

C-peptide in diabetes diagnosis and therapy

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1. ABSTRACT

C-peptide is known for several decades. It is released in equimolar amounts together with insulin from the pancreatic beta cells. Still there has been quite remarkable lack of interest in C-peptide. C-peptide is rarely used to classify type of diabetes although it seems self-evident that it is important to estimate the function of those cells which do not function good enough and therefore causes a syndrome which requires life-long treatment and leads to serious complications. Not until recent years C-peptide is accepted as a relevant outcome in trials aiming at preservation of beta cell function, although it is known for decades that some C-peptide is associated with less complications in type 1 diabetes (T1D). Preservation of beta cell function is important to make diabetes milder, and when beta cell function can be preserved before clinical manifestation of T1D, we are on our way to prevent that disease. Residual C-peptide/insulin secretion can be of value in classification of diabetes in different types. C-peptide may give valuable clinical information on why patients are more or less stable/labile in their blood glucose and more or less easy to treat. It explains why patients with T1D have different tendency to develop severe acute complications, both severe hypoglycaemia and diabetic keto-acidosis (DKA). Longstanding C-peptide may decrease risk of developing severe late complications. Finally, although still under debate, C-peptide seems to have several effects on different organs suggesting that it is an important hormone, interesting per se, and not only as a reflection of insulin secretion.

2. INTRODUCTION

Pancreatic beta cells produce insulin. We know that this peptide is a hormone which is necessary for survival. However for each molecule of insulin another peptide is also released, the so called connecting peptide (C-peptide), a 31 amino acid segment that links the A and B-chain of the insulin molecule and together make up the proinsulin molecule (Figure 1) (1). C-peptide has a role to promote the correct structure and cleavage of proinsulin. Some proinsulin is secreted when the beta cells are stimulated (2), not least during meals, but most proinsulin is cleaved into insulin and C-peptide, which is secreted in equimolar amounts. While insulin has a short half-life of a few minutes, and most goes to and remains in the liver, C-peptide passes liver, has a half life of half an hour, and is finally catabolised by the kidneys, and some is secreted in the urine.

3. C-PEPTIDE A MARKER FOR ENDOGENOUS INSULIN SECRETION

While patients with the most common form of diabetes, type 2 diabetes (T2D), still produce insulin, patients with T1D have usually lost all or most of their capacity to secrete insulin. Thus, patients with T1D need to receive exogenous insulin and it would therefore be difficult to estimate own insulin secretion by measuring insulin in serum. This would be even more difficult as insulin is transported to the liver and most of it never passes this organ. However, as C-peptide is secreted in equimolar

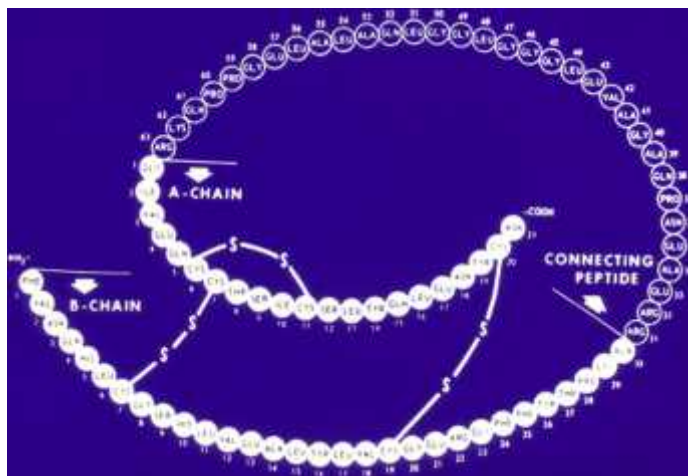


Figure 1. The pro-insulin molecule consists of insulin (an A-chain and a B-chain kept together by sulfid bridges) and a connecting peptide (C-peptide). The beta cells usually secrete only a small fraction of pro-insulin, but most is split into equimolar amounts of insulin and C-peptide.

amounts, C-peptide will reflect insulin secretion. Furthermore, as mentioned C-peptide passes the liver, has a longer half-life and is secreted into the urine. In 1974, Lise Heding published a RIA-method for determination of C-peptide (3), which increased our knowledge of beta cell function in insulin-treated diabetes. It was shown that even children with classical T1D have some residual insulin secretion (4), the more the older and the longer the patients were at onset of the disease. A proportion of proinsulin was not split but, especially in connection to meals, proinsulin was also released (2). The decline of beta cell function seems to have a certain course, more rapid in younger children, and this picture has not changed in recent decades (5, 6).

Fasting serum C-peptide has been a common way of trying to standardize the evaluation of beta cell function, but there has been increasing interest not only in this basal capacity but also in how the beta cells respond to a stimulus. However, the opinions have differed how this beta cell response should be estimated. Many researchers used stimulation with e.g. glucagon or arginin, which was regarded as most reliable. However, meal tests e.g. the standardized breakfast load, was used at our clinic (5), with the argument that the response to a natural load, a meal, would be more clinically relevant. Later we got to know much more about the different hormones involved in beta cell stimulation and understand the mechanisms why a meal test is more physiological. Recent studies have also proven that a meal test is most reliable and give the best information (7).

However, a Mixed Meal Tolerance Test (MMTT) is invasive which may be a problem for children, it is laborious and it requires some hours at hospital, a problem for adults but also for school children and children who need to be accompanied by their parents. Long ago urinary C-peptide has been used (8), but not until recently we have tried to validate urinary C-peptide more carefully. These studies show that a urine sample produced over a meal is

highly correlated to the area under the curve (AUC) of a MMTT, which in turn is very well correlated to the maximal peak C-peptide response after stimulation. Thus determination of C-peptide in urine collected over a meal may give quite valuable information (9) and may sometimes probably be enough to get the information needed for instance if we are dealing with a patient with complete lack of insulin, a classic T1D, or a patient with e.g. Maturity Onset Diabetes in the Young (MODY) or T2D with more residual insulin secretion. Furthermore it will give valuable information in the decision if efforts should be made to preserve residual insulin secretion. Table 1 summarizes arguments for the relevance to determine C-peptide, which will be further discussed below.

4. C-PEPTIDE AS A DIAGNOSTIC TOOL

Residual insulin secretion has been proposed as a mean of classifying diabetes. The heterogeneity of diabetes at clinical onset along with the increasing incidence in children and adolescents (10) makes it of interest to elucidate if C-peptide levels in newly-diagnosed children to test if C-peptide may improve the classification. The clinical diagnosis of diabetes in routine pediatric practice is based on symptoms and signs of the patient, history e.g. family history, and blood glucose level. In the great majority of T1D, an autoimmune process is thought to explain the loss of the pancreatic islet beta cells. However, auto-antibodies are not always detectable (11). DKA is a feature of T1D but the frequency varies among populations. In Sweden pediatric patients diagnosed with diabetes had less than 17% DKA at diagnosis (12). T2D is a heterogeneous disease with a more protracted course than T1D, very slowly leading to insufficient secretion of insulin. Most patients are obese, with signs of insulin resistance. Usually these patients are supposed to have no autoimmune destruction of the beta cells (13). Still, some patients classified as having T2D have auto-antibodies, especially against GAD65 (14, 15), and they may also show signs of cell-mediated immunity (16). There is a genetic predisposition in T1D, but all patients not

Table 1. Arguments for the relevance and value of C-peptide determination

C-peptide is secreted in equimolar amounts of insulin, and can therefore be used for estimation of residual insulin secretion in patients receiving exogenous insulin
Residual C-peptide/insulin secretion can be of great value in classification of diabetes in different types, with very low secretion in classic T1D, and often high values in T2D and in MODY. C-peptide <0.2 nmol/l in a random sample at diagnosis is a strong support for the diagnosis of T1D
C-peptide may give valuable clinical information on why patients are more or less stable/labile in their blood glucose and more or less easy to treat
Degree of C-peptide explains why patients with T1D have different tendency to develop severe acute complications, both severe hypoglycaemia and DKA
Longstanding C-peptide may decrease risk of developing severe late complications
C-peptide is used as one of the main criteria for inclusion in trials aiming at preserving beta cell function
C-peptide is used to estimate and follow the result of islet transplantation
C-peptide seems to counteract the hexamer form of insulin, thereby increasing the availability of biologically active insulin
C-peptide seems to have several effects on different organs suggesting that it is an important hormone, thus interesting <i>per se</i> , and not only as a reflection of insulin secretion

even in a high incidence countries have risk-HLA (17, 18), and, although T2D has an even stronger genetic predisposition, there is no clear genetic marker. Thus even though determination of HLA and auto-antibodies may improve the classification of diabetes, these markers are not always specific for a certain type of diabetes, not even in young people. Furthermore, these determinations may not even be available in many parts of the world.

Lack of residual insulin is the classical sign of T1D, while patients with T2D often have a quite good insulin secretion, although with relative lack of insulin in relation to their increased demand. Thus, determination of C-peptide should be the natural laboratory complement to clinical signs and symptoms, when diabetes should be classified.

In a nationwide study in Sweden (BDD; Better Diagnosis of Diabetes) of all newly-diagnosed diabetic children C-peptide was measure in the first blood sample taken at diagnosis before first insulin injection (19). In this large patient population (ca 3000 patients) the clinical classification of diabetes, before any information on HLA, islet autoantibodies or C-peptide was known, was T1D in 93%, T2D in 1.9%, MODY in 0.8% secondary diabetes (0.6 %), while 3.3 % could not be classified. Thus, the great majority of the patients assigned a clinical diagnosis of T1D had classic symptoms with polyuria, thirst, weight loss, accompanied by high blood glucose values. Patients with high BMI and no or mild clinical symptoms, often with T2D in the family, were classified as T2D. MODY also less often had clinical symptoms and more often a strongly positive family history (i.e. 3 generations) of diabetes but without other autoimmune diseases among relatives.

However, the diagnosis was still not always evident. A few patients were initially believed to have diabetes, although it was later shown that they actually had no diabetes. The future will show if these patients in fact had diabetes but went spontaneously into complete remission, and if so whether they will relapse sometime in the future. Some patients were classified as T2D or MODY although they had classic symptoms of T1D, high blood glucose, and even keto-acidosis. Thus in the individual patient the diagnosis is difficult which explains that a few per cent of patients were not classified at all.

The most typical characteristic of T1D should be lack of insulin, irrespective of aetiology, genes, autoimmune signs or not, while T2D usually is characterized by insulin resistance with a quite good, sometimes even increased

insulin secretion. The MODY forms may differ regarding beta cell function, but many patients with MODY have a rather low requirement of exogenous insulin (20). Remarkably enough, although diabetes is a disease of the insulin producing beta cells there is no tradition to measure C-peptide neither as a basis for diagnosis, nor as an estimation of the clinical course.

It is known for very long time that children with higher age at onset of diabetes tend to have higher C-peptide values (4) and there is reason to believe that when manifestation of the disease is precipitated by an increased insulin demand e.g. because of obesity or puberty, the C-peptide levels are higher. Even though such factors may obscure the interpretation of C-peptide measurements the BDD study showed interesting results regarding C-peptide measured at the very diagnosis, when the patients have their beta cells stimulated by high glucose concentrations (19): In a random, non-fasting serum sample at diagnosis, 56% of the patients had a C-peptide value <0.2 nmol/l (Figure 2). Children classified as T2D had the highest mean C-peptide (1.83 ± 1.23 nmol/l) followed by MODY (1.04 ± 0.71 nmol/l) and T1D (0.28 ± 0.25 nmol/l). Only 1/1037 children who at diagnosis had C-peptide <0.2 nmol/l was classified with a type of diabetes other than T1D. Predictive value of C-peptide >1.0 nmol/l. for the classification of either T2D or MODY was 0.46 (CI 0.37-0.58).

1) Most children with newly-diagnosed diabetes had measurable C-peptide and about half of them good enough to be of clinical significance (not fasting > 0.2nmol/L). 2) Among patients with low C-peptide (<0.2 nmol/L stimulated value) there was a <2/1000 chance that the patient has another type of diabetes than T1D.

Thus serum C-peptide <0.2 nmol/L in a random sample at diagnosis is a strong support for the diagnosis of T1D. At the other end of the C-peptide spectrum, a value 1.0 nmol/L suggests that there is a chance of at least 1 in 2 that the patient does not have T1D but another type, most often T2D.

Most of those 3.3% of the patients not initially classified could be correctly classified, many of them thanks to C-peptide determination, where those with very high C-peptide (>1.0 nmol/l) often had T2D, some MODY, while those with very low C-peptide could be classified as T1D.

In summary, even in Sweden, a country where the incidence of T1D among children is second only to Finland

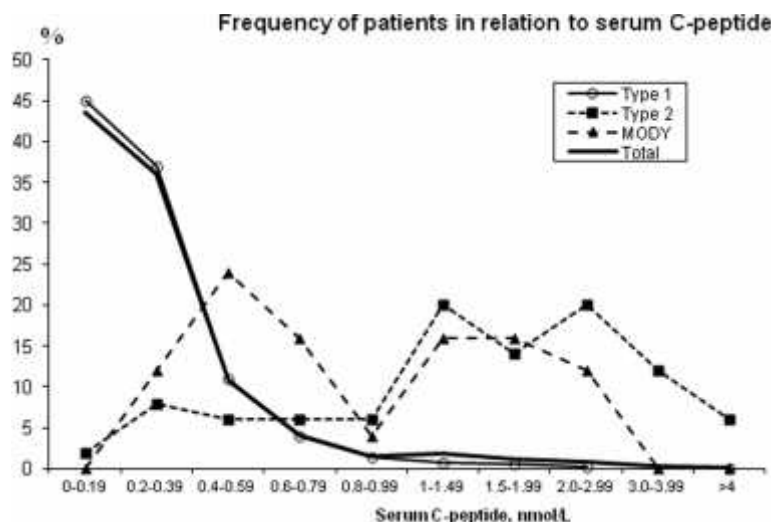


Figure 2. When patients in a random serum sample have C-peptide <0.2 nmol/l the chance is minimal (1/1000) that the diagnosis is something else than T1D.

and the care centralized to hospitals with paediatric clinics, a significant number of newly diagnosed diabetic children could not easily be clinically classified. This group of patients will probably increase with increasing prevalence of obesity. Patients with any type of diabetes may have similar symptoms and signs, and neither HLA typing nor determination of auto-antibodies may always allow a correct classification. A random C-peptide taken at diagnosis of diabetes helps to classify diabetes, and as diabetes is a disease of the beta cells, a regular evaluation of beta-cell function is self-evident. A random C-peptide will also be informative when planning for possible future prevention clinical trials, which are aiming to preserve beta-cell function.

5. CLINICAL VALUE EVEN OF A LIMITED BETA CELL FUNCTION

During weeks or months after diagnosis patients with T1D tend to get decreasing insulin requirement. Some may become very stable in their blood glucose and need less than 0.5 U/kg, 24 hrs, which has been regarded as partial remission (21, 22). Even though the insulin demand increases parallel to decreasing residual insulin secretion, some patients may keep some residual insulin secretion for many years (5, 6). It is a well-known clinical experience that patients with residual insulin secretion have a more stable blood glucose, the disease is easier to treat. It is shown that a slight insulin secretion tends to protect against ketoacidosis (23-25) and it also has been found to diminish the risk of serious hypoglycaemia (26). These effects mean a clear improvement of quality of life even though this is difficult to measure scientifically. Furthermore, the DCCT-trial showed that even a quite small residual insulin secretion (peak C-peptide after a meal stimulation > 0.20 nmol/l) significantly decreased not only serious hypoglycaemia but also development of late vascular complications (27). A slight residual beta cell function seems to be of clinical value (28).

Islet transplantation with restoration of some beta cell function has led to improvement of vascular complications (29, 30), and very low residual beta cell function (fasting serum C-peptide > 0.6 nmol/l) seems to prevent late vascular complications (31), which may explain why those patients who had survived for more than 50 years of insulin dependent diabetes often had residual insulin secretion (32).

6. EFFORTS TO PRESERVE BETA CELL FUNCTION

The increasing evidence that residual beta cell function is important, together with increasing support for the concept that T1D is an autoimmune disease, made immune interventions at the onset of diabetes relevant and justified.

Thus, when autoantibodies against islet of Langerhans were discovered (33, 34) it was a natural step to try to remove these autoantibodies. This led to a trial with plasmapheresis at diagnosis (35), the world's first immune intervention in T1D. Already then C-peptide was used as an outcome.

Treatment with cyclosporin (36, 37) had such a clear effect on preservation of beta cell function that it has been regarded as a proof of concept: immune intervention may work to preserve beta cell function. However, the adverse events made it difficult to justify this treatment in clinical use, and several other interventions showed no or limited effect but some of them risks and adverse events (38-43). This led to that the interest for preservation of residual insulin secretion in already diabetic patients rather tended to decrease for some years when the hope for transplantation and/or closed loop systems (artificial pancreas) looked more promising than interventions at onset of the disease.

But when neither transplantation nor artificial pancreas solved the problems, the interest for preservation of residual beta cell function has regained interest. In recent years there has been some encouraging results in Phase II trials both with use of monoclonal antibodies (mAbs), especially anti-CD3 antibodies (44-47), anti-CD20 (48), other forms of immune intervention although sometimes with adverse events and serious risks (49-54) and not least with auto-antigen therapy with GAD (55, 56) and Diapep277 (57), easy to administrate and with no adverse events.

The drug authorities have required clinically relevant outcomes of such studies, and asked for reduction of insulin dose and/or improved metabolic control (HbA1c). Beta cell function *per se* was not regarded as important enough. However unless reduced to very low requirement with 1-2 daily injections or no requirement at all, reduction of insulin dose is not extremely interesting for the patients, and gradually also some authorities have understood that residual insulin secretion, C-peptide, is enough clinically relevant. C-peptide has become the accepted endpoint of immune intervention trials (58).

Several treatment modalities efficacious in Phase II, have unfortunately not been confirmed in Phase III trials (59). Thus, anti-CD3 mAb failed to reach primary endpoint in two recent Phase III trials. Thus the Defend using a low dose regimen completely failed (60) to show efficacy in spite of enough dose to provoke adverse events. Another Phase III trial, Protegé showed that the arm with the highest dose during the longest time (14 days x 2) seemed to have some positive effects on preserving residual insulin secretion and reducing insulin requirement (61). GAD-vaccine showed no effect in a Phase II trial in US testing three injections and also failed to meet primary endpoint after 15 months in a European Phase III trial, even though the effect was rather good with statistically significant efficacy in certain pre-specified subgroups (62). As this treatment so far has shown no treatment related adverse event and is extremely easy to perform and tolerable for the patients, future studies of auto-antigen therapy is needed (63). Just during revision of this paper preliminary data on the Phase III Diapep 277 trial were presented at the IDF congress (64). Out of 457 included patients (302 males and 155 females, 16-45 years old, with a mean age of 27 years, who had fasting C-peptide >0.22 nmol/l at inclusion, 169 in resp active and placebo arm had been evaluated after 24 months. Patients in the actively treated arm, who had received Diapep 277 every 3 months up to 21 months, had a 23 % better preservation of C-peptide (AUC) then in the placebo arm ($p=0.037$). These results are very encouraging, although the effect may be restricted to mild cases of T1D in adults.

7. C-PEPTIDE *per se* AS HORMONE THERAPY

As no function of C-peptide could be found in the early studies, it became generally accepted that the role of C-peptide is just to keep the proinsulin molecule in its correct shape, and the interest of C-peptide was limited to becoming a marker for residual insulin secretion. As even a

slight residual insulin secretion may decrease the risk of both acute and late complications. These effects may be explained by residual insulin secretion, reflected by the C-peptide, but studies in recent years have shown increasing evidence that C-peptide is a hormone in itself (65).

C-peptide seems to cause release of Nitric Oxide (NO) in vitro (66-68), and increased blood flow in vivo both in skin (69), kidney (70), peripheral nerve (71), and myocardium (72). Furthermore, C-peptide seems to improve the defect erythrocyte deformability seen in T1D (73). C-peptide also may influence endothelial function of importance for the immune function. Thus, there are experimental studies suggesting that C-peptide influences inflammatory agents (67), leukocyte adhesion to endothelial cells (74) and leucocyte infiltration in damaged tissue (75). These and other experimental findings suggesting a special effect on nerve function (71, 76, 77) have lead to clinical trial with C-peptide therapy. Thus C-peptide was given in physiological concentrations in a randomized double-blind placebo controlled trial to T1D patients with long duration of diabetes (78). Sensory nerve conduction velocity was improved and vibration perception threshold decreased. Similar results have been confirmed in other randomized double-blind placebo controlled studies (79). This effect seemed to exist irrespective of glucose control. Also autonomic nerve function seems to be ameliorated by C-peptide therapy (80). The mechanisms for this effect is unknown, but the abovementioned effect on blood-flow may cause an improvement of blood flow also to nerves.

C-peptide also seems to influence the kidney function in patients with T1D. Thus C-peptide infusion in physiological concentrations have been shown to decrease glomerular filtration rate (70), and in T1D patients with sign of nephropathy C-peptide treatment lead to not only decreased glomerular filtration rate compared to placebo-treated patients, but also reduction of albumin excretion (81, 82). These clinical studies have been supported by studies in experimental animals where both functional and morphological improvements have been observed (83). C-peptide has been found to elicit a constriction of the afferent glomerular arteriole and a relaxation of the efferent arteriole (84, 85) as well as inhibition of tubular reabsorption of sodium (84).

8. MECHANISMS FOR C-PEPTIDE ACTION

Although studies suggest that C-peptide has its own effects in physiological concentrations, both in animals and in humans, the mechanism is not clear. No receptor on the cell membrane has been identified, although there are suggestions on how signalling occurs (86). Some studies indicate that C-peptide has an effect in nucleoli where it promotes transcription of genes encoding RNA (87). Renal cells show elevation of intracellular calcium when exposed to C-peptide in physiological concentrations (88, 89), and it also elicits phosphorylation (90). Insulin like effects have been found in that C-peptide stimulates glucose utilization in myoblasts (91) and in skeletal muscle (92). Cross-talks between insulin and C-peptide ligand

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receptors cannot be excluded. Another interesting effect of C-peptide seems to be that C-peptide counteracts the hexamer form of insulin, increasing the availability of biologically active insulin (93).

9. CONCLUSION

C-peptide is part of function of the pancreatic beta cells, and therefore of self-evident interest in T1D, when beta cell function disappears. It may also be of interest in T2D as this disease also requires decreasing beta cell function to become manifest. Still there has been quite remarkable lack of interest in C-peptide. C-peptide is rarely used to classify type of diabetes although it seems self-evident that it is important and worthwhile to classify as well as follow for estimation of beta cell function in a syndrome where lack of function of these cells requires life-long heavy expensive treatment and leads to serious complications. It should be as natural to estimate the C-peptide and insulin secretion, as it is to estimate hormones in other hormonal diseases.

Not until in recent years C-peptide is accepted as a relevant outcome in trials aiming at preservation of beta cell function, although it is known for decades that some C-peptide is associated with less complications in T1D. Preservation of beta cell function is important to make diabetes milder, and when beta cell function can be preserved before clinical manifestation of T1D, we are on our way to prevent that disease.

A big question remains to be answered whether C-peptide is just a connecting peptide, a bi-product in the release of insulin, or whether C-peptide is a hormone. Many experimental studies suggest that C-peptide does have effects in physiological concentrations, and there are also some clinical studies which support this idea. However, still these studies are short-term and rather small. More studies are needed, and ought to be done, to confirm or reject the hypothesis. If C-peptide is a hormone, it should reasonably be replaced in patients with insufficient beta cell function.

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