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A REVIEW ARTICLE

# C-Peptide: From Physiology to Clinical Use

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*ABSTRACT*

***Lack of C-peptide, along with lack of insulin, is the main feature of type 1 diabetes mellitus (T1DM) and is also observed in advanced stages of type 2 diabetes mellitus (T2DM) punctuated by progressive β-cell loss. Therapeutic approaches to control high blood sugars have largely failed in stemming the onslaught of diabetic vasculopathy, and alternative therapeutic strategies become the need of the hour to target both the glucose fluctuations and the diabetic complications.***

## INTRODUCTION

In the year 1967, Steiner DF and colleagues identified and explained that a larger protein proinsulin is cleaved into insulin and C-peptide. The two are stored in the secretory granules of the pancreatic beta-cells and finally released together in equimolar concentration. C-peptide facilitates the correct folding of insulin and formation of its disulphide bridges. Following its discovery, several futile attempts were made to detect insulin-like effects of C-peptide,[1] making scientific community believe that C-peptide had no major physiological role, except for its potential use as a biomarker of beta-cell activity.

Very exciting new information on C-peptide physiology has emerged during the past 20 years. C-peptide appears to bind specifically to cell membranes, elicits intracellular signalling via G-protein and $Ca^{2+}$-dependent pathways, results in increased expression of endothelial nitric oxide synthase, $Na^{+}$, $K^{+}$-ATPase and several transcription factors of importance for anti-inflammatory, anti-oxidant, and cell protective mechanisms. Clinical trials in patients with T1DM have shown that C-peptide in replacement doses exerts preventive effects on early stages of diabetes-induced damage to peripheral nerves, kidneys, and the retina.

## PHYSIOLOGY OF C-PEPTIDE

C-peptide is a 31-amino acid peptide. The amino acid sequence of C-peptide shows considerable variability between species, in contrast to the well-preserved molecular structure of insulin. (Fig. 1) Of these, Glu27 and Gln31 are considered important for interaction between C-peptide and cell membranes.[2]

The plasma concentration of C-peptide in the overnight fasted state is 0.3–0.6 nM in healthy subjects, and postprandial levels may rise to 1–3 nM. Higher levels are observed in overweight individuals.[3] Its biological half-life is 30 minutes in healthy individuals and longer in subjects with T2DM.[4]

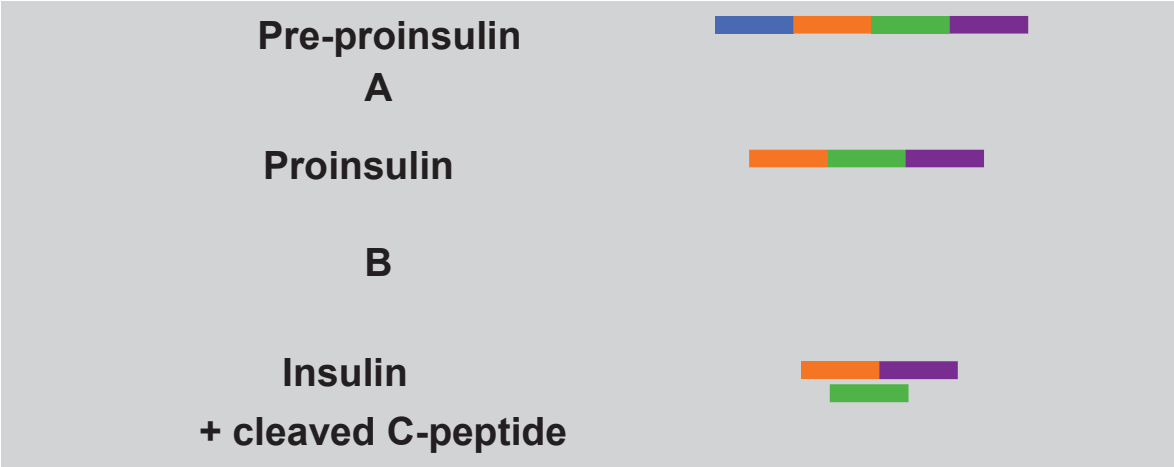


*Fig. 1: STRUCTURE OF PRE-PROINSULIN, PROINSULIN, AND INSULIN*

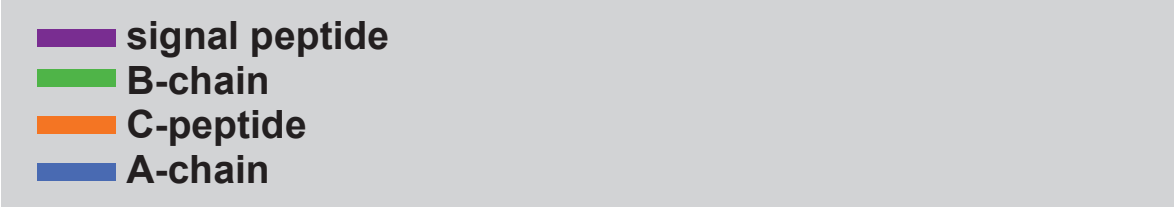


*Fig. 2: Structure of Proinsulin*

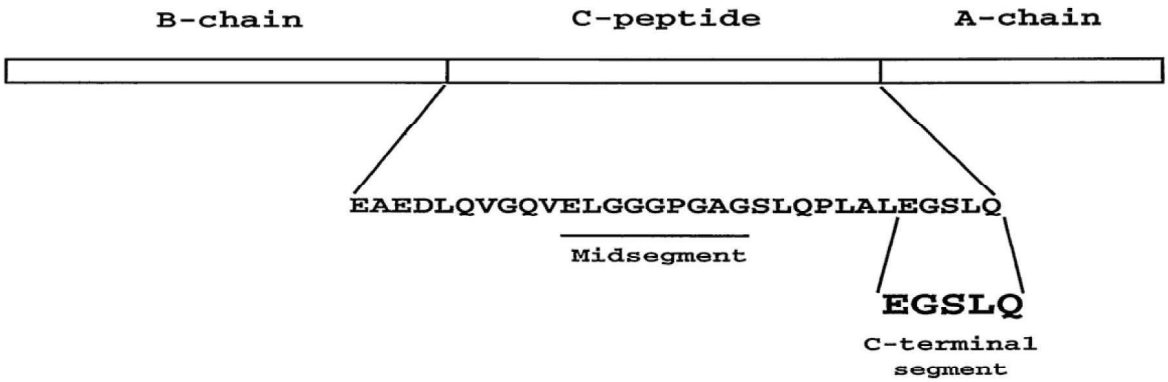


*Fig. 3:*

Pre-proinsulin is transcribed as a 100-amino acid chain and contains a terminal signal sequence (blue). (Fig 2 and 3)

Linear representation of human proinsulin indicating amino acid sequence of C-peptide and showing position of COOH-terminal pentapeptide,

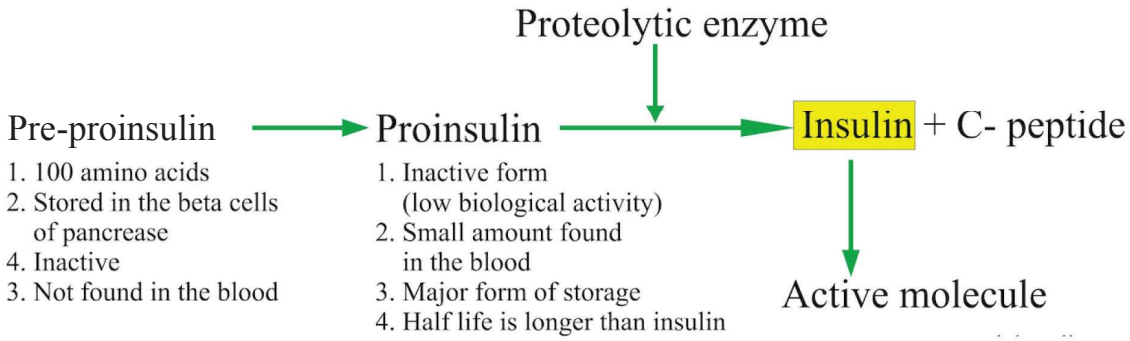


*Fig. 4:*

which mimics action of C-peptide in assays of binding and $Na^+$-$K^+$-ATPase activity.[5]

After the signal peptide of pre-proinsulin is removed in the rough endoplasmic reticulum, proinsulin is post-translationally modified in the Golgi apparatus and the secretory granules of pancreatic β-cells, resulting in the production of insulin and C-peptide (31 amino acids).[5,6]

Proinsulin is cleaved by proteolytic converting enzymes that remove the connecting peptide (C-peptide) and the lysine-arginine (Lys-Arg) and arginine-arginine (Arg-Arg) sequences of dibasic amino acids, thus forming the mature insulin molecule, which consists of A- and B-chains connected by disulphide bonds. (Fig. 4)

## SCHEMATIC REPRESENTATION OF CELLULAR EFFECTS OF C-PEPTIDE, PARTICULARLY IMPORTANT IN MEMBRANE INTERACTIONS AND SIGNALLING

As shown above,[5] C-peptide binds to cell membrane receptors coupled to a pertussis toxin-sensitive G protein. (Fig. 5 and 6) The G protein activates $Ca^{2+}$ channels, resulting in an increased

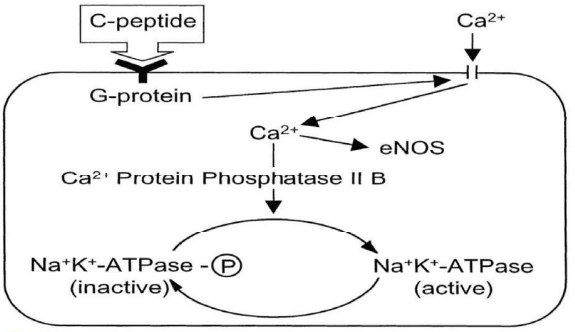


*Fig. 5:*

intracellular $Ca^{2+}$ concentration and activation of both endothelial nitric oxide synthase (eNOS) and $Ca^{2+}$- calmodulin-dependent protein phosphatase 2B (PP2B). PP2B finally transforms the phosphorylated form of $Na^+$-$K^+$- ATPase into its dephosphorylated, active form.

Unlike insulin, C-peptide is able to bypass hepatic retention and is ultimately metabolised primarily by the renal cortex[7,8] with only a small fraction being excreted in the urine.

C-peptide generated by proinsulin proteolysis is not only a chaperone for insulin in β-cells, but also a signalling molecule, which impacts many physiological and biochemical processes via specific C-peptide receptors. C-peptide facilitates

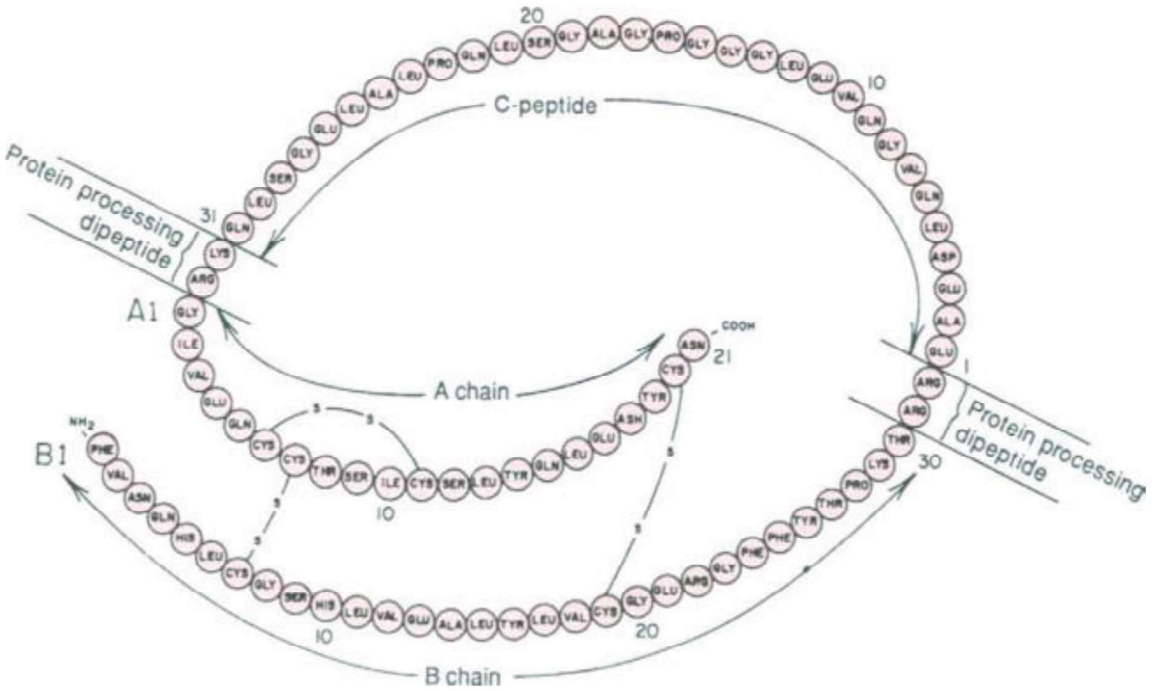


*Fig. 6:*

***Table 1:*** *Differences between C-peptide and insulin.*

| Functions | C-peptide | Insulin |
|---|---|---|
| Diabetic complications; retinopathy, nephropathy, neuropathy, impaired wound healing | Beneficial Impact | Not Involved |
| Glucose uptake | Indirect involvement | Direct effect |
| AMPK regulation | Activation | Inhibition |
| ROS production | Inhibition | Activation |
| Na+/K+ ATPase regulation | Activation as well as expression | Only Activation |
| NO production | Activation | Activation |
| Inflammation | Inhibition, possible aggravation at higher concentrations | Inhibition, possible aggravation at higher concentrations |

the intracellular transport, sorting, and proteolytic processing of proinsulin into biologically active insulin in the maturing secretory granules of the β-cells.

## WHAT IS THE RELATIONSHIP BETWEEN C-PEPTIDE AND INSULIN?

Elevations in plasma glucose levels are the physiological stimuli responsible for simultaneous release of insulin and C-peptide from the same secretory vesicles. Despite this, C-peptide does not appear to directly alter glucose metabolism. However, it does appear that C-peptide and insulin-initiated signalling cascades interact and that such interactions are important for the normal functioning of both peptides, particularly in erythrocytes.[9]

## C-PEPTIDE TESTING

Venous blood c-peptide levels can be measured in the random, fasting, or stimulated state. Stimulation methods include using glucagon, intravenous/oral glucose, sulphonylurea, and glucose-like peptide 1, amino acids, or a mixed meal.[1]

## INTERPRETATION

In a person with insulin-treated diabetes, a stimulated blood C-peptide of <0.6 nmol/L (fasting < 0.25 nmol/L and or post-meal urinary C-peptide:creatinine ratio < 0.6 nmol/mmol) is suggestive of marked insulin deficiency and T1DM.

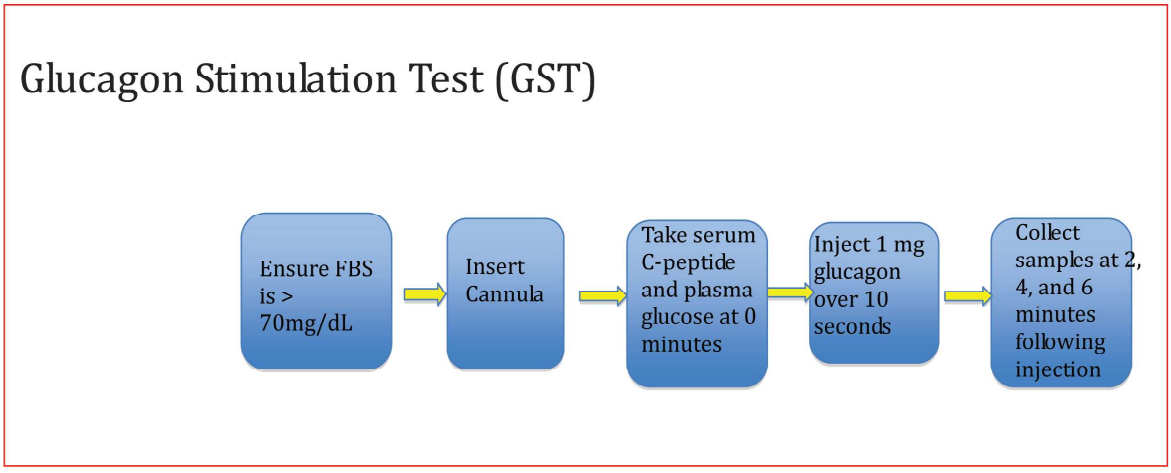


*Fig. 7: Glucagon Stimulation Test*

Persistence of C-peptide above these levels after 3–5 years from diagnosis is suggestive of T2DM or monogenic diabetes. A stimulated blood C-peptide < 0.2 nmol/L (fasting < 0.08 nmol/L and or post-meal urinary C-peptide:creatinine ratio < 0.2 nmol/mmol) confirms absolute insulin deficiency and absolute insulin requirement. (Fig.7) Causes of a high C-peptide level include insulinomas, insulin resistance, kidney disease, and Cushing syndrome. In healthy subjects, the plasma concentration of C-peptide in the overnight fasted state is 0.3–0.6 nM and in postprandial levels may rise to 1–3 nM. Higher levels are observed in overweight individuals. Its biological half-life is 30 minutes in healthy individuals but longer in subjects with T2DM.

## C-PEPTIDE AND DIABETIC NEUROPATHY

Six months of C-peptide replacement resulted in improvements in sensory (sural) nerve conduction velocity (NCV), clinical scores of neuropathy impairment, and vibration perception. Both, the C-peptide- and placebo-treated arms displayed glycaemic equipoise throughout the study.[10]

## ROLE OF C-PEPTIDE IN ENDOTHELIAL PHYSIOLOGY

C-peptide's anti-oxidant, anti-inflammatory, and anti-apoptotic effects

(AMP: adenosine monophosphate; ATP: adenosine triphosphate; ENOS: endothelial nitric oxide synthase; ICAM: intercellular adhesion molecule; MCP: monocyte chemotactic protein; PDE3: phosphodiesterase 3; PKC: protein kinase C; RBC: red blood cell; ROS: reactive oxygen species; VCAM: vascular cell adhesion molecule; WBC: white blood cell)

C-peptide and insulin exert opposing actions on the activity of PDE3 in the red blood cell (RBC), which ultimately lead to increased ATP release, which has both direct and indirect effects on vasodilation. Inhibitory actions of both C-peptide and insulin decrease cytokine release from leukocytes (WBC) that result in decreases in endothelial expression of cell adhesion molecules needed for WBC migration out of blood vessel. C-peptide also reduces formation of reactive oxygen species (ROS) and activity of caspase-3, in the endothelial cells which results in

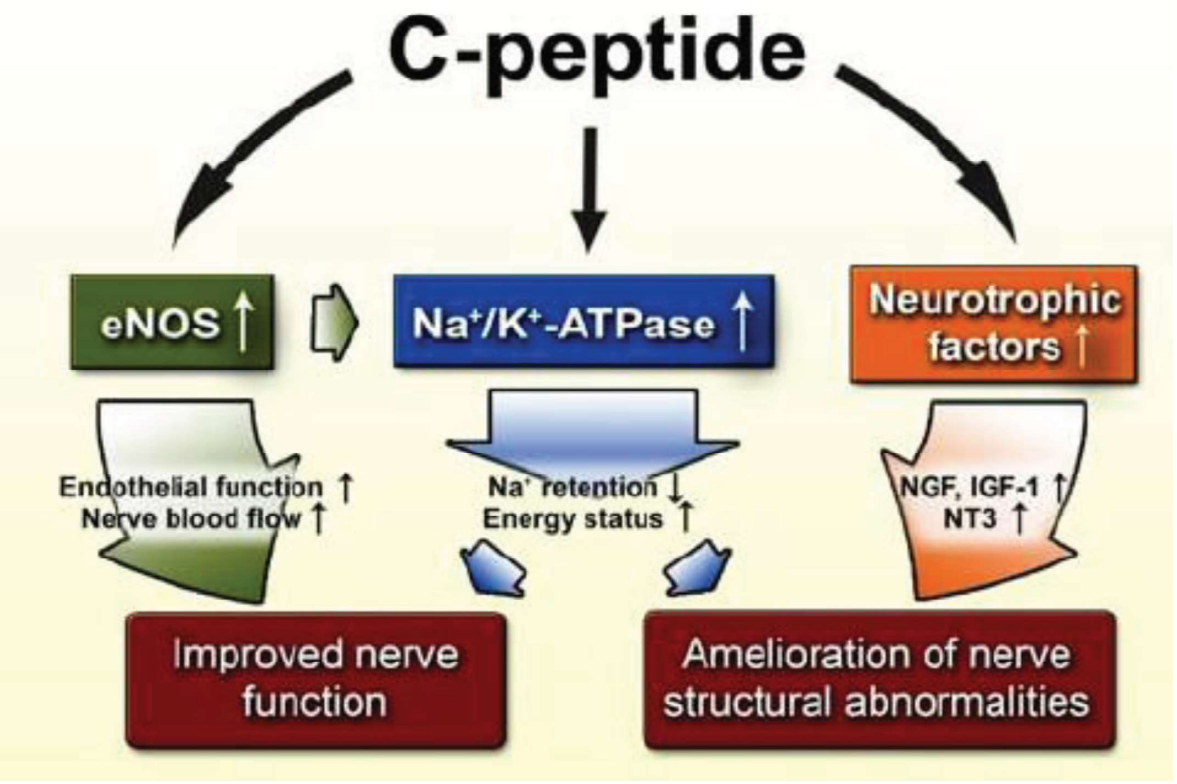


*Fig. 8:*

(eNOS: endothelial nitric oxide synthase; IGF: insulin-like growth factor; NGF: nerve growth factor)

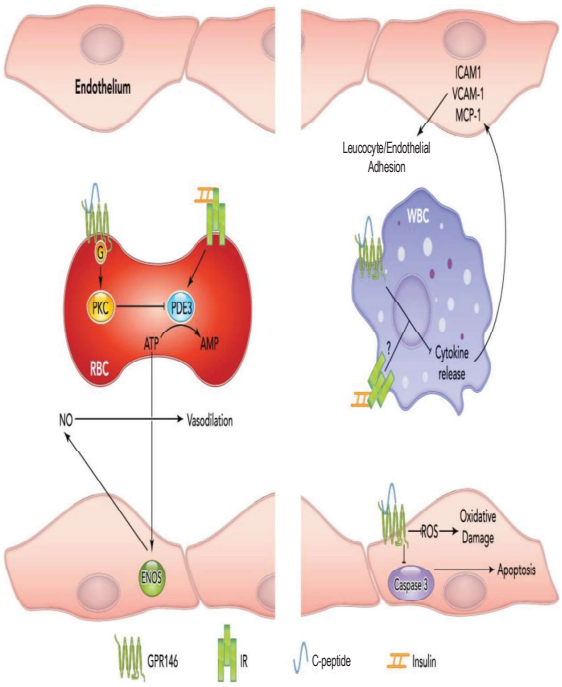


*Fig. 9: C-PEPTIDE IN ENDOTHELIAL PHYSIOLOGY*

an anti-apoptotic effect. (Fig 8 and 9)

Loss of adequate C-peptide levels, as in T1DM, would eliminate this tuning of insulin signalling, which, presumably, would not result in immediate deleterious effects but over time could lead to an accumulation of cellular defects (due to the unopposed actions of insulin) and ultimately to endothelial and subsequent microvascular dysfunction.

## DOES C-PEPTIDE HALT OR REVERSE THE PROGRESSION OF DIABETIC KIDNEY DISEASE?

C-peptide exerts reno-protective effects in T1DM via inhibiting tubular sodium reabsorption, reducing afferent arteriolar diameter and glomerular permeability, preventing and attenuating the progression of glomerular hyperfiltration, hypertrophy, and microalbuminuria, renal inflammation, glomerulosclerosis, and tubulointerstitial fibrosis. C-peptide also reduces renal cortical inflammation, apoptosis and preserves the renal microvascular architecture.[11]

C-peptide may also have blood glucose-lowering effects; however, it appears that C-peptide exerts its reno-protective effects independently of blood glucose regulation.

## KNOWN/UNKNOWN RENO-PROTECTIVE EFFECTS OF C-PEPTIDE (Fig.10)

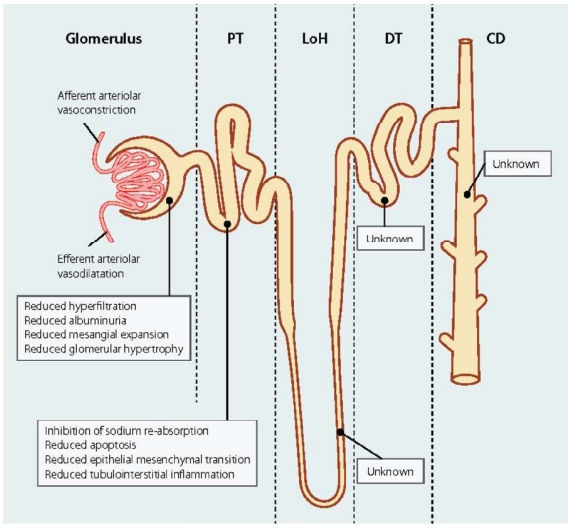


*Fig. 10:*
*(CD: collecting duct; DT: distal tubule; LoH: loop of Henle; PT: proximal tubule)*

## MULTIPLE ACTIONS OF C-PEPTIDE IN DIABETES (Fig.1)

There is evidence to suggest that C-peptide is capable of impacting the development of diabetic complications via the following outlined mechanisms. Several laboratory studies[12,13] have proved that C-peptide directly inhibits endothelial cell ROS formation. Other studies[14,15] have convincingly demonstrated stimulation and increased expression of both eNOS and $Na^+$, $K^+$-ATPase activities in several tissues and an inhibitory effect on nuclear factor kappa B (NF-κB) which results in diminished diabetes-mediated expression of cytokines, chemokines, and cell adhesion molecules.[16,17] C-peptide triggers anti-apoptotic mechanisms by inhibiting TG-2 and caspase-3.[12] Thus, C-peptide antagonises the hazards of hyperglycaemia by improving microcirculation, alleviating endothelial dysfunction, and inhibiting endothelial apoptosis.

It can hence be considered that a normal physiological role of C-peptide may be to prevent or diminish the formation of ROS and other oxidant species that accompany the modest

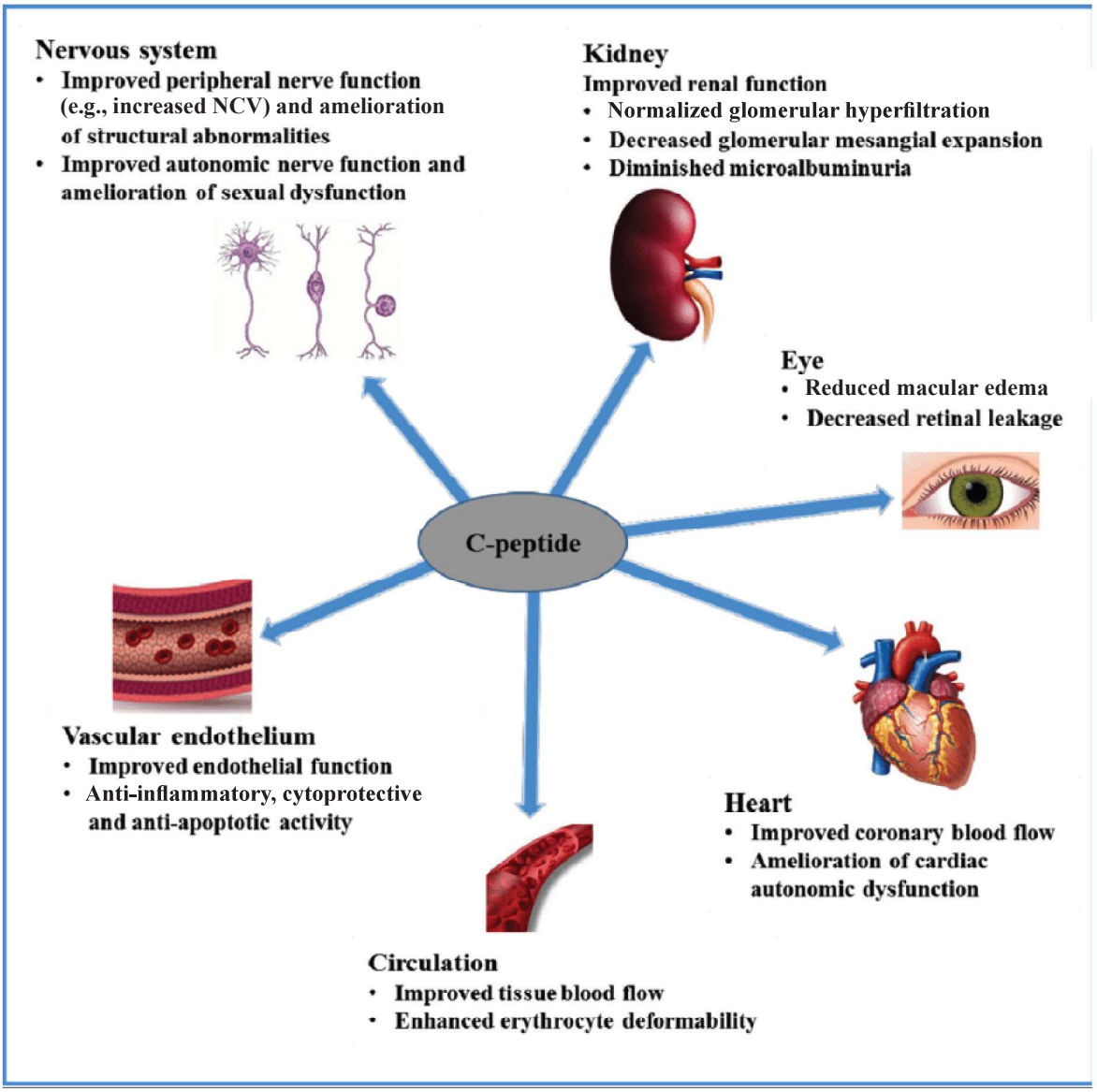


*Fig. 11: Effects of C peptide on different organs.*
*(NCV: nerve conduction velocity)*

elevations of blood glucose levels following intake of carbohydrate meals. If so, it becomes apparent that patients with diabetes, especially T1DM, are particularly vulnerable. They lack both C-peptide and insulin and are perennially exposed to persistently elevated sugar levels.[18]

## SUMMARY

Numerous studies, including the Diabetes Control and Complications Trial and experimental studies, have suggested a preventive role of C-peptide in diabetic complications in animal models and T1DM patients.[19] C-peptide probably interacts with a GPCR, perhaps GPR146, to exert anti-inflammatory, anti-apoptotic, vasodilatory, and anti-oxidant effects in the vascular endothelium through both direct actions on the endothelial cells and indirectly through interaction with erythrocytes and immune cells. The vasoprotective mechanisms of C-peptide involve the maintenance of vascular function, as well as the prevention of endothelial cell death, microvascular permeability, inflammation, and neointima formation.[20]

C-peptide plays a physiologically relevant role in the tuning of insulin signalling. Thus, loss of C-peptide could have important implications for

endothelial physiology and could account for the microvascular dysfunction observed in diabetes.

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