ARTÍCULO ORIGINAL

Gac Méd Caracas 2020;128(4):537-546
DOI: 10.47307/GMC.2020.128.4.9

# C-peptide as a biomarker in preeclampsia

Drs. Elsa Camacho[1], María Gabriela Matos[1], Rafael Cortéz[2], Anita Israel[1*]

**SUMMARY**

*Preeclampsia (PE) is a syndrome exclusive to human pregnancy and responsible for high perinatal morbidity and mortality, whose manifestations include hypertension, proteinuria, and edema. There is evidence that PE incidence is four times higher in diabetic type 1 women than in non-diabetic women; and increases in women with metabolic syndrome and insulin resistance. C-peptide is a 31 amino acid residue polypeptide part of the proinsulin, which is enzymatically cleaved into insulin and C-peptide molecule. Both are simultaneously secreted in equimolar concentrations into blood, thus serum C-peptide level might reflect real insulin production. Little is known about the relationship between circulating levels of C-peptide and the increase in blood pressure in PE. For this reason, we assessed serum levels of C-peptide, and insulin in women with normal pregnancy and with PE, in a population of 30 Venezuelan women. Serum samples were evaluated by multiplex microsphere analysis (Bio-Plex Pro Assays). Our results show increased insulin and C-peptide serum levels in women with PE. Spearman correlation analysis in all subjects showed a positive association between SBP and C-peptide (r= 0.4841; P<0.0251) and insulin (r= 0.4386; P<0.0221), associated with a positive correlation between SBP and proteinuria and glucose. Our results suggest that an increase in C-peptide could be associated with the development of hypertension and insulin resistance in PE. Therefore, the quantification of this peptide could be a promising biomarker to predict the onset of pregnancy-induced hypertension.*

**Key words**: *Preeclampsia, C-peptide, Insulin resistance, hypertension.*

**RESUMEN**

*La preeclampsia (PE) es un síndrome exclusivo de la gestación humana y responsable de una alta morbi-mortalidad perinatal, cuyas manifestaciones incluyen la hipertensión arterial, proteinuria y edema. Existe evidencia que su incidencia es cuatro veces mayor en mujeres diabéticas tipo 1 que en las mujeres no diabéticas; y también incrementa en mujeres con síndrome metabólico y resistencia a la insulina. El péptido C es un polipéptido de 31 aminoácidos, un subproducto del proceso de la biosíntesis de la insulina, que resulta del clivaje de la proinsulina. Tanto la insulina como el péptido C son secretados en cantidades equimolares y liberados al torrente sanguíneo. Se conoce poco acerca de la relación que existe entre los niveles circulantes del péptido C y los incrementos de la presión arterial en la PE. Por ello, en el presente estudio se cuantificaron los niveles plasmáticos del péptido C e insulina en mujeres*

DOI: https://doi.org/10.47307/GMC.2020.128.4.9

Elsa Camacho: 000-0003-1270-6230, elsacamacho500@gmail.com
María Gabriela Matos: 0000-0001-7290-5237, gbrielamatosu@gmail.com
* Anita Israel: 0000-0003-1812-0759, astern88@gmail.com

[1]Laboratorio de Neuropéptidos, Facultad de Farmacia, Universidad Central de Venezuela.
[2]Servicio de la Ginecología del Hospital Clínico Universitario de Caracas. Caracas, Venezuela.

**Recibido:** 25 de agosto de 2020
**Aceptado:** 05 de octubre de 2020

*con embarazo normal y con PE, pertenecientes a una población de 30 mujeres venezolanas. Se evaluaron muestras de suero mediante el análisis multiplex de microesferas (Bio-Plex Pro Assays). Los resultados muestran incrementos significativos de los niveles plasmáticos del péptido C y la insulina en las mujeres con PE. Mediante el análisis de correlación de Spearman en todas las participantes, la PAS mostró una asociación positiva con el péptido C (r= 0,4841; P<0,0251) y con la insulina (r= 0,4386; P<0,0221), estos asociados a una correlación positiva entre PAS, proteinuria y glucosa. Los resultados sugieren que el incremento del péptido C podría estar asociado al desarrollo de hipertensión y resistencia a la insulina en pacientes con PE. Igualmente, indican que la cuantificación de este péptido circulante podría constituir un biomarcador prometedor para predecir la aparición de la hipertensión inducida por el embarazo.*

**Palabras clave***: Preeclampsia, péptido C, resistencia a la insulina, hipertensión.*

## INTRODUCTION

Preeclampsia (PE) is a complex disease, exclusive to human pregnancy, being the main cause of fetal and maternal morbidity and mortality. For this reason, it has been called the disease of multiple theories, in which both environmental and genetic factors have been associated with its development. It is characterized by new-onset hypertension and proteinuria, which usually arises after 20 weeks of gestation, more frequently in the third trimester and reverses in the postpartum period (1-4). In women with pre-gestational diabetes mellitus (type 1 or type 2), the risks for preeclampsia and pregnant-induced hypertension (PIH) is elevated about four times compared to non-diabetic women (5). Likewise, women with metabolic syndrome are also at high risk of suffering PE (6). Metabolic syndrome and type 2 diabetes are known to be associated with insulin resistance (7). In effect, insulin sensitivity in late normal pregnancy is 45 to 70 percent lower than that of nonpregnant women, and pregnancy itself induces a state of peripheral insulin resistance, the purpose of which is likely to ensure a sustained postprandial supply of glucose to the fetus (8). Indeed, insulin sensitivity in late normal pregnancy is 45 to 70 percent lower than that of nonpregnant women, and it is restored postpartum (9). Several lines of evidence suggest mid-trimester maternal insulin resistance is associated with a significantly increased risk of subsequent preeclampsia. Furthermore, the fact that PE usually begins late in pregnancy, when insulin resistance is highest (10), supports this possible association. However, the mediators responsible for the possible relationship between PE and insulin resistance have not been fully established. Recently, researchers have focused on several new potential mediators of insulin resistance in gestational diabetes mellitus (GDM), including C-peptide, which could be involved in the regulation of insulin resistance, kidney dysfunction, and the increase in blood pressure during pregnancy.

The connector peptide, or C-peptide, is a 31 amino acid residues that result from cleavage of proinsulin in its middle portion, between the segment A and B chains of insulin (11,12). Both C-peptide and insulin are stored within the secretory granules of pancreatic beta cells and are released into the bloodstream in equimolar amounts in response to glucose stimulation (13,14). Its deficiency, together with that of insulin, is the main characteristic of type 1 diabetes mellitus (T1DM), and of the late phase of type 2 diabetes mellitus (T2DM); therefore, quantification of circulating C-peptide in clinical practice is a useful and widely used method to monitor pancreatic beta-cell function, discrimination between T1DM and T2DM, detection of absolute insulin deficiency and the identification of patients with diabetes of adult-onset (15).

C-peptide has been considered for many years as an inactive peptide; however, numerous studies reveal that this molecule plays a physiological role in different cell types (16-18). There is evidence that indicates that C-peptide can suppress several molecular mechanisms involved in the pathophysiology of diabetic nephropathy and, therefore, prevents the onset and progression of diabetes-induced kidney failure (17,20). Likewise, studies in animal models of T1DM reveal that C-peptide has positive effects in the early phase of nephropathy, retinopathy, and neuropathy (21-23), also having a positive impact on the kidneys, retina, and nerves in patients with T1DM and T2DM (24-26). Yasuhi et al. (27) reported that during the second trimester of pregnancy there is a strong correlation

between circulating levels of C-peptide and glucose and the subsequent development of gestational hypertension. However, little is known about the relationship between blood pressure values and circulating levels of C-peptide in PE. Therefore, and to establish the possible correlation between the increase in blood pressure in PE and the alterations in the circulating levels of C-peptide, in this study serum levels of this peptide were determined in a group of Venezuelan healthy pregnant women and PE pregnant women.

## MATERIALS AND METHODS

### Subjects

A descriptive cross-sectional study was conducted in healthy pregnant women and in women with PE who meet the inclusion requirements. The sample consisted of 30 pregnant women (17 healthy pregnant women and 13 pregnant women with PE), aged between 17 and 40 years old and with gestational ages between 28 and 40 weeks, or in the last trimester of pregnancy, who attended the Gynecology Service of the Hospital Universitario de Caracas, Caracas-Venezuela, between the dates of June 2014 to July 2015 were selected. Pre-eclamptic pregnant women were defined as those who presented proteinuria (≥0.3 g/24-hour urine) and/or systolic blood pressure (SBP) values equal to or greater than 140 mmHg, or diastolic blood pressure (DBP) equal to or greater than 90 mmHg, measured twice, 4-6 hours apart.

Exclusion criteria were established: chronic arterial hypertension, autoimmune diseases, type 1 and type 2 diabetes mellitus, angiopathy, mental retardation, psychiatric diseases, neurological disorders, multiple pregnancies, chronic kidney disease, patients with a body mass index equal to or greater than 30 kg/m$^2$, maternal or fetal infection, and congenital anomalies of the fetus. The selected patients underwent a physical examination, blood pressure measurement with the use of a mercury sphygmomanometer, afterward biological samples were taken.

All the volunteers signed and dated the informed consent which was written simply, and carefully read and orally explained by trained personnel, indicating what the study consisted of. Likewise, the participants clarified their doubts with the investigator in charge.

All the procedures used were reviewed and approved by the Bioethics Committee of the Hospital Universitario de Caracas, Caracas-Venezuela, and complied with the Declaration of Helsinki for experimentation studies with human beings (1975 and revised in 1983).

### Biological samples

The pre-analytical conditions were the ones recommended worldwide for this type of assessment: Blood samples were obtained after 8-12 hours of fasting and without a regular diet. The blood sample was obtained by direct venipuncture in the antecubital region with multiple needles (Venojet®), using tubes with a coagulation accelerator (Vacutainer®). Immediately, they were centrifugated at 3000 rpm for 15 minutes. Serum was separated and concentrations of insulin and C-peptide, BUN, and creatinine were determined. Serum aliquots were stored at a temperature of -80 °C until further analysis.

### Blood pressure determination

In the sitting position, the systolic and diastolic blood pressure was determined with the use of a mercury sphygmomanometer, which was placed on the left arm.

### Biochemical methods

Blood glucose values were determined by an enzymatic method, using the commercial kit (Stanbio) with reference values being 70–105 mg/dL. Serum creatinine was determined by a colorimetric method using a commercial kit (Laboratorios Biogamma C.A., Caracas, Venezuela). The results were expressed in mg/dL. Urinary proteins were determined using a commercial kit and the results were expressed as g/24h (0.05-0.15 g/24h) (STAT FAX 3300 Chemistry Analyzer).

### Serum diabetes markers determination

All serum samples were evaluated in duplicate by multiplex microsphere analysis (Bio-Plex Pro Assays, Life Science Group, BIORAD). Briefly, the Bio-Plex® system is based on three technological cores. The first uses up to 100 polystyrenes (5.6 μm) or magnetic (8 μm) microspheres, fluorescently stained encoded with a spectral code (xMAP Technology), which allows the simultaneous detection of 100 different molecules in one of the wells of the 96-well microplate. The second is a flow cytometer with two laser beams associated with an optical system that allows the different molecules attached to the surface of the microspheres to be quantified. The third is a high-speed digital signal processor that handles fluorescence data with high efficiency. With this technique concentrations of C-peptide and insulin were assessed and expressed as pg/mL.

### Statistic analysis

The data were expressed as the mean ± standard error of the mean (S.E.M.). Data distribution was evaluated using the Shapiro-Wilk, Kolmogorov-Smirnov, Jarque-Bera normality tests. The data were analyzed using the GraphPad Instat program 5.0 version (GraphPad Software, Inc). The Mann Whitney U test was used to compare the values of the variables of the groups under study. The correlations between variables were made by the Spearman correlation test. A value of $P<0.05$ was considered statistically significant.

## RESULTS

### Characteristics of the patients

The clinical characteristics of the patients are described in Table 1. No significant differences were found in age, weight, height, gestational age and body mass index between the two groups studied. In contrast, SBP and DBP values were significantly higher in women with PE when compared with control pregnant women.

Table 1

Clinical characteristics of pregnant control and preeclamptic patients

| Parameter | Healthy pregnant women | Preeclamptic patients | P |
|---|---|---|---|
| N=30 | N= 17 | N= 13 | |
| Age (years) | 26.47 ± 0.92 | 28.62 ± 2.70 | NS |
| Weight (kg) | 71.80 ± 2.97 | 74.24 ±2.46 | NS |
| Size (m) | 1.60 ± 0.02 | 1.59 ± 0.01 | NS |
| BMI (kg/m$^2$) | 28.4 ± 0.91 | 29.33± 0.99 | NS |
| Gestational age (weeks) | 35.5 ± 1.08 (28 - 40) | 35.38 ± 0.99 (29 - 41) | NS |
| SBP (mmHg) | 112.90 ± 2.26 | 153.58 ± 2.74*** | <0.0001 |
| DBP (mmHg) | 74.20 ± 1.81 | 103.16 ± 3.61**** | <0.0001 |

NS: no significant

### Laboratory parameters

Table 2 shows significant increases in blood glucose, blood urea nitrogen (BUN), proteinuria, and creatinine in preeclampsia pregnant women when compared to pregnant controls of the same gestational age.

Table 2

Laboratory parameters of healthy and preeclamptic pregnant women

| Parameter | Healthy pregnant women | Preeclamptic patients | P |
|---|---|---|---|
| N=30 | N=17 | N=13 | |
| Glycemia (mg/dL) | 73.50 ± 2.46 | 86.23 ± 5.2* | 0.0305 |
| BUN (mg/dL) | 11.40 ± 1.23 | 17.61 ± 2.10* | 0.0123 |
| Proteinuria (g/24h) | 0.25 ± 0.10 | 4.0 ± 2.4 *** | 0.0001 |
| Serum Creatinine (mg/dL) | 0.54 ± 0.02 | 0.66 ± 0.05* | 0.042 |

### Serum C-peptide and insulin levels in pregnant women with preeclampsia or controls

As seen in Figure 1, a significant increase in serum levels of C-peptide and insulin was observed in the group of PE compared to the control group.

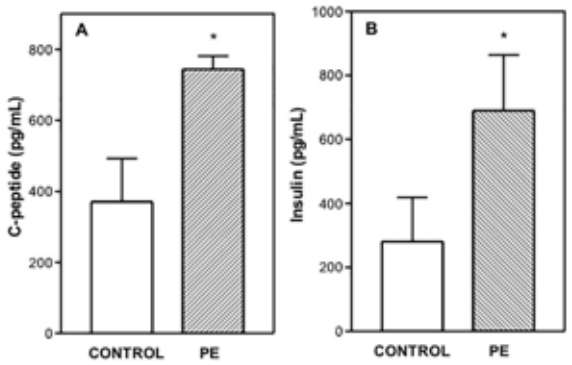


**Figure 1**. Serum levels of C-peptide C (A) and insulin (B) in pregnant control or preeclampsia (PE) women. *P<0.05.

### Correlation between SBP and clinical laboratory parameters

Spearman correlation analysis in all subjects shows that there is a significant positive correlation between SBP vs. proteinuria, glycemia, C-peptide, and insulin (Table 4).

Table 4

Spearman's correlation analysis

| | r | P |
|---|---|---|
| SBP & Proteinuria | 0.8192 | 0.0001 |
| SBP & Glycemia | 0.4150 | 0.0281 |
| SBP & C-peptide | 0.4082 | 0.0251 |
| SBP & Insulin | 0.4386 | 0.0221 |

## DISCUSSION

In the present study, we demonstrate an increased serum level of C-peptide in Venezuelan women in their third trimester of pregnancy

with PE, when compared with those of pregnant normotensive control, which was associated with a significant increase in serum glucose and insulin. Furthermore, SBP values of all the population of the pregnant woman were positively correlated with serum levels of C-peptide, insulin, glucose, and proteinuria, suggesting the coexistence of PE with possible maternal insulin resistance in the last trimester of pregnancy. In this regard, it was shown that in the second trimester of pregnancy, there is a strong correlation between C-peptide levels and fasting and postprandial glucose, and the probability of subsequent development of gestational hypertension, being this association independent of maternal obesity and blood pressure during mid-pregnancy (27). These findings may reflect an amplified response of pancreatic beta-cells to glycemic stimulus, similar to those found in states of insulin resistance. Likewise, in a preliminary study in 301 patients from sub-Saharan Africa, dysregulation of C-peptide was evaluated in the presence of PE and HIV infection, and it was found that serum C-peptide was elevated in PE when compared to non-pregnant and normotensive pregnant women, while the alteration of C-peptide levels was not affected by HIV status (28). Contrary to our findings, El Sayed et al. (29) in a study carried out on 60 pregnant women in Alexandria, and in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cooperative Research Group (30), it was reported that serum C-peptide and insulin levels were significantly lower in women with severe preeclampsia than in women with mild preeclampsia and normotensive pregnant women. Similarly, Valensise et al. (31) reported higher insulin levels, but not higher C-peptide values, in pregnant women undergoing oral glucose tolerance tests who develop gestational hypertension. These disagreements could be due to the difference in the methods used to assess serum C-peptide levels and the involvement of different ethnic groups in the different studies.

Currently, the possible role of the C-peptide in PE has not yet been clarified. PE is a hypertensive disorder that occurs mainly due to placental maladjustment, which involves endothelial dysfunction, which consistently affects the kidney producing proteinuria (32), morphological evidence of glomerular endotheliosis (33), decreased renal plasma flow (RPF) and glomerular filtration rate. Therefore, the increased C-peptide levels in PE could be associated with its renoprotective effect, as its beneficial actions have been described in T1DM complications since it is capable of improving glomerular hyperfiltration and hypertrophy, and proteinuria (34-36), and by improving erythrocytes deformability in T1DM patients by increasing Na+/K+ATPase activity (37,38). Indeed, acute or long-term infusion of C-peptide has been shown to reduce early disturbances of diabetic nephropathy such as hyperfiltration, glomerular hypertrophy, and microalbuminuria. Likewise, in a randomized study in 21 normotensive patients with T1DM and incipient nephropathy, who received combined treatment with C-peptide and insulin for 3 months, it was shown a reduction of albuminuria, indicating that C-peptide can improve renal function in patients with type 1 diabetes mellitus (39). In addition, it was shown in experimentally induced diabetic nephropathy that C-peptide administration reverted the higher fasting serum glucose and lower insulin levels and decreased renal injury markers such as serum urea, creatinine, tumor necrosis factor-alpha (TNF-$\alpha$), angiotensin II, malonyl dialdehyde, total antioxidant, Bcl-2, and NO in renal tissue. Beneficial effects of C-peptide were partially antagonized by L-NAME, indicating that NO partially mediates C-peptide effects, and suggesting that C-peptide deficiency could contribute to renal and metabolic alterations in T1DM (40).

C-peptide exerts important physiological effects on different cells such as endothelial, beta-pancreatic, renal tubule, and fibroblast cells (16,25). There is evidence indicating that plasma C-peptide concentrations provide an indirect measure of insulin secretion reserve (41,42), and have been suggested as an indicator of pancreatic beta-cell function (43). Therefore it has increased the interest in this peptide due to its unique property as an independent marker of insulin biosynthesis and secretion, because unlike insulin, C-peptide is subjected to an insignificant liver first-pass metabolism, making it a special diagnostic tool in diabetes (44). Insulin resistance is known to be a common feature linking

hypertension and hyperinsulinemia (45). Indeed, in cross-sectional studies in fasting patients in the third trimester of pregnancy (46), with postprandial hyperinsulinemia (47), with insulin resistance (48), are conditions identified as being associated with high blood pressure in late pregnancy. Several lines of evidence suggest that PE may be associated with higher degrees of insulin resistance than those that characterize normal pregnancy. Postulated mechanisms, through which insulin resistance could increase blood pressure in pregnancy, as occurs in essential hypertensive patients, include activation of the sympathetic nervous system (49,50), renal sodium retention (51), increased cation transport (52) and association with endothelial dysfunction (53). Similarly, it has been reported that there are higher risks of PE with various conditions associated with insulin resistance including gestational diabetes, polycystic ovary syndrome, obesity, and weight gain (54).

Even though serum C-peptide has been established as a marker of insulin resistance and obesity, specifically in T2DM and basal levels of C-peptide are significantly elevated in patients with metabolic syndrome and diabetes (55), the most recent studies have shown that C-peptide serum levels are strongly and positively associated with stroke events, independent of serum insulin level in diabetics (56). In this sense, it has been postulated that C-peptide levels constitute a better predictor of total mortality or mortality related to cardiovascular events, than serum insulin itself and other measurements derived from insulin resistance in non-diabetic individuals; however, it is not clear what the mechanism of such association (57). Likewise, C-peptide has been postulated as a risk factor for cardiovascular disease (CVD) since its serum levels were significantly and negatively associated with serum levels of high-density lipoproteins (HDL-C) in individuals without diabetes, indicating that C-peptide serum levels are associated with CVD death whose cause, at least in part, may be due to low HDL-C levels (56,58). In a study by Cabrera et al. (59) in which they analyze the association between serum C-peptide and coronary heart disease, it was shown that elevated C-peptide is associated with the incidence of myocardial infarction and coronary heart disease in the general population, in consequence, it could be a predictor of coronary events earlier than impaired fasting glucose. Furthermore, evidence-based clinical, epidemiological, and experimental studies have shown that hyperinsulinemia is associated with different cardiovascular risk factors, such as hypertension (60). Indeed, it is known that hyperinsulinemic and insulin-resistant individuals have increased sympathetic nervous system activity and salt sensitivity, factors which increase the probability that they will develop hypertension. Therefore, since elevated insulin and C-peptide levels coexist in PE, C-peptide may exhibit an insulin-like mechanism promo-ting hypertension during pregnancy (11). That is why pregnancy provides an opportunity to examine the association between increased beta-cell secretion and high blood pressure in subjects with no history of previous hypertension. It could then be inferred that the increase in C-peptide secretion and therefore its circulating levels described in the present study, may constitute a compensatory response to the increase in blood pressure during the last trimester of pregnancy, so its measurement could be an early marker of risk for pregnancy-induced hypertension.

In conclusion, we report that a population of Venezuelan pregnant women with 28-40 weeks of gestation presents higher serum C-peptide levels when compared with normotensive pregnant women. These findings support the concept that serum C-peptide levels could serve as an early predictor of preeclampsia and insulin resistance in pregnant women. However, large-scale and prospective studies are required to assess whether the association between increased blood pressure, proteinuria, and increased C-peptide levels are early markers to predict the development of PE.

## ACKNOWLEDGMENTS

This work was subsidized by the Popular Ministry of Science, Technology and Industries, Science Mission Project, Sub-project 7, ECCV No. 2007001585, the Project for Research Stimulation PEII-20122000760, and the Scientific and Humanistic Development Council with the CDCH Project PI-06-7368-2008-1 and CDCH-UCV AIA-06.8402.2012.

## CONFLICT OF INTEREST

The authors have no conflict of interest.

### REFERENCES


1. Hutcheon JA, Lisonkova S, Joseph KS. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. Best Pract Res Clin Obstet Gynaecol. 2011;25(4):391-403.
2. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122-1131.
3. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20(1):IX–XIV.
4. Roberts JM, Gammill HS. Preeclampsia: Recent insights. Hypertension. 2005;46(6):1243-1249.
5. Persson M, Norman M, Hanson U. Obstetric and perinatal outcomes in type 1 diabetic pregnancies: A large, population-based study. Diabetes Care. 2009;32(1):2005-2009.
6. Briana DD, Malamitsi-Puchner A. Reviews: Adipocytokines in normal and complicated pregnancies. Reprod Sci. 2009;16(10):921-937.
7. Schindler K, Vila G, Hoppichler F, Lechleitner M, Luger A, Anderwald C, et al. The impact of type 2 diabetes on circulating adipokines in patients with metabolic syndrome. Obes Facts. 2012;5: 270-276.
8. Hauth JC, Clifton RG, Roberts JM, Myatt L, Spong CY, Leveno KJ, et al. Maternal insulin resistance and preeclampsia. Am J Obstet Gynecol. 2011;204(4): 327.e321–327.e6.
9. Kühl C. Glucose metabolism during and after pregnancy in normal and gestational diabetic women. 1. Influence of normal pregnancy on serum glucose and insulin concentration during basal fasting conditions and after a challenge with glucose. Acta Endocrinol (Copenh). 1975;79(4):709-719.
10. Kühl C. Insulin secretion and insulin resistance in pregnancy and GDM: Implications for diagnosis and management. Diabetes. 1991;40(Suppl 2):18-24.
11. Wahren J, Shafqat J, Johansson J, Chibalin A, Ekberg K, Jörnvall H. Molecular and cellular effects of C-peptide–New perspectives on an old peptide. Exp Diabesity Res. 2004;5(1):15-23.
12. Wahren J. C-peptide: New findings and therapeutic implications in diabetes. Clin Physiol Funct Imaging. 2004;24(4):180-189.
13. Bhatt MP, Lim YC, Ha KS. C-peptide replacement therapy as an emerging strategy for preventing diabetic vasculopathy. Cardiovasc Res. 2014;104(2):234-244.
14. Ghorbani A, Shafiee-Nick R. Pathological consequences of C-peptide deficiency in insulin-dependent diabetes mellitus. World J Diabetes. 2015;6(1):145-150.
15. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013;30(7):803-817.
16. Zhong Z, Kotova O, Davidescu A, Ehrén I, Ekberg K, Jörnvall H, et al. C-peptide stimulates $Na^+$,$K^+$-ATPase via activation of ERK1/2 MAP kinases in human renal tubular cells. Cell Mol Life Sci. 2004;61(21):2782-2790.
17. Wahren J, Larsson C. C-peptide: New findings and therapeutic possibilities. Diabetes Res Clin Pract. 2015;107(3):309-319.
18. Williams KV, Becker DJ, Orchard TJ, Costacou T. Persistent C peptide levels and microvascular complications in childhood-onset type 1 diabetes of long duration. J Diabetes Complications. 2019;33(9):657-661.
19. Brunskill N. C peptide and diabetic kidney disease. J Intern Med. 2017;281(1):41-51.
20. Recio C, Lazaro I, Oguiza A, Lopez-Sanz L, Bernal S, Blanco J, et al. Suppressor of cytokine signaling-1 peptidomimetic limits progression of diabetic nephropathy. J Am Soc Nephrol. 2017;28(2):575-585.
21. Samnegard B, Jacobson SH, Jaremko G, Johansson BL, Ekberg K, Isaksson B, et al. C-peptide prevents glomerular hypertrophy and mesangial matrix expansion in diabetic rats. Nephrol Dial Transplant. 2005;20(3):532-538.
22. Sjöquist M, Huang W, Johansson BL. Effects of C-peptide on renal function at the early stage of experimental diabetes. Kidney Int. 1998;54(3):758-764.
23. Lim YC, Bhatt MP, Kwon MH, Park D, Lee S, Choe J, et al. Prevention of VEGF-mediated microvascular permeability by C-peptide in diabetic mice. Cardiovasc Res. 2014;101(1):155-164.
24. Shaw JA, Shetty P, Burns KD, Fergusson D, Knoll GA. C-peptide as a therapy for kidney disease: A systematic review and meta-analysis. PLoS One. 2015;10(5):e0127439.
25. Wahren J, Foyt H, Daniels M, Arezzo JC. Long-acting C-peptide and neuropathy in type 1 diabetes: a 12-month clinical trial. Diabetes Care. 2016;39(4):596-602.
26. Qiao X, Zheng H, Zhang S, Liu S, Xiong Q, Mao F, et al. C-peptide is independent associated with diabetic peripheral neuropathy: A community-based study.

Diabetol Metab SynDr. 2017;9:12.

27. Yasuhi I, Hogan JW, Canick J, Sosa MB, Carpenter MW. Mid pregnancy serum C-peptide concentration and subsequent pregnancy-induced hypertension. Diabetes Care. 2001;24(4):743-747.
28. Thakoordeen S, Moodley J, Naicker T. The role of C-peptide in HIV associated pre-eclampsia. Gynecol Obstet. 2018;8 (Conference procedings). doi: 10.4172/2161-0932-C1-025.
29. El Sayed MA, Abdel Gayed AM, El Shalakany AH, Murad YM. Serum insulin and C-peptide levels as markers of pre-eclampsia in pregnant women. Menoufia Med J. 2015;28(1):233-238.
30. HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcome (HAPO) study: Preeclampsia. Am J Obstet Gynecol. 2010;202(3):255. 255.e1–255.e7.
31. Valensise H, Larciprete G, Vasapollo B, Novelli GP, Menghini S, di Pierro G, et al. C-peptide and insulin levels at 24–30 weeks gestation: An increased risk of adverse pregnancy outcomes? Eur J Obstet Gynecol Reprod Biol. 2002;103(2):130-135.
32. Shah DM. Role of the renin-angiotensin system in the pathogenesis of preeclampsia. Am J Physiol Renal Physiol. 2005;288(4):F614-F625.
33. Munkhaugen J, Vikse BE. New aspects of pre-eclampsia: Lessons for the nephrologist. Nephrol Dial Transplant. 2009;24(10):2964-2967.
34. Nordquist L, Moe E, Sjöquist M. The C-peptide fragment EVARQ reduces glomerular hyperfiltration in streptozotocin-induced diabetic rats. Diabetes Metab Res Rev. 2007;23(5):400-405.
35. Kamiya H, Zhang W, Ekberg K, Wahren J, Sima AA. C-Peptide reverses nociceptive neuropathy in type 1 diabetes. Diabetes. 2006;55(12):3581-3587.
36. Ekberg K, Brismar T, Johansson BL, Lindström P, Juntti-Berggren L, Norrby A, et al. C-peptide replacement therapy and sensory nerve function in type 1 diabetic neuropathy. Diabetes Care. 2007;30(1): 71–76.
37. Kunt T, Schneider S, Pfützner A, Goitum K, Engelbach M, Schauf B, et al. The effect of human proinsulin C-peptide on erythrocyte deformability in patients with Type I diabetes mellitus. Diabetologia. 1999;42:465-471.
38. Starzyk D, Korbut R, Gryglewski RJ. Effects of nitric oxide and prostacyclin on deformability and aggregability of red blood cells of rats *ex vivo* and *in vitro*. J Physiol Pharmacol. 1999;50(4):629-637.
39. 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):181-189.
40. Elbassuoni EA, Aziz NM, El-Tahawy NF. Protective effect of C-peptide on experimentally induced diabetic nephropathy and the possible link between C-peptide and Nitric Oxide. Appl Physiol Nutr Metab. 2018;43(6):617-624
41. Faber OK, Binder C. C-peptide: An index of insulin secretion. Diabetes Metab Rev. 1986;2(3-4):331-345.
42. Sherry NA, Tsai EB, Herold KC. Natural history of beta-cell functions in type 1 diabetes. Diabetes. 2005;54(Suppl 2):S32-S39.
43. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006;116(7):1793-1801.
44. Brandenburg D. History and diagnostic significance of C-peptide. Exp Diabetes Res. 2008;576862:7.
45. Bevier WC, Jovanovic-Peterson L, Burns A, Peterson CM. Blood pressure predicts insulin requirement and exogenous insulin is associated with increased blood pressure in women with gestational diabetes mellitus. Am J Perinatol. 1994;11(5):369-373.
46. Kaaja R, Tikkanen MJ, Viinikka L, Ylikorka O. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. Obstet Gynecol. 1995;85(3):353-356.
47. Bauman WA, Maimen M, Langer O. An association between hyperinsulinemia and hypertension during the third trimester of pregnancy. Am J Obstet Gynecol. 1988;159(2):446-450.
48. Caruso A, Ferrazzani S, De Carolis S, Lucchese A, Lanzone A, De Santis L, et al. Gestational hypertension but not preeclampsia is associated with insulin resistance syndrome characteristics. Hum Reprod. 1999;14(1):219-223.
49. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. Diabetes. 1981;30(3):219-225.
50. DeFronzo RA, Cooke C, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, calcium, potassium, and phosphate in man. J Clin Invest. 1975;55(4):845-855.
51. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities: The role of insulin resistance and the sympathoadrenal system. N Engl J Med. 1996;334(6):374-381.
52. Doria A, Fioretto P, Avogaro A, Carraro A, Morocutti A, Trevisan R, et al. Insulin resistance is associated with high sodium-lithium countertransport in essential hypertension. Am J Physiol Endocrinol Metab. 1991;261(6):E684-E691.
53. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. N Engl J Med. 1994;330:1431-1438.
54. Solomon CG, Seely EW. Brief review: Hypertension

in pregnancy a manifestation of the insulin resistance syndrome? Hypertension. 2001;37(2):232-239.

55. Chan WB, Tong PC, Chow CC, So WY, Ng MC, Ma RC, et al. The associations of body mass index, C peptide and metabolic status in Chinese Type 2 diabetic patients. Diabet Med. 2004;21(4):349-353.
56. Li Y, Meng L, Li Y, Sato Y. Associations of serum C-peptide level with body fat distribution and ever stroke in nondiabetic subjects. J Stroke Cerebrovasc Dis. 2014;23(3):e163-169.
57. Min JY, Min KB. Serum C-peptide levels and risk of death among adults without diabetes mellitus. CMAJ. 2013;185(9):E402-408.
58. Li Y, Li Y, Meng L, Zheng L. Association between serum C-peptide as a risk factor for cardiovascular disease and high-density lipoprotein cholesterol levels in nondiabetic individuals. PLoS One. 2015;10(1):e112281.
59. Cabrera de Leon A, Oliva JG, Marcelino I, Almeida D, Alemán J, Brito B, et al. C-peptide as a risk factor of coronary artery disease in the general population. Diab Vasc Dis Res. 2015;12(3):199-207.
60. Reaven GM. Insulin resistance, the insulin resistance syndrome, and cardiovascular disease. Panminerva Med. 2005;47(4):201-210.