

BIOLOGICAL EFFECTS OF C-PEPTIDE AND PROINSULIN: WE JIBE TOGETHER

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ABSTRACT

Discovery of Insulin marked a paradigm shift in the management of diabetes patients. Over the years as research in different types of insulin advanced the management of diabetes improved. However there were still many unanswered question pertaining to the use of insulin and its precursors. One such intriguing compound is C-peptide. Recently the role of C-peptide has opened many avenues in the treatment of diabetes. Large number of studies has demonstrated that lack of C-peptide may exacerbate diabetes. In a large group of diabetes the replacement with C-peptide, lead to marked amelioration of diabetes complications. Research has shown marked improvement in endoneural blood flow with C-peptide replacement. C-peptide previously was considered as an inert molecule, however with the discovery of large number of receptors of C-peptide the action of the hormones has turned out to be enigmatic. Further research is needed to ensure some of the question particularly the therapeutic implication of C-peptide is prevention of diabetes related complications.

Keywords: Proinsulin, C-peptide, Type-1 Diabetes, Diabetes neuropathy, Diabetes Nephropathy

INTRODUCTION

The German anatomy student Paul Langerhans first described in 1869 the "islands of clear cells" distributed throughout the pancreas (1) but he did not realize the physiologic significance of these cell clusters, which are today known as islets of Langerhans. Islets are the endocrine compartment of the pancreas, comprising around 2 – 3% of the total pancreatic volume.

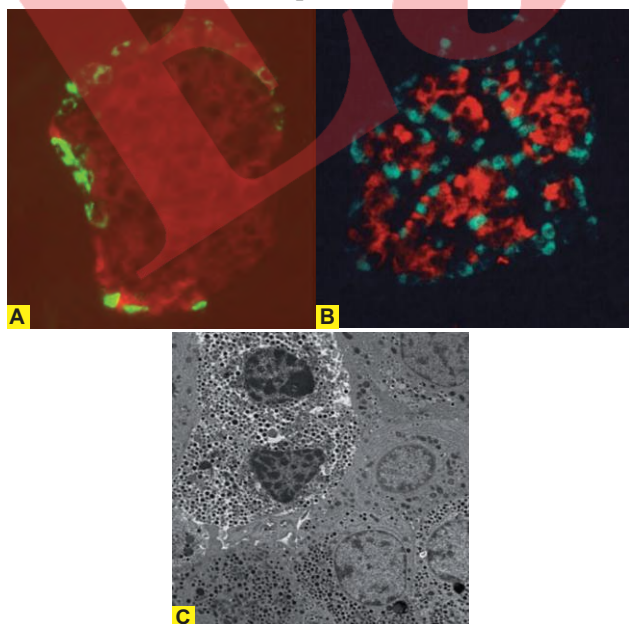


Fig 1: Anatomy of The Islet of Langerhans

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(A) This figure details a section of mouse pancreas and green, red immunofluorescence indicates glucagon and insulin secreting cells, Image courtesy of V. Foot, King's College London.

(B) Human islet. This Figure shows a section of human pancreas and in contrast to mouse islet cells they are less organized. In human islets β - cells comprise approximately 50 – 60% of the endocrine cell mass. Scale bar is 10 μ m. Image courtesy of V. Foot, King's College London.

(C) Transmission electron micrograph of human islet cells. The figure shows a transmission electron micrograph of several cells within a human islet. The two cells in the top left with the electron dense secretory granules surrounded by a clear halo are β - cells. The cells in the lower half of the micrograph are α - cells. Scale bar is 2 μ m. Data from Jones & Persaud, unpublished.

1. Insulin Biosynthesis

The Beta cells are highly specialized for both the production and storage of Insulin. This is a well orchestrated act in which the insulin is rapidly secreted on metabolic demand despite slow process of producing Insulin. The short arm of chromosome 11 houses the gene responsible for the production of Preproinsulin, a precursor of Insulin. Within a minute of its formation the Preproinsulin is discharged in the endoplasmic reticulum and the proteolytic enzymes cleave and form Proinsulin. Proinsulin is a 9 - kDa

peptide, containing the A and B chains of insulin (21 and 30 amino acid residues, respectively) joined by the C peptide (30–35 amino acids).

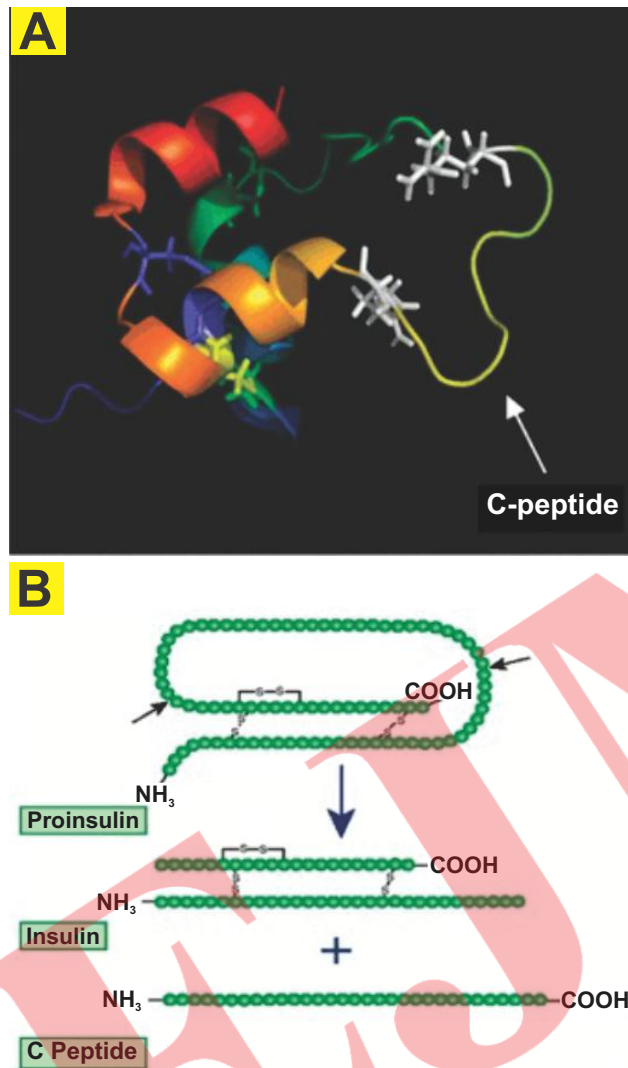


Fig 2: Proinsulin C-peptide Molecule

C-peptide is the peptide segment connecting insulin A and B chains, and a product of proinsulin cleavage in the secretory granules, generated in the pancreatic β -cells as part of normal insulin production.

(A) This figure is a image of proinsulin molecule, in which C-peptide is cleaved by endoproteases at the level of the two Arginine residues linking the B-chain and C-peptide. After the cleavage C-peptide is secreted in equimolar amount with insulin.

(B) a schematic view of the proinsulin molecule is shown. The black arrows indicate sites of cleavage by proteases at the level of arginine residues. C-peptide circulates at low nanomolar concentrations in healthy individuals, but is absent in most patients with type 1 diabetes (T1D). (1)

1.1 Structural Integrity of C-Peptide and Physiological Role of C-Peptide

C-peptide is a 31-amino acid peptide. It is negatively charged and not reported to show an ordered tertiary structure under physiological conditions (2). The amino acid sequence of C-peptide shows considerable variability between species, in contrast to the well-preserved molecular structure of insulin. However, in mammals the eight residues at positions 1, 3, 6, 11, 12, 21, 27, and 31 of C-peptide are conserved or vary in only one species. Of these, Glu27 and Gln31 have been ascribed special importance for interaction between C-peptide and cell membranes (3). A similar structural variability exists for relaxin (4), and the possibility may be considered that both relaxin and C-peptide have taken on hormonal roles late in the course of evolution by affecting specific cellular processes needed only in higher mammals. The plasma concentration of C-peptide in the overnight-fasted state is about 0.3–0.6 nM in healthy subjects, and postprandial levels is about 1–3 nM. Higher levels are observed in overweight individuals (5). Its biological half-life is ~30 min in healthy individuals and longer in subjects with type 2 diabetes (6). Unlike insulin, C-peptide escapes hepatic retention and is eventually catabolized primarily by the renal cortex (7, 8), with only a small fraction being excreted in the urine.

C peptide, Proinsulin connecting peptide is a cleavage product of insulin synthesis and is co secreted by pancreatic beta cells along with Insulin. Quite intriguing is the fact that the C-peptide is not associated with recombinant Insulin and a large no of preliminary studies have found out that lack of C peptide may exacerbate diabetes associated complications. C peptide may have a role in prevention of a large no of Diabetic complications. Previously thought to be biologically inert C-peptide may have varied role to play and it may correct some vascular and neurological complications associated with diabetes.

There were compelling evidences of indirect biological effects of C peptide. Thus, it is a clinical observation that subjects with type 1 diabetes who retain low plasma concentrations of C-peptide are less prone to develop microvascular complications than those in whom β -cell function has ceased completely (9,10). In addition, pancreas or β -cell transplantation, which results in restoration of endogenous insulin and C-peptide concentrations, is accompanied by significant amelioration of diabetes-induced abnormalities, both structural and functional, of the peripheral nerves and the kidneys (11, 12).

In the early 1990s, the growing interest in C-peptide led to the reevaluation of the possible relation between

C-peptide and insulin. A series of studies were undertaken involving administration of the peptide to patients with type 1 diabetes, who lack C-peptide. It soon became apparent that replacement of C-peptide in physiological concentrations resulted in significant improvements of several diabetes-induced functional abnormalities (13-16). These findings prompted a renewed interest in C-peptide physiology, and during the past 20 years a steadily increasing number of reports on new aspects of C-peptide physiology have emerged. The information available today includes studies of the peptide's interaction with cell membranes and its intracellular signaling properties (17, 18). In vivo studies in animal models of type 1 diabetes have defined a beneficial influence of C-peptide on diabetes-induced functional and structural abnormalities of the kidney, peripheral nerves, and the central nervous system. In addition, several clinical studies describing positive effects of C-peptide replacement therapy on nerve and kidney function in type 1 diabetic patients have been reported (19). The wealth of information now available supports the hypothesis that C-peptide, contrary to previous views, does exert important physiological effects. These effects and their underlying mechanisms of action are discussed in this review.

2. C-Peptide and Nerve Impairment in Type 1 Diabetes

Foot ulceration and limb amputation is the most dreaded complication of Diabetic neuropathy. It starts as a simple sensory loss in the extremity and can have drastic consequences if left untreated. Autonomic dysfunction involving problems with gastric emptying, urinary bladder dysfunction, or erectile dysfunction occurs later in the spectrum of disorders.

Direct measurements of nerve blood flow have shown that endoneurial blood flow is substantially reduced in diabetes (20, 21). Administration of C-peptide in replacement doses resulted in a marked improvement of the endoneurial perfusion deficit, possibly a result of C-peptide-elicited stimulation of endothelial nitric oxide synthase (eNOS) and augmented NO availability (21). C-peptide in physiological concentrations improves nerve function in type 1 diabetes via a NO-sensitive neurovascular mechanism, mediating dilation of the vasa vasorum. C-peptide effects on both endoneurial blood flow and NCV were abrogated by an eNOS blocker, indicating that C-peptide in physiological concentrations improves nerve function in type 1 diabetes via a NO-sensitive neurovascular mechanism, mediating dilation of the vasa nervorum.

One of the hallmark features in type 1 diabetes is also

Decreased Na^+, K^+ -ATPase activity in peripheral nerve tissue (22). It is associated with inactivation of Na^+ channels, intra-axonal sodium accumulation, and swelling of the paranodal region during the early phase of the disorder. It is again intriguing to observe that C-peptide in physiological concentrations prevents or partially corrects the diabetes-induced reduction in nerve Na^+, K^+ -ATPase activity in diabetes (22, 23), thereby contributing to diminished Na^+ retention and partial correction of nerve structural abnormalities, paranodal swelling in particular.

Painful neuropathy is a debilitating consequence of diabetes, which is at least partly due to damage to the unmyelinated and the small myelinated nociceptive fibers (24). High firing frequencies and spinal sensitization are features of degeneration of these fibers and this clinically manifests as hyperalgesia. It is interesting to note that replacement of C-peptide from the onset of diabetes in rats completely prevents thermal hyperalgesia as well as degeneration and loss of unmyelinated fibers (25). These findings are accompanied by improved regulation via the transcription factors c-fos and c-jun of gene expression of several neurotrophic factors, e.g., NGF, NT3, and IGF-I, and their receptors. All these findings and several other clinical studies have propounded this idea that C-peptide has a role to play in prevention of development of painful diabetic neuropathy.

A C-peptide beneficial effect in animal models has been confirmed by few clinical studies. A double-blind placebo-controlled study in patients with type 1 diabetes with early-stage neuropathy showed that C-peptide replacement over a 3-mo period resulted in a gradual increase in sural NCV, reaching 2.7 m/s, amounting to 80% correction of the initial nerve conduction deficit as well as improvements in vibration perception (26). These results have subsequently been confirmed and extended in a larger study involving patients with clinically manifested neuropathy and type-1 diabetes (27). Another astonishing feature is that six months of C-peptide replacement resulted in improvements in sensory (sural) NCV, clinical scores of neuropathy impairment, and vibration perception. Levels of glycemic control were similar in C-peptide- and placebo-treated patients and unchanged during the study.

Abnormal cardiac autonomic nerve function is associated with cardiac arrhythmias and sudden death. The condition may be evaluated as reduced heart rate variability (HRV) during deep breathing, a measurement that primarily reflects vagal nerve function. In patients with type 1 diabetes, a 2-h infusion of C-peptide is reported to significantly increase HRV, whereas no change was seen after saline

infusion (27). The heart rate brake index after a tilting manoeuvre was also improved after C-peptide infusion. A similar, albeit less-marked improvement was seen after 3 mo of C-peptide administration in patients with type 1 diabetes (28). Improved parasympathetic tone may be the reason in subjects with erectile dysfunction who reported significant improvement after C-peptide treatment for 6 months. To sum up we can say that the available experimental and clinical evidence demonstrates that C-peptide, by stimulating endoneurial eNOS, increasing nerve blood flow, augmenting nerve Na^+, K^+ -ATPase, and stimulating nerve trophic factors exerts beneficial effects on the mechanisms underlying nerve dysfunction in type 1 diabetes.

3. C-Peptide and the End Stage Renal Disease in Diabetes

Diabetes is the single leading cause of end-stage renal disease (ESRD) in the Western world and is associated with increased cardiovascular risk, high morbidity, and mortality (29). It's a specific set of structural and functional abnormalities of kidneys due to diabetes insults. The structural abnormality manifests as hypertrophy of kidneys, an increase in glomerular basement membrane thickness, nodular and diffuse glomerulosclerosis. The functional abnormality manifests as increase in glomerular filtration rate and intraglomerular hypertension subsequent proteinuria, systemic hypertension and eventual loss of renal function. Despite the improvements in clinical management and treatment of ESRD, the prevalence of diabetic nephropathy (i.e., ESRD due to diabetes) is likely to continue to rise due to the increasing prevalence of diabetes and obesity worldwide. As a result, diabetic nephropathy will remain a significant economic and health burden unless novel treatments can be identified. The earliest feature of Diabetic Nephropathy is microalbuminuria and is defined as Urinary albumin excretion of 30-299 mg/24 hrs in a 24 hrs urinary collection on at least two occasions within a 3 to 6 months period. Now as the modalities of treatment of Coronary artery disease in diabetes patients improve more and more no of people with diabetes kidney diseases would prop up.

The conventional strategies for the treatment of diabetic nephropathy include glycemic control, blood pressure lowering, and blockade of the renin-angiotensin system as a means of blood pressure control, but also direct preservation of renal function. However, despite overall improvements in treatment, diabetic nephropathy remains associated with high morbidity and mortality, indicating the need for the development of novel treatments, and in recent years, C-peptide has started to emerge as a potential novel

therapeutic treatment for type 1 diabetes-associated end-organ complications, including diabetic nephropathy (30). A number of studies have demonstrated that acute and long term infusion of C-peptide reduces hallmark of early diabetes nephropathy changes namely glomerular hyperfiltration, glomerular hypertrophy and microalbuminuria. A randomized study was performed in 21 normotensive patients with microalbuminuria that received, along with their regular insulin regimen, either subcutaneous injections of C-peptide (600 nmol/day) or placebo over 3 mo. Patients receiving C-peptide showed a decline in urine albumin excretion compared with baseline in a time-dependent manner, but no effects on GFR were observed (28). Usually in STZ-induced diabetic rat there is development of glomerular hyperfiltration within days of induction of diabetes, however patients included in this particular study had normal GFR at baseline. Thus, these data suggest that C-peptide has no effect on GFR when GFR is normal. So there is substantial evidence of beneficial effects of C peptide in early prevention of Diabetes nephropathy. In a no of studies c-peptide administration has attenuated Glomerular hyperfiltration, renal cortical inflammation and as a end result there is reduced microalbuminuria.

The role of C peptide in type 2 diabetes or non diabetic renal disease remains largely unexplored. Most of the studies examining the renoprotective effects of C-peptide have focused solely on experimental models or humans with type 1 diabetes. A study in patients with type 2 diabetes reported that higher baseline levels of C-peptide are associated with a reduced risk of incident microvascular complications (31), indicating that treatment with C-peptide in these patients may potentially afford renoprotection.

The major mechanisms of renoprotection by C-peptide is mainly preventing and attenuating the progression of glomerular hyperfiltration, hypertrophy, and permeability, reducing afferent arteriolar diameter, microalbuminuria and renal inflammation, and inhibiting tubular sodium reabsorption. Some of the studies have pointed that C-peptide may have a Glucose lowering effects however; its renoprotective effects appear to be independent of glucose regulation. Finally, it will be sufficient to say that spectrum of renoprotective effects of c-peptide on Type 1 diabetes patients may well extend to the group of type 2 diabetes patients.

Microvascular Complications of Diabetes and Role of C-peptide

The effect of C-peptide includes a positive influence

on long term complication in Type-1 and Type-2 Diabetes. Surprisingly C-peptide administration has been found to improve microvascular complications of diabetes. At the cellular level c-peptide has been shown to increase capillary blood flow. C-peptide has an impact on diabetic neuropathy via improvement in endoneurial blood flow and exonal swelling (32). Most of the complications of diabetes are due to underlying molecular level changes. The basic mechanism of development of complication is hyperglycemia and this hyperglycemia is the basis of eight different types of defects in diabetes which are described as ominous octet. It was first propounded by Ralph de Fronzo at the Banting lecture at American diabetes association in 2008. The persons destined to develop Type 2 Diabetes inherit a particular set of genes that make them resistant to the effects of insulin. These core defects are the underlying mechanisms of all diabetes complications. Interestingly this ominous octet is also the basis of Diabetes treatment modalities.

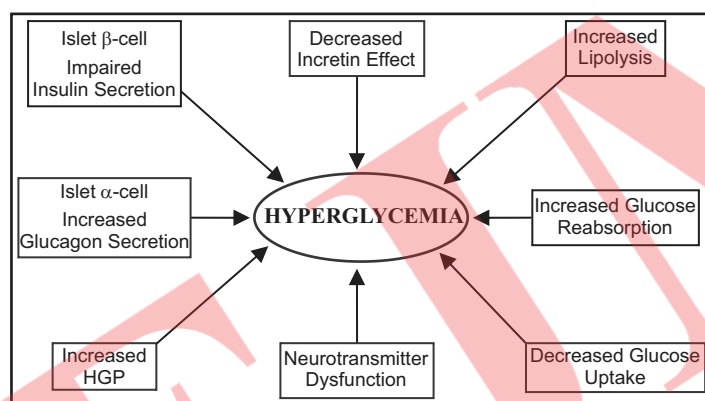


Fig 2: The Ominous Octet

Dietary advice on dealing with diabetes has formed a long and tedious task close to half a century. Diets evolved from Allen, Joslin, and Mediterranean and later it was realized that diabetes diet is a healthy-eating plan that's naturally rich in nutrients and low in fat and calories. Key elements are fruits, vegetables and whole grains. In fact, a diabetes diet is the best eating plan for most everyone.

A "unifying hypothesis" that seeks to explain the biochemical mechanisms behind the development of microvascular complications of diabetes was put forward by Brownlee (32). The hypothesis, now widely accepted, focuses on the fact that the metabolic abnormalities of diabetes, primarily the elevated blood glucose levels and the lack of insulin, cause formation of ROS in vascular endothelial cells, generating oxidative stress. The increased ROS formation leads to a series of downstream metabolic disturbances such as stimulation of glucose metabolism along the polyol pathway, augmented

intracellular formation of advanced glycation end products and their receptors, activation of PKC isoforms, and increased activity of the hexosamine pathway. These metabolic changes, in turn, give rise to suppression of endothelial eNOS and Na⁺,K⁺-ATPase activities of endothelial cells and several tissues as well as upregulation of proinflammatory genes via NF-κB-mediated mechanisms, resulting in augmented formation of cytokines, chemokines, cell adhesion molecules, VEGF, and TGF-β. This panoply of detrimental events results in the gradual development of microvascular circulatory abnormalities, impairment of endothelial function, and endothelial cell apoptosis, cornerstones of what is clinically known as microcirculatory complications.

Carbohydrate meals results in modest elevations of blood glucose levels and normal physiological role of C-peptide may be to prevent or diminish the formation of ROS and other oxidant species that accompany increased blood glucose levels. It is now apparent that C-peptide in conjunction with regular insulin therapy serves as an endogenous antioxidant and prevents the generation of hyperglycemia-mediated oxidative stress. In addition, the peptide is capable of directly retarding the deleterious effects of several of the downstream metabolic abnormalities resulting from hyperglycemia, thereby further emphasizing the potential therapeutic benefits from C-peptide therapy.

CONCLUSION

The way the c-peptide and Insulin interacts across multiple tissues is an enigma. There are still many unanswered questions. The earlier conviction that C-Peptide acts as a biologically inert molecule now seems to have yielded and now we see a wide range of sub-cellular interactions and the discovery of C-peptide receptors has further jeopardized the understanding of the action of this molecule. Evidence rightly points out that the C-peptide signaling ultimately by a complex series of intra-molecular actions leads to protection against hyperglycemia associated microvascular complications. The therapeutic implications of C-peptides needs to be further explored. Does C-peptide sensitizer or high affinity agonists have a role to play in the prevention of diabetes related complications? This is a question that needs to be further explored.

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