

C-Peptide and Vascular Complications in Type 2 Diabetic Subjects

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C-peptide is a small peptide comprised of 31 amino acids, with a short half-life of approximately 30 minutes. It was first identified by Steiner et al. [1] as a by-product of proinsulin and its main role is in assisting in the arrangement of the correct structure of insulin. Proinsulin consists of an A chain, connecting peptide (C-peptide), and B chain. C-peptide has a central glycine-rich region that allows the correct positioning of the A and B chains for insulin to achieve its tertiary structure [1]. It is secreted into the bloodstream in equimolar amounts together with insulin in response to glucose stimulation. C-peptide has been long considered an inactive peptide; however, over the last two decades, numerous studies have revealed that C-peptide displays a physiological role in different cell types [2,3]. The C-terminal pentapeptide of C-peptide obtains the full activity of intact C-peptide in stimulating Na^+/K^+ -ATPase [4]. The amino acid sequence of C-peptide can vary by species, although it has several conserved sequences, such as its N-terminal acidic region, glycine-rich central segment, and C-terminal pentapeptide [5]. Moreover, evidence indicates that C-peptide is not merely an inactive by-product of insulin biosynthesis but also a hormonally-active peptide itself [3,4].

The delta C-peptide value, which postprandial serum C-peptide levels minus fasting serum C-peptide levels, correlates closely with glucagon-stimulated C-peptide concentrations and decreases progressively as diabetic duration increases [5,6]. Approximately 5% of pancreatic C-peptide is excreted in the urine [7]. Twenty-four-hour urine collections have been

shown to correlate well with serum C-peptide measurements; however, they are cumbersome and prone to incomplete urine collection [8]. Post-meal urine C-peptide creatinine ratios have been shown to have similar sensitivity and specificity to glucagon-stimulated serum C-peptide values in classifying diabetes by insulin requirement, but have otherwise been studied little [9]. Urine C-peptide collected in boric acid has recently been shown to be stable for 72 hours at room temperature with no decline in C-peptide levels over this time [10]. The urine C-peptide creatinine ratio may thus have the potential to provide a simple practical measurement of insulin secretion for use in routine clinical practice.

The Diabetes Control and Complications Trial showed that the residual secretion of serum C-peptide decreased the incidence of diabetic retinopathy and nephropathy in subjects with type 1 diabetes [11]. In a recent cross sectional study, 471 type 1 diabetic patients were followed from 1994 to 2004 [12]. Those subjects with the lowest fasting C-peptide levels were found to have the highest rate of microvascular complications. No association was observed between C-peptide levels and macrovascular complications. There is increasing evidence that, in type 1 diabetic patients, the conservation of residual beta cell function slows microvascular complications, by improving blood glucose control and by the preservation of residual C-peptide secretion.

The effects of C-peptide include a positive influence on long-term complications in type 1 diabetic patients. Some groups

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have shown that C-peptide administration in type 1 diabetes results in amelioration of diabetes-induced renal and nerve dysfunction. C-peptide treatment for 6 months has been shown to improve sensory nerve function [13,14]. Furthermore, beneficial effects by C-peptide replacement on renal function and structure in type 1 diabetes have been reported [15,16]. Given that C-peptide increases capillary blood flow in type 1 diabetic patients, Wallerath et al. [17] demonstrated *in vitro* that C-peptide stimulates the release of nitric oxide (NO) from endothelial NO synthase (eNOS) in endothelial cells, and that this effect is mediated by inducing a Ca^{2+} influx into the cells. In the kidney, C-peptide supplementation suppresses diabetes-induced abnormal renal eNOS expression. This downregulation may explain C-peptide's beneficial effects on diabetic nephropathy [18]. C-peptide has an impact on diabetic neuropathy via improvements to endoneural blood flow and axonal swelling [19], or its improvements to decreased blood flow in the extremities [20]. Several studies have proposed a direct role of endogenous insulin and C-peptide in the improvement of endothelial dysfunction [21]. Moreover, C-peptide increases NO production through ERK1/2 MAP kinase-dependent up-regulation of eNOS gene transcription [22].

In contrast to these studies in type 1 diabetes, several studies [23-25] have reported conflicting results on the association between serum C-peptide levels and vascular complications in type 2 diabetes. Yoon et al. [26] reported that the stimulated C-peptide value response to glucagon is associated with microvascular complications to a greater extent than the basal C-peptide level in subjects with type 2 diabetes. Recently, Bo et al. [27] analyzed a representative cohort of 2,113 subjects with type 2 diabetes mellitus, and a subgroup of 931 individuals from this cohort without chronic complications at baseline, recruited from a diabetes clinic. They found that higher baseline C-peptide levels were associated with a reduced risk of incident microvascular complications, but imparted no survival benefit to subjects with type 2 diabetes mellitus. Further large prospective studies are needed to clarify the association between C-peptide and microvascular complications in type 2 diabetic subjects.

Subjects with metabolic syndrome or type 2 diabetes exhibit a greater propensity for the development of a scattered and extensive pattern of arteriosclerosis [28]. Typically, these insulin resistant subjects demonstrate higher serum levels of insulin and C-peptide. Recent data suggest that C-peptide binds to specific, yet unidentified, cell surface receptors. First reports

about C-peptide deposition in the vessel wall came from Marx et al. [29], when they demonstrated the deposition of C-peptide in the subendothelial space in the thoracic aorta in diabetic subjects. In that study, C-peptide deposition was found in the intima of the vessel wall in the thoracic aorta of diabetic subjects. Among 21 subjects with C-peptide deposition, 77% showed infiltration of monocytes/macrophages and 57% infiltration of CD4+ lymphocytes [29]. These data suggest that C-peptide deposition may precede monocyte and T cell migration into the vessel wall. Based on this observation, the hypothesis was raised that C-peptide may deposit in the vessel wall during early atherogenesis, and then through chemotactic effects may promote the recruitment of monocytes and CD4+ lymphocytes. In addition to its chemotactic effect, interesting data exists that suggest that in monocyte-like ThP1 cells, C-peptide increases the expression of the PPAR γ -regulated gene CD36, an important scavenger receptor for the macrophage uptake of oxidized low density lipoprotein in arteriosclerotic lesions [30]. These data suggest that C-peptide may also promote the differentiation of monocyte/macrophages towards foam cells, thus representing another potential proatherogenic effect of C-peptide. Patients with early type 2 diabetes and insulin resistance show greater levels of C-peptide in the blood. Together with increased endothelial dysfunction, this leads to the deposition of C-peptide in the intima of the vessel wall. According to *in vitro* results, C-peptide may have a chemotactic effect on the inflammatory cells involved in the onset of the atherosclerosis, such as monocytes/ macrophages and CD4+ lymphocytes. Further, C-peptide has an effect on the proliferation of smooth muscle cells in the media. These cells migrate into developing atheroma and together with inflammatory cell recruitment represent an initial step in the development of atherosclerosis.

Vascular smooth muscle cells (VSMCs) play a critical role in the development of arteriosclerotic plaques by proliferating and subsequently moving from the media into early lesions and fatty streaks [31]. VSMCs are also important in restenosis formation after coronary intervention. After vascular injury, these cells start to proliferate and then they migrate into the developing neointima. Thus, they become the major cellular substrate for restenotic tissue [32,33]. Several mechanisms, such as platelet-derived growth factor release from activated platelets, secretion of cytokines, and growth factors from inflammatory cells, have been shown to induce VSMC proliferation during atherogenesis and restenosis formation [31]. Since

C-peptide also co-localizes with VSMCs in the media of early arteriosclerotic lesions in some diabetic subjects, it has been suggested that C-peptide could also exhibit biological activity in these cells [34]. Walcher et al. [35] showed that high levels (10 nmol/L) of C-peptide induces proliferation of human and rat smooth muscle cells in a concentration-dependent manner, as assessed by Ki-67 assay and thymidine incorporation assay. In addition, C-peptide induces phosphorylation of protein tyrosine kinase (Src) and PI-3 kinase and induces activation of ERK1/2 MAP kinase. VSMC proliferation by extracellular stimuli takes place in the mid-to-late G1 phase of the cell cycle, where D-type cyclins promote G1- to S-phase transition leading to Rb phosphorylation [36]. Walcher et al. [35] showed that C-peptide increases cyclin D1 expression and Rb phosphorylation, suggesting that C-peptide acts via similar signaling pathways. In another study, insulin did not alter endothelial cell (EC) proliferation or migration, whereas C-peptide (10 nmol/L) stimulated EC proliferation by 40% [37].

Serum levels of C-peptide are associated with metabolic syndrome in subjects with type 2 diabetes and in subjects with nephropathy and vascular disease [38]. C-peptide is eliminated from the body by the kidneys [38]. In the period of insulin resistance and early type 2 diabetes, elevated levels of C-peptide circulate through the glomeruli and could deposit in the juxtaglomerular apparatus, and from there could have a mitogenic effect on mesangial cells.

The majority of data described above suggest that C-peptide may promote lesion development in subjects with type 2 diabetes and insulin resistance, while the application of C-peptide in type 1 diabetic subjects who lack C-peptide has been shown to improve diabetic microvascular complications such as diabetic neuropathy. The potential proatherogenic action of C-peptide is not in conflict with the clinical benefits of C-peptide treatment in subjects with type 1 diabetes. Comparing situations, supplementation of C-peptide in type 1 diabetic subjects may be beneficial, whereas in subjects with insulin resistance and type 2 diabetes, increased levels of C-peptide may be harmful. Further studies in animal models of arteriosclerosis are warranted to examine whether the hypothesis of C-peptide's proatherogenic effects holds true *in vivo*. Moreover, additional work is needed to identify the C-peptide receptor to better understand and modulate the physiological function of C-peptide.

In conclusion, C-peptide might contribute to the initiation and progression of vascular lesions in diabetic patients. Fur-

ther understanding of these mechanisms may pave the way for future therapies that target vascular disease in subjects with diabetes.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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