takes no more than 10 minutes to be completed) and precision (the tool is valid and reproducible). Developed since the 1980s in the United States as a generic and multi-dimensional questionnaire, it includes 36 questions that allow the patient to assemble 8 different parameters, summarizing the overall assessment with respect to Physical (ISF) and Mental health (ISM). A final score ranging from 0 to 100, where zero corresponds to the worse general health status and 100 to the best health status estimates patient’s self health status perception. All SF-36 questions, except one, refer to a period of four weeks prior to completing the questionnaire.

4.4 Results

4.4.1 Quality of life

Treatment satisfaction, self-perception of glycaemic variations, percetion of good or poor state of health, disease acceptance, fear oh hypoglycaemia, fear of complications of diabetes, feeding-related frustration, pain, anxiety or depression, social implications of the disease, life-style implications of the disease (for example sport) and difficulty in

working were assessed as parameters of quality of life through questionnaires. The CSII group showed higher treatment-related satisfaction (84.8% vs 52.8%, p = 0.005), and better disease acceptance (84.8% vs 52.8%, p = 0.012) compared with patients on MDI, despite similar age (MDI mean age 38 ± 12.5, CSII 41 ± 11.6). However, no differences among the other cathegories were observed, in particular for what concers feeding-related frustration, social implications of the disease and percetion of good or poor state of health.

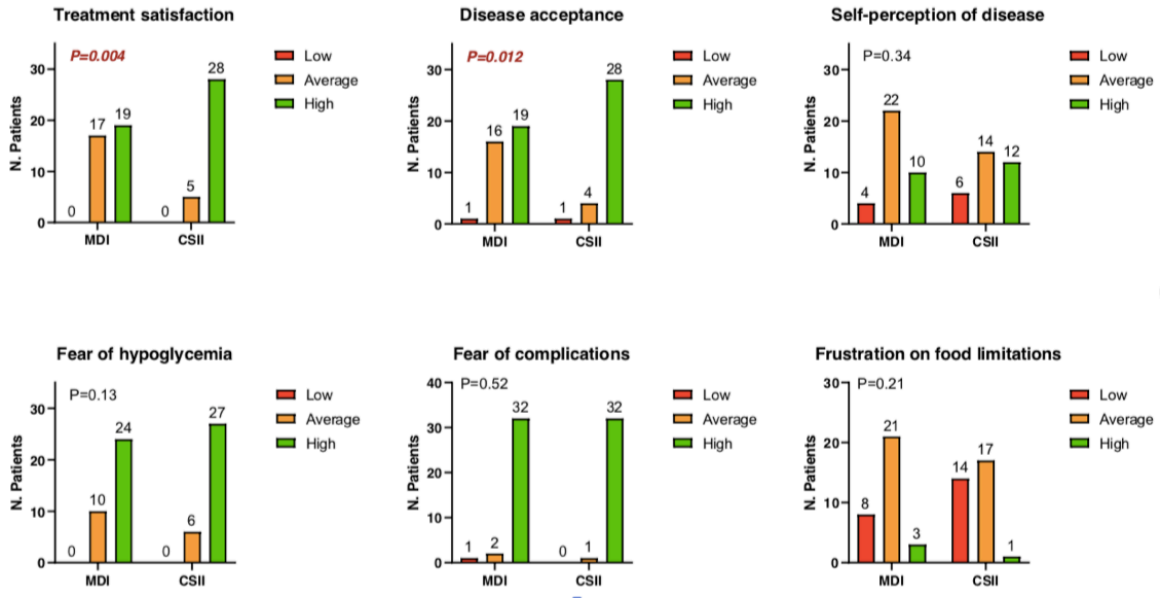


Figure 30: patient's quality of life perception

4.4.2 Glucose control

TIR resulted higher in the CSII group than in the MDI group (p=0.001). No differences were observed among different CSII devices (p = ns) and among TAR and TBR. The Dexcom G6 group had higher TIR values than the Freestyle (p=0.03) group, but similar to the Medtronic (p=0.12) group. TAR and TRB did not differ among CGM devices.

Table 9: study population features

	Mean value ± DS
Age (years)	39,3 ± 12,09
Duration of disease (years)	21,59 ± 11,19
BMI (Kg/m^2)	26,05 ± 4,63
HbA1c %	7,43% ± 1%
TIR %	58% ± 21%
TAR %	36% ± 19%
TBR %	6% ± 6,9%

Table 10: study population features, MDI group

	Mean value ± DS
Age (years)	38 ± 12,52
Duration of disease (years)	20,4 ± 11,69
BMI (Kg/m^2)	25,31 ± 3,64
HbA1c %	7,45% ± 1%
TIR %	47% ± 21%
TAR %	41% ± 17%
TBR %	10% ± 1%

Table 11: study population features, CSII group

	Mean value ± DS
Age (years)	40,78 ± 11,72
Duration of disease (years)	22,84 ± 10,65
BMI (Kg/m^2)	26,83 ± 5,44
HbA1c %	7,41% ± 1%
TIR %	63% ± 19%
TAR %	33% ± 20%
TBR %	3% ± 3%

Table 12: TIR, TAR and TBR mean values

	TIR	TAR	TBR
Freestyle	50% + 0.23	40% + 0.18	10% + 0.1
Dexcom G6	67% + 0.18	29% + 0.19	4% + 0.03
Medtronic (Enlite or Guardian sensor)	55% + 0.21	42% + 0.21	3% + 0.03
Dexcom + Medtronic	62% + 0.2	34% + 0.2	4% + 0.03

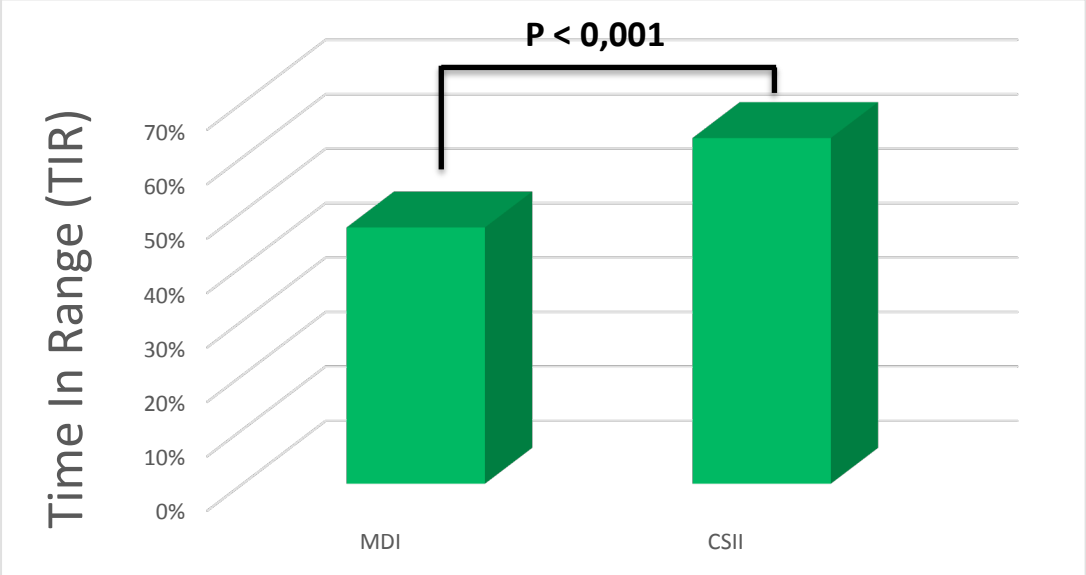


Figure 31: Comparison between MDI and CSII groups TIR. CSII group TIR was significantly higher than MDI group one

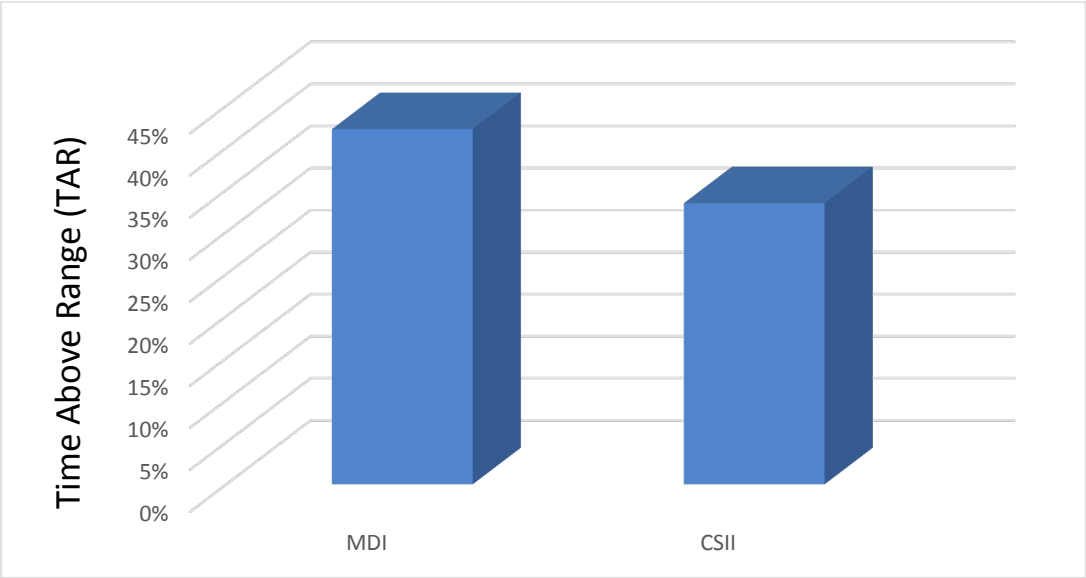


Figure 32: Comparison between MDI and CSII groups TAR. No significantly difference was observed between the two groups.

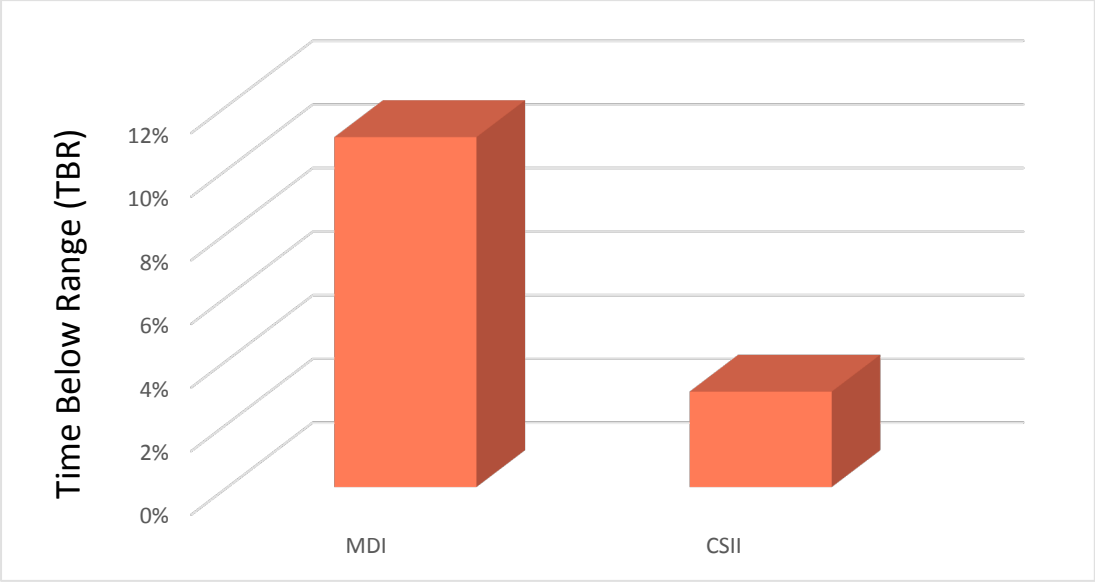


Figure 33: Comparison between MDI and CSII groups TBR. No significantly difference was observed between the two groups.

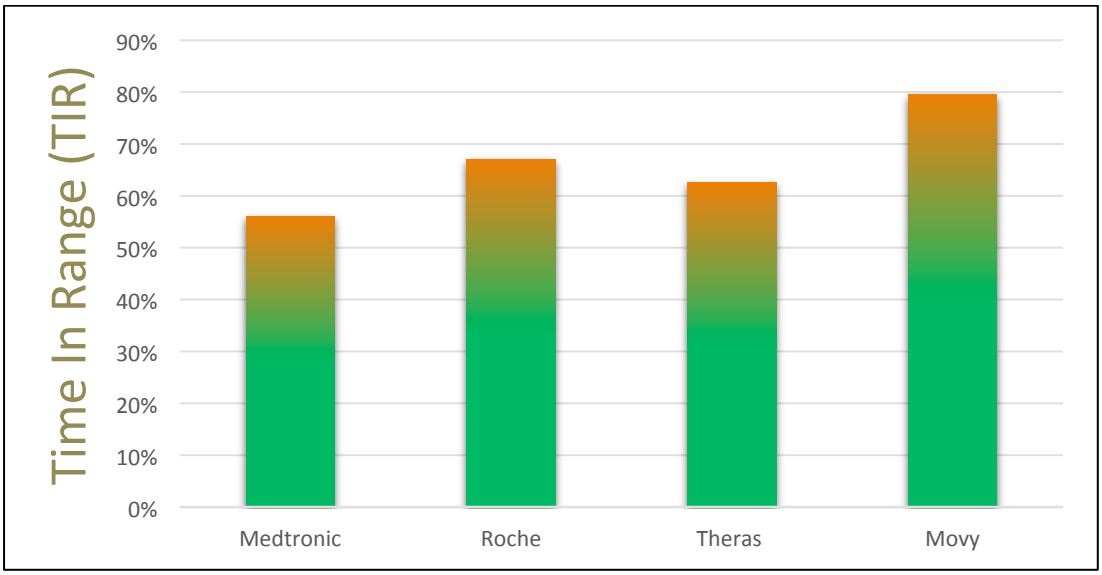


Figure 34: Comparison between Medtronic, Roche, Theras and Movy CSII devices TIR. No significantly difference was observed.

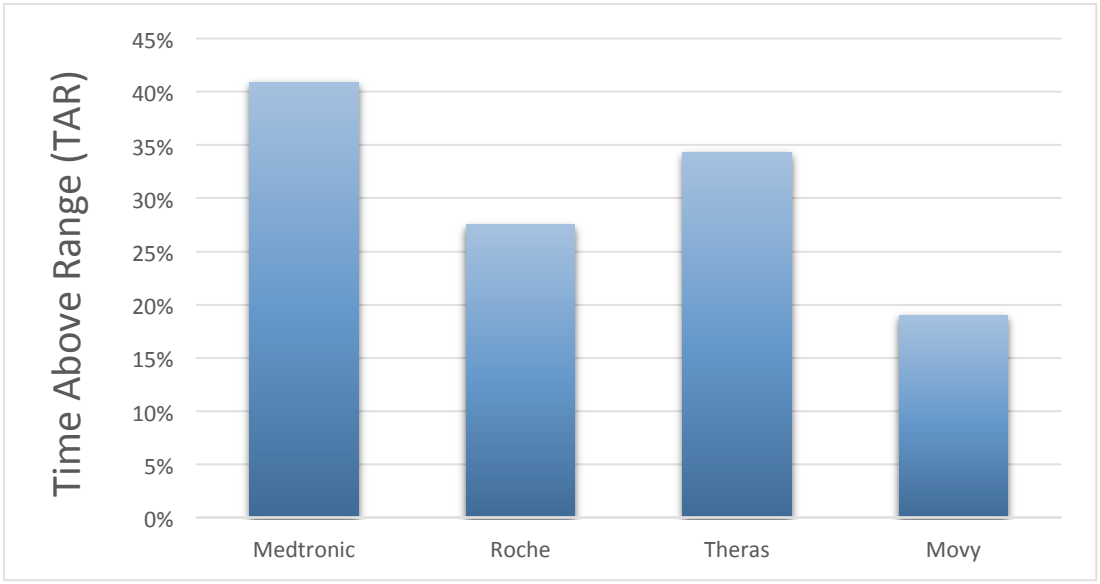


Figure 35: Comparison between Medtronic, Roche, Theras and Movy CSII devices TAR. No significantly difference was observed.

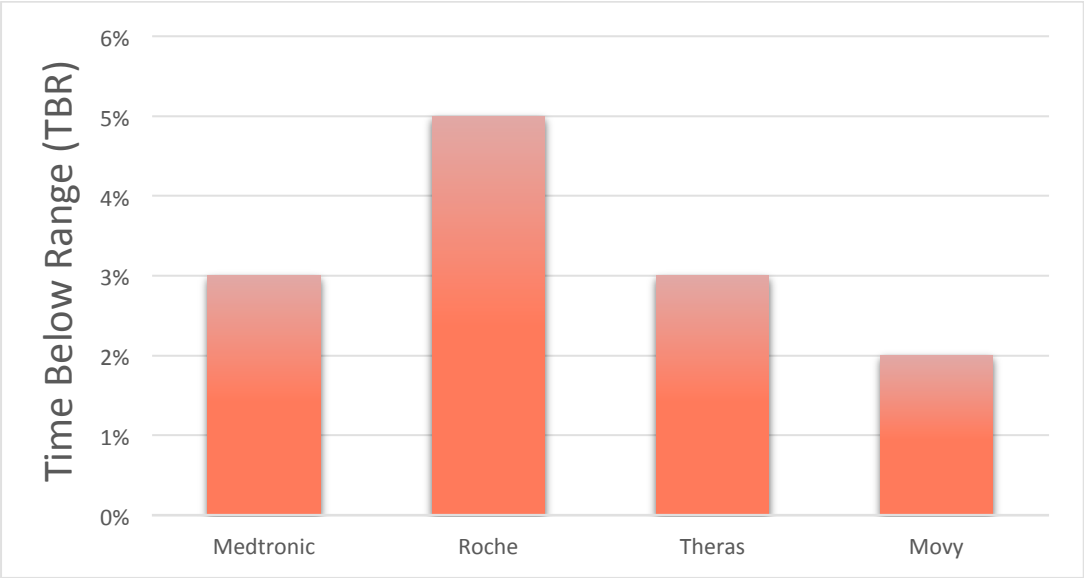


Figure 36: Comparison between Medtronic, Roche, Theras and Movy CSII devices TBR. No significantly difference was observed.

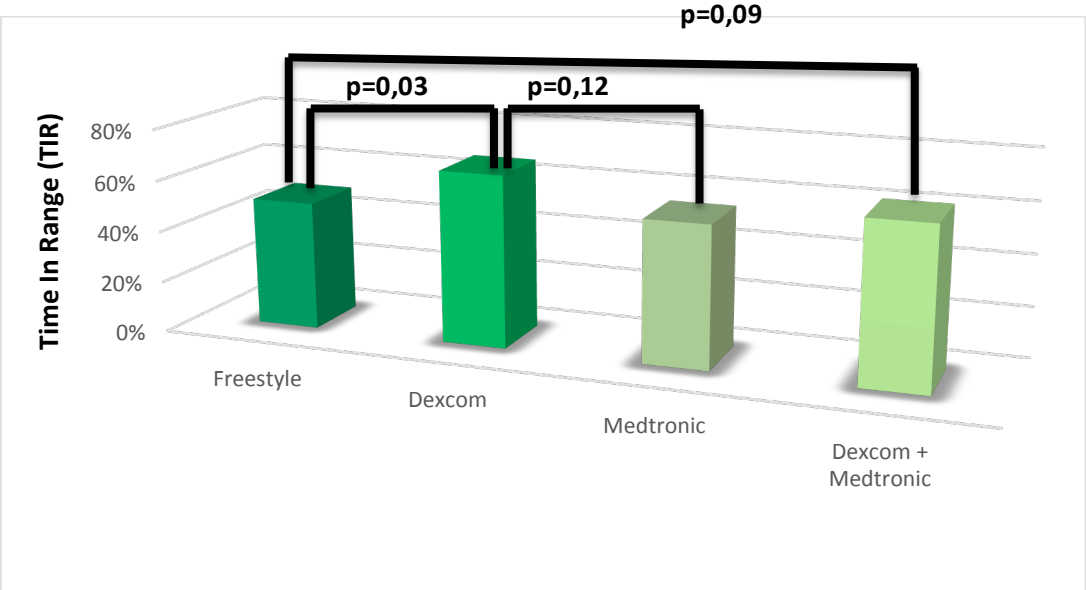


Figure 37: Comparison between Freestyle Libre-1, Dexcom and Medtronic sensor devices TIR. No significantly difference was observed.

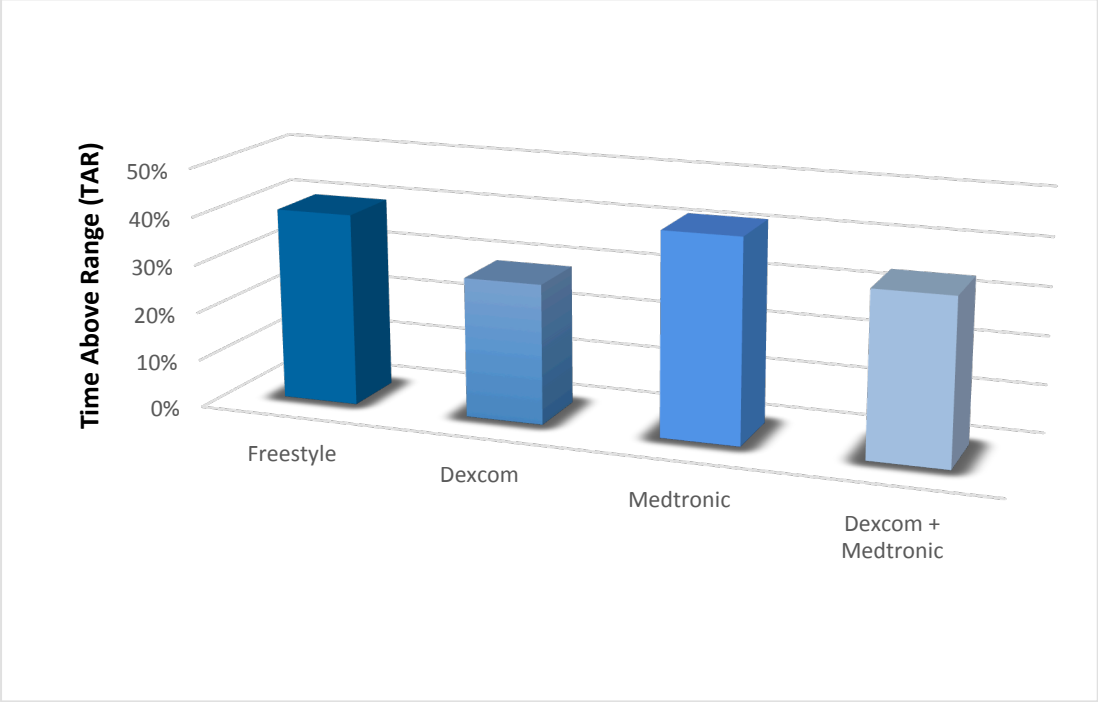


Figure 38: Comparison between Freestyle Libre-1, Dexcom and Medtronic sensor devices TAR. No significantly difference was observed.

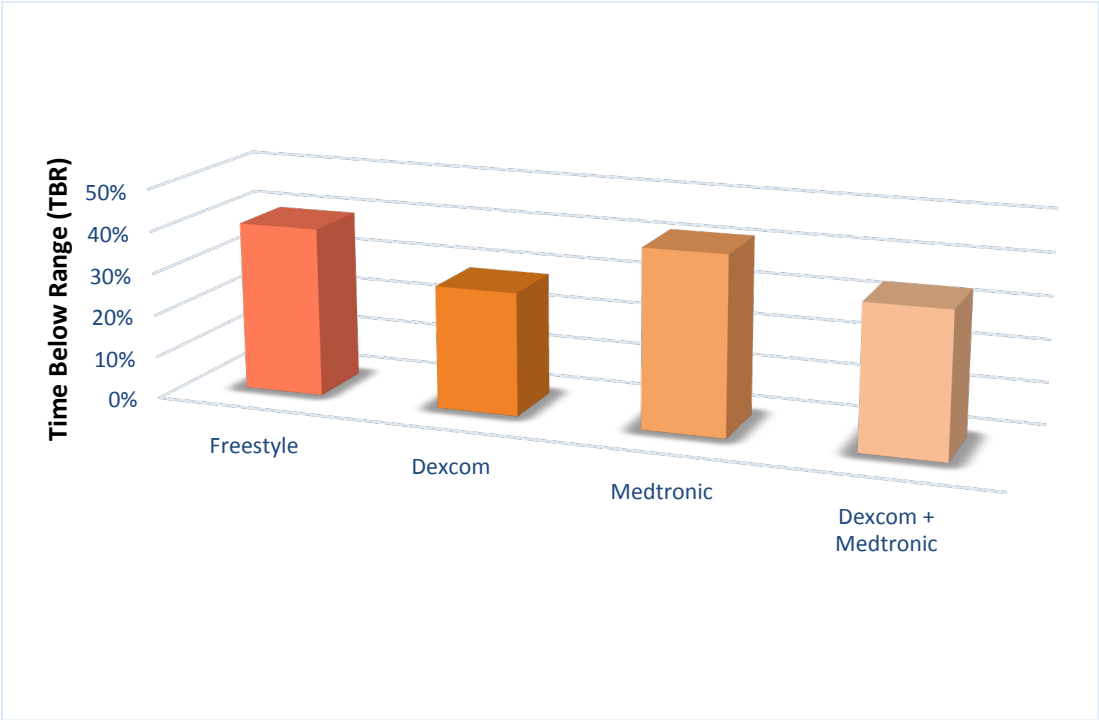


Figure 39: Comparison between Freestyle Libre-1, Dexcom and Medtronic sensor devices TBR. No significantly difference was observed.

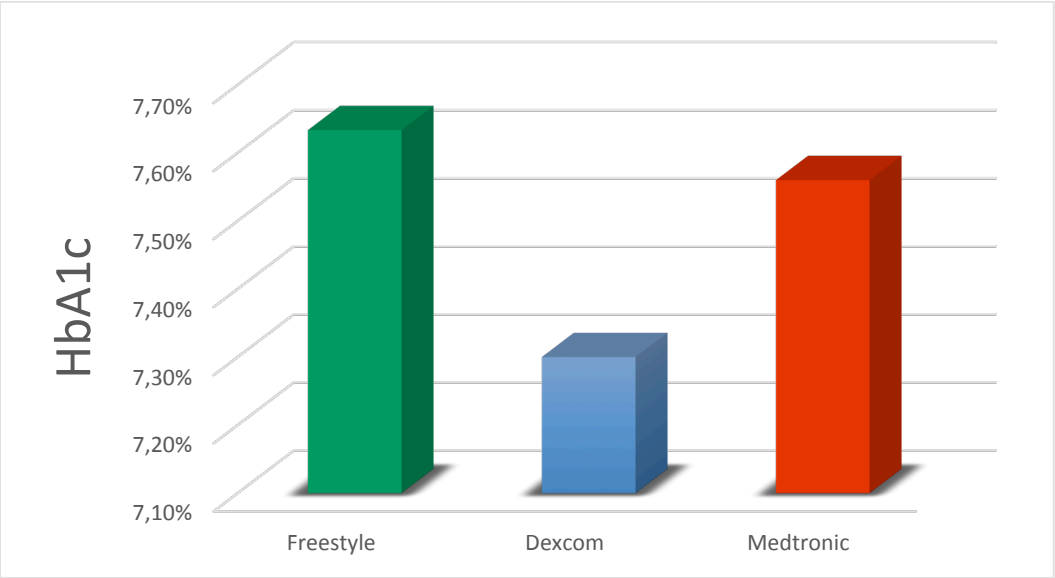


Figure 40: Comparison between Freestyle Libre-1, Dexcom and Medtronic sensor devices HbA1c. No significantly difference was observed.

4.5 Discussion

Treatment-related satisfaction resulted higher in patients on CSII therapy in comparison to patients on MDI regimen. This result is probably due to the major life-style flexibily guaranteed by CSII and CGM devices, concerning in particular the possibility to avoid multiple injections and finger glucose measurments [128,141,143,144]. Despite this, self quality of life perception didn't show significant differences between the two groups so that it is likely to deduce that, even if CSII therapy improves patient's management of T1D, it doesn't impact on everyday life coexistence with the disease. TIR resulted significantly higher in patients belonging to the CSII group in comparison with patients belonging to the MDI group, with consequently lower TAR and TBR in the CSII group in comparison with the MDI one. No differences between HbA1c were observed comparing CSII therapy group and MDI regimen group. This result confirms how HbA1c cannot be considered a reliable glucose control marker in all clinical cases because of the interference of glucose variabily. Focusing on CSII different device brands (Medtronic, Theras, Roche and Movy), no differences were observed both in self quality of life perception and glucose control. This result is likely due to the high performance of all the devices included in this study. However, our data should be read

with caution given the relatively small number of patients in each CSII device subgroup. Comparing CGM devices, instead, TIR resulted significantly higher in patients using Dexcom G6 in comparison with Freestyle Libre and Medtronic. This result likely finds explanation in the higher MARD (Mean Absolute Relative Difference or rather the correspondance between actual glycaemia and glycaemia detected by the device) of Dexcom G6 in comparison with other devices [145,146].

Bibliography

1. Maahs, D. M., West, N. A., Lawrence, J. M. & Mayer-Davis, E. J. Epidemiology of Type 1 Diabetes. *Endocrinol. Metab. Clin. North Am.* **39**, 481–497 (2010).

2. Soltesz, G., Patterson, C., Dahlquist, G., & EURODIAB Study Group. Worldwide childhood type 1 diabetes incidence ? what can we learn from epidemiology? *Pediatr. Diabetes* **8**, 6–14 (2007).

3. Rewers, M. & Ludvigsson, J. Environmental risk factors for type 1 diabetes. *Lancet Lond. Engl.* **387**, 2340–2348 (2016).

4. Wang, Z., Xie, Z., Lu, Q., Chang, C. & Zhou, Z. Beyond Genetics: What Causes Type 1 Diabetes. *Clin. Rev. Allergy Immunol.* **52**, 273–286 (2017).

5. Concannon, P., Rich, S. S. & Nepom, G. T. Genetics of Type 1A Diabetes. *N. Engl. J. Med.* **360**, 1646–1654 (2009).

6. Petrone, A. *et al.* Similar incidence of type 1 diabetes in two ethnically different populations (Italy and Slovenia) is sustained by similar HLA susceptible/protective haplotype frequencies: Petrone et al : Incidence of T1DM and HLA haplotype frequencies. *Tissue Antigens* **60**, 244–253 (2002).

7. Thomson, G. *et al.* Relative predispositional effects of HLA class II DRB1-DQB1 haplotypes and genotypes on type 1 diabetes: a meta-analysis. *Tissue Antigens* **70**, 110–127 (2007).

8. Polychronakos, C. & Li, Q. Understanding type 1 diabetes through genetics: advances and prospects. *Nat. Rev. Genet.* **12**, 781–792 (2011).

9. Bennett, S. T. *et al.* Insulin VNTR allele-specific effect in type 1 diabetes depends on identity of untransmitted paternal allele. The IMDIAB Group. *Nat. Genet.* **17**, 350–352 (1997).

10. Pugliese, A. *et al.* The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat. Genet.* **15**, 293–297 (1997).

11. Pugliese, A. The insulin gene in type 1 diabetes. *IUBMB Life* **57**, 463–468 (2005).

12. Pozzilli, P., Strollo, R. & Barchetta, I. Natural history and immunopathogenesis of type 1 diabetes. *Endocrinol. Nutr. Organo Soc. Espanola Endocrinol. Nutr.* **56 Suppl 4**, 50–52 (2009).

13. Bottazzo, G., Florin-Christensen, A. & Doniach, D. ISLET-CELL ANTIBODIES IN DIABETES MELLITUS WITH AUTOIMMUNE POLYENDOCRINE DEFICIENCIES. *The Lancet* **304**, 1279–1283 (1974).

14. Bluestone, J. A., Herold, K. & Eisenbarth, G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* **464**, 1293–1300 (2010).

15. Taplin, C. E. & Barker, J. M. Autoantibodies in type 1 diabetes. *Autoimmunity* **41**, 11–18 (2008).

16. Bonifacio, E. *et al.* Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *The Lancet* **335**, 147–149 (1990).

17. Gorsuch, A. N. *et al.* EVIDENCE FOR A LONG PREDIABETIC PERIOD IN TYPE I (INSULIN-DEPENDENT) DIABETES MELLITUS. *The Lancet* **318**, 1363–1365 (1981).

18. Irvine, W. J., Gray, R. S., Mccallum, C. J. & Duncan, L. J. P. CLINICAL AND PATHOGENIC SIGNIFICANCE OF PANCREATIC-ISLET-CELL ANTIBODIES IN DIABETICS TREATED WITH ORAL HYPOGLYCÆMIC AGENTS. *The Lancet* **309**, 1025–1027 (1977).

19. Berson, S. A. & Yalow, R. S. QUANTITATIVE ASPECTS OF THE REACTION BETWEEN INSULIN AND INSULIN-BINDING ANTIBODY. *J. Clin. Invest.* **38**, 1996–2016 (1959).

20. Palmer, J. P. *et al.* Insulin Antibodies in Insulin-Dependent Diabetics Before Insulin Treatment. *Science* **222**, 1337–1339 (1983).

21. the BABYDIAB-BABYDIET Study Group, Ziegler, A.-G. & Bonifacio, E. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. *Diabetologia* **55**, 1937–1943 (2012).

22. Vardi, P. *et al.* Concentration of Insulin Autoantibodies at Onset of Type I Diabetes: Inverse Log-Linear Correlation With Age. *Diabetes Care* **11**, 736–739 (1988).

23. Ziegler, R. *et al.* Specific Association of HLA-DR4 With Increased Prevalence and Level of Insulin Autoantibodies in First-Degree Relatives of Patients With Type I Diabetes. *Diabetes* **40**, 709–714 (1991).

24. Graham, J. *et al.* Genetic Effects on Age-Dependent Onset and Islet Cell Autoantibody Markers in Type 1 Diabetes. *Diabetes* **51**, 1346–1355 (2002).

25. Nanclares, G. P., Bilbao, J. R. & Castaño, L. No Association of *INS* -VNTR Genotype and IAA Autoantibodies. *Ann. N. Y. Acad. Sci.* **1037**, 127–130 (2004).

26. Bingley, P. J., Bonifacio, E., Mueller, P. W., & Participating Laboratories. Diabetes Antibody Standardization Program: First Assay Proficiency Evaluation. *Diabetes* **52**, 1128–1136 (2003).

27. Pihoker, C., Gilliam, L. K., Hampe, C. S. & Lernmark, Å. Autoantibodies in Diabetes. *Diabetes* **54**, S52–S61 (2005).

28. Wenzlau, J. M. *et al.* The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc. Natl. Acad. Sci.* **104**, 17040–17045 (2007).

29. Achenbach, P. *et al.* Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. *Diabetologia* **52**, 1881–1888 (2009).

30. Viskari, H. *et al.* Humoral beta-cell autoimmunity is rare in patients with the congenital

rubella syndrome. *Clin. Exp. Immunol.* **133**, 378–383 (2003).

31. Savola, K. *et al.* IA-2 antibodies--a sensitive marker of IDDM with clinical onset in childhood and adolescence. Childhood Diabetes in Finland Study Group. *Diabetologia* **41**, 424–429 (1998).

32. West, I. C. Radicals and oxidative stress in diabetes. *Diabet. Med. J. Br. Diabet. Assoc.* **17**, 171–180 (2000).

33. Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813–820 (2001).

34. Griffiths, H. R. Is the generation of neo-antigenic determinants by free radicals central to the development of autoimmune rheumatoid disease? *Autoimmun. Rev.* **7**, 544–549 (2008).

35. Trigwell, S. M. *et al.* Islet glutamic acid decarboxylase modified by reactive oxygen species is recognized by antibodies from patients with type 1 diabetes mellitus. *Clin. Exp. Immunol.* **126**, 242–249 (2008).

36. Szkudelski, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol. Res.* **50**, 537–546 (2001).

37. Steiner, D. F. New Aspects of Proinsulin Physiology and Pathophysiology. *J. Pediatr. Endocrinol. Metab.* **13**, (2000).

38. Steiner, D. F., Terris, S., Chan, S. J. & Rubenstein, A. H. Chemical and biological aspects of insulin and proinsulin. *Acta Med. Scand. Suppl.* **601**, 55–107 (1976).

39. Blanter, M., Sork, H., Tuomela, S. & Flodström-Tullberg, M. Genetic and Environmental Interaction in Type 1 Diabetes: a Relationship Between Genetic Risk Alleles and Molecular Traits of Enterovirus Infection? *Curr. Diab. Rep.* **19**, 82 (2019).

40. Madden, D. L. *et al.* Juvenile onset diabetes mellitus in pregnant women: Failure to associate with coxsackie B1–6, mumps, or respiratory syncytial virus infections. *J. Pediatr.* **92**, 959–960 (1978).

41. Granados, H. M. *et al.* Programmed cell death-1, PD-1, is dysregulated in T cells from children with new onset type 1 diabetes. *PloS One* **12**, e0183887 (2017).

42. Scherm, M. G. & Daniel, C. miRNA Regulation of T Cells in Islet Autoimmunity and Type 1 Diabetes. *Curr. Diab. Rep.* **20**, 41 (2020).

43. Infante, M. *et al.* Influence of Vitamin D on Islet Autoimmunity and Beta-Cell Function in Type 1 Diabetes. *Nutrients* **11**, E2185 (2019).

44. Kyvik, K. O., Green, A., Svendsen, A. & Mortensen, K. Breast feeding and the development of type 1 diabetes mellitus. *Diabet. Med. J. Br. Diabet. Assoc.* **9**, 233–235 (1992).

45. Frederiksen, B. *et al.* Infant exposures and development of type 1 diabetes mellitus: The Diabetes Autoimmunity Study in the Young (DAISY). *JAMA Pediatr.* **167**, 808–815 (2013).

46. Schmid, S., Buuck, D., Knopff, A., Bonifacio, E. & Ziegler, A. G. BABYDIET, a

feasibility study to prevent the appearance of islet autoantibodies in relatives of patients with Type 1 diabetes by delaying exposure to gluten. *Diabetologia* **47**, 1130–1131 (2004).

47. Hummel, S., Pflüger, M., Hummel, M., Bonifacio, E. & Ziegler, A.-G. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. *Diabetes Care* **34**, 1301–1305 (2011).

48. Kibirige, M., Metcalf, B., Renuka, R. & Wilkin, T. J. Testing the Accelerator Hypothesis. *Diabetes Care* **26**, 2865–2870 (2003).

49. American Diabetes Association. 10. Microvascular Complications and Foot Care: *Standards of Medical Care in Diabetes—2018*. *Diabetes Care* **41**, S105–S118 (2018).

50. Boyle, P. J. Diabetes Mellitus and Macrovascular Disease: Mechanisms and Mediators. *Am. J. Med.* **120**, S12–S17 (2007).

51. Beckman, J. A., Creager, M. A. & Libby, P. Diabetes and Atherosclerosis: Epidemiology, Pathophysiology, and Management. *JAMA* **287**, 2570 (2002).

52. Kannel, W. B. Diabetes and cardiovascular disease. The Framingham study. *JAMA J. Am. Med. Assoc.* **241**, 2035–2038 (1979).

53. Haffner, S. M., Lehto, S., Rönnemaa, T., Pyörälä, K. & Laakso, M. Mortality from Coronary Heart Disease in Subjects with Type 2 Diabetes and in Nondiabetic Subjects with and without Prior Myocardial Infarction. *N. Engl. J. Med.* **339**, 229–234 (1998).

54. Buse, J. B. *et al.* Primary Prevention of Cardiovascular Diseases in People With Diabetes Mellitus. *Diabetes Care* **30**, 162–172 (2007).

55. Lehto, S., Rönnemaa, T., Pyörälä, K. & Laakso, M. Predictors of Stroke in Middle-Aged Patients With Non–Insulin-Dependent Diabetes. *Stroke* **27**, 63–68 (1996).

56. Nathan, D. M. & for the DCCT/EDIC Research Group. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 Years: Overview. *Diabetes Care* **37**, 9–16 (2014).

57. Laing, S. P. *et al.* Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* **46**, 760–765 (2003).

58. Lindholm, L. H. *et al.* Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *The Lancet* **359**, 1004–1010 (2002).

59. Colhoun, H. M. *et al.* Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *The Lancet* **364**, 685–696 (2004).

60. Frick, M. H. *et al.* Helsinki Heart Study: Primary-Prevention Trial with Gemfibrozil in Middle-Aged Men with Dyslipidemia. *N. Engl. J. Med.* **317**, 1237–1245 (1987).

61. Koskinen, P. *et al.* Coronary Heart Disease Incidence in NIDDM Patients In The Helsinki Heart Study. *Diabetes Care* **15**, 820–825 (1992).

62. Manninen, V. *et al.* Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. *JAMA* **260**, 641–651 (1988).

63. Keenan, H. A. *et al.* Clinical Factors Associated With Resistance to Microvascular Complications in Diabetic Patients of Extreme Disease Duration. *Diabetes Care* **30**, 1995–1997 (2007).

64. Photocoagulation treatment of proliferative diabetic retinopathy: the second report of diabetic retinopathy study findings. *Ophthalmology* **85**, 82–106 (1978).

65. Palmberg, P. *et al.* The natural history of retinopathy in insulin-dependent juvenile-onset diabetes. *Ophthalmology* **88**, 613–618 (1981).

66. Nathan, D. M., Singer, D. E., Godine, J. E. & Perlmuter, L. C. Non-insulin-dependent diabetes in older patients. Complications and risk factors. *Am. J. Med.* **81**, 837–842 (1986).

67. Fowler, M. J. Microvascular and Macrovascular Complications of Diabetes. *Clin. Diabetes* **26**, 77–82 (2008).

68. Nathan, D. M. Long-Term Complications of Diabetes Mellitus. *N. Engl. J. Med.* **328**, 1676–1685 (1993).

69. Gross, J. L. *et al.* Diabetic Nephropathy: Diagnosis, Prevention, and Treatment. *Diabetes Care* **28**, 164–176 (2005).

70. Kussman, M. J., Goldstein, H. & Gleason, R. E. The clinical course of diabetic nephropathy. *JAMA* **236**, 1861–1863 (1976).

71. Krolewski, A. S., Warram, J. H., Christlieb, A. R., Busick, E. J. & Kahn, C. R. The changing natural history of nephropathy in type I Diabetes. *Am. J. Med.* **78**, 785–794 (1985).

72. Mogensen, C. E. Microalbuminuria Predicts Clinical Proteinuria and Early Mortality in Maturity-Onset Diabetes. *N. Engl. J. Med.* **310**, 356–360 (1984).

73. Mauer, S. M. *et al.* Structural-functional relationships in diabetic nephropathy. *J. Clin. Invest.* **74**, 1143–1155 (1984).

74. Feldt-Rasmussen, B., Hegedüs, L., Mathiesen, E. R. & Deckert, T. Kidney volume in type 1 (insulin-dependent) diabetic patients with normal or increased urinary albumin excretion: effect of long-term improved metabolic control. *Scand. J. Clin. Lab. Invest.* **51**, 31–36 (1991).

75. Østerby, R., Gundersen, H. J. G., Nyberg, G. & Aurell, M. Advanced Diabetic Glomerulopathy: Quantitative Structural Characterization of Nonoccluded Glomeruli. *Diabetes* **36**, 612–619 (1987).

76. Kimmelstiel, P. & Wilson, C. Intercapillary Lesions in the Glomeruli of the Kidney. *Am. J. Pathol.* **12**, 83-98.7 (1936).

77. Bilous, R. W., Mauer, S. M., Sutherland, D. E. & Steffes, M. W. Mean glomerular volume and rate of development of diabetic nephropathy. *Diabetes* **38**, 1142–1147 (1989).

78. Said, G., Goulon-Goeau, C., Slama, G. & Tchobroutsky, G. Severe early-onset

polyneuropathy in insulin-dependent diabetes mellitus. A clinical and pathological study. *N. Engl. J. Med.* **326**, 1257–1263 (1992).

79. Factors in development of diabetic neuropathy. Baseline analysis of neuropathy in feasibility phase of Diabetes Control and Complications Trial (DCCT). The DCCT Research Group. *Diabetes* **37**, 476–481 (1988).

80. Ellenberg, M. Diabetic neuropathic cachexia. *Diabetes* **23**, 418–423 (1974).

81. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. *Diabetes Care* **43**, S14–S31 (2020).

82. Marre, M. *et al.* Converting enzyme inhibition and kidney function in normotensive diabetic patients with persistent microalbuminuria. *Br. Med. J. Clin. Res. Ed* **294**, 1448–1452 (1987).

83. Cook, J. *et al.* Angiotensin converting enzyme inhibitor therapy to decrease microalbuminuria in normotensive children with insulin-dependent diabetes mellitus. *J. Pediatr.* **117**, 39–45 (1990).

84. Feldt-Rasmussen, B., Mathiesen, ElisabethR. & Deckert, T. EFFECT OF TWO YEARS OF STRICT METABOLIC CONTROL ON PROGRESSION OF INCIPIENT NEPHROPATHY IN INSULIN-DEPENDENT DIABETES. *The Lancet* **328**, 1300–1304 (1986).

85. The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. The DCCT Research Group. *Diabetes* **35**, 530–545 (1986).

86. Callaghan, B. C., Price, R. S. & Feldman, E. L. Distal Symmetric Polyneuropathy: A Review. *JAMA* **314**, 2172–2181 (2015).

87. Franceschi, R. *et al.* A systematic review of the prevalence, risk factors and screening tools for autonomic and diabetic peripheral neuropathy in children, adolescents and young adults with type 1 diabetes. *Acta Diabetol.* (2022) doi:10.1007/s00592-022-01850-x.

88. Dos Santos, T. J., Donado Campos, J. de M., Fraga Medin, C. A., Argente, J. & Rodríguez-Artalejo, F. New insulin delivery devices and glycemic outcomes in young patients with type 1 diabetes: a protocol for a systematic review and meta-analysis. *Syst. Rev.* **8**, 259 (2019).

89. Fisher, L. K. The selection of children and adolescents for treatment with continuous subcutaneous insulin infusion (CSII). *Pediatr. Diabetes* **7**, 11–14 (2006).

90. Hanas, R. Selection for and Initiation of Continuous Subcutaneous Insulin Infusion. *Horm. Res. Paediatr.* **57**, 101–104 (2002).

91. Claxton, K. *et al.* Methods for the estimation of the National Institute for Health and Care Excellence cost-effectiveness threshold. *Health Technol. Assess.* **19**, 1–504 (2015).

92. Fu, S., Li, L., Deng, S., Zan, L. & Liu, Z. Effectiveness of advanced carbohydrate

counting in type 1 diabetes mellitus: a systematic review and meta-analysis. *Sci. Rep.* **6**, 37067 (2016).

93. Kaul, K., Tarr, J. M., Ahmad, S. I., Kohner, E. M. & Chibber, R. Introduction to Diabetes Mellitus. in *Diabetes* (ed. Ahmad, S. I.) vol. 771 1–11 (Springer New York, 2013).

94. Wang, Z., Du, Z. & Zhu, F. Glycosylated hemoglobin is associated with systemic inflammation, hypercoagulability, and prognosis of COVID-19 patients. *Diabetes Res. Clin. Pract.* **164**, 108214 (2020).

95. Dasu, M. R., Devaraj, S., Zhao, L., Hwang, D. H. & Jialal, I. High Glucose Induces Toll-Like Receptor Expression in Human Monocytes. *Diabetes* **57**, 3090–3098 (2008).

96. Hu, R., Xia, C.-Q., Butfiloski, E. & Clare-Salzler, M. Effect of high glucose on cytokine production by human peripheral blood immune cells and type I interferon signaling in monocytes: Implications for the role of hyperglycemia in the diabetes inflammatory process and host defense against infection. *Clin. Immunol.* **195**, 139–148 (2018).

97. Zhou, F. *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* **395**, 1054–1062 (2020).

98. Guan, W. *et al.* Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* **382**, 1708–1720 (2020).

99. Sattar, N., McInnes, I. B. & McMurray, J. J. V. Obesity Is a Risk Factor for Severe COVID-19 Infection: Multiple Potential Mechanisms. *Circulation* **142**, 4–6 (2020).

100. Akbar, D. H. Bacterial pneumonia: comparison between diabetics and non-diabetics. *Acta Diabetol.* **38**, 77–82 (2001).

101. Simonnet, A. *et al.* High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus□2 (SARS□CoV□2) Requiring Invasive Mechanical Ventilation. *Obesity* **28**, 1195–1199 (2020).

102. Chen, X. *et al. Hypertension and Diabetes Delay the Viral Clearance in COVID-19 Patients*. http://medrxiv.org/lookup/doi/10.1101/2020.03.22.20040774 (2020) doi:10.1101/2020.03.22.20040774.

103. Libby, P. & Simon, D. I. Inflammation and Thrombosis: The Clot Thickens. *Circulation* **103**, 1718–1720 (2001).

104. Chen, N. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet* **395**, 507–513 (2020).

105. Guo, X. *et al.* Serum levels of immunoglobulins in an adult population and their relationship with type 2 diabetes. *Diabetes Res. Clin. Pract.* **115**, 76–82 (2016).

106. Jennbacken, K., Ståhlman, S., Grahnemo, L., Wiklund, O. & Fogelstrand, L. Glucose impairs B-1 cell function in diabetes. *Clin. Exp. Immunol.* **174**, 129–138 (2013).

107. Geerlings, S. E. & Hoepelman, A. I. M. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol. Med. Microbiol.* **26**, 259–265 (1999).

108. Erener, S. Diabetes, infection risk and COVID-19. *Mol. Metab.* **39**, 101044 (2020).

109. Volti, S. L. *et al.* Hyporesponsiveness to intradermal administration of hepatitis B vaccine in insulin dependent diabetes mellitus. *Arch. Dis. Child.* **78**, 54–57 (1998).

110. Strollo, R. *et al.* Antibodies to post-translationally modified insulin in type 1 diabetes. *Diabetologia* **58**, 2851–2860 (2015).

111. Strollo, R. *et al.* HLA-dependent autoantibodies against post-translationally modified collagen type II in type 1 diabetes mellitus. *Diabetologia* **56**, 563–572 (2013).

112. Weiss, M., Steiner, D. F. & Philipson, L. H. Insulin Biosynthesis, Secretion, Structure, and Structure-Activity Relationships. in *Endotext* (eds. Feingold, K. R. et al.) (MDText.com, Inc., 2000).

113. Leighton, E., Sainsbury, C. A. & Jones, G. C. A Practical Review of C-Peptide Testing in Diabetes. *Diabetes Ther. Res. Treat. Educ. Diabetes Relat. Disord.* **8**, 475–487 (2017).

114. Sims, E. K. *et al.* Elevations in the Fasting Serum Proinsulin–to–C-Peptide Ratio Precede the Onset of Type 1 Diabetes. *Diabetes Care* **39**, 1519–1526 (2016).

115. Sims, E. K. *et al.* Proinsulin Secretion Is a Persistent Feature of Type 1 Diabetes. *Diabetes Care* **42**, 258–264 (2019).

116. Ježek, P., Jabůrek, M. & Plecitá-Hlavatá, L. Contribution of Oxidative Stress and Impaired Biogenesis of Pancreatic β-Cells to Type 2 Diabetes. *Antioxid. Redox Signal.* **31**, 722–751 (2019).

117. Gerber, P. A. & Rutter, G. A. The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus. *Antioxid. Redox Signal.* **26**, 501–518 (2017).

118. Syeda, K., Mohammed, A. M., Arora, D. K. & Kowluru, A. Glucotoxic conditions induce endoplasmic reticulum stress to cause caspase 3 mediated lamin B degradation in pancreatic β-cells: Protection by nifedipine. *Biochem. Pharmacol.* **86**, 1338–1346 (2013).

119. American Diabetes Association. 4. Lifestyle Management: *Standards of Medical Care in Diabetes—2018*. *Diabetes Care* **41**, S38–S50 (2018).

120. Jenkins, D. J. *et al.* Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am. J. Clin. Nutr.* **34**, 362–366 (1981).

121. Deeb, A., Al Hajeri, A., Alhmoudi, I. & Nagelkerke, N. Accurate Carbohydrate Counting Is an Important Determinant of Postprandial Glycemia in Children and Adolescents With Type 1 Diabetes on Insulin Pump Therapy. *J. Diabetes Sci. Technol.* **11**, 753–758 (2017).

122. Enander, R., Gundevall, C., Strömgren, A., Chaplin, J. & Hanas, R. Carbohydrate counting with a bolus calculator improves post-prandial blood glucose levels in children and adolescents with type 1 diabetes using insulin pumps: Carbohydrate counting in children. *Pediatr. Diabetes* **13**, 545–551 (2012).

123. Kawamura, T. *et al.* The factors affecting on estimation of carbohydrate content of meals in carbohydrate counting. *Clin. Pediatr. Endocrinol.* **24**, 153–165 (2015).

124. American Diabetes Association. Nutrition Recommendations and Interventions for Diabetes. *Diabetes Care* **31**, S61–S78 (2008).

125. Krebs, J. D. *et al.* The Diabetes Excess Weight Loss (DEWL) Trial: a randomised controlled trial of high-protein versus high-carbohydrate diets over 2 years in type 2 diabetes. *Diabetologia* **55**, 905–914 (2012).

126. Te Morenga, L. & Mann, J. The role of high-protein diets in body weight management and health. *Br. J. Nutr.* **108**, S130–S138 (2012).

127. American Diabetes Association. 5. Facilitating Behavior Change and Well-being to Improve Health Outcomes: *Standards of Medical Care in Diabetes—2020*. *Diabetes Care* **43**, S48–S65 (2020).

128. Yeh, H.-C. *et al.* Comparative effectiveness and safety of methods of insulin delivery and glucose monitoring for diabetes mellitus: a systematic review and meta-analysis. *Ann. Intern. Med.* **157**, 336–347 (2012).

129. Cummins, E. *et al.* Clinical effectiveness and cost-effectiveness of continuous subcutaneous insulin infusion for diabetes: systematic review and economic evaluation. *Health Technol. Assess.* **14**, (2010).

130. Alfonsi, J. E. *et al.* Carbohydrate Counting App Using Image Recognition for Youth With Type 1 Diabetes: Pilot Randomized Control Trial. *JMIR MHealth UHealth* **8**, e22074 (2020).

131. Anthimopoulos, M. *et al.* Computer Vision-Based Carbohydrate Estimation for Type 1 Patients With Diabetes Using Smartphones. *J. Diabetes Sci. Technol.* **9**, 507–515 (2015).

132. DAFNE Study Group. Training in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes: dose adjustment for normal eating (DAFNE) randomised controlled trial. *BMJ* **325**, 746–746 (2002).

133. Schmidt, S. *et al.* Use of an Automated Bolus Calculator in MDI-Treated Type 1 Diabetes. *Diabetes Care* **35**, 984–990 (2012).

134. for the T1D Exchange Clinic Network, the DPV Initiative, and the National Paediatric Diabetes Audit and the Royal College of Paediatrics and Child Health registries *et al.* Use of insulin pump therapy in children and adolescents with type 1 diabetes and its impact on metabolic control: comparison of results from three large, transatlantic paediatric registries. *Diabetologia* **59**, 87–91 (2016).

135. Bonfanti, R. *et al.* Survey on the use of insulin pumps in Italy: comparison between pediatric and adult age groups (IMITA study). *Acta Diabetol.* **53**, 403–412 (2016).

136. Lepore, G. *et al.* Continuous subcutaneous insulin infusion is more effective than multiple daily insulin injections in preventing albumin excretion rate increase in Type 1 diabetic patients. *Diabet. Med.* **26**, 602–608 (2009).

137. Crossen, S., Xing, G. & Hoch, J. S. Changing costs of type 1 diabetes care among US children and adolescents. *Pediatr. Diabetes* **21**, 644–648 (2020).

138. Joish, V. N. *et al.* Estimation of Annual Health Care Costs for Adults with Type 1 Diabetes in the United States. *J. Manag. Care Spec. Pharm.* **26**, 311–318 (2020).

139. EQuality1 Study Group--Evaluation of QUALITY of Life and Costs in Diabetes Type 1 *et al.* Quality of life and treatment satisfaction in adults with Type 1 diabetes: a comparison between continuous subcutaneous insulin infusion and multiple daily injections. *Diabet. Med. J. Br. Diabet. Assoc.* **25**, 213–220 (2008).

140. Faria, H. T. G. *et al.* Qualidade de vida de pacientes com diabetes mellitus antes e após participação em programa educativo. *Rev. Esc. Enferm. USP* **47**, 348–354 (2013).

141. Polonsky, W. H., Hessler, D., Layne, J. E. & Zisser, H. Impact of the Omnipod ® Insulin Management System on Quality of Life: A Survey of Current Users. *Diabetes Technol. Ther.* **18**, 664–670 (2016).

142. Iturralde, E. *et al.* Expectations and Attitudes of Individuals With Type 1 Diabetes After Using a Hybrid Closed Loop System. *Diabetes Educ.* **43**, 223–232 (2017).

143. Cummins, E. *et al.* Clinical effectiveness and cost-effectiveness of continuous subcutaneous insulin infusion for diabetes: systematic review and economic evaluation. *Health Technol. Assess. Winch. Engl.* **14**, iii–iv, xi–xvi, 1–181 (2010).

144. Garg, S. K. *et al.* Glucose Outcomes with the In-Home Use of a Hybrid Closed-Loop Insulin Delivery System in Adolescents and Adults with Type 1 Diabetes. *Diabetes Technol. Ther.* **19**, 155–163 (2017).

145. Guillot, F. H. *et al.* Accuracy of the Dexcom G6 Glucose Sensor during Aerobic, Resistance, and Interval Exercise in Adults with Type 1 Diabetes. *Biosensors* **10**, E138 (2020).

146. Akturk, H. K., Dowd, R., Shankar, K. & Derdzinski, M. Real-World Evidence and Glycemic Improvement Using Dexcom G6 Features. *Diabetes Technol. Ther.* **23**, S21–S26 (2021).