High serum copeptin may be a marker of an increased carotid intima-media thickness in asymptomatic patients with type 1 diabetes.

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Research Letter

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Abstract

Copeptin is a stable peptide derived from cleavage of the precursor of arginine-vasopressin (AVP), [1,2] that can be easily measured by using a simple rapid assay. Copeptin has been related with an increased incidence of stroke and cardiovascular mortality in individuals with diabetes, mainly type 2 [3,4]. It is also a risk predictor for end-stage renal disease, coronary heart disease, and all-cause mortality in people with type 1 diabetes (T1DM) [5]. However, we have recently reported that copeptin does not appear to be a reliable marker for the screening of asymptomatic peripheral arterial disease in a population of patients with T1DM [6]. We here explore the putative relationships between serum copeptin level and carotid intima-media thickness (cIMT) in asymptomatic T1DM-patients comprehensively investigated for subclinical carotid atherosclerosis.

Introduction

Copeptin is a stable peptide derived from cleavage of the precursor of arginine-vasopressin (AVP), [1,2] that can be easily measured by using a simple rapid assay. Copeptin has been related with an increased incidence of stroke and cardiovascular mortality in individuals with diabetes, mainly type 2 [3,4]. It is also a risk predictor for end-stage renal disease, coronary heart disease, and all-cause mortality in people with type 1 diabetes (T1DM) [5]. However, we have recently reported that copeptin does not appear to be a reliable marker for the screening of asymptomatic peripheral arterial disease in a population of patients with T1DM [6]. We here explore the putative relationships between serum copeptin level and carotid intima-media thickness (cIMT) in asymptomatic T1DM-patients comprehensively investigated for subclinical carotid atherosclerosis.

Design And Methods

Study Population

We conducted an observational cross-sectional study including 60 asymptomatic T1DM-patients, from a larger cohort (*clinicaltrials.gov Identifier: NCT02910271*) designed to comprehensively address their subclinical atherosclerosis profile [7]. T1DM required age at onset of diabetes <30 yr-old, previous episode of ketoacidosis or diabetic autoimmunity, and mandatory use of insulin for survival, as defined by ADA criteria. Exclusion criteria were: i) symptomatic intermittent claudication according to Edinburgh Claudication Questionnaire [8]; ii) previous diagnosis of peripheral artery disease, diabetic foot or leg amputation; iii) previous diagnosis of cerebrovascular disease iv) end-stage renal disease; and v) ongoing pregnancy. A detailed description of this trial has been reported elsewhere [7]. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and was approved by Ramón y Cajal ethics committee (Date of approval: January 22, 2016; Reference number: 464/15). Written consent has been obtained from each subject after full explanation of the purpose and nature of all procedures used.

Clinical, biochemical variables and copeptin assay

We review the medical history and recorded clinical parameters related to T1DM and cardiovascular risk factors of all patients at recruitment. Then, participants were submitted to a complete anthropometric evaluation that included weight, height, waist circumference, and hip circumference measurements. A fasting blood sample and urine collection were collected to assess renal function and to measure the urinary albumin-to-creatinine ratio, serum lipids, and HbA_{1c}. Technical characteristics of assays used for biochemical measurements have been described elsewhere in detail [7].

Copeptin concentration was measured in fasting serum samples. Blood samples were left to clot for one hour and then centrifuged at 1500 $\times g$ for 10 min. Then, serum was kept frozen at -80°C until assayed. After thawing, serum copeptin concentrations were measured in duplicate using a commercial ELISA kit from a single manufacturer and assay lot (*High Sensitive ELISA kit for Copeptin, CPP HEA365Hu Cloud-Clone, USA*) The lower limit of detection of the assay was 2.63 pmol/L and its intra- and inter-assay coefficients of variation were below 10% and 12%, respectively. The technician in charge of these assays was blinded to patient's features.

Assessment of carotid ultrasound examination

All study participants underwent a carotid ultrasound examination. Patients rested in supine position for at least 10 min before measurements were taken. Vascular tests were conducted under standardized conditions after an overnight fasting to avoid the possible interference of a postprandial surge in glucose levels. cIMT was calculated for both common carotid arteries of each patient, and the means of such measurements was used for analysis. A Toshiba Nemio model SSA-550A Basic Diagnostic Ultrasound System (*Toshiba Medical System S.A., Alcobendas, Madrid, Spain*) with a 7.5-MHz probe was used in these assessments. Common carotid, internal carotid, external carotid, and vertebral arteries were also scanned for the presence of carotid plaques (CP), defined as IMT $\geq$ 1.5 mm protruding into the lumen [7].

Statistical analysis

Data are shown as means $\pm$ SD and counts (percentages). Normality of continuous variables was assured as needed by applying logarithmic transformation and checked using the Kolmogorov-Smirnov test. Because copeptin concentrations were not normally distributed, their results were expressed as medians [interquartile range]. We used χ^2 or Fisher's exact tests for categorical variables, and Student's *t* or Mann–Whitney *U* tests for continuous variables as appropriate. We used Spearman's correlations to evaluate the association between clinical and biochemical variables, and copeptin concentrations. We used Pearson's correlations between cIMT and continuous variables of interest.

Finally, a multiple stepwise linear regression model was used to explore the main determinants of cIMT levels among those variables with a *P* value < 0.10 in univariate analyses. Statistical significance was set at a *P* value < 0.05.

Participants were classified as a function of their normal ($\leq 95^{\text{th}}$ percentile) or abnormal ($>95^{\text{th}}$ percentile) carotid IMT values – using normative values from the healthy Spanish population stratified by age and sex [9] – and into high copeptin (HighCp) and low copeptin (LowCp) subgroups – using 13 pmol/L concentration as cut-off value, which is the upper limit of normality of copeptin levels in healthy adults under normoosmotic conditions [2].

Results

The clinical and biochemical characteristics of the study cohort are summarized in the Table 1. The study population's age was 43 ± 10 yrs, the duration of T1DM was 28 ± 9 yrs, and had a mean HbA_{1c} of $7.7 \pm 1.2\%$ (60 ± 13 mmol/mol). Eight patients (13%) showed asymptomatic CP in the internal carotid artery. Median copeptin level of the whole population of study participants was 14.0 [11.3] pmol/L. Those individuals with abnormal cIMT values had higher body mass index (BMI), waist circumference (WC), diastolic blood pressure (BP) and low-density lipoprotein (LDL)-cholesterol concentrations than their counterparts with normal cIMT values. Considering all patients as a whole, cIMT correlated with age ($r = 0.423$, $P = 0.001$), duration of T1DM ($r = 0.379$, $P = 0.003$), BMI ($r = 0.385$, $P < 0.003$), WC ($r = 0.409$, $P = 0.002$), fat mass percentage with respect to total body weight ($r = 0.323$, $P = 0.014$), and systolic BP ($r = 0.328$, $P = 0.012$).

Patients in the HighCp group were older, had higher triglyceride concentrations, and HDL-cholesterol concentrations and serum sodium levels than those subjects in the LowCp group. In addition, copeptin levels showed an inverse correlation with serum sodium ($r = -0.281$, $P = 0.032$). HighCp-patients showed greater cIMT values than LowCp subjects (0.74 ± 0.15 vs 0.66 ± 0.13 mm, respectively, $P = 0.036$). Age, duration of T1DM, copeptin levels subgroups (coded as: low = 0 / high = 1), BMI, systolic BP, the percentage of fat mass and LDL-cholesterol concentrations were introduced as independent variables into a multivariate regression analysis. The stepwise model (R^2 : 0.277; $P < 0.001$) retained systolic BP [β : 0.321 (95%CI: 0.095 to 0.542)], duration of T1DM [β : 0.332 (95%CI: 0.104 to 0.546)], and copeptin subgroup [β : 0.272 (95%CI: 0.090 to 0.998)] as significant predictors of the variability in cIMT in our study population.

Discussion

In our series, serum copeptin is associated with subclinical carotid atherosclerosis in asymptomatic adult patients with T1DM. In conceptual agreement, copeptin has been previously related to atherosclerosis and all-cause mortality in T1DM [5]. We cannot determine by our study design if the relationship between copeptin and carotid atherosclerosis is causal or results from an unknown confounder factor. However, a potential pathophysiological link between copeptin and carotid disease is plausible. First, carotid baroreceptor reflex regulates short-term variations in BP through autonomic adjustments in heart rate, cardiac output, and peripheral resistance. Second, copeptin derives from cleavage of AVP, and AVP is mostly released in response to the decrease in BP detected by carotid baroreceptors. Lastly, an increased cIMT reduces carotid baroreflex sensitivity, even after adjusting for BP, age, BMI, sex, and smoking history, in healthy individuals.[10]

Limitations of our study such as its small sample size and cross-sectional design warrant larger studies. However, if our results were confirmed, serum copeptin may improve the stratification of cardiovascular risk in patients with T1DM. Further research is needed to determine the value in identifying carotid disease of this biochemical marker in patients with T1DM.

Declarations

Compliance With Ethical Standards

- **Funding:** This study was funded by grants from Fondo de Investigación Sanitaria (PI1400649, PI151686, PIE1600050 & PI1801122) of the Instituto de Salud Carlos III, Spanish Ministry of Economy and Competitiveness. The funding organizations played no role on the study design, collection, analysis, and interpretation of data, on the writing of the report; nor on the decision to submit the report for publication.
- **Conflicts of interest:** All the authors do not declare any conflict of interest. The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.
- **Ethical approval:** The study protocol was approved by Ramón y Cajal ethics committee (Date of approval: January 22, 2016; Reference number: 464/15). All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.
- **Informed consent:** Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1. Demographic and clinical characteristics of all study participants, and as a function of carotid IMT values and copeptin concentrations.

Variable	Carotid IMT					Copeptin levels		
	<i>All patients</i> (n = 60)	* _a	Abnormal (n = 26)	Normal (n = 34)	<i>P value</i>	** _a	High (n = 33)	Low (n = 27)
Women / Men, <i>n</i> (%)	19 (32) / 41 (68)		6 (23) / 20 (77)	a 13 (38) / 21 (62)	0.211		12 (36) / 21 (64)	7 (26) / 20 (74)
	(21–44) / (56–79)		(11–42) / (58–89)	(24–55) / (45–76)			(22–53) / (47–78)	(13–45) / (55–88)
Age, <i>years</i>	43 ± 10		44 ± 9	43 ± 11	0.551		46 ± 11	40 ± 7
	(40–46)		(40–48)	(39–47)			(42–50)	(37–43)
Duration of diabetes, <i>years</i>	28 ± 9		29 ± 8	26 ± 9	0.152		29 ± 10	26 ± 7
	(26–30)		(25–31)	(23–29)			(25–33)	(23–29)
Microangiopathy, <i>n</i> (%)	30 (50)		13 (50)	17 (50)	1.000		17 (52)	13 (48)
	(38–62)		(32–70)	(34–66)			(35–68)	(31–66)
Cardiovascular disease, <i>n</i> (%)	3 (5)		2 (8)	1 (3)	0.574		3 (9)	0 (0)
	(4–18)		(2–24)	(1–15)			(3–24)	(0–12)
Smoking habit, <i>n</i> (%)	26 (43)		9 (35)	17 (50)	0.233		14 (42)	12 (44)
	(32–56)		(19–54)	(34–66)			(27–59)	(28–63)
Antiaggregant therapy, <i>n</i> (%)	9 (15)		4 (15)	5 (15)	1.000		9 (27)	0 (0)
	(8–26)		(6–34)	(7–30)			(15–44)	(0–12)
Statin therapy, <i>n</i> (%)	32 (53)		16 (62)	16 (47)	0.265		19 (58)	13 (48)
	(41–65)		(43–78)	(31–63)			(41–73)	(31–66)
Antihypertensive therapy, <i>n</i> (%)	21 (35)		11 (42)	10 (29)	0.299		12 (36)	9 (33)
	(24–48)		(26–61)	(17–46)			(22–53)	(19–52)
Use of ACE-I or ARB, <i>n</i> (%)	21 (35)		11 (42)	10 (29)	0.299		12 (36)	9 (33)
	(24–48)		(26–61)	(17–47)			(22–53)	(19–52)
Use of diuretics, <i>n</i> (%)	8 (13)		5 (19)	3 (9)	0.275		5 (15)	3 (11)
	(7–24)		(9–38)	(3–23)			(7–31)	(4–28)
Body mass index, <i>kg/m²</i>	26 ± 4		27 ± 3	25 ± 4	0.011		26 ± 4	25 ± 4
	(27–29)		(26–28)	(24–26)			(25–27)	(23–27)
Obesity, <i>n</i> (%)	7 (12)		5 (19)	2 (6)	0.223		6 (18)	1 (4)
	(6–22)		(9–39)	(2–19)			(9–34)	(1–18)
Waist circumference, <i>cm</i>	97 ± 12		93 ± 9	85 ± 12	0.008		90 ± 12	88 ± 11
	(94–100)		(89–97)	(81–89)			(86–94)	(84–92)
Fat mass, %	25 ± 8		26 ± 6	24 ± 9	0.402		26 ± 8	23 ± 8
	(23–27)		(24–28)	(21–27)			(23–29)	(20–26)
Systolic BP, <i>mmHg</i>	125 ± 16		130 ± 18	122 ± 15	0.076		124 ± 15	127 ± 18
	(121–129)		(123–137)	(117–127)			(119–129)	(120–134)
Diastolic BP, <i>mmHg</i>	73 ± 9		76 ± 9	71 ± 8	0.047		71 ± 10	76 ± 7
	(71–75)		(72–80)	(68–74)			(67–75)	(73–79)
Heart rate, <i>bpm</i>	76 ± 12		76 ± 12	77 ± 12	0.741		74 ± 12	79 ± 12
	(73–79)		(71–81)	(73–81)			(70–78)	(74–84)

eGFR, mL/min/1.73 m ²	89 ± 19	87 ± 22	91 ± 17	0.483	85 ± 21	96 ± 16
	(84–94)	(78–96)	(85–97)		(78–92)	(90–102)
Plasma urea, mmol/L	5.7 ± 2.3	5.9 ± 2.9	5.5 ± 1.5	0.794	5.8 ± 2.7	5.5 ± 1.5
	(5.2–6.3)	(4.8–7.2)	(4.9–5.9)		(4.8–6.8)	(4.8–6.2)
Serum sodium, mEq/L	139 ± 2	139 ± 3	139 ± 3	0.425	138 ± 3	140 ± 2
	(138–140)	(138–140)	(138–140)		(137–139)	(139–141)
UACR, mg/g	305 ± 1900	582 ± 2080	94 ± 296	0.760	478 ± 2554	94 ± 326
	(0–7958)	(0–13920)	(0–1970)		(0–13837)	(0–2229)
UACR stages, n (%)						
Normoalbuminuria	45 (76)	21 (81)	24 (73)	0.363	24 (73)	21 (78)
	(63–84)	(62–92)	(54–83)		(56–85)	(59–89)
Microalbuminuria	10 (17)	4 (15)	6 (18)		6 (18)	4 (15)
	(9–28)	(6–33)	(8–34)		(9–34)	(6–32)
Macroalbuminuria	4 (7)	1 (4)	3 (9)		2 (6)	2 (7)
	(3–16)	(1–19)	(3–23)		(2–20)	(2–23)
HbA _{1c} , mmol/mol	60 ± 13	63 ± 13	59 ± 12	0.231	61 ± 12	60 ± 13
	(57–63)	(58–68)	(55–63)		(57–65)	(55–65)
HbA _{1c} , %	7.7 ± 1.2	7.9 ± 1.2	7.5 ± 1.1		7.7 ± 1.1	7.6 ± 1.2
	(7.4–8.0)	(7.4–8.4)	(7.1–7.9)		(7.3–8.1)	(7.1–8.1)
Total cholesterol, mmol/L	4.5 ± 0.8	4.7 ± 0.9	4.3 ± 0.7	0.058	4.5 ± 0.8	4.5 ± 0.8
	(4.3–4.7)	(4.3–5.1)	(4.1–4.7)		(4.2–4.8)	(4.2–4.8)
HDL-cholesterol, mmol/L	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.548	1.3 ± 0.3	1.5 ± 0.4
	(1.3–1.5)	(1.2–1.7)	(1.3–1.5)		(1.2–1.4)	(1.3–1.7)
LDL-cholesterol, mmol/L	2.6 ± 0.7	2.8 ± 0.8	2.5 ± 0.5	0.043	2.6 ± 0.7	2.6 ± 0.7
	(2.4–2.8)	(2.5–3.1)	(2.3–2.7)		(2.4–2.9)	(2.3–2.9)
Triglycerides, mmol/L	1.0 ± 0.6	1.1 ± 0.6	0.9 ± 0.5	0.221	1.1 ± 0.7	0.8 ± 0.3
	(0.8–1.2)	(0.85–1.34)	(0.7–1.1)		(0.9–1.3)	(0.7–0.9)
Fibrinogen, mg/dL	339 ± 106	360 ± 103	322 ± 106	0.257	351 ± 103	324 ± 109
	(311–366)	(318–402)	(285–359)		(315–388)	(281–367)
ESR, mm/h	16 ± 18	21 ± 22	13 ± 13	0.042	20 ± 22	13 ± 10
	(11–21)	(12–30)	(8–18)		(12–28)	(9–17)
Homocysteine, mU/L	10 ± 3	10 ± 2	10 ± 4	0.887	9 ± 2	10 ± 4
	(9–11)	(9–11)	(9–11)		(8–10)	(8–12)
hs-CRP, mg/L	3.3 ± 2.8	3.7 ± 2.1	3.0 ± 3.3	0.092	3.1 ± 2.3	3.7 ± 3.4
	(2.6–4.0)	(2.9–4.5)	(1.8–4.2)		(2.3–3.9)	(2.4–5.0)
IMT, mm	0.70 ± 0.15	0.83 ± 0.10	0.61 ± 0.10	<0.001	0.74 ± 0.15	0.66 ± 0.15
	(0.66–0.74)	(0.79–0.87)	(0.58–0.64)		(0.69–0.79)	(0.61–0.71)
Carotid plaques, n (%)	8 (13)	4 (15)	4 (12)	0.717	4 (12)	4 (15)
	(7–24)	(6–34)	(5–27)		(5–27)	(6–32)
Serum copeptin, pmol/L	14.0 [11.3]	14.4 [9.9]	13.6 [14.4]	0.720	18.7 [12.3]	8.1 [5.3]

Data are expressed as means $\pm$ SD, medians [interquartile range], or counts (percentage). Figures below those statistics denote 95% confidence intervals. Differences between groups were analyzed using Student t or Fisher's exact tests. $P < 0.05$ was considered as statistically significant.

Abbreviations ACE-I, ACE inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; eGFR, estimated glomerular filtration rate computed by the MDRD-4 formula; ESR, erythrocyte sedimentation rate; HDL, high density-lipoprotein; hs-CRP, high-sensitivity C reactive protein; IMT, carotid intima-media thickness; LDL, low density-lipoprotein; UACR, urinary albumin-to-creatinine ratio.