doi: 10.5455/medscience.2013.02.8067

Copeptin: May It Be a Novel Biomarker for Insulin Theraphy in Subjects with Diabetes?

Ece Harman[1], Yavuz Dodurga[2], Gulsah Gundogdu[3], Ceylan Ayada[4], Gulten Erken[5], Vural Kucukatay[3], Cigir Biray Avci [6], Osman Genc [4]

[1] Izmir Katip Celebi University, Ataturk Training and Research Hospital, Department of Endocrinology, İzmir-Turkey

[2] Pamukkale University School of Medicine, Department of Medical Biology, Denizli, Turkey

[3] Pamukkale University School of Medicine, Department of Physiology, Denizli, Turkey

[4] Dumlupinar University School of Medicine, Department of Physiology, Kütahya, Turkey

[5] Balikesir University School of Medicine, Department of Physiology, Balikesir, Turkey

[6] EgeUniversity School of Medicine, Department of Medical Biology, İzmir, Turkey

Abstract

Copeptin is a marker about prognosis of acute illnesses, generally. It may be also an indicator associated with treatment of chronic diseases. We aimed to evaluate copeptin levels in rat models with stress, diabetes, diabetes+insulin. Healthy male Wistar rats, about 3 months old, weighing 200–250 g, were obtained from University Animal House. They were housed in small cages at standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12 h light/dark cycle and were fed ad libitum with standard rat chow and tap water. Rats were divided into 4 groups: 8 control (C), 8 diabetic (D), 8 diabetic+insulin (DI) and 8 stress (S) rats. Quantitative measurement of Copeptin was performed using the ELISA method (Uscn Life Sciences, USA), according to the manufacturer's instructions. Copeptin level was statistically significant decreased in D+I groups. There was no difference in copeptin level between the S, D, and C groups. Copeptin may be considered as a new tool for the comparison of the efficiencies of new therapeutic modalities in diabetes.

Key Words: Copeptin, insulin, diabetes

(Rec. Date: Jan 24, 2013 - Accept Date: Mar 07, 2013)

Corresponding Author: Ece Harman, Izmir Katip Celebi University, Ataturk Training and Research Hospital, Department of Endocrinology, Izmir, Turkey.
E-mail: ecarmu@gmail.com, **Phone**: +90 232 2444444 1376; +90 537 2545328

Introduction

Copeptin, which was described for the first time by Holwerda in 1972, is a glycosylated 39–amino acid long peptide with a leucine-rich core segment [1]. Copeptin is co-synthesized with vasopressin, also known as antidiuretichormone, thereby directly mirroring vasopressin levels, but it is very stable in the serum or plasma at room temperature and is easy and robust to measure [2,3].

As a prognostic marker, copeptin levels were independent predictors of survival in critically ill patients suffering from hemorrhagic and septic shock. Copeptin levels also have prognostic implications in diseases other than infections [4]. The underlying mechanism is thought to be the role of copeptin as a measure of a high individual stress level. Interestingly, exercise seems to elicit a significant increase in circulating copeptin concentrations within minutes [5]. The prognostic accuracy of copeptin has been analyzed in sepsis, pneumonia, lower respiratory tract infections, stroke and other acute illnesses [6]. Thereby, copeptin was found to accurately mirror disease severity and to discriminate patients with unfavorable outcomes from patients with favorable outcomes. Copeptin improves the prognostic information provided by commonly used clinical scoring instruments. An accurate prognostic assessment has the potential to guide interventions and effectively plan and monitor rehabilitation and, thus optimize the management of individual patients and the allocation of limited health care resources. Future intervention studies must prove the value of copeptin in clinical decision making and in improving the overall medical management of patients with acute illnesses [7].

Chronic psychosocial stress is frequently accompanied by increased plasma levels of arginine vasopressin (AVP), which is an amplifier of the hypothalamic pituitary-adrenal (HPA) axis along with corticotropin-releasing hormone (CRH) [8]. AVP action has been linked to liver glycogenolysis and insulin and glucagon secretion [9,10].

Copeptin is a cleavage product of the C-terminal part of the AVP precursor that is produced in equimolar amounts with AVP, a process similar to the generation of insulin and C peptide. In contrast to AVP, copeptin is stable, has a long half-life, and is not bound to platelets [11]. Thus, using a validated sandwich assay to measure copeptin in plasma, we tested the hypothesis that plasma copeptin would be associated with stress, diabetes, and treatment of diabetes. Results of this study may further answer important questions in terms of function(s) of copeptin.

Material and Methods

Animal groups

Healthy male Wistar rats, about 3 months old, weighing 200–250 g, were obtained from University Animal House. They were housed in small cages at standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12 h light/dark cycle and were fed ad libitum with standard rat chow and tap water. All experimental procedures in animals were performed under appropriate regimes with veterinary services and licensed projects. Rats were divided into 4 groups: 8 control (C), 8 diabetic (D), 8 diabetic+ insülin (DI) and 8 stress (S) rats.

Stress group: The rats were kept in transparent plastic cages (30x15x10 cm), each containing only one rat, exposed to a 12:12 light/dark cycle. The rats were exposed to movement restriction, without anesthesia for two hours in a day during ten days.

Diabetes mellitus group: Diabetes was induced in rats (200–250 g) with a single intraperitoneal injection of Streptozotocin (STZ) (60 mg/kg body weight, Sigma, St. Louis, USA) dissolved in a phosphate buffer. After three days of STZ administration, blood was collected from tail vein and samples were analyzed for blood glucose by using a glucometer (Aqua-Check, Roche, Basel, Switzerland). Animals with fasting blood glucose levels (BGLs) greater than 300 mg/dL were considered as diabetic.

Diabetes mellitus+insulin group: Diabetes+insulin [T1DM + I; treated with single dose of 60 mg/kg of STZ (i.p.) and insulin (3 UI/day/rat) (i.p.)] groups. Insulin (3 UI/day/rat; i.p) was administered to T1DM + I rats for two weeks. No additional treatments were given to all rats. Blood was collected from tail vein and samples were analyzed for blood glucose by using a glucometer once a week.

Control group: In this group, animals were not done any manipulation.

After two weeks, the study was terminated. At termination, blood samples of each animal was collected for all animals in whole groups which were anaesthetized via intraperitoneal injection of xylazine (5 mg/kg) and ketamine (90 mg/kg) and all serum samples were stored at -80 °C.

Copeptin Measurement via ELISA

Blood samples were collected into plain tubes (1.5 mL) and serum was separated via centrifugation at 1500 rpm for 15 min. Samples were stored at -86 °C until analysis. In order to reduce inter assay variance all samples were analyzed in 1 assay; repeated freeze-thaw cycles were avoided. Quantitative measurement of Copeptin was performed using the ELISA method (Usen Life Sciences, USA), according to the manufacturer's instructions. A value of the triplicate readings for every standard, and the sample and control groups was calculated. Absorbance was measured using a computer-controlled spectrophotometric plate reader (Multiscan FC-ThermoScientific) at a wave length of 450 nm. The concentration of Copeptin in the serum samples was interpolated from a standard curve drawn with the assistance of logarithmic transformation.

Statistical analysis

All values are expressed as the mean ± SD. The data were evaluated by one-way analysis of variance and post hoc least significant difference (LSD) for multiple comparisons of the means. p values less than 0.05 were considered significant.

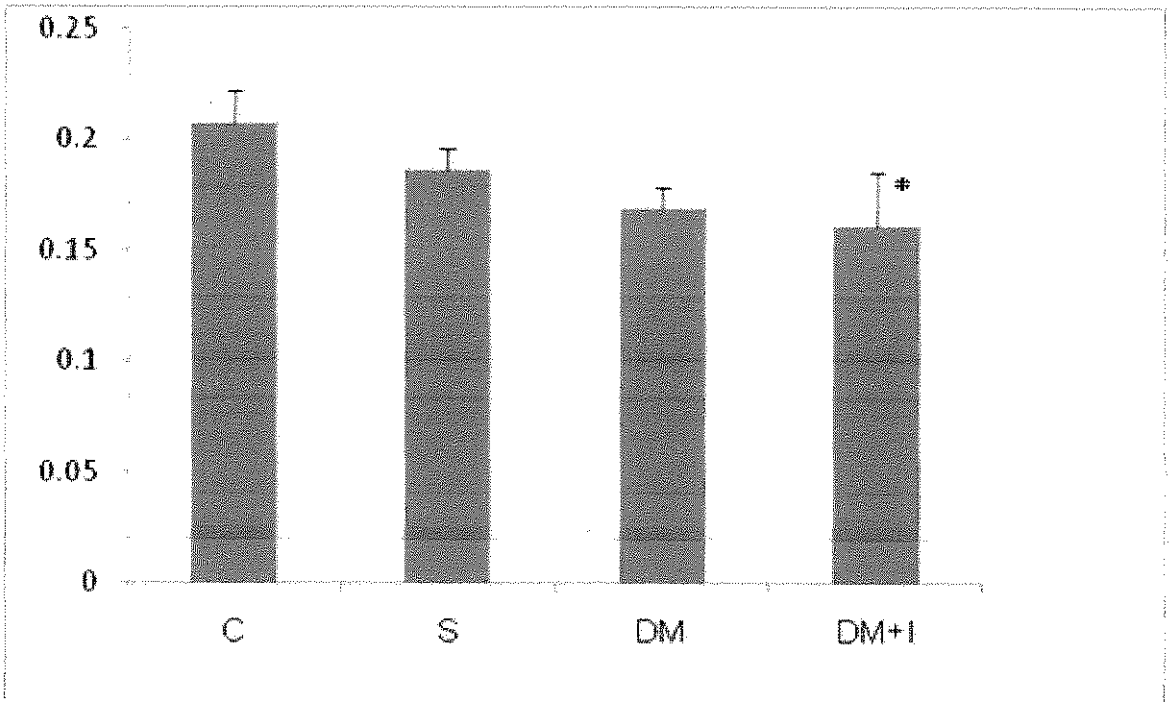
Results

We measured rat copeptin level in the rat serum from four groups as S, D, D+I and C. The mean serum concentrations of copeptin in groups are presented in Table 1. Copeptin level was statistically significantly low in D+I groups compared with control samples ($p<0.05$) (Figure 1). Copeptin levels were similar in the S, D, and C groups.

Table 1. The mean serum concentrations of copeptin in groups

	N	Mean (pmol/L)	Std. Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
S	8	0.186	0.013972	0.153085359	0.219164641
DM	8	0.169	0.009921	0.145540285	0.192459715
DM+I	8	0.161	0.009153	0.139106675	0.182393325
C	8	0.208	0.024358	0.150027141	0.265222859
TOTAL	32	0.180	0.006706	0.166685485	0.193814515

Figure 1. Copeptin levels in the groups



Discussion

Diabetes is a multifactorial disease characterised by chronic hyperglycaemia resulting from defects in insulin secretion and/or insulin action [12]. These defects have detrimental effects on the utilization of glucose and free fatty acids (FFA) by muscle, liver and adipose tissue. Factors that influence the development of chronic hyperglycaemia include genetic abnormalities; environmental causes, such as nutritional excess and lack of activity; increased hepatic production of glucose; increases in visceral fat; atherogenicdyslipidaemias; increased adiposity of muscle and liver; β-cell dysfunction; and, an imbalance of oxidation and inflammation, natural processes involved in maintaining a physiologic state. Understanding pathophysiologic processes that trigger the development of diabetes mellitus provides opportunities for designing pharmacologic interventions to target mediators of these processes. Modulation of oxidative-inflammatory cascade mechanism involved in metabolic and immune processes can improve glucose metabolism,insulin resistance, improve vascular function [13].

There are some alternative agents for treatment of diabetes. In type 2 diabetic patients with baseline HbA1C 8.5-9.0%, monotherapy with metformin (4.5) or sulfonylureas (6.7) reduces the HbA1c by~ 1.5-2%. Studies in which bedtime insulin is added to a sulfonylurea alone [14]

or to combined metformin/sulfonylurea treatment have also been shown to be effective [15,16]. In some studies, it was shown effectiveness of the antihyperglycemic treatment on oxidative stress and inflammation [17].

AVP is a key neurohormone in the human body, with numerous important physiologic activities including regulation of blood pressure, fluid volume, and serum osmolality. Copeptin is considered to be a reliable and clinically useful surrogate marker for AVP. Enhorning S et al found elevated copeptin to be associated with incident diabetes mellitus independently of all clinically used diabetes mellitus confounders including plasma glucose and insulin [18]. Further, in the same population, they found elevated copeptin to be cross-sectionally associated with the metabolic syndrome, obesity, hypertension, high C-reactive protein [18]. Prevention studies in humans and animals suggested a role for AVP in diabetic nephropathy, microalbuminuria and renal failure, metabolic syndrome [19, 20, 21, 22]. Moreover, copeptin is associated with cardiovascular events among patients with end-stage renal disease and type 2 diabetes [23]. In other study, copeptin levels strongly associated with stroke, sudden death, combined cardiovascular events, and mortality in hemodialysis patients with type 2 diabetes. The fact that copeptin was elevated many years before the development of overt diabetes mellitus and abdominal obesity, and remained significantly associated independently of a broad range of potential confounders, suggests a primary role for the AVP system in the pathophysiology of diabetes mellitus and abdominal obesity.

In this preliminary study, we aimed to evaluate copeptin levels in rat models with stress, diabetes, diabetes+insulin.Our data suggest that activity of the AVP system has potential importance in the treatment of diabetes mellitus. Results of this study may further answer important questions in terms of function(s) of copeptin.

Conclusion

Copeptin is an indicator for chronic diseases other than acute illness, too. Copeptin may be considered as a new tool for the comparison of the efficiencies of new therapeutic modalities in diabetes.

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

1. Holwerda DA. A glycopeptide from the posterior lobe of pig pituitaries. Isolation and characterization. Eur J Biochem. 1972;28(3):334–9.
2. Struck J, Morgenthaler NG, Bergmann A. Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides. 2005;26(12):2500–4.
3. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem. 2006; 52(1):112–9.
4. KatanM, Müler B, Crain MC. Copeptin: A new and promising diagnostic and prognostic marker. Critical Care. 2008;12(2):117-8.
5. Katan M, Morgenthaler N, Widmer I, Puder JJ, Konig C, Muller B, Christ-Crain M. Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. Neuro Endocrinol Lett. 2008;29(3):341-6.
6. Morgenthaler NG, Struck J, Jochberger S, Dunser MW. Copeptin: Clinical use of a new biomarker. Trends in endocrinology and metabolism: TEM. 2008,19(2):43-9.
7. Katan M, Fluri F, Morgenthaler NG, Schuetz P, Zweifel C, Bingisser R, MüllerK, Meckel S, Gass A, Kappos L, Steck AJ, Engelter ST, Müller B, Christ-Crain M. Copeptin: a novel, independent prognostic marker in patients with ischemic stroke. Ann Neurol. 2009:66(6):799-808.
8. Antoni FA. Vasopressinergic control of pituitary adrenocorticotropin secretioncomes of age. Front Neuroendocrinol. 1993;14(2):76-122.
9. Keppens S, de Wulf H. The nature of the hepatic receptors involved in vasopressin-induced glycogenolysis. Biochim Biophys Acta. 1979;588(1):63-9.
10. Abu-Basha EA, Yibchok-Anun S, Hsu WH. Glucose dependency of argininevasopressin-induced insulin and glucagon release from the perfused rat pancreas.Metabolism. 2002;51(9):1184-90.
11. Struck J, Morgenthaler NG, Bergmann A. Copeptin, a stable peptide derived fromthe vasopressin precursor, is elevated in serum of sepsis patients. Peptides. 2005;26(12):2500-4.
12. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003;26(Suppl. 1):S5-20.
13. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115(5):1111-9.

14. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, CreagerMA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J,Vogel R. International Brachial Artery Reactivity Task Force. Guidelines for theultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. J Am Coll Cardiol. 2002;39:257-65.

15. Mazzone T, Meyer PM, Feinstein SB, Davidson MH, Kondos GT, D'Agostino RB, Sr, Perez A, Provost JC, Haffner SM. Effect of pioglitazone compared with glimepirideon carotid intima-media thickness in type 2 diabetes: a randomized trial. JAMA. 2006;296(21):2572-81.

16. Musi N, Fujii N, Hirshman MF, Ekberg I, Fröberg S, Ljungqvist O, Thorell A, Goodyear LJ. AMP-activated protein kinase (AMPK) is activated in muscle ofsubjects with type 2 diabetes during exercise. Diabetes. 2001;50(5):921-7.

17. Joya-Galeana J, Fernandez M, Cervera A, Reyna S, Ghosh S, Triplitt C,. Musi N, DeFronzo RA, Cersosimo E. Effects of insulin and oral anti-diabetic agents onglucose metabolism, vascular dysfunction and skeletal muscle inflammation in type2 diabetic subjects. Diabetes Metab Res Rev. 2011;27(4):373-82.

18. Enhörning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G, Struck J, Morgenthaler NG, Bergmann A, Lindholm E, Groop L, Lyssenko V, Orho-Melander M, Newton-Cheh C, Melander O. Plasma copeptin and the risk of diabetes mellitus. Circulation. 2010;121(19):2102-8.

19. Enhörning S, Bankir L, Bouby N, Struck J, Hedblad B, Persson M, Morgenthaler NG, Nilsson PM, Melander O. Copeptin, a marker of vasopressin, in abdominalobesity, diabetes and microalbuminuria: the prospective Malmö Diet and CancerStudy cardiovascular cohort. Int J Obes (Lond). 2012 May 22. doi:10.1038/ijo.2012.88. [Epub ahead of print] PubMed PMID: 22614056.

20. Bardoux P, Bichet DG, Martin H, Gallois Y, Marre M, Arthus MF, Lonergan M,Ruel N, Bouby N, Bankir L. Vasopressin increases urinary albumin excretion inrats and humans: involvement of V2 receptors and the renin-angiotensin system.Nephrol Dial Transplant. 2003;18(3):497-506.

21. Bardoux P, Martin H, Ahloulay M, Schmitt F, Bouby N, Trinh-Trang-Tan MM, Bankir L. Vasopressin contributes to hyperfiltration, albuminuria, and renalhypertrophy in diabetes mellitus: study in vasopressin-deficient Brattleborорats. Proc Natl Acad Sci U S A. 1999;96(18):10397-402.

22. Enhörning S, Struck J, Wirfält E, Hedblad B, Morgenthaler NG, Melander O.Plasma copeptin, a unifying factor behind the metabolic syndrome. J Clin Endocrinol Metab. 2011;96(7):E1065-72.

23. Fenske W, Wanner C, Allolio B, Drechsler C, Blouin K, Lilienthal J, Krane V; German Diabetes, Dialysis Study Investigators. Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus. J Am Soc Nephrol. 2011;22(4):782-90.