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Running head: Diabetes and copeptin predict low cognitive speed

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The data have been presented as a poster with published abstract at the Vas-Cog Conference, Lille, France, September 11–14, 2011.

Keywords: diabetes mellitus, copeptin, executive function, dementia, AQT, cognitive processing speed, arginine vasopressin

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ABSTRACT

Background/Aims

We examined the potential impact of vascular risk factors including copeptin—a robust surrogate marker of arginine vasopressin associated with the metabolic syndrome and diabetes risk—on future cognitive abilities in a population-based cohort.

Methods

Participants (n = 933) were investigated using baseline data, including copeptin levels, and data collected 16 years later using A Quick Test of Cognitive Speed (AQT) and the Mini-Mental State Examination (MMSE).

Results

Logistic regression showed that diabetes (OR 1.86, *P*<0.05) and higher copeptin levels (OR 1.19, *P*<0.05) were independently associated with an increased risk of low AQT performance.

Conclusion

Prevalence of diabetes mellitus and elevated copeptin levels in middle age predict lower cognitive speed after long-term follow-up.

INTRODUCTION

Dementia is a very demanding disease for patients, family members and caregivers. Additionally, it is a growing socio-economic problem. The development of different treatment alternatives inherently requires understanding the pathogenesis of the disease, and more attention should be paid to the pre-dementia state. Growing evidence supports a vascular aetiology for cognitive impairment and dementia [1]. Not only does this concern the vascular and mixed variants of dementia, but also Alzheimer's disease (AD) [1-3]. Hypertension in midlife is a well-established risk factor for dementia [4,5] that has also been connected to cognitive decline [6,7]. Dyslipidaemia [7], insulin resistance, type 2 diabetes [8-10] and obesity [11,12] have also been identified as risk factors in this context.

Arginine vasopressin (AVP), also known as anti-diuretic hormone (ADH), is a neurohypophysial hormone secreted specifically upon increased plasma osmolality and decreased plasma volume [13,14]. AVP is unstable in the plasma, but is released from a precursor peptide along with copeptin, a stable and easily measured biomarker that is released in an equimolar ratio and closely mirrors AVP levels [15]. Recent studies recognized copeptin as a prognostic biomarker of acute illness, such as heart disease and ischaemic stroke [16]. Moreover, copeptin has been suggested as a unifying factor of the main components of the metabolic syndrome [17] and elevated levels of copeptin strongly predict the subsequent development of diabetes mellitus [18]. The potential connection between copeptin or AVP levels and cognitive impairment has not been studied previously. The aim of the present study was to investigate the possible future impact of vascular and metabolic factors, including copeptin, on early signs of cognitive impairment in the elderly.

MATERIAL AND METHODS

Subjects

Data originated from the Malmö Diet and Cancer Study (MDCS) [19], a population-based, prospective cohort study that is part of the European Prospective Investigation into Diet and Cancer (EPIC). Between 1991 and 1996, residents of Malmö, the third-largest city of Sweden, were invited to partake in a comprehensive health examination. 28 449 inhabitants were enrolled, which corresponded to a participation rate of ~40% [19,20]. In a cardiovascular cohort, focusing on carotid artery disease and cardiovascular risk factors, fasting plasma samples from 5 405 individuals were collected [21]. A re-examination of this cohort is currently taking place and, in 2008, the cognitive screening tools Mini-Mental State Examination (MMSE) [22] and A Quick Test (of cognitive speed) (AQT) [23] were added to the research protocol. The present study consisted of data from 1 053 individuals from the cardiovascular cohort who, at the time of the study, had completed this protocol after a mean follow-up time of 15.8 years. Complete data on all variables included were available for 933 participants.

Procedure

Blood samples were drawn at baseline after overnight fasting. Plasma levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides, insulin, cystatin C and copeptin, as well as blood glucose levels, were analysed at the Department Of Clinical Chemistry, University Hospital of Malmö, according to a national standardization and quality control system. LDL cholesterol concentration was calculated according to the Friedewald formula [21]. Blood glucose levels were measured in whole blood using a hexokinase-glucose-6-phosphate dehydrogenase method. The plasma levels of copeptin were measured using a commercially available assay in the chemiluminescence/coated-tube format

(Thermo Fisher Scientific, BRAHMS GmbH, Hennigsdorf, Germany) [24]. Cystatin C levels were measured using a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, Illinois). Diabetes mellitus was defined by self-report of a diagnosis set by a physician, the use of diabetes medication, or a fasting blood glucose $\geq$ 6.1 mmol/L. Blood pressure was measured twice in the supine position using an Omron M5-I IntelliSense after 5 min of rest. Individuals born abroad were labelled with "foreign nationality". Education was divided into two categories: an education of 8 years or less ("low education level") and 9 years or more ("high education level"). The diagnosis of hypertension was based on ongoing anti-hypertensive treatment at baseline. Smoking at baseline (yes/no) was also included in the logistic regression analyses because of the possible influence of smoking on vasopressin levels [25]. A homeostatic assessment model (HOMA), which is a robust epidemiological method for assessing B-cell function and insulin resistance, was calculated from the formula HOMA1-IR = (fasting plasma insulin (mU/L) × fasting plasma glucose (mmol/L)) / 22.5, where blood levels of glucose were used [26]. Body mass index (BMI) was calculated from the formula BMI = mass (kg) / height (m^2). Cognitive screening tests were administered at follow-up by a trained nurse, biomedical scientist or assistant nurse.

Cognitive tests

The MMSE [22] and AQT [23] are two cognitive screening tests with proven validity [22,27,28]. The MMSE tests orientation, memory, attention, naming ability, ability to follow written and oral instructions and copying of pentagons, with a total score of 30 points. It is generally accepted that a score below 24 indicates cognitive impairment [22,29]. AQT consists of three parts: colour, form and (performed last in the sequence) colour–form, to which we refer when presenting AQT results in this study. AQT colour–form is a dual-dimension naming test where the naming time of different coloured and formed (shaped)

items on a test grid is used as the objective measure. A naming time of more than 70 seconds (s) is considered pathological [30]. The AQT colour–form tests cognitive speed, including attention/executive function and verbal automaticity, and performance of the test activates temporo-parietal cortical regions of the brain—areas that are affected at an early stage of AD. The AQT has a documented high sensitivity for detecting cognitive impairment and AD [23,27,28]. AQT colour–form results have been shown to be increased by 1 second per decade in healthy subjects aged 15 to 95 years [31]. Age thus has a minimal effect on AQT colour–form naming time.

Ethics

The study was approved by the Research Ethics Committee of Lund University, Sweden. Written informed consent was provided by all participants in the study.

Statistics

Statistical analysis was performed using the SPSS 19.0 statistical package. A probability value below 0.05 was regarded as statistically significant. Copeptin and HOMA (see below) were not normally distributed; therefore, they were transformed using the natural logarithm when analysed as continuous variables. Univariate ANOVA was used to compare groups regarding continuous variables and the chi-squared test was used to compare categorical variables. In ANOVA analyses, age and cystatin C levels were treated as covariates (with interaction). Logistic regression models were created to examine how the performances on AQT and MMSE, were affected by the following multiple independent variables (including vascular/metabolic risk factors stemming from the definition of the metabolic syndrome): age, sex, nationality, education level, diabetes mellitus, HOMA (transformed using the natural logarithm), plasma levels of triglycerides, HDL and LDL cholesterol, systolic blood pressure,

anti-hypertensive treatment, cystatin C levels, BMI and plasma copeptin levels (transformed using the natural logarithm). Inclusion of the variable smoking (yes/no) in the models was an additional adjustment performed thereafter. In the comparison of groups in ANOVA analyses and in logistic regression models, the cut-offs for the cognitive tests were established from the reference values of each test: 70 s on the AQT and 24 points on the MMSE. Individuals with AQT results > 70 s and an MMSE score < 24 points were classified as "low AQT performance" and "low MMSE performance", respectively, and those with AQT results ≤ 70 s and an MMSE score ≥ 24 points were classified as "normal AQT performance" and "normal MMSE performance", respectively.

RESULTS

Characteristics at baseline

Among the 1 053 participants, data on all variables included in statistical analyses were available for 933 subjects. Hence, 120 subjects (11%) were excluded from the statistical analyses because of missing data on any of the investigated variables. There were no significant differences between included and excluded individuals regarding mean age, sex distribution, nationality or education level (data not shown). In addition, there were no differences in the prevalence of diabetes, smoking habits, anti-hypertensive treatment, results of the MMSE and AQT, systolic blood pressure, BMI, cystatin C levels, copeptin levels, HOMA or LDL (data not shown). However, higher mean levels of triglycerides and lower levels of HDL were observed among excluded individuals (data not shown).

The baseline characteristics of the investigated sample are presented in Table 1. Forty per cent of the participants were men. The mean age (SD) at baseline was 57.5 (5.7) years and 56% of individuals had studied for 9 years or more. Nine per cent were born abroad, 7% had

diagnosed diabetes mellitus at baseline and nearly 20% of the cases received anti-hypertensive treatment. One hundred and ninety-two individuals (21%) were current smokers at baseline. The mean (SD) level of HDL was 1.38 (0.37) mmol/L, the mean (SD) BMI was 26.0 (3.7) kg/m^2 and the mean (SD) level of copeptin was 6.46 (5.57) pmol/L.

→ *Table 1*

Cognitive test results at follow-up

The mean age (SD) at follow-up was 73.5 (5.7) years, the mean (SD) AQT result was 75.7 (21.2) s and the mean (SD) MMSE score was 28.1 (1.8) points (Table 1). Twenty-eight persons (3%) scored below 24 points on the MMSE ("low MMSE performance"), indicating the presence of cognitive impairment, whereas 498 (53%) persons had a reading time on the AQT over 70 s ("low AQT performance").

Differences at baseline between individuals with normal and low cognitive performance at follow-up

Univariate ANOVA analyses revealed the presence of significant differences in baseline age and cystatin C levels between individuals with normal and low AQT performance 15.8 years later: the mean age (SD) was 56.2 (5.7) years for normal AQT performance and 58.6 (5.5) years for low AQT performance ($P = 0.000$). The mean levels of cystatin C were 0.753 (0.123) mg/L for normal AQT performance and 0.789 (0.138) mg/L for low AQT performance ($P = 0.000$).

Statistically significant differences in baseline age, but not in cystatin C levels, were observed in univariate ANOVA analyses between individuals with normal and low MMSE

performance: the mean age (SD) was 57.4 (5.7) years for normal MMSE performance and 60.7 (5.4) years for low MMSE performance ($P = 0.003$). The mean level of cystatin C was 0.771 (0.131) mg/L for normal MMSE performance and 0.792 (0.152) mg/L for low MMSE performance ($P = 0.418$).

→ *Table 2*

→ *Table 3*

Regarding low versus normal AQT performance at the follow-up examination, further statistically significant differences were observed between the groups at baseline. The group of individuals with low AQT performance was of foreign nationality to a greater extent (12 vs 5%) and had a higher prevalence of diabetes mellitus (10 vs 5%), lower education level (53% vs 35%), lower levels of HDL (mean value 1.35 vs 1.42 mmol/L), higher BMI (26.4 vs 25.5 kg/m^2), higher copeptin levels (7.00 vs 5.86 pmol/L) and higher HOMA (2.04 vs 1.72) at baseline (Table 2).

A significantly higher proportion of foreign individuals (21 vs 8%), as well as persons with lower education level (68% vs 44%), was found in the low MMSE performance group compared with the high MMSE performance group (Table 3).

Logistic regressions

The multivariate logistic regression analysis showed that older age (OR, 1.07; 95% CI, 1.05–1.10; $P < 0.001$ per year), lower education level (OR, 1.97; 95% CI, 1.49–2.60; $P < 0.001$), foreign nationality (OR, 3.37; 95% CI, 1.96–5.82; $P < 0.001$), presence of diabetes mellitus

(OR, 1.86; 95% CI, 1.06–3.27; $P < 0.05$) and higher copeptin levels (OR, 1.19; 95% CI, 1.03–1.37; $P < 0.05$ per SD increase) increased the risk of low cognitive performance on AQT 15.8 years later, whereas higher HDL levels (OR, 0.68; 95% CI, 0.47–0.98; $P < 0.05$ per mmol/L) reduced the risk of low performance (Table 4). Additional adjustment for smoking only marginally changed these relationships: age, education level, nationality, diabetes mellitus, and copeptin and HDL levels remained significant predictors of AQT outcome (data not shown). To control the results further, individuals of foreign nationality were excluded from the AQT logistic regression model: older age (OR, 1.07; 95% CI, 1.04–1.10; $P < 0.001$ per year), lower education level (OR, 1.97, 95% CI, 1.48–2.62, $P < 0.001$), presence of diabetes mellitus (OR, 1.87; 95% CI; 1.06–3.30; $P < 0.05$) and higher copeptin levels (OR, 1.20; 95% CI, 1.03–1.40; $P < 0.05$ per SD increase) remained significant predictors. No other variable investigated had a statistically significant impact on AQT outcome (data not shown).

→ *Table 4*

Regarding the MMSE, older age (OR, 1.12; 95% CI, 1.03–1.21; $P < 0.05$ per year), lower education level (OR, 2.48; 95% CI; 1.10–5.60; $P < 0.05$) and foreign nationality (OR, 3.56; 95% CI, 1.37–9.27; $P < 0.05$) increased the risk of low cognitive performance on MMSE 15.8 years later (Table 5). Inclusion of smoking in the model did not change these relationships (data not shown). No significant impact of the other variables investigated on MMSE was found (Table 5).

→ *Table 5*

To further explore the data, a cut-off was created at MMSE 28 points, dichotomizing the material into two groups: 49 % scored 28 points or less and 51 % scored more than 28 points.

A new multivariate logistic regression analysis showed that older age (OR, 1.05; 95% CI, 1.02–1.07; *P* < 0.001 per year) and lower education level (OR, 2.39; 95% CI; 1.82–3.14; *P* < 0.001) increased the risk of low cognitive performance on MMSE (cut-off 28 p) 15.8 years later. Copeptin levels were not significant (OR, 1.09; 95% CI; 0.95–1.26; *P* = 0.23 per SD increase) and including smoking in the model did not change the relationships (data not shown).

DISCUSSION

The major finding of this study was that, in addition to demographic factors, diabetes mellitus and elevated plasma levels of copeptin—a marker of AVP associated with the key components of the metabolic syndrome [17,18] that was previously shown to predict diabetes mellitus development—predicted significantly lower performance in processing speed after 16 years. This was independent of other vascular and metabolic factors investigated, as well as demographic factors. Even after controlling for the effects of smoking and specifically excluding individuals with foreign nationality, the results remained significant.

Copeptin levels increased the risk of low AQT performance by 1.2 times per standard deviation increase and the presence of diabetes mellitus yielded an almost twofold increase in this risk. It has been suggested that higher levels of vasopressin, as measured based on the levels of copeptin, might contribute to insulin resistance and the development of diabetes via mechanisms that act at the AVP receptor level [17]. Several theories regarding how insulin resistance and type 2 diabetes are related to dementia have been reviewed. Some concern the undeviating brain toxic effects caused by higher plasma glucose levels [9], whereas others deal with hyperinsulinemia and secondary effects on the turnover of β-amyloid [8,9]. It has also been discussed whether disturbed insulin signalling could be related to

hyperphosphorylation of the tau protein [32]. However, many studies have focused on the diabetic effects of atherosclerosis, vascular damage and ischaemic stroke on the brain [8,9]. Insulin resistance has been associated with lower brain volume and dysexecutive functions [33]. Moreover, the existence of an increased volume of white matter hyperintensities (WMH) in the brain has been associated with diabetes [34]. Specifically, such changes can interfere with executive functioning [35], attention and processing speed [36], which are measured by the AQT.

Higher HDL cholesterol levels significantly reduced the risk of low AQT performance by 32% per mmol/L. Accordingly, higher levels of HDL cholesterol seem to act protectively for the preservation of normal cognitive speed. Higher BMI had a negative influence on AQT results ($P < 0.05$). These results are in agreement with the known impact of vascular and metabolic factors on vascular damage, possibly leading to brain injury through atherosclerosis and/or white matter hyperintensities [37,38]. Higher levels of cystatin C at baseline were found in subjects with low AQT performance at follow-up. However, this is likely a result of an underlying correlation between age and cystatin C in the low AQT performance group (i.e. the renal function is decreasing with age). Since the multivariate regression model could not identify cystatin C as a significant predictor we refrain from further interpretation of the possible role of cystatin C in this context.

Age, education level and nationality were epidemiological factors of importance for cognitive speed, according to our results. Age itself is the strongest risk factor for dementia. We found that older age increased the risk of a low AQT performance time by 1.1 times per year. Previously, it was demonstrated that age has only a minimal effect on AQT colour–form results [31]. More studies are needed to evaluate whether age has a stronger effect on

cognitive processing speed measured by AQT than was previously known. Lower education level was associated with an almost twofold increased risk for low AQT performance in our study. Education level has a known impact on AQT, with a well-recognized dichotomized cut-off at 8 years of education [27]. Foreign nationality increased the risk of low AQT performance by 3.4 times. This effect was interpreted not as an incapacity for the Swedish language (all participants spoke Swedish and no interpreters were necessary), but to some extent as a difficulty in verbal automaticity and fluency in a second language in a stressed situation. Therefore, we created a second logistic regression model excluding foreigners to ensure that our results were robust. Copeptin levels and diabetes mellitus remained significant predictors of AQT performance at the same significance level for subjects born in Sweden (n = 853), thus reinforcing the independent association with the AQT results.

Traditional vascular and metabolic factors did not seem to be related with low cognitive performance on the MMSE. Regarding this test, age, education level and nationality were the only variables investigated that yielded significant results. Older age increased the risk of low MMSE performance by 1.1 times per year. It has been shown that older age influences MMSE results negatively [39]. Lower education level, which is known as an important factor that affects the results of the MMSE [39], increased the risk of pathological MMSE results by 2.5 times. Nationality also had a great impact on MMSE results, with a 3.6 times increased risk of low MMSE performance observed for non-native subjects. However, one must be careful when drawing conclusions from the MMSE statistical model, as it was imbalanced because of the division of the cohort into low (3%) and normal (97%) MMSE groups. We chose to accept that division to be able to retain the established cut-offs for both cognitive tests used in the study. Nonetheless, exploring the data further with MMSE cut-off 28 points – which was also the mean value for the MMSE – revealed that older age and lower education

level again increased the risk of low MMSE performance. The effect observed for nationality at the lower MMSE cut-off might be unreliable due to the existing imbalance in this model, as discussed above, since it is not retained for MMSE cut-off 28. The results together suggest that the MMSE might not be a sensitive screening test for identification of subtle cognitive deficits resulting from minor vascular changes in the brain in a healthy population.

Further research is needed to define the exact pathogenetic mechanisms involved; however, here we showed that the reserve capacity for faster neuronal traffic in the brain might be reduced by vascular and metabolic factors in the long-term. The present findings emphasise the importance of vascular risk factors in the deterioration of cognitive abilities. We suggest that the cognitive impairment caused by traditional vascular and metabolic risk factors is best and earliest identified using tests such as the AQT, which measure executive functions in which cognitive speed and attention ability are prominent. It is likely that the MMSE is not the optimal cognitive screening test for capturing deficits resulting from vascular damage in the brain, as it does not assess these abilities. Therefore, screening tests that evaluate such abilities are appropriately administered together with the MMSE in the initial cognitive evaluation. AQT is easy to administer and its performance requires a minimal amount of time.

Nevertheless, it is remarkable that 53% of the participants exhibited low cognitive performance according to the AQT, whereas only 3% had pathological values on the MMSE. Even though it is expected that the AQT is more efficient than the MMSE in identifying signs of early vascular damage in the brain, we believe that there is a need for further studies and possibly also a revision of the reference value of the AQT.

We have strengthened the support for the impact of vascular and metabolic factors on cognitive speed. Moreover, our results were derived from a well-established and large cohort. Few studies have examined vasopressin and copeptin levels in relation to human cognition, and none have studied them in the context of vascular and metabolic factors. Nonetheless, one limitation of this study stemmed from the fact that copeptin levels may be influenced by heart and renal failure, which can give rise to higher levels of the biomarker in the plasma. We performed adjustments for renal function by using cystatin C in logistic regression models, although we could not adjust for the presence of heart failure. In addition, the participants of the MDCS are known to be, to some extent, healthier than the background population [20]. Thus, our finding of an increased risk of low cognitive performance due to vascular risk factors, such as elevated levels of copeptin, may be an underestimation.

Conclusion

We showed that higher levels of copeptin and prevalence of diabetes during middle age predicted low cognitive speed among the elderly after long-term follow-up. There is growing evidence of a metabolic and vascular role in the pathogenesis of cognitive impairment and dementia, and this study lends additional support to these findings. Further studies are needed to evaluate the role of copeptin in this context. Nevertheless, the present study draws attention to the overall importance of metabolic and vascular control in preventing future CNS events leading to impaired cognitive functioning.

Agreement on Medical Training and Clinical Research between Skåne County Council and

Lund University.

(Table 1). Baseline and follow-up characteristics

Baseline data	
n	933
Women, number (%)	564 (60.5)
Age, years, mean (SD)	57.5 (5.7)
Low education level (8 years or less), number (%)	414 (44.4)
Born abroad, number (%)	80 (8.6)
Diabetes mellitus, number (%)	69 (7.4)
Systolic blood pressure, mmHg, mean (SD)	142 (19)
Anti-hypertensive treatment, number (%)	182 (19.5)
Current smoker, number (%)	192 (21)
Triglycerides, mmol/L, mean (SD)	1.33 (0.64)
HDL, mmol/L, mean (SD)	1.38 (0.37)
LDL, mmol/L, mean (SD)	4.2 (1.01)
BMI, kg/m^2, mean (SD)	26.0 (3.7)
Copeptin, pmol/L, mean (SD)	6.46 (5.57)
HOMA, mean (SD)	1.89 (1.50)
Cystatin C, mg/L, mean (SD)	0.772 (0.132)
Follow-up data	
Age, years, mean (SD)	73.5 (5.7)
MMSE, points, mean (SD)	28.1 (1.8)
AQT, s, mean (SD)	75.7 (21.2)

(Table 2). Vascular and demographic variables at baseline in the AQT groups

Variable	AQT ≤ 70 s	AQT > 70 s	P
Women, number (%)	276 (63.4)	288 (57.8)	0.08[*]
Low education level (8 years or less), number (%)	151 (34.7)	263 (52.8)	0.000[*]
Foreign nationality (born abroad), number (%)	20 (4.6)	60 (12)	0.000[*]
Diabetes mellitus, number (%)	21 (4.8)	48 (9.6)	0.005[*]
Systolic blood pressure, mmHg, mean (SD)	140 (19)	143 (19)	0.529[**]
Anti-hypertensive treatment, number (%)	74 (17)	108 (21.7)	0.072[*]
Triglycerides, mmol/L, mean (SD)	1.27 (0.60)	1.39 (0.67)	0.085[**]
HDL, mmol/L, mean (SD)	1.42 (0.38)	1.35 (0.37)	0.018[**]
LDL, mmol/L, mean (SD)	4.2 (1.03)	4.3 (0.99)	0.646[**]
BMI, kg/m^2, mean (SD)	25.5 (3.6)	26.4 (3.8)	0.007[**]
Copeptin, pmol/L, mean (SD)	5.86 (4.10)	7.00 (6.55)	0.004[**,***]
HOMA, mean (SD)	1.72 (1.16)	2.04 (1.72)	0.037[**, ***]

[*] χ^2 test

[**] ANOVA, age and cystatin C levels were included as covariates

[***] ANOVA performed on logarithmic-transformed variables

(Table 3). Vascular and demographic variables at baseline in the MMSE groups

Variable	MMSE≥ 24 points	MMSE< 24 points	*P*
Women, number (%)	546 (60.3)	18 (64.3)	0.416[*]
Low education level (8 years or less), number (%)	395 (43.6)	19 (67.9)	0.010[*]
Foreign nationality (born abroad), number (%)	74 (8.2)	6 (21.4)	0.027[*]
Diabetes mellitus, number (%)	68 (7.5)	1 (3.6)	0.372[*]
Systolic blood pressure, mmHg, mean (SD)	142 (19)	146 (18)	0.814[**]
Anti-hypertensive treatment, number (%)	177 (19.6)	5 (17.9)	0.526[*]
Triglycerides, mmol/L, mean (SD)	1.33 (0.64)	1.35 (0.49)	0.858[**]
HDL, mmol/L, mean (SD)	1.38 (0.37)	1.35 (0.34)	0.560[**]
LDL, mmol/L, mean (SD)	4.2 (1.01)	4.6 (0.92)	0.122[**]
BMI, kg/m^2, mean (SD)	26.0 (3.7)	25.8 (3.1)	0.598[**]
Copeptin, pmol/L, mean (SD)	6.46 (5.62)	6.56 (3.85)	0.510[**, ***]
HOMA, mean (SD)	1.89 (1.51)	1.83 (0.97)	0.925[**, ***]

[*] χ^2 test

[**] ANOVA/GLM, age and cystatin C levels were included as covariates

[***] ANOVA performed on logarithmic-transformed variables

(Table 4). Logistic regression using an AQT cut-off of 70 s as a dependent variable

	OR	**95% C. I. for OR**	*P*
Age	1.07	1.05–1.10	< 0.001
Education level	1.97	1.49–2.60	< 0.001
Nationality	3.37	1.96–5.82	< 0.001
Diabetes mellitus	1.86	1.06–3.27	< 0.05
HDL cholesterol	0.68	0.47–0.98	< 0.05
lnCopeptin (Z-score)[*]	1.19	1.03–1.37	< 0.05

[*] OR is expressed per 1 SD increase.

Variables entered on step 1: ZlnCopeptin, cystatin C, triglycerides, HDL, LDL, diabetes mellitus, BMI, systolic blood pressure, anti-hypertensive treatment, nationality, age, sex, education level and lnHOMA

Note: Hosmer–Lemeshow Test for the last step sig = 0.54.

(Table 5). Logistic regression using an MMSE cut-off of 24 points as a dependent variable

	OR	**95% C. I. for OR**	*P*
Age	1.12	1.03–1.21	< 0.05
Education level	2.48	1.10–5.60	< 0.05
Nationality	3.56	1.37–9.27	< 0.05
Diabetes mellitus	0.34	0.04-2.80	0.31
HDL cholesterol	0.59	0.16-2.12	0.42
lnCopeptin (Z-score)*	1.16	0.72-1.86	0.55

* OR is expressed per 1 SD increase.

Variables entered on step 1: ZlnCopeptin, cystatin C, triglycerides, HDL, LDL, diabetes mellitus, BMI, systolic blood pressure, anti-hypertensive treatment, nationality, age, sex, education level and lnHOMA

Note: Hosmer–Lemeshow Test for the last step sig = 0.84

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