

C-peptide: Roles in diabetes, insulinoma, and hypoglycemia

C-peptide measurements are proving to be a useful aid in the diagnosis of diabetes mellitus, hypoglycemia, and insulinoma. This article provides a concise review of C-peptide production and physiology, explores its application in diabetes testing, and reviews its well-established role in hypoglycemia and insulinoma. A comparison of C-peptide assays on the Siemens Immunoassay systems is also provided.

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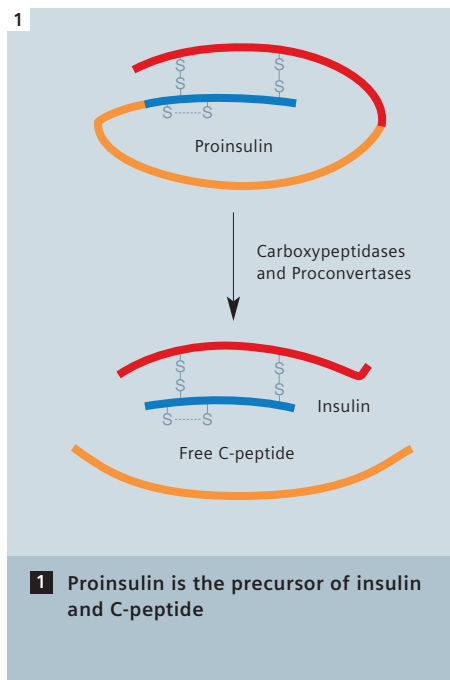
C-peptide was initially thought to be just a by-product of insulin production and biologically inert; however, research findings indicate that it does have important biological activity, especially in relation to diabetes mellitus.

Studies have linked low levels of C-peptide to diabetes mellitus complications, and evidence suggests that maintaining higher levels of C-peptide is especially beneficial for type 1 diabetics.¹ C-peptide measurements have also been used to classify diabetes mellitus and as a marker of pancreatic β -cell function.

C-peptide production

C-peptide is produced by a series of enzymatic cleavages of the precursor molecules preproinsulin and proinsulin. Preproinsulin, a precursor of proinsulin, is produced in the endoplasmic reticulum of pancreatic β -cells in response to elevated blood glucose levels in healthy individuals; it is then cleaved by microsomal enzymes into proinsulin.²

Proinsulin is the precursor of insulin and C-peptide. It consists of the α -chain and β -chain of insulin linked together by C-peptide (Figure 1). C-peptide is composed of 31 amino acids (Figure 2) and facilitates the correct folding of proinsulin to allow the cysteine disulfide bridges between the α -chain and the β -chain of insulin to form. Enzymatic cleavage of proinsulin by proconvertases and carboxypeptidases produces insulin and C-peptide, which are released in equimolar amounts from β -cells into the portal circulation (Figure 1). C-peptide is cleared by the kidney and has a half-life of about 20 to 30 minutes compared to insulin which is cleared through the liver and has a half-life of about 3 to 5 minutes.² The longer half-life, renal clearance, and equimolar release of C-peptide make it an attractive proxy for estimating insulin secretion and β -cell function.



Mechanisms of action and biological activity

While the physiological functions of insulin are well established and understood, the functions of C-peptide are still being established and investigated. C-peptide has been shown to bind to specific cell surface receptors in cultured cells derived from human renal tubules, mesangium, skin fibroblasts, and saphenous vein endothelium.² Data suggest that it acts through G-protein-coupled receptors to activate calcium-dependent signaling pathways via the C-terminal pentapeptide portion of the molecule.³ C-peptide activation of calcium signaling is thought to increase the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$, which has been found to be reduced in patients with the advanced microvascular complications of diabetes mellitus (nephropathy and retinopathy).² The middle segment of

the peptide has also been shown to activate $\text{Na}^+\text{-K}^+\text{-ATPase}$, but not as strongly as the full peptide or the pentapeptide sequence (Figure 2).³ C-peptide's beneficial effects on the microvascular complications of diabetes mellitus are thought to be mediated through endothelial nitric oxide synthase.² In addition to receptor-mediated effects, C-peptide has been shown to produce non-receptor-mediated effects.⁴

Clinical research studies: C-peptide replacement in diabetes mellitus

In addition to the biological activity shown in the laboratory studies, clinical studies have found that replacement of C-peptide in type 1 diabetic patients improves many of the complications associated with this disease. Complications frequently associated with diabetes include retinopathy, nephropathy (characterized by glomerular hyperfiltration and albuminuria), neuropathy, and cardiac disease. Johansson et al.⁵⁻⁷ have performed multiple studies evaluating the effects of the short-term replacement of C-peptide in type 1 diabetic patients. They investigated renal function and whole body glucose uptake in type 1 diabetic patients and found that low-dose C-peptide infusion as compared with sodium chloride infusion significantly increased whole body glucose utilization by 25 percent and decreased glomerular hyperfiltration by 6 percent.⁵ In a follow-up study, they found that administration of C-peptide for one month in type 1 diabetics decreased HbA1c by 9 to 16 percent vs. controls, which suggested that C-peptide improved glycemic control in these patients.⁶ They also observed 40 percent and 55 percent decreases in albuminuria at 2 and 4 weeks, respectively, indicating that C-peptide ameliorated the diabetic nephropathy.⁶

2



2 Sequence for human C-peptide. The pentapeptide sequence is highlighted in blue³



In another follow-up study of type 1 diabetics, they compared the effects of subcutaneous injections of insulin and C-peptide or insulin and placebo for three months. During C-peptide treatment, albuminuria decreased, signs of autonomic neuropathy decreased as evidenced by increased respiratory heart rate variability, and sensory perception improved as evidenced by improved thermal thresholds. These changes were statistically significant as compared with the insulin plus placebo treatment period.⁷ These studies consistently show that C-peptide replacement with or without insulin ameliorates several of the common complications experienced by diabetic patients.

Also supporting the role of C-peptide replacement for the amelioration of diabetic neuropathy are the results of a randomized double-blind placebo controlled

study by Ekberg et al.⁸ This study found that in type 1 diabetic patients, administration of C-peptide for three months improved sensory nerve conduction and vibration perception.⁸ Diabetics are also at high risk for heart disease and often have diastolic dysfunction and myocardial perfusion abnormalities. The effect of C-peptide replacement on myocardial function was evaluated in a study by Hansen et al.⁹ This was a randomized double-blind crossover study that compared the effect of C-peptide infusion with saline infusion on myocardial function in type 1 diabetic patients. They used healthy volunteers as controls. Compared to age-matched controls, diabetic patients had reduced diastolic velocities and myocardial blood volume at baseline. During administration of C-peptide, however, the diastolic velocities and myocardial blood flow of the diabet-

ics increased to the levels observed in the healthy controls. These findings suggest that C-peptide exerts a beneficial effect on myocardial function.⁹ C-peptide clearly has several important biological actions and more research is needed to fully delineate its functions and to characterize the effects and the role of long-term replacement therapy for diabetes mellitus.

Rationale for C-peptide measurement

Since C-peptide and insulin are released in equimolar amounts from the β -cells of the pancreas, the measurement of C-peptide has been used as a marker of β -cell function and an index of insulin secretion. Direct measurements of endogenous insulin by immunoassay are



problematic in patients undergoing insulin therapy because assays can cross-react with the insulin analogs that the patient is taking. Moreover, these assays are affected by the presence of anti-insulin antibodies.^{10, 11} One study found that 36 percent of patients on human insulin developed autoantibodies after 12 months of treatment.¹² Thus, C-peptide measurements represent a better alternative index of insulin secretion and residual β -cell function. In addition, the extensive and variable hepatic extraction of insulin also makes it difficult to accurately estimate insulin secretion from peripheral insulin concentrations.¹³ Monitoring residual β -cell function through the measurement of C-peptide is a strategy endorsed by the American Diabetes Association (ADA), and it recommends the use of C-peptide measurements as the outcome measure in clinical trials investigating methods to preserve β -cell function.^{1, 14}

Additionally, the use of C-peptide and insulin measurements together provides valuable information for the evaluation of hypoglycemia and the diagnosis of insulinoma, and will be examined later in this article.

C-peptide and the classification of diabetes mellitus

The diagnosis of diabetes mellitus is based on the finding of glucose levels measured on two separate occasions exceeding the designated cutoff. Typically, patient history, age of onset, and physical characteristics like body mass index (BMI) are used to determine what type of diabetes a patient has; however, relying on these characteristics for accurate classification can be misleading. Type 1 accounts for 5 to 10 percent, and type 2 accounts for 90 to 95 percent of all diabetics.

Misclassification of patients can lead to a delay in appropriate treatment and unnecessary patient morbidity: a type 1 diabetic not receiving insulin or a type 2 diabetic taking insulin unnecessarily.¹⁵ Type 1 diabetes mellitus is characterized by β -cell destruction, which leads to very low or undetectable levels of C-peptide. In contrast, type 2 diabetes mellitus is associated with insulin resistance and typically initially has normal or elevated levels of C-peptide, which can decrease over the course of the disease.¹⁶

C-peptide levels are typically measured in serum or plasma and can also be

measured in urine. Urine measurements generally require a 24-hour collection and are not as convenient as serum or plasma measurements. The testing protocols used for serum or plasma C-peptide measurements include fasting, random, and stimulated protocols. In the stimulation protocols, the stimulant mimics the rise in glucose induced by meals and gives an estimate of the β -cell function. C-peptide can be stimulated by a glucose load, mixed meal, or glucagon. In type 1 diabetics, little or no increase would be expected above baseline in a C-peptide stimulation test, but type 2 diabetics would be expected to show an increase in levels over baseline. Agreement between C-peptide immunoassay methods is poor, so measurements for comparison should be done with a single method at a single institution.¹⁷

The inaccuracy of clinical diagnosis and the usefulness of C-peptide in the classification of diabetes mellitus are illustrated in a study by Wright-Pascoe et al.¹⁸ on a small series of diabetic patients treated with insulin. Patients were classified on the basis of the criteria of the National Diabetes Data Group and the World Health Organization (WHO) that relies on glucose levels and patient history. This study found that using fasting C-peptide

Some studies have shown that C-peptide replacement with or without insulin ameliorates some complications experienced by diabetic patients.



measurements, only 5 of 13 patients (38 percent) initially classified as having type 1 diabetes mellitus actually had the disease.¹⁸ In a similar study also using C-peptide measurements, 31 percent of type 2 diabetics (diagnosed by American Diabetic Association [ADA] and WHO criteria) were determined to be misclassified as type 1 diabetics.¹⁹ Shiraj et al.²⁰ found that 84 percent of type 1 diabetics, 7 percent of type 2 diabetics, and 4 percent of healthy controls had basal C-peptide levels below a cutoff of 0.6 ng/ml (0.2 nmol/l), indicating that C-peptide can discriminate between these populations.

Similar percentages were obtained for glucagon-stimulated C-peptide below a cutoff of 0.32 nmol/l: 86 percent of type 1 diabetics, 8 percent of type 2 diabetics, and 0 percent of healthy controls had levels below the cutoff.²⁰

Other clinical studies have also shown that C-peptide measurements can be used to aid in the classification of diabetes mellitus.^{21–23} Berger et al.²¹ compared classification of diabetes by random, fasting, and glucagon-stimulated C-peptide measurement with classification by a prior diagnosis of type 1 or type 2 diabetes mellitus made without C-peptide measurements. Patients diagnosed with

type 1 diabetes mellitus had to be on insulin for at least 3 years after diagnosis and patients with type 2 diabetes mellitus had to have been managed without insulin for 3 or more years after initial diagnosis. In this study, 1093 patients and 1449 samples were analyzed. Using receiver operating characteristics (ROC) curve analysis, all three methods were able to distinguish between type 1 and type 2 diabetics (Figure 3).²¹ Random C-peptide also correlated well with both fasting and glucagon-stimulated C-peptide, with *r* values of 0.78 and 0.77, respectively. These data show that a random C-peptide was slightly, but not significantly, better than fasting and glucagon-stimulated C-peptide for the classification of diabetes mellitus.

The optimum cutoffs identified in this study were 1.5 ng/ml (0.5 nmol/l) for random C-peptide, 1.27 ng/ml (0.42 nmol/l) for fasting C-peptide, and 1.81 ng/ml (0.6 nmol/l) for glucagon-stimulated C-peptide.²¹

Consistent with the Berger et al. study, Bakhtadze et al.²², in another diabetes classification study, found that patients diagnosed with type 1 diabetes mellitus had significantly lower ($p < 0.0001$) median fasting plasma C-peptide than patients with type 2 diabetes mellitus or diabetes mellitus that was unclassified. The median values were 0.73 ng/ml (0.24 nmol/l) for type 1 diabetes mellitus, 2.24 ng/ml (0.74 nmol/l) for type 2 diabetes mellitus, and 1.45 ng/ml (0.48 nmol/l) for unclassified diabetes mellitus.

C-peptide protocol	ROC curve (AUC)	Confidence interval (95%)
Random	0.98	0.97 to 0.99
Fasting	0.91	0.89 to 0.93
Glucagon-stimulated	0.92	0.87 to 0.96

3 Receiver operating characteristics (ROC) curve analysis results of random, fasting, and glucagon-stimulated C-peptide tests²¹
AUC, area under the curve



Type 1 diabetes patients who are able to maintain C-peptide levels have fewer complications and better glycemic control.

In this study, they also tested for the presence of islet cell antibodies and HLA-DRQB1 genotypes, both of which are associated with type 1 diabetes. Type 1 diabetic patients were 3.6 times more likely to have islet cell antibodies (autoantibodies) than were type 2 diabetics. Moreover, patients with islet cell antibodies had markedly lower mean fasting plasma C-peptide levels than those without islet cell antibodies (0.73 ng/ml [0.24 nmol/l] vs. 2.1 ng/ml [0.69 nmol/l], $p < 0.0001$). The patients with HLA-DRQB1 genotypes, which are associated with a risk for type 1 diabetes mellitus and negative islet cell antibodies, also had lower median levels of C-peptide than those without the risk-associated genotypes (1.54 ng/ml [0.51 nmol/l] vs. 2.24 ng/ml [0.74 nmol/l]). The authors propose that low C-peptide levels and the presence of a HLA-DRQ1 genotype may be used to differentiate idiopathic type 1 diabetes mellitus (negative for autoantibodies) from type 2 diabetes mellitus, which would not have the HLA-DRQ1 genotype and would have normal or high levels of C-peptide.²² Clearly, although there is some overlap, C-peptide levels can be used to discriminate between type 1 and type 2 diabetes mellitus.

C-peptide and subclassification of type 1 diabetes

C-peptide levels may also be able to subclassify type 1 diabetes. C-peptide levels were found by Tanaka et al.²³ to distinguish a subtype of type 1 diabetes mellitus called fulminant type 1 diabetes mellitus. This condition is characterized by an abrupt onset of symptoms, marked hyperglycemia, severe diabetic ketoacidotic coma, normal or near normal HbA1c levels, absence of autoantibodies, and involvement of the exocrine pancreas with elevated levels of serum pancreatic enzymes. They recruited 125 patients who met the criteria for type 1 diabetes mellitus as defined by the ADA. A subset of these patients ($n = 25$) who were negative for autoantibodies against pancreatic antigens and had HbA1c levels of less than 8.3 percent were diagnosed as having fulminant type 1 diabetes mellitus. The remaining patients were labeled as type 1 diabetes mellitus. They found that the integrated C-peptide levels (Σ C-peptide taken at 0, 30, 60, 90, and 120 minutes during an oral glucose tolerance test) were lower in patients with fulminant type 1 diabetes mellitus at onset and at 1 and 2 years after onset than in patients with type 1 diabetes mellitus.

Consistent with these results, fasting C-peptide levels at onset for fulminant type 1 diabetics were also lower than fasting C-peptide levels for type 1 diabetes mellitus. ROC curve analysis found an area under the curve (AUC) for integrated C-peptide of 0.974 and fasting C-peptide of 0.973 for distinguishing between these two types of diabetes mellitus. The optimum cutoff for integrated C-peptide was less than or equal to 1.63 ng/ml (0.540 nmol/l) and for fasting C-peptide was less than or equal to 0.1 ng/ml (0.033 nmol/l).²³ Several studies present consistent evidence that supports the use of C-peptide as an aid in the classification of diabetes mellitus and suggests that it might be a useful tool in the management of this disease.

C-peptide used as indicator of glycemic control and declining β -cell function

The Diabetes Control and Complications Trial (DCCT) found that C-peptide levels correlate with overall glycemic control. They analyzed patients on the basis of a mixed meal-stimulated C-peptide levels. Patients with stimulated C-peptide greater than 0.6 ng/ml (0.2 nmol/l) had

significantly lower HbA1c levels and were treated with less insulin, indicating better overall glycemic control, as compared with those with levels less than 0.6 ng/ml (0.2 nmol/l) (Figure 4). The DCCT found that there was less retinopathy and nephropathy with preserved β -cell function.¹ Thus, in addition to good glycemic control, the preservation of β -cell function is now a therapeutic goal for type 1 diabetes mellitus, and monitoring β -cell function in these patients via C-peptide levels is a rational approach because insulin measurements would be complicated by the presence of exogenous insulin.

While type 1 diabetes mellitus is associated with markedly decreased β -cell function, following β -cell function in type 2 diabetics may also be important since it can decline over the course of the disease. Kim et al.¹⁶ found that type 2 diabetic patients positive for glutamic acid decarboxylase antibodies (GADA) experienced a decline in C-peptide values over 2 years, indicating a decrease in β -cell function. Fasting C-peptide levels decreased from 1.24 ng/ml (0.41 nmol/l) to 0.95 ng/ml (0.31 nmol/l) and stimulated C-peptide from 1.99 ng/ml (0.66 nmol/l) to 1.61 ng/ml (0.53 nmol/l). In patients without GADA, there was no decrease in C-peptide, indicating no decrease in β -cell function.¹⁶ This suggests that a GADA screen in combination with C-peptide measurements can be used to identify a subset of type 2 patients whose disease is progressing and who may need to be treated more aggressively.

Lower C-peptide levels in type 2 diabetics are also associated with insulin treatment. Siraj et al. found lower basal and glucagon-stimulated C-peptide levels in type 2 diabetic patients treated with insulin than in type 2 diabetics not treated with insulin.²⁰ These data suggest that C-peptide levels might be able to identify type 2 diabetic patients that would be managed better with insulin.

C-peptide and transplant failure

In severe cases of diabetes that have not been managed successfully with insulin, transplantation may be warranted (pancreas or β -cell transplant). After transplantation, monitoring graft function by

monitoring β -cell function has been used to detect transplant failure. Transplant failure is typically monitored with measurements of insulin, glucose, and C-peptide, and in some protocols, graft failure has been defined by a specific level of C-peptide.^{24, 25}

C-peptide levels can be predictive of autoantibodies

Törn et al.²⁶ have found that C-peptide levels can predict the presence of autoantibodies (defined as the presence of at least one of the following: islet cell antibodies, GADA, or tyrosine phosphatase antibodies). They determined that a random C-peptide value of below 0.91 ng/ml (0.3 nmol/l) had a 94 percent positive predictive value for patients with autoantibodies and a value above 2.42 ng/ml

(0.8 nmol/l) had a 72 percent positive predictive value for patients without autoantibodies.²⁶

Hypoglycemia

Hypoglycemia is a symptom associated with a wide range of conditions, and a careful history in addition to the appropriate laboratory tests are necessary for its evaluation (Figure 5). The population most at risk for hypoglycemia is diabetics, particularly type 1 diabetics. It has been estimated that 10 to 30 percent of these patients have one episode of hypoglycemia requiring third-party assistance per treatment year.²⁷ In these patients, the cause of hypoglycemia is usually due to insulin overdose, and C-peptide levels would be low in comparison to insulin levels. Thus, C-peptide measurements

Characteristic	C-peptide ≥ 0.6 – 1.51 ng/ml ≥ 0.2 – 0.5 nmol/l	C-peptide < 0.6 ng/ml < 0.2 nmol/l	p value
n	138	274	
Age (years)	28.2 \pm 6.7	26.1 \pm 7.4	< 0.007
HbA1c	8.3 \pm 1.6	9.2 \pm 1.6	< 0.001
Insulin (U/kg/day)	0.49 \pm 0.20	0.69 \pm 0.24	< 0.001

4 A comparison of type 1 diabetics on the basis of levels of stimulated C-peptide in the Diabetes Control and Complications Trial (DCCT) intensive treatment group¹

Type	Examples
Organ related	Insulinoma, nesidioblastosis, multiple endocrine neoplasia, noninsulinoma pancreatogenic hypoglycemia, severe liver disease, end-stage kidney disease, dialysis, congestive heart failure, acute respiratory failure
Endocrine disorders	Generalized hypopituitarism and hypothalamic deficiency, adrenal failure, hypothyroidism, after removal of pheochromocytoma
Inborn errors of metabolism	Glycogen storage disease, hereditary fructose intolerance, galactosemia, carnitine deficiency, disorders of gluconeogenesis, disorders of mitochondrial β -oxidation
Nonislet tumor	Insulin-like growth factor-secreting tumor (mesenchymal tumors, hemangiopericytomas), lymphoma, myeloma, leukemias
Miscellaneous	Sepsis, starvation, total parenteral nutrition, severe excessive exercise
Drug-induced and dietary toxins	Insulin, sulfonylurea, repaglinide, salicylates, paracetamol, quinine, beta-blockers, alcohol, mushrooms, unripe ackee fruit
Reactive hypoglycemia	Post-gastric surgery, alcohol induced

5 Causes of adult hypoglycemia (modified from reference 31)

can be used to confirm the diagnosis. In the evaluation of a nondiabetic patient with hypoglycemia, C-peptide can be a useful aid in narrowing the differential diagnosis.

C-peptide measurements in the evaluation of hypoglycemia are performed in the context of the 72-hour (prolonged) fast, the gold standard procedure. The prolonged fast, generally an inpatient procedure, involves repeated measurements of insulin, C-peptide, and glucose (every 6 hours) until glucose levels fall to 40 to 45 mg/dL or symptoms of hypoglycemia develop or until 72 hours have elapsed.²⁸⁻³⁰ Elevated C-peptide and insulin levels at the end of the fast point to an insulinoma or sulfonylurea ingestion

as the cause for the hypoglycemia, whereas low C-peptide levels and high insulin levels point to exogenous insulin administration as the cause; low glucose with low insulin and C-peptide levels indicate a non-insulin-mediated hypoglycemia. Sulfonylurea drug use can be determined by measurement of the drug in plasma or urine.

Insulinoma

An insulinoma is a tumor of the β -cells of the pancreas and it is the most common cause of hypoglycemia due to endogenous hyperinsulinism. Age of onset is generally 40 to 60 years, but

insulinomas may present as early as 6 weeks and as late as 70 years. These tumors are typically solitary adenomas and are rarely metastatic. Most are small with 90 percent less than 2 cm in diameter, but they can be as large as 15 cm. Endoscopic ultrasound, transabdominal ultrasound, and intraoperative ultrasound are the preferred imaging modalities, but CT, MRI, and in some cases PET imaging have also been used. These tumors are hard to localize because of their small size and anatomic location and are typically treated by surgical resection.³²

Patients present with symptoms of hypoglycemia at irregular intervals and of varying duration. Symptoms include but are not limited to light-headedness, altered mental status, and abnormal behavior. They commonly occur in the morning after overnight fast, after skipping a meal, or after exercise. However, symptoms can occur at other times as well, and patient presentations are variable. Diagnosis of insulinoma typically involves the 72-hour fast.

The C-peptide suppression test can also be used to confirm the diagnosis since elevated insulin during the test is suggestive of insulinoma.^{27, 28} This test involves the infusion of insulin and the measurement of glucose and C-peptide during the infusion. The basis of the test is the administration of exogenous insulin to suppress endogenous insulin and C-peptide production. Lack of suppression (percent below baseline) is suggestive of endogenous hyperinsulinism likely due to an insulinoma.²⁷

Key features	ADVIA Centaur® Assay	IMMULITE® 2000 Assay
Sample size	50 μ l	25 μ l
Assay format	Two-site murine monoclonal sandwich	Two-site murine monoclonal sandwich
Time to first result	35 minutes	35 minutes
Working range	0.5–30 ng/ml	0.1–15 ng/ml
Expected range (95% confidence interval)		
Serum	0.81 to 3.85 ng/ml	0.9 to 7.1 ng/ml
Urine	<156.46 μ g/day	3.6 to 253 μ g/day
Within-run CV	3.7–4.7%	1.7–2.3%
Total CV	5.1–9.5%	3.3–4.8%

6 Features of C-peptide assays from Siemens Healthcare Diagnostics
CV, Coefficient of variation

Summary

C-peptide is an important, biologically active molecule. Short-term replacement of C-peptide improves some of the complications associated with diabetes mellitus. In addition, type 1 patients who are able to maintain C-peptide levels have fewer complications and better glycemic control. C-peptide measurements play a key role in the evaluation of hypoglycemia and insulinoma, are a useful aid in the classification of diabetes mellitus, and may play a larger role in its management in the future.





References

- 1 Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. *Diabetes mellitus* 2004; 53(1):250-64.
- 2 Marques RG, Fontaine MJ, Rogers J. C-peptide: much more than a byproduct of insulin biosynthesis. *Pancreas* 2004; 29(3):231-8.
- 3 Wahren J, Ekberg K, Johansson J, et al. Role of C-peptide in human physiology. *Am J Physiol Endocrinol Metab* 2000; 278(5):E759-68.
- 4 Ido Y, Vindigni A, Chang K, et al. Prevention of vascular and neural dysfunction in diabetic rats by C-peptide. *Science* 1997; 277(5325):563-6.
- 5 Johansson BL, Sjoberg S, Wahren J. The influence of human C-peptide on renal function and glucose utilization in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1992; 35(2):121-8.
- 6 Johansson BL, Kernell A, Sjoberg S, Wahren J. Influence of combined C-peptide and insulin administration on renal function and metabolic control in diabetes type 1. *J Clin Endocrinol Metab* 1993; 77(4):976-81.
- 7 Johansson BL, Borg K, Fernqvist-Forbes E, Kernell A, Odergren T, Wahren J. Beneficial effects of C-peptide on incipient nephropathy and neuropathy in patients with Type 1 diabetes mellitus. *Diabet Med* 2000; 17(3):181-9.
- 8 Ekberg K, Brismar T, Johansson BL, Jonsson B, Lindstrom P, Wahren J. Amelioration of sensory nerve dysfunction by C-peptide in patients with type 1 diabetes. *Diabetes* 2003; 52(2):536-41.
- 9 Hansen A, Johansson BL, Wahren J, von Bibra H. C-peptide exerts beneficial effects on myocardial blood flow and function in patients with type 1 diabetes. *Diabetes* 2002; 51(10):3077-82.
- 10 Owen WE, Roberts WL. Cross-reactivity of three recombinant insulin analogs with five commercial insulin immunoassays. *Clin Chem* 2004; 50(1):257-9.
- 11 Sapin R. Insulin assays: previously known and new analytical features. *Clin Lab* 2003; 49(3-4):113-21.
- 12 Lauritano AA, Clements RS Jr, Bell D. Insulin antibodies in non-insulin-dependent diabetes: effect of treatment with semisynthetic human insulin. *Clin Ther* 1989; 11(2):268-77.
- 13 Kjems LL, Christiansen E, Volund A, Bergman RN, Madsbad S. Validation of methods for measurement of insulin secretion in humans in vivo. *Diabetes* 2000; 49(4):580-8.
- 14 Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 2003; 26(3):832-6.
- 15 Armitage M, Frier BM, Duncan LJ. A misplaced medal? 50 years of unnecessary insulin treatment. *Br Med J (Clin Res Ed)* 1983; 286(6368):844.
- 16 Kim CS, Nam JH, Nam JS, et al. Clinical and biochemical characteristics of nonobese type 2 diabetic patients with glutamic acid decarboxylase antibody in Korea. *Metabolism* 2006; 55(8):1107-12.
- 17 Wiedmeyer HM, Polonsky KS, Myers GL, et al. International comparison of C-peptide measurements. *Clin Chem* 2007; 53(4):784-7.
- 18 Wright-Pascoe R, Mills J, Choo-Kang E, Morrison EY. The role of C-peptide in the classification of diabetes mellitus. *West Indian Med J* 2000; 49(2):138-42.
- 19 Borg H, Arnqvist HJ, Bjork E, et al. Evaluation of the new ADA and WHO criteria for classification of diabetes mellitus in young adult people (15-34 yrs) in the Diabetes Incidence Study in Sweden (DISS). *Diabetologia* 2003; 46(2):173-81.
- 20 Shiraj E, Reddy S, Scherbaum WA, Abdulkadir J, Hammel JP, Faiman C. Basal and postglucagon C-peptide levels in Ethiopians with diabetes. *Diabetes Care* 2002; 25:453-57.
- 21 Berger B, Stenstrom G, Sundkvist G. Random C-peptide in the classification of diabetes. *Scand J Clin Lab Invest* 2000; 60(8):687-93.
- 22 Bakhtadze E, Borg H, Stenstrom G, et al. HLA-DQB1 genotypes, islet antibodies and beta cell function in the classification of recent-onset diabetes among young adults in the nationwide Diabetes Incidence Study in Sweden. *Diabetologia* 2006; 49(8):1785-94.
- 23 Tanaka S, Endo T, Aida K, et al. Distinct diagnostic criteria of fulminant type 1 diabetes based on serum C-peptide response and HbA1c levels at onset. *Diabetes Care* 2004; 27(8):1936-41.
- 24 Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; 355(13):1318-30.
- 25 Coppelli A, Giannarelli R, Vistoli F, et al. The beneficial effects of pancreas transplant alone on diabetic nephropathy. *Diabetes Care* 2005; 28(6):1366-70.
- 26 Törn C, Landin-Olsson M, Scherstén B. Predictability of C-peptide for autoimmune diabetes in young adult diabetic patients. *Pract Diab Int* 2001; 18(3):83-8.
- 27 Guettier JM, Gorden P. Hypoglycemia. *Endocrinol Metab Clin North Am* 2006; 35(4):753-66, viii-ix.
- 28 Finlayson E, Clark OH. Surgical treatment of insulinomas. *Surg Clin North Am* 2004; 84(3):775-85.
- 29 Service FJ. Hypoglycemic disorders. *N Engl J Med* 1995; 332(17):1144-52.
- 30 Service FJ. Hypoglycemia. In: *ACP Medicine Online* (Dale DC, Federman, eds), 2003. American College of Physicians, New York.
- 31 Gama R, Teale JD, Marks V. Best practice No 173: clinical and laboratory investigation of adult spontaneous hypoglycaemia. *J Clin Pathol* 2003; 56(9):641-6.
- 32 Virally ML, Guillausseau PJ. Hypoglycemia in adults. *Diabetes Metab* 1999; 25(6):477-90.