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C-peptide and residual β-cell function in pediatric diabetes – state of the art

C-peptyd i resztkowa funkcja komórek β trzustki w cukrzycy u dzieci – aktualny stan wiedzy

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Abstract

C-peptide, the molecule produced in an equimolar concentration to insulin, has become an established insulin secretion biomarker in diabetic patients. Measurement of C-peptide level can be helpful in clinical practice for assessing insulin-producing β-cells residual function, especially in the patients who have already started exogenous insulin therapy. Advances in assays have made measurement of C-peptide more reliable and inexpensive. Traditionally, C-peptide is widely used to differentiate between type 1, type 2 and monogenic types in diabetic patients of all ages, both when the diabetes occurs and even months and years after the initial diagnosis. Moreover, in the patients with type 1 diabetes, the C-peptide secretion can become a reliable predictor of the clinical partial remission in the first months after diagnosis, although noteworthy, its' any specified level is not included in the definition of this phase of the disease. Many other clinical factors such as age, use of innovative technologies, the intensity of physical activity or body mass influence the concentration of C-peptide as well as diabetes remission occurrence and duration. They may interfere the interpretation of C-peptide level in the diabetes course. There is a great need to assess the new, adjusted C-peptide levels in these situations. A multitude novel therapies including immunomodulative factors and stem cell transplants can also use C-peptide in the patient selection and post-therapeutic monitoring of the outcome in researches aimed in extension of remission period. Recent research proves C-peptide presence and preserved function and being the possible important player in better metabolic control in long-lasting diabetes type 1. These findings may open the area for trials to regenerate β-cells and save endogenous insulin secretion for many years after diagnosis. Last but not the least, C-peptide presents its own physiological effect on other tissues, among others on the endothelial function, thus participates in inhibiting micro- and macrovascular diabetes complications. The idea of C-peptide as a new, additional to insulin cure remains as much attractive as elusive.

Key words:

type 1 diabetes, C-peptide, partial remission, residual insulin secretion.

Streszczenie

C-peptyd – cząsteczka produkowana w równym stężeniu z insuliną, stał się uznanym biomarkerem wydzielania insuliny u osób chorujących na cukrzycę. Pomiar jego stężenia może być pomocny w praktyce klinicznej w ocenie resztkowej funkcji komórek β produkujących insulinę, zwłaszcza u pacjentów, którzy rozpoczęli już terapię insuliną egzogenną. Postęp w dziedzinie metod pomiarowych sprawił, że pomiar C-peptydu stał się z czasem coraz bardziej wiarygodny oraz niedrogi. Tradycyjnie C-peptyd jest szeroko stosowany do różnicowania między typem 1, typem 2 i typami monogenowymi u pacjentów z cukrzycą w każdym wieku, zarówno w momencie wystąpienia cukrzycy, jak nawet miesiące czy lata po jej rozpoznaniu. Co więcej, u chorych na cukrzycę typu 1 wydzielanie C-peptydu może stać się wiarygodnym predyktorem częściowej remisji klinicznej w pierwszych miesiącach po rozpoznaniu choroby, chociaż, co warte podkreślenia, jego poziom nie mieści się w definicji tej fazy choroby. Wiele innych czynników klinicznych, takich jak wiek, stosowanie innowacyjnych technologii, intensywność aktywności fizycznej czy masa ciała, wpływa na stężenie C-peptydu oraz na wystąpienie i czas trwania remisji cukrzycy. Mogą one zaburzać interpretację stężenia C-peptydu w przebiegu choroby. Istnieje zatem istotna potrzeba oceny nowych, skorygowanych wyników pomiaru C-peptydu w tych sytuacjach. Wiele nowych terapii, w tym z użyciem czynników immunomodulacyjnych i przeszczepów komórek macierzystych w badaniach mających na celu wydłużenie okresu remisji, może również wykorzystywać C-peptyd w doborze pacjentów i monitorowaniu wyników terapii. Najnowsze badania dowodzą, że C-peptyd jest obecny i zachowuje swoją funkcję, a także może odgrywać ważną rolę w lepszej kontroli metabolicznej także w długotrwałej cukrzycy typu 1. Wyniki te mogą otworzyć pole do badań mających na celu regenerację komórek β i zachowanie endogennego wydzielania insuliny przez wiele lat po rozpoznaniu choroby. Wreszcie, C-peptyd wykazuje własne fizjologiczne działanie na inne tkanki, między innymi na funkcję śródbłonka, uczestnicząc w ten sposób w hamowaniu mikro- i makronaczyniowych powikłań cukrzycy. Idea C-peptydu jako nowej, dodatkowej, poza insuliną, metody leczenia pozostaje tyleż atrakcyjna, co nieuchwytna.

Słowa kluczowe:

cukrzyca typu 1, C-peptyd, częściowa remisja, resztkowa sekrecja insuliny.

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Introduction

Insulin, the peptide hormone produced in β-cells of pancreatic islets, plays the key role in carbohydrate metabolism. In the process of insulin production and secretion, the precursor molecule called preproinsulin transforms to proinsulin by the signal peptide cleavage. Then proinsulin maturates into active insulin by the action of cellular endopeptidases, releasing C-peptide and leaving the insulin A- and B- chains connected by two disulfide bonds. C-peptide was first discovered in 1967 [1], and at that time was considered to be only an inactive by-product of insulin synthesis. C-peptide is highly resistant to plasma peptidases and has a reasonably stable and long half-life time that makes its concentration a marker of beta-cell function and insulin secretion. Further investigations showed that this molecule can be also a biologically active hormone with a wide spectrum of actions [2, 3]. In current time, some questions are being stated like: what factors influence residual beta-cell function at diabetes onset and in forthcoming years with the disease, is there residual β-cell function existing after many years of recognition DMT1 – using modern, ultrasensitive assays, is there a clinical significance of low-levels of C-peptide and is preserved insulin secretion beneficial for reduction of complications rates [4]. Recent advances in assays, ultrasensitive C-peptide assays among them, have made assessment of insulin secretion using C-peptide cheaper, more reliable and broadly available. The importance of C-peptide and its role in the diagnosis, monitoring and treatment of diabetes in children and adolescents are discussed in this review.

Methods of C-peptide measurement

In last years, there have been a great improvement in the C-peptide methods of assessment. Early assays were time–consuming, expensive and not-repeatable. New ones, highly sensitive and specific, using monoclonal antibodies, reduced costs of the assays, improved detection limits and reproducibility. A cautious attention is needed for interpretation of c-peptide values obtained from research studies, where different methods might have been used. This may be of particular importance when patients results are close to threshold value for a clinical decision and creates an important matter to resolve in pediatric diabetology, where there is a great space of research to prolong C-peptide/endogenous insulin secretion with a number of immunomodulation studies or other new treatment possibilities. An additional hassle remains a lack of reliable reference ranges for different clinical situations [5].

C-peptide can be measured in a fasting, non-fasting ("random") blood sample or in stimulations tests. Both fasting and random measurements remain easy to perform in clinical practice as they are less expensive, however their potential to detect subtle C-peptide levels is limited. When interpreting the results the clinician must be aware that β-cell stimulation in the fasting state may be altered by hypoglycaemia, and insulin administration, if was needed. Therefore stimulation tests seem advantageous [5]. Correlations between fasting and post-stimulation C-peptide are high in insulin treated patients, so stimulated C-peptide, including non-fasting "random" sample appears to propose better clinical utility. Non-fasting sampling is the simplest method to test in outpatients settings and correlates with fasting and stimulated C-peptide. For formal post-stimulation test a numerous methods are used. The best evidence is for glucagon stimulation test (GST-measurement of c-peptide 6 minutes after 1 mg glucagon intravenous injection, given in fasting state), and mix-meal tolerance test (MMTT), where c-peptide is measured 90–120 minutes after standardized liquid meal. C-peptide can also be measured during the 75 g oral glucose tolerance test [6]. A MMTT has been established to be the "gold standard" of stimulation testing to evaluate residual insulin secretion with excellent sensitivity [6] as it produces higher concentrations of C-peptide than glucagon stimulation test. For GST nausea was reported in the majority studies, particularly in the young age-group, making this test preferred by the patients. However, MMTT is preferred for the assessment of β-cell function in therapeutic trials for T1D [7] and in a research due to its increased peak response, but in everyday clinical practice a 120 minutes test seems to be too time consuming. In such a situation a GST, may play an important role owing to its short duration leading to less patient inconvenience with satisfactory sensitivity and reproducibility [6].

Another potentially attractive, non-invasive β-cell function evaluation is urine C-peptide measurement, which also can be performed in an outpatients settings. C-peptide is excreted in urine through glomerular filtration and peritubular capillary uptake with 10–20 times higher concentration than in plasma. However 24 h urinary C-peptide sample collection (24 h UCP) is time consuming and inconvenient for the patient. Correction for creatinine adjusts urine C-peptide concentration for variation in urine concentration and enables the use of single urine sample replacement of 24 h urine collection. Urinary C-peptide to creatinine ratio (UCPCR) correlates well with 24 h UCP [5]. The usability of the UCPCR was also reported in pediatric population, where Besser *et al.* showed high correlation with the 90 minutes stimulated C-peptide [8, 9]. Modern ultrasensitive C-peptide assays allow to detect C-peptide levels as low as 0.0015-0.0025 nmol/l like in the study by Wang *et al*. [10]. Those new assays revealed that C-peptide production persists for decades after disease onset and remains functionally responsive [10, 11].Some new possibilities to monitor β-cell function with more convenient, less costly measures are developed, with some model-estimates C-peptide average. A particular model based on disease duration, BMI, insulin dose, HbA_{1c} and both fasting plasma C-peptide and glucose (CP_{est}) has been proposed to be a convenient and economical alternative to use in everyday practice and as a primary estimation in clinical trials of T1D novel therapies [12]. A similar model for estimated C-peptide including: age, gender, BMI, HbA_{1c} and insulin dose enabled to predict 90 minute stimulated C-peptide measurements at 6 months post-diagnosis in children with diabetes type 1 [13].

C-peptide as an insulin secretion marker

C-peptide physiology makes it suitable for insulin secretion assessment. Its half-life in longer than insulin and it circu-

lates at concentrations five times higher in systemic circulation. C-peptide is used when assessing β-cells function in clinical practice is needed, in preference to insulin concentration. In patients treated already with exogenous insulin, C-peptide is the only option, as exogenous insulin is detected with insulin assays. Peripheral C-peptide levels also more precisely reflects portal insulin secretion than measurement of peripheral insulin, which is extensively metabolised by the liver during first-pass. That makes C-peptide an excellent biomarker of endogenous insulin secretion in patients with T1D already treated with insulin – and has been used this way even since 1973 [14].

Moreover, insulin peripheral clearance is variable. Of important note are observations providing that there is a great impact of individuals' insulin resistance on C-peptide concentration. An obese or overweight insulin-resistant person may have normal or high C-peptide at disease presentation, even with autoimmunity typical for type 1, and still will develop absolute insulin deficiency. The understanding of this problem is of extremely important, especially among teenagers with excess of body weight being recognised with diabetes. The differential diagnosis may be difficult, and treatment for "double diabetes" may be needed. The opposite situation must be regarded in very physically active diabetic children and adolescents. Being very insulin sensitive, their C-peptide level may be underestimated, not meaning the depletion the of β-cells (discussed extensively below in remission, C-peptide and physical activity section) [5].

C-peptide in different types of diabetes

Type 1 diabetes

An important clinical meaning of C-peptide is surely a differentiation between type 1 and type 2 diabetes. Absolute insulin deficiency development is a key feature of T1D where a rapid fall of insulin/C-peptide levels after diagnosis is usually observed. This utility is considered to be even greater in long-lasting diabetes as a substantial overlap of C-peptide levels between type 1 and type 2 at the time of diagnosis may be observed, however after 3–5 years vast majority of type 1 patients will present very low C-peptide secretion, undetectable with routine methods. New clinical researches, considering use of ultra-sensitive C-peptide assays, and new data reporting the presence and clinical meaning of low-levels of C-peptide in long lasting T1D are described below.

What is important, most but not all "insulin dependent" diabetes diagnosed in children are confirmed as type 1 with positive autoantibodies. Anti-β-cells autoantibodies are strongly recommended for differential diagnosis in children, and C-peptide should not determine the type of the disease [15]. Positive result confirms autoimmune origin of diabetes and the need for lifelong insulin therapy. Positive autoantibodies are also used in modern diabetology, especially for research studies, to screen for diabetes in preclinical phase of the disease, when C-peptide levels are still completely within the normal range, yet this issue exceeds the subject of this review. Back to topic, low C-peptide level at recognition suggests insulin-dependency and orientates the recognition as type 1, even in these rare incidents of negative autoimmunity. Although the differentiation diagnosis with monogenic diabetes may be needed in some cases.

During a pre-symptomatic phase of already existing autoimmunity, β-cell loss may first be observed as β-cell secretory capacity reduction manifested as impaired first-phase insulin response to glucose and abnormal glucose tolerance. Such a phenomenon progresses in time until the remaining β-cell secretion can no longer meet the demand for insulin to control glycemia. A functional β-cell mass of approximately 25% of normal is recognized to be required to avoid T1D symptoms, but is already associated with dysregulated glucagon secretion [16].

Typically T1D is mostly diagnosed when the disease symptoms occur, and approximately 80-90% of β-cells have already been destroyed, with detectable but below normal range C-peptide levels. confirming insulin deficiency. Higher C-peptide results should be interpreted with a great caution, particularly in the obese ones, or may simply reflect the earlier stage of β-cells destruction. A clinician should keep in mind that not all the patients with T1D are diagnosed at the same point of the disease course, therefore C-peptide spectrum at the diagnosis may vary, including even patients with its levels within a normal range. The SEARCH for Diabetes in Youth study revealed that up to 40% of newly-diagnosed T1D patients were within the 5th percentile of C-peptide in healthy peers in the National Health and Nutrition Examination Survey (NHANES) study, and 10% were within the 50th percentile [17]. Generally, younger and prepubertal children present lower levels of C-peptide at the time of diagnosis, comparing to the older ones. Most common concept to explain this pattern, is that the final β-cell mass is reached not earlier than at early adolescence [18]. Another factor related to C-peptide levels at disease diagnosis is also the possibility of insulin resistance. Those with higher body mass index usually present with higher C-peptide levels at time of diagnosis [3]. Some of the patients experience preserved insulin secretion of the remaining β-cells in the preclinical phase of the disease (asymptomatic autoimmunization) and in first months after the initiation of diagnosis - so called remission or „honeymoon phase". During the first year post-diagnosis the rate of stimulated c-peptide reduction is about 40% in patients aged 7-45 [19].

Insulin resistance and type 2 diabetes

Although initially type 2 diabetes (T2D) was described as „the adult type", recent years show a progressing rise of obesity, T2D and metabolic syndrome incidence in the pediatric population. In those patients, insulin resistance proceeds to compensatory rise of insulin secretion, and therefore elevated C-peptide levels [20]. Nevertheless, T1D is still known to be more likely diagnosed in children, even with greater body mass, and in those patients the clinical measure of C-peptide is useful to differentiate the type of diabetes, when hyperglycaemia occur. Additionally, in the patients previously diagnosed with T2D and successfully treated with oral antidiabetic drugs, C-peptide can be useful to monitor the function of β-cells within the years, allowing to recognize the need of starting exogenous insulin therapy.

MODY diabetes

Some of the newly-diagnosed diabetes in children and young adults (according to different studies – 1–5%) may be incorrectly diagnosed as T1D or T2D, whereas the hyperglycaemia is caused by monogenic diabetes. Different therapeutic approach makes the differentiation between those diseases even more important. Maturity onset diabetes of the young (MODY) refers to hereditary forms of diabetes due to mutations in an autosomal dominant gene. MODY it is not caused by β-cell destruction, therefore C-peptide secretion is not impaired in those patients. That is why every patient diagnosed with T1D presenting no positive antibodies, low insulin demand in longer observation and without obesity nor insulin resistance symptoms, should be considered as MODY, especially GCK-MODY and those caused by HNF1A and HNF4A mutations. The first mentioned does not require pharmacological treatment, two last ones may be treated with sulphonylourea with improvement in glycaemic control. Patients with mitochondrial diabetes and these with neonatal diabetes develop severe insulin insufficiency with very low, or undetectable C-peptide level, so other diagnostic methods should be used. Besser found, that home post-meal urine C-peptide : creatinine ratio > 0.2 nmol/mmol sustained over 5 years after diabetes diagnosis presents high sensitivity and specificity (97% and 96% respectively) to differentiate HNF1A/4A MODY from type 1 diabetes [8]. There is a need of C-peptide monitoring in the first years after diabetes diagnosis to recognize those patients and to perform specific genetic testing [21, 22]. When the diabetes subtype is not clear to determine, C-peptide measurement may play an important role to establish a proper diagnosis and appropriate treatment. However, it should be remembered that regardless of the classification/aetiology of diabetes, the awareness of an absolute insulin deficiency in a patient (usually defined as C-peptide < 0.2 nmol/l after MMTT or < 0.08 nmol/l fasting) is crucial in clinical management. This values may become adapted as a cut-off value to predict a poor β-cell reserve with a probable requirement of insulin therapy implementation, preferably in form of intensive insulin therapy [5, 6]. Guidelines from Scientific Societies limit the use of C-peptide in the diabetes diagnosis only to unclear cases, or when there is a serious doubt over the diagnosis of T1D or T2D [23, 24]. Thus, C-peptide is still felt to be more important in research than in daily clinical practice. It does however provide an excellent marker of residual β-cell activity and may be of clinical significance as is associated in longitudinal studies with HbA_{1c} [25] .

Remission period in the course of type 1 diabetes – the position of C-peptide

Type 1 diabetes remission can be complete (when the patient does not require insulin therapy at all) or partial (when patient achieves a good metabolic control with low doses of insulin). In the remission phase glucose levels become relatively stable due to transient recovery of islet β-cells and improved insulin sensitivity in the target tissues. That leads to better glycaemic control and lower insulin demand. Complete remission is rather rare, and usually defined as insulin independence with normal glycaemic control (HbA_{1c} < 6%). Partial remission (PR) attracts more attention in the studies as it a more common phenomenon, however its definition also vary due to use of different clinical parameters [26]. According to the ISPAD (International Society of Paediatric and Adolescent Diabetes) Consensus Guidelines, PR is defined as insulin requirement < 0.5 U/kg body weight/24 h with HbA_{1c} level < 7% [15], while some studies propose to use a lower dose of insulin at 0.3 U/kg/day [27]. According to guidelines provided by Diabetes Poland, the PR phase should be defined as an insulin demand below 0.3 U/kg/day together with C-peptide values > 0.5 ng/ml and proper glycaemic control [24]. Recently, another indicator of remission was proposed – insulin daily dose (IDD)-adjusted glycated haemoglobin index [HbA_{1c} (%) + 4 × DDI (U/kg body weight/24 h)] [28]. There are also studies using C-peptide level as an independent factor in a definition of partial remission [11]. Stimulated C-peptide > 300 pmol/l was proposed by Bonfanti 1998, as the only determining factor for the PR diagnosis [27]. Although C-peptide remains a "gold standard" indicator of β-cell function and should be included in PR phase definition, most of already used PR definitions rely solely on HbA1c [26].

The prevalence of PR reported by different researches vary widely between 11% up to 90% [29] with duration estimated for 9 months. Generally, the peak prevalence of PR occurs between 3 and 6 months after diagnosis and insulin therapy administration. Subsequently, its rate declines with disease duration up to 20% at 6 month, and only 10% at 12 months [30]. Different clinical and metabolic factors have been described to influence the PR phase rate and duration. Severe ketoacidosis, younger age at onset (< 5), female sex and a presence of multiple diabetes associated antibodies had a 73% predictive value in patients who did not experience a PR phase [31]. In some reports male patients presented better tendency to develop PR and to maintain it for a longer time [32]. On the other hand, in study by Szypowska, girls had higher C-peptide at diagnosis [33]. However, in most studies the role of gender in the PR phase still remains unclear. Younger children, mainly those with diabetes onset below 5 years of age, are reported to be less likely to enter the PR phase [34]. On the other hand, in some reports younger age at diagnosis was predictive for PR [35]. Remission rates in children with diabetes onset after puberty have been shown to be significantly higher than those in prepuberal age [32, 33]. Patients diagnosed in severe diabetic ketoacidosis show a low prevalence of PR and a study by Chobot *et al.* indicated only pH at onset as an independent PR predictor [36].

Some studies evaluated C-peptide level at the beginning of diabetes and the chance of PR occurred to be unrelated. The reason is unclear, but underestimated initial C-peptide due to glucotoxicity taking place straight after disease recognition might be a possible explanation [26]. Strict glycaemic control with insulin pumps, is postulated to have a positive influence on remission rate, as sensor-augmented pump therapy from diagnosis was connected with less marked fall in fasting C-peptide, especially in older children [37, 38]. Moreover, the co-existing insulin resistance observed in obese patients may

play a role in C-peptide secretion, as those with higher BMI are more likely to present higher C-peptide at the diabetes onset [33, 35]. Children followed and diagnosed in the TEDDY study, compared to community diagnosed controls, had significantly higher C-peptide values at recognition and throughout the first years post-diagnosis, with lower total daily insulin demand and better metabolic control [39].

Similarly, the rate of C-peptide secretion decline after the diagnosis depends on multiple factors. Again, younger age at the diagnosis predisposes to a faster loss of C-peptide secretion afterwards [34, 40, 41]. It has also been suggested that worsening of β-cell function could be augmented by poor glycaemic control, but the evidence is not clear – while some of the studies confirm that theory [42], the other show no correlation between diabetes control and C-peptide [43, 44].

Physical activity, remission and C-peptide in type 1 diabetes

Interestingly, there are several studies trying to reveal possible mechanisms of the effect of exercise on preserved β-cell function measured by C-peptide level [45, 46]. In such a context, β-cell mass might be possibly preserved through two mechanisms: reduced β-cell death and increased β-cell proliferation. There is growing evidence, that exercise influences on both of these mechanisms [46]. Physical activity (PA) induces elevation of circulating GH, IGF-1, GLP-a, IL-6, IL-1, which are believed to have a positive effect on β-cell mass. Mechanisms proposed to explain how exercise may reduce β-cell death include visceral fat (fat derived cytokines source) mass reduction, decrease of circulating concentration of proinflammatory leptin and TNF-α, and increase of anti-inflammatory adiponectin. Such a switch in the cytokine environment may potentially modulate immune processes that are responsible for β-cell destruction in T1D. Exercise modulates also innate immunity. PA leads to a significant elevation in T regulatory cells, decreased immunoglobulin secretion and produces a shift in the Th1/Th2 balance to decrease Th1 cell production. Finally, exercise helps to normalize glucose plasma and serum lipids which chronic elevation is already known to induce β-cell death [47–49].

In healthy children physical activity improves insulin sensitivity and decreases the need of C-peptide over time. Research by Huus *et al*. showed, that high PA was related to lower C-peptide in children aged 8–12 years. Longitudinal follow-up showed that reduced PA increased insulin resistance and β-cell load, and thus might increase the risk for both T1D and T2D [50].

Of importance, assessment of β-cell function using stimulated C-peptide in a situation of stable insulin sensitivity presents a great clinical utility, however it may become underestimated in the context of exercise. Physiological increase of insulin sensitivity occurring during and after PA results in better insulin action, accompanied by specific β-cell response: reduction of fasting and stimulated insulin secretion. Therefore, assessment of β-cell function in the context of exercise is still challenging and requires to consider those compensatory changes of insulin production. New models, such as „disposition index" have been proposed to reflect β-cell function in situations of changed insulin sensitivity [46].

Studies have already presented that PA preserves β-cell function both in healthy humans and at different stages of the natural history of T2D and its positive effect appears to be greater in T2D patients with significant pre-existing β-cell function. Therefore the use of physical exercise as a potential therapy aimed for β-cell preservation seems appealing also in patients newly diagnosed with T1D, who are likely to still present residual insulin secretion [46]. A randomised controlled pilot trial in adults with newly recognised T1D was performed to address the hypothesis that exercise preserves β-cell function. Participants were assigned to two groups: control (usual care) or intervention (exercise consultation every month) for 12 months. In the intervention group better insulin sensitivity and decreased insulin demand was observed. The β-cell function loss rate measured by C-peptide appeared similar between the groups, however the improved insulin sensitivity may have affected this results. There is indeed a great need to find and incorporate more appropriate measures of β-cell function, related to the influence of PA [51].

In our recent studies performed in pediatric T1D patients, we also have observed a great potential of regular PA in preserving β-cell function – results showed that children and adolescents who exercised regularly before diabetes onset were admitted to the hospital in better clinical condition and presented higher fasting and stimulated C-peptide levels than non-active ones. During one year follow up those patients also presented better metabolic control with lower daily insulin demand. In our another study, observation of newly diagnosed T1D pediatric patients revealed that those who were physically active during the course of the disease, presented significantly higher prevalence of partial remission than non-active peers 2 years after diagnosis (44% vs. 13%), with better HbA_{1c} levels and lower insulin demand during the whole observation time. In both studies, the influence of exercise on C-peptide secretion was marked. [52, 53]

PA related health benefits in T1D are indisputable, and it should be advised as a part of routine management in all patients since diabetes onset. Exercise not only promotes fitness but also reduces daily insulin demand, improves lipid profile, endothelial function and overall well-being, finally resulting in reduction of cardiovascular risk and mortality. Although, studies clearly show, that people with T1D do not exercise regularly, and that PA is not sufficiently promoted and supported at the time of diagnosis. Forementioned studies could become a strong argument to implement it much earlier in the therapy. Its attraction lies in the possibility to be applied alone, or as a combination therapy for β-cell preservation in T1D.

C-peptide in long-term diabetes type 1

The Eisenbarth model of T1D postulated immune-mediated linear loss of β-cells. The model assumed that all T1D individuals rapidly and inevitably progress to absolute insulin deficiency [54]. It was believed that most people with long standing

disease, within 1-2 years of diagnosis, would show little or no residual C-peptide production, thus clinicians often considered the presence of residual insulin secretion as rare phenomenon in this population. Although this model remains largely true, there is a growing accumulating evidence about sustained β-cell function in those with long duration T1D [55–57]. Numerous recent studies describe at least detectable C-peptide levels in patients with a long history of the disease [58–61].

In the Diabetes Control and Complications Trial (DCCT) study, in a group of intensive-treated subjects, those who had > 0.2 pmol/ml C-peptide initially or sustained over 12 months presented significantly less complications, and a 79% decrease in the risk of retinopathy [62, 63]. Interestingly, all these benefits have been observed together with a reduction in hypoglycaemic events. DCCT established a fasting C-peptide level > 0.23 ng/ml as „clinically significant" and as an evidence of preserved insulin secretion. Newer reports presented an association between even lower (0.03 pmol/ml) levels of C-peptide and clinical benefits [62]. In the DCCT study diabetes duration was associated with C-peptide value: with 48% patients with duration less than 5 years and only 8% of those with longer duration (5–15 years) having a MMTT stimulated C-peptide at least at a level of 0.2 nmol/l (corresponding with preserved β-cell function) [64]. Data obtained in this study supported the relationship of persistent β-cell function, tight diabetes control and less frequency of both micro- and macroangiopathies [65]. In another, very recent study, for symptomatic T1D, stimulated C-peptide > 0.6 ng/ml has been shown to indicate the presence of clinically important residual β-cell function for contributing to glycaemic control [16].

The Joslin 50-yr Medalist Study demonstrated at least 0.03 pmol/ml random serum C-peptide levels in 67% of patients with T1D duration of 50 years and more [58]. Of these individuals 2.6% presented even higher C-peptide of > 0.2 pmol/ml. To date, Joslin study remains the evidence of the highest significant residual β-cell preservation in patients with a long lasting T1D, suggesting that C-peptide preservation might contributed in the long term survival of those patients. In comparison, the DCCT reported that only 11% of patients screened by MMTT with mean duration of 2.3 yr had a comparable to Medalist Study level of C-peptide [63].

A number of studies so far demonstrated low, but still detectable C-peptide secretion a long time after the diagnosis. The majority of long-duration T1D patients have detectable C-peptide, especially, when ultrasensitive methods are used. The majority of them are insulin "microsecretors" and some maintain clinically relevant endogenous secretion for many years after diagnosis. Low level C-peptide was functionally responsive, as revealed in 80% of patients with diabetes duration of 30 years responding to a mixed meal by a rise in C-peptide secretion [10, 60]. In study by Davis et al, one third of patients 3 to over 50 years from diagnosis had detectable C-peptide, with percentage varying according to disease duration or age. At all durations of disease, diagnosis during adulthood was associated with greater frequency and higher values of C-peptide [61]. These findings (with C-peptide measured by ultrasensitive methods) suggest that interventions to preserve insulin and C-peptide secretion might lead to positive results and prevention of chronic complications also in patients with long lasting disease, who were thought to already lose their β-cell function [10].

Recent studies confirmed that the rate of C-peptide secretion fall over time is significantly related to the age of disease onset, with younger age predisposing to far more rapid C-peptide decline. Those diagnosed over 15–18 years of age are found with higher and longer persisting C-peptide [61, 66]. Lower levels of C-peptide have been associated with poorer glycaemic control and increased HbA1c values [62]. And in opposite, in DCCT study, "intensive treatment group" had higher and longer sustained C-peptide levels. Some new data support two clear phases of C-peptide secretion fall: an initial expotential fall over the first 7-year period, followed by a prolonged stabilization, where C-peptide levels no longer decline [67]. Genotypic risk score for T1D was found to be inversely associated with detectable C-peptide secretion - C-peptide persistence occurs to be influenced by variants in the HLA region, that are different from those determining early-onset T1D risk [66].

A number of studies concentrated particularly on C-peptide levels in long-lasting T1D pediatric populations. Among youths within 1st year of diagnosis, four of five with autoantibody positive diabetes had still clinically significant amount of residual β-cell function, and about one-third had fasting C-peptide levels above the 5th percentile of a healthy adolescent population. Then, even 5 years after diagnosis 10% of them had fasting C-peptide above clinically significant threshold [17]. In our own observation 34% of young patients < 18 years of age with mean disease duration of 5 years presented prolonged, detectable C-peptide secretion at clinically relevant level. Those patients appeared to come from the group with longer clinical remission, and still had lower insulin daily demand., therefore residual β-cell function seemed to be beneficial for metabolic control [68]. Our results stay in agreement with the newest publication by Gronberg et al., where authors report detectable C-peptide in 38% of patients below 25 years of age, with at least 10 years of disease. In their observation patients with preserved residual β-cell function had better HbA1c during the first 3 years after diagnosis [69].

C-peptide and novel and future therapies

Nowadays, the aim of the therapy of T1D became not only maintaining normoglycaemia with newer and better insulin supply (new insulin pumps, continuous glucose monitoring systems, hybrid closed-loop technologies), but also to inhibit the autoimmune process of β-cell destruction. So far, no therapy has been approved to be effective enough as a T1D prevention both in the pre-symptomatic stage and after the disease clinical diagnosis. However, the most recent reports of the possible underlying mechanisms enabled to develop new therapeutic concepts aiming to inhibit or even reverse the autoimmune islet cell destruction at its early phase. There are intervention trials ongoing all around the world, that have a potential to preserve β-cell function. They are well worth striving for because of a possibility to lead to better metabolic control and less complications [70].

Many of the trials attempting to preserve β-cell function conducted over past 30 years tried to use agents acting as "suppression" of autoimmune inflammatory processes. Unfortunately some side effects of immunosuppression are described like: neoplasms and re-activation of previous infections. To be remembered, these therapies are also very expensive, difficult to organize, and require strictly chosen patients.

Among the possible therapeutic approaches, we can find cellular therapies with patient's own multiplicated regulatory T-lymphocytes (Tregs) [71]. Such a therapy is a promising opportunity to prolong remission phase in newly-diagnosed T1D children, as already reported results of insulin secretion maintenance for longer time and higher c-peptide levels have been observed in treated versus control group [71, 72]. The follow-up for one year confirmed the positive effect of the therapy, with maintained increase in C-peptide in the intervention group [73].

Several published clinical trials implemented umbilical cord stem cells [74], immunomodulatory therapies with biological drugs (abatacept, teplizumab, rituximab) or granulocyte-colony-stimulating factor (G-CSF) and combined therapies [75, 76]. Immune ablation and autologous hematopoietic stem cell transplantation (AHSCT) is another possible therapeutic strategy to inhibit the immune process in T1D, however this therapy presents significant adverse event risk [77, 78]. Stem cells have unique immunomodulatory capacities and have been considered as a promising interventional strategy for T1D [79]. Recently published systematic review and meta-analysis showed that stem cell therapy could significantly increase fasting C-peptide levels and reduce both HbA_{1c} levels and insulin doses [79].

Unfortunately, only few of the new therapeutic programmes are allowed to be performed in pediatric patients: the ones who present less advanced pancreatic cell destruction and who therefore could benefit better outcome from the novel therapies. Nevertheless, the β-cell function assessed by C-peptide monitoring seems to be the most reliable method to assess the effectiveness of those therapies. Moreover, C-peptide level can help recognize the patients with partially preserved insulin secretion and thus with potentially better response to the therapy.

The clinical and therapeutic potential of C-peptide replacement

So far, the major possibility to prevent diabetic complications is to achieve and maintain strict glycaemic control. However, even when therapeutic glycaemic goals are achieved, the risk for complications is not ceased completely. New therapeutic options are looming, and one of such alluring possibilities may be C-peptide administration [80]. As mentioned before, DCCT data showed the relationship of diabetes control, β-cell function and frequency of both micro- and macroangiopathies. Studies performed in last decades suggest, that C-peptide level may be connected with diabetes complications not only as an insulin secretion marker, but also as an independent factor, with its own physiological effect on the endothelial function and intracellular signalling [81–83]. The effects of C-peptide on inhibition of endothelial ROS formation [84], NF-κB [85] and increased expression of eNOS and Na+K+-ATPase activities [86], as well as on activating antiapoptotic mechanisms [87] results in protection against destructive effect of hyperglycaemia. Type 1 diabetes patients who present remaining β-cell activity are described in several studies as less liable to develop microvascular complications when compared to totally C-peptide deficient individuals [88]. Thus, C-peptide may play an important role on its own [89] and research on its administration to T1D patients who lack this molecule, were undertaken. The results revealed significant improvement in several diabetes vascular abnormalities. Recent clinical and experimental evidence demonstrates that C-peptide replacement exerts beneficial effects on diabetic neuropathy, nephropathy, atherosclerosis and cardiovascular complications [90–92].

C-peptide was proved to bind to several human cells, including endothelial, skin, renal tubular cells and fibroblasts – with full binding saturation occurring at concentration of 0.9 nmol/l. This remains consistent with no observed response to exogenous C-peptide in healthy people, who present physiological levels of their own C-peptide with all its binding sites already fully occupied. The cell membrane structure to which C-peptide seems to interact with is postulated to depend on G-protein coupled receptor [91, 93]. Unfortunately, attempts to identify a specific C-peptide receptor have not yielded reliable results. Some studies reported internalization of C-peptide after binding to the cell membrane (in human endothelial cells, or umbilical artery smooth muscle cells). C-peptide can exert transcriptional effects and research findings demonstrate that the peptide may exert growth factor activity [91, 93]. Activation of specific intracellular pathways occur in a variety of cell types in response to C-peptide exposure. It may result in Ca^{2+} intracellular concentration elevation and increased phosphorylation of phospholipase C (PLC), protein 3-kinase C (PKC) isoforms and phophatidylinositol 3-kinase. Important cellular end-effects mediated by C-peptide are connected with its regulatory influence on Na^{+},K^{+}-ATPase activity mediated via PLC, PKC isoforms and MAPK signalling. Another C-peptide cellular end-effect is its activating influence on endothelial nitric oxide synthase (eNOS) in endothelial cells resulting in vascular smooth muscle relaxation and increased blood flow [86]. Additionally C-peptide was reported to increase transcription factors involved in cell growth, migration inflammatory responses and even apoptosis. And last but not least, C-peptide may interfere with the insulin signalling pathway at the level of insulin receptor leading to GLUT mobilization [91–93].

Patients with T1D show increased levels of different inflammatory markers, already from the early age, also in childhood. Inflammation is recognised as an important risk factor contributing to vascular damage. In this concept C-peptide has been found to reduce endothelial cell surface expression of adhesion molecules, to attenuate leucocyte-endothelium interaction and also to reduce glucose-induced secretion of chemokines and interleukins via endothelial cells and monocytes. C-peptide in physiological concentration exerts a protective effect against migration of vascular smooth muscle cells. Some reports demonstrate, that C-peptide is even able to suppress both glucose-

induced and TNF-α mediated activation of NF-kB via reduced generation of reactive oxygen species [94]. Summarising, C-peptide antagonizes pro-inflammatory responses in endothelial cells and leucocytes exposed to various stress and inflammation-related factors [95].

Microvascular and macrovascular complications in diabetes are with no doubts connected with endothelial dysfunction. C-peptide influences both release of NO from vascular endothelium and altered blood reology in T1D. Administration of C-peptide in replacement dose in experimental models and patients resulted in prompt increase in blood flow and about 30% increase in left ventricular myocardial blood flow [91, 93]. Several studies showed that C-peptide may cause beneficial effect of diabetes related renal dysfunction. Replacement of C-peptide in diabetic patients decreased glomerular hyperfiltration and urinary albumin excretion compared with placebo-treated ones. These results support the meaning of C-peptide in diminishing the diabetes-induced kidney damage and were recently confirmed on animal model [80, 96-98]. Experimental studies performed on diabetic animal models show that C-peptide in replacement doses has the ability to improve peripheral nerve function and prevent or reverse the development of nerve structural changes [99, 100]. This effect has been confirmed also in some clinical studies [99]. First to mention, 3-month C-peptide replacement in early stage neuropathy patients resulted in nerve conduction improvement in double-blind placebo-controlled study. Also, short or 3-month C-peptide administration improved heart rate variability in patients with recognized cardiac autonomic innervation. Finally, C-peptide administration study was performed for 12 months in clinical trial to evaluate its influence on peripheral neuropathy: long-acting C-peptide administered once-weekly resulted in marked improvement of vibration perception threshold in large, 250 patients group of T1D [101].

It might be considered that T1D is a disease of bi-hormonal deficiency. C-peptide administration together with regular insulin therapy, may be beneficial in the prevention and treatment of microvascular complications [102]. Considering pediatric patients diagnosed with T1D who are going to live with the disease for a life-long time, maintaining preserved C-peptide secretion or developing the new therapeutic possibilities with C-peptide replacement additionally to insulin from the very beginning, seems to be crucial to achieve a better life quality in further life.

Conclusions

This review confirms that C-peptide, initially believed to be just an useless by-product of insulin production, has been in fact widely used in diabetology. As an excellent biomarker of insulin secretion, it allows to distinguish between insulin sufficient and insulin deficient individuals with diabetes. It is used in the diagnosis of diabetes and in differentiating the type of the disease in uncertain clinical situations. Its concentration is not affected by exogenous insulin treatment, so we can use C-peptide to assess the preserved insulin secretion in individuals with T1D and better define the partial remission stage. C-peptide is also useful in the monitoring of the effect of novel and experimental therapies aiming to inhibit or even reverse the autoimmune β-cell destruction. Finally, C-peptide itself shows its own physiological effect on endothelial function and there is a great need to preserve or replace its secretion in diabetic patients to protect them from long-term complications of the disease. As a promising particle in diabetology, C-peptide deserves in-depth research in many clinical trials involving the whole spectrum of diabetic research projects.

However, C-peptide still seems to be a more important tool in scientifical research rather than in everyday clinical practice. Given the raising evidence of multiple meanings of this molecule, we suspect that C-peptide in the future will play more remarkable role in guidelines relating to diabetes diagnosis, prognosis, management and morbidity prediction.

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