Thomsen, C. F., Dreier, R., Goharian, T. S., Goetze, J. P., Andersen, L. B., Faber, J. … Jeppesen, J. L. (2019). Association of copeptin, a surrogate marker for arginine vasopressin secretion, with insulin resistance: Influence of adolescence and psychological stress. *Peptides, 115*, 8-14.

Association of copeptin, a surrogate marker for arginine vasopressin secretion, with insulin resistance: influence of adolescence and psychological stress

Camilla F. Thomsen[a*], Rasmus Dreier[a,b], Tina S. Goharian[a], Jens P. Goetze[c], Lars B. Andersen[d,e], Jens Faber[f,g], Mathias Ried-Larsen[h], Anders Grøntved[h], Jørgen L. Jeppesen[a,f]

[a]Department of Medicine, Amager and Hvidovre Hospital in Glostrup, University of Copenhagen, Glostrup, Denmark; [b]Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet Glostrup, University of Copenhagen, Glostrup, Denmark; [c]Department of Clinical Biochemistry, Rigshospitalet Blegdamsvej, University of Copenhagen, Copenhagen, Denmark; [d]Faculty of Education, Arts and Sport, Western Norway University of Applied Sciences, Campus Sogndal, Norway; [e]Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, Norway; [f]Department of Medicine O, Endocrine Unit, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark; [g]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [h]Research Unit for Exercise Epidemiology, Department of Sport Science and Clinical Biomechanics, Centre of Research in Childhood Health, University of Southern Denmark, Odense, Denmark.

Correspondence to Camilla F. Thomsen, Department of Medicine, Amager and Hvidovre Hospital in Glostrup, University of Copenhagen, Valdemar Hansens Vej 1-23, DK-2600, Glostrup, Denmark. E-mail: allimac_fjord@hotmail.com
Declarations of interest: none.

ABSTRACT
In middle-aged and elderly individuals, circulating copeptin concentrations, a surrogate marker for arginine vasopressin (AVP) secretion, associates with insulin resistance (IR). Whether this association is present in adolescents and young adults is unclear. Because psychological stress associates with higher circulating copeptin concentrations and IR, it has been speculated that increased AVP secretion could be a link between psychological stress and IR. We measured plasma copeptin concentrations in 351 14-16-year-old adolescents and 617 20-28-year-old young adults from the Danish site of the European Youth Heart Study, a population-based cardiovascular risk factor study in adolescents and young adults. IR was determined by the homeostatic model assessment method. Among the young adults, we used symptoms of depression, evaluated by means of the Major Depression Inventory (MDI) scale, as a measure of psychological stress. We applied linear regressions to examine associations, expressed as unstandardized regression coefficients (*B*) with 95% confidence intervals (CIs), between variables of interest, stratified by age group and adjusting for age, sex and Tanner stages. Copeptin and IR were log-transformed. Among the young adults, copeptin associated with IR (*B* (95%CI) =0.19 (0.11 to 0.27), *P*<0.001). This association was not found among the adolescents (*B*=-0.01 (-0.12 to 0.09), *P*=0.78). MDI score associated with IR (*B*=0.010 (0.004 to 0.016), *P*<0.001) and copeptin (*B*=0.010 (0.004 to 0.015); *P*<0.002) in the young adults. Adjusted for copeptin, the strength of the association between MDI score and IR somewhat diminished (to

B=0.008). In conclusion, adolescence and psychological stress appear to influence the association between copeptin and IR.
Key words: Arginine vasopressin; Copeptin; Depression; Insulin; Insulin resistance; Psychological stress

1. Introduction

Arginine vasopressin (AVP) is well known for its role in the regulation of extracellular fluid, vasoconstriction and the endocrine stress response [1]. However, AVP is difficult to measure, owing to its short half-life and complex pre-analytical requirements [1]. Therefore copeptin, the stable, biologically inactive, C-terminal portion of pro-vasopressin, is often used as a surrogate marker for AVP secretion [1].

Recently, it has become clear that AVP could have additional metabolic effects in humans. Thus, many cross-sectional and prospective studies, performed in overall generally healthy middle-aged and elderly individuals, have found circulating copeptin concentrations to be associated with insulin resistance (IR) as well as glucose intolerance, hyperinsulinemia and dyslipidaemia [2-8], the well-known metabolic abnormalities closely linked to IR [9]. However, the relationship between AVP, IR and the aforementioned metabolic abnormalities remains to be fully clarified [8].

Psychological stress, for example related to childhood maltreatment, written exams and social stress tests, as well as stressful mental states, such as suicidal behaviour, have been associated with higher circulating copeptin concentrations [10-13]. This suggests that an increased AVP secretion, because of stressful mental brain activity, could be involved in the development of IR and the metabolic abnormalities related to IR [14-16]. A possible mediating factor, in addition to potential direct actions of AVP on target organs [1,8], could be AVP-stimulated increased pituitary-adrenal axis activity [8], leading to hypercortisolaemia followed by the development of a dysmetabolic state [17].

Thus far, it is unclear whether the association between circulating copeptin concentrations, IR and the metabolic disturbances related to IR, found in studies of middle-aged and elderly individuals [2-8], is also present in children, adolescents and young adults. A few studies have addressed this issue, but the results have been inconsistent [18-20]. In addition, more knowledge regarding a possible link between psychological stress, increased AVP activity and IR and the metabolic abnormalities related to IR is warranted [8].

On this basis, we initiated the present study with two specific goals in mind: 1) we wanted to clarify whether circulating copeptin concentrations would be associated with IR and the metabolic abnormalities related to IR in healthy adolescents and young adults from the general population; and 2) we wanted to clarify whether increased AVP secretion, as reflected by higher circulating copeptin concentrations, could be a mediating factor between psychological stress and IR and the metabolic abnormalities related to IR. To achieve these goals, we studied a large sample of adolescents and young adults from the Danish site of the European Youth Heart Study (EYHS), which is an international population-based study of cardiovascular risk factors in children, adolescents and young adults [21,22]. The EYHS includes measurements of insulin, glucose, lipids, blood pressure (BP), and anthropometric variables, assessment of psychological stress as well as a bio-bank, which enabled us to measure copeptin in plasma [22].

2. Materials and methods

2.1. Study population

This cross-sectional study is based on the Danish EYHS data [22]. The EYHS protocol has been provided elsewhere [21]. In addition to the variables listed above, the EYHS also includes information about smoking habits, alcohol consumption and physical fitness [21-23]. In 2009–2010, 709 14–16-year-old healthy adolescents, 469 20–22-year-old healthy adults and 658 26–28-year-old healthy adults were re-invited to participate in a new wave of the Danish part of the EYHS. The participation rate was 59% (*n*=399) for the adolescents and 46% (*n*=650) for the young adults. In this study, the two adult groups were collapsed into one group. Exclusion criteria included pregnancy (*n*=12), diabetes (*n*=3) and non-fasting or no information about fasting state (*n*=67), leaving 351 adolescents (55.8% girls, age range: 14-16 years) and 617 young adults (52.4% women, age range: 20-28 years) for the study. In the situation of missing data, these were handled with the pairwise deletion technique, which involves deleting a case when it is missing a variable necessary for a particular analysis but including that case in analyses for which all necessary variables are present. Because of this, total number of participants varies in the various calculations. In analyses evaluating IR, calculated by the Homeostatic Model Assessment 2 for IR (HOMA2-IR) [24], we excluded, as prescribed, individuals with fasting glucose <3.5 mmol/L and with fasting insulin outside the range of 20–400 pmol/L (*n*=3 glucose <3.5 mmol/L, *n*=83 insulin <20 pmol/L and *n*=2 insulin >400 pmol/L), leaving 873 individuals for the IR analyses. The study was approved by the Regional Scientific Ethical Committee for Southern Denmark, and data was collected according to the Declaration of Helsinki. All participants gave their written informed consent, and regarding the adolescents, written consent of the child's parent or legal guardian was also obtained.

2.2. Maturity and anthropometric measurements

We measured weight, height and waist circumference (WC) while the participants were wearing light clothing without shoes. WC was measured midway between the lower rib margin and the iliac crest at the end of gentle expiration. Pubertal status was assessed according to Tanner stages, as described elsewhere [25]. Maturity stage among the adolescents was almost exclusively stage 3, 4 and 5, thus, we collapsed maturity into a 3-point ordinal variable (Tanner stages 2-3, Tanner stage 4 and Tanner stage 5).

2.3 Blood biomarkers measurements

Venous blood samples were drawn in the morning after at least eight hours of fasting. Plasma copeptin was analyzed with an automated immunofluorescence assay on a KRYPTOR platform (Thermo Fischer, Henningsdorf, Germany). Specifications of the copeptin assay were as follows: detection limit 0.9 pmol/L, intra-assay coefficient of variation (CV) <15%, and inter-assay CV <17%. Glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using an Olympus AU600 random access analyser. Insulin was analysed using an enzyme immunoassay (Dako Diagnostics, Ely, U.K.).

2.4. BP measurements

Systolic BP (SBP) and diastolic BP (DBP) were measured with the participants in the sitting position after resting for five minutes, using a Dinamap monitor (Dinamap model XL-Kivex/Critikron, Tampa, FL, US), which has previously been validated in children and adolescents against direct radial artery readings, as described elsewhere [25]. Five measurements were done with two-minute intervals, and the mean of the last three measurements was used.

2.5. Physical fitness measurements

Because level of physical activity has been shown to be inversely associated with both circulating copeptin concentrations [2,3,6] and degree of IR [9], we also included the Danish EYHS physical fitness measurements in the present study. Among the adolescents, aerobic fitness was determined by an indirect maximal cycle ergometer test—the watt-max test. The protocol has been described in detail elsewhere [21]. The maximal power output was scaled to body mass (Watts/kg). Among the young adults, cardiorespiratory fitness was assessed directly using a maximal exercise test on a cycle ergometer (Monark 839E, Varberg, Sweden). The protocol has been described in detail elsewhere [23]. During the test, pulmonary oxygen uptake (VO_2), expired carbon dioxide (CO_2) and pulmonary ventilation were analysed using indirect calorimetry (AMIS 2001, Innovision, Odense Denmark). Maximal VO_2 consumption (VO_2-max) was estimated as the average of VO_2 during the last 15 secs of the test divided by body weight and expressed as ml/(kg·min).

2.6. Assessment of psychological stress using symptoms of depression

To investigate a possible link between psychological stress and circulating copeptin concentrations, we included data regarding symptoms of depression using the well-established Major Depression Inventory (MDI) scale among the young adults [23]. Although it can be difficult to distinguish what comes first depression or psychological stress, depressed persons typically experience psychological stress [26], so we thought depression overall is a reasonable measure for psychological stress. The MDI scale includes10 ICD-10 symptoms of depression, and the 10 symptoms are based on 12 items on a six-point Likert scale (0 to 5). As a severity measure, the MDI score ranges from 0 to 50. In our depression analyses, we express the level of depression as a continuous variable.

2.7. Statistical analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, US). Continuous data are presented as mean ± standard deviation (SD) or as median (interquartile range), depending on normal or non-normal distribution, and categorical data are presented as frequency percent (Table 1). Group comparisons were performed using t-test for continuous data and chi-squared test for categorical data. To fulfil the model

assumptions of the t-test, non-normally distributed variables (copeptin, triglycerides, insulin and HOMA2-IR) were (natural) log-transformed prior to group comparisons. Multivariable linear regression was used to examine the associations, expressed as unstandardized regression coefficients (*B*) with 95% confidence intervals (CIs), of plasma copeptin concentrations (independent variable) with various variables of interest (dependent variables) stratified according to age group and further adjusting for age and sex and Tanner stages (Table 2). The data in Table 2 were subsequently adjusted for fitness. In the regression models, the non-normally distributed variables listed above were (natural) log-transformed to fulfil the model assumptions of linear regression. We specifically compared the strength of the age-group dependent associations between copeptin and the metabolic variables by testing interactions, as well as we tested for interactions with age, sex, smoking habit and alcohol intake. Finally, also using multivariable regression, we studied the association of depression score (independent variable) with plasma copeptin concentrations and HOMA2-IR (dependent variables) among the young adults, as well as we studied the association of depression score (independent variable) with HOMA2-IR with successive adjustments for candidate mediators (Table 3) with possible attenuation of the *B*-estimates in focus. Statistical tests were 2-sided, and $P<0.05$ was considered significant.

3. Results

3.1. Group comparisons

General characteristics of the study population stratified by age group are shown in Table 1. The two groups had similar sex distribution. As expected, our participants had overall normal BP and lipids levels. Regarding the anthropometric, hemodynamic, lipid and metabolic variables, body mass index (BMI), WC, BP, TC, triglycerides, HDL-C and LDL-C were significantly lower whereas insulin, HOMA2-IR and copeptin were significantly higher among the adolescents comparing to the young adults ($P<0.005$). Furthermore, the male participants had higher plasma copeptin concentrations than the female participants (adolescents (median (interquartile range)): 7.77 (5.70-10.5) pmol/L versus 6.19 (4.19-8.98) pmol/L; adults: 7.27 (4.47-9.33) pmol/L versus 5.13 (2.82-6.68), $P<0.001$).

3.2. Metabolic and hemodynamic associations

The associations of plasma copeptin concentrations with metabolic and hemodynamic variables of interest are summarized in Table 2. Among the adolescents, all anthropometric, hemodynamic, lipid (except for LDL-C) and metabolic variables were negatively associated with copeptin, but the strength of the associations was not statistically significant, except for BMI ($P<0.014$). Regarding the young adults, all variables were positively associated with copeptin (except for HDL-C), and the strength of the positive associations reached statistical significance for DBP, triglycerides, glucose, insulin and HOMA2-IR. Furthermore, the strength of the associations between copeptin and insulin and HOMA2-IR was stronger among the adults compared with the adolescents, as reflected by $P<0.007$ and $P<0.006$ in the age-group interaction analyses.

In a mechanistic perspective, DBP, triglycerides and glucose are known to be physiologically linked to IR and compensatory hyperinsulinemia [9]. Therefore, we tested if

these variables would still be significantly associated with copeptin in models that in addition to age and sex also included HOMA2-IR or insulin. In such models, none of the three factors were significantly associated with copeptin (*P*>0.22), whereas HOMA2-IR and insulin were (P<0.001).

Finally, we found no interactions between copeptin and sex (*P*>0.28) or age (*P*>0.98) in any of the regression models in Table 2.

3.3. Physical fitness and copeptin

The mean level of fitness was 4.09 +/- 0.72 watt/kg among the adolescents and 39.13 +/-8.67 ml/(kg·min) among the young adults. In the adolescent group (n=286), we found no significant relationship between fitness and copeptin in models including adjustments for age, sex and Tanner stages (*P*=0.32), and further adjustment for fitness did not materially affect the results presented in Table 2 in the adolescent group (data not shown). However, among the young adults (n=549), we found a statistically significant inverse relationship between fitness and copeptin adjusted for age and sex (copeptin as independent variable: *B*=-2.850 (-4.024 to -1.676), *P*<0.001; fitness as independent variable: *B*=-0.0140 (-0.020 to -0.008), *P*<0.001). In addition, further adjustment for fitness markedly attenuated the strength of the association between copeptin and HOMA2-IR (*B*=0.19 (0.11 to 0.27), *P*<0.001, to *B*=0.099 (0.025 to 0.173), *P*<0.010), but otherwise this further adjustment did not essentially affect the other results shown in Table 2 in the young adult group (data not shown).

3.4. Psychological stress and copeptin

With respect to a link between psychological stress and copeptin, data for depression were available for analysis for 582 young adults. The mean MDI score was 9.65 ± 7.93 for females (n=305) and 7.45 ± 6.64 for males (n=277). Adjusted for age and sex, MDI-score was significantly associated with copeptin (*B*=0.010 (0.004 to 0.015); *P*<0.0016). We found no evidence of a sex-dependence regarding this association *(P*=0.63 for interaction). MDI score was also associated with HOMA2-IR (*B*=0.010 (0.004 to 0.016), *P*<0.0001). However, when including MDI score and copeptin in the same model, the strength of the association between MDI score and HOMA2-IR diminished but remained significant (*B*=0.008 (0.002 to 0.014), *P*<0.005), which was also the case for copeptin (*B*=0.17 (0.10 to 0.25); P<0.001).

Finally, in an extended model, including adjustments for age and sex but also smoking habits and alcohol intake, factors associated with depression [23], we looked for possible combined mediating roles of copeptin and fitness between psychological stress and IR with successive inclusion of these variables in the extended model (Table 3). Addition of copeptin reduced the strength of the association between MDI score and HOMA2-IR, and after addition of both copeptin and fitness, MDI score was no longer significantly associated with HOMA2-IR, whereas copeptin was. However, inclusion of fitness substantially decreased the strength of the association between copeptin and HOMA2-IR. Finally, in the fully adjusted extended model, we found no sex-dependent interactions (*P*>0.32).

3.4. Supplemental analyses

Circulating copeptin concentrations have been reported to be increased by smoking and high alcohol use in one study [27], but not in other studies [2,3,6]. Therefore, we performed further analyses in both groups adjusting for these variables and including interaction terms with these variables. However, these analyses did not materially affect any of the associations presented in Table 2 (data not shown). In this context, it is important that none of the adolescents and only 1.9% (n=12) of the young adults consumed alcohol daily.

4. Discussion

4.1. Major findings

It was a major finding of this study that among the young adults, plasma copeptin concentrations were associated with IR, whereas such an association was not found in the adolescents. It was a major finding, too, that among the young adults, MDI score was associated with plasma copeptin concentrations. It was also a major finding that the strength of the association between MDI score and IR was somewhat diminished when adjustment was made for plasma copeptin concentrations. The latter finding suggests that AVP could be a mediating factor between psychological stress and IR, although the level of fitness could also play a major mediating role regarding psychological stress, AVP and IR.

4.2. Copeptin, IR and metabolic variables in children and adolescents

There are only a few non-population-based studies published in children and adolescents regarding blood copeptin concentrations and metabolic variables [18-20]. The results have been inconsistent, and insulin was not measured in these studies [18-20].

In a study in a paediatric university clinic, including 30 girls and 54 boys, aged 11–18 years [18], serum copeptin concentrations were found to correlate with SBP, DBP, triglycerides, the triglyceride/HDL-C ratio and BMI but not glucose [18]. In multivariate analysis, only SBP and BMI were found to be significantly associated with copeptin [18]. However, apparently no adjustment was made for sex in the multivariate analysis, which makes the results difficult to interpret because the boys had higher copeptin concentrations and higher BP levels than the girls [18].

Another study, in a university obesity clinic, included 51 obese children (10.8±3.2 years, 39% male, 45% pre-pubertal) and 24 lean children of similar age, sex and pubertal stage [19]. The study focused on the relationship between serum copeptin concentrations, puberty and metabolic variables [19], which were, however, only measured in the obese children [19]. Serum copeptin concentrations were significantly (P=0.047) higher in the obese children (5.8±2.8 pmol/L) compared with the lean children (4.6±2.2 pmol/L) [18]. In the obese and normal-weight children combined, copeptin was significantly associated with BMI, adjusted for age, sex and pubertal stage [19]. In the obese children, copeptin was significantly associated with BMI, adjusted for sex and pubertal stage, but none of the metabolic variables [19]. Why copeptin, in contrast to the findings above, was negatively associated with BMI in our adolescents is not clear.

Finally, a study in a university diabetes clinic, including 141 individuals: 80 patients with type 1 diabetes (13.0±3.4 years, HbA1c 7.85±1.42%) and 61 healthy controls (12.4±2.8 years) [20], found that despite higher fasting blood glucose concentrations (8.2±3.8 mmol/L vs. 4.7±0.5 mmol/L; *P* <0.01), the patients with type 1 diabetes and the healthy controls had similar plasma copeptin concentrations (4.75±3.46 pmol/L vs. 5.56±3.15 pmol/L; *P*=0.24) [20]. However, the patients with type 1 diabetes were lean (BMI (kg/m^2): 19.7±3.8) with no evidence of metabolic or hemodynamic disarrangement other than higher glucose levels. In both groups, no correlations were found between plasma copeptin concentrations and metabolic factors other than glomerular filtration rate (negative correlation) [20].

4.3. Copeptin in a pathophysiological perspective

Briefly, before further pathophysiological discussions, it is important to mention that the copeptin concentrations reported to be associated with metabolic disorders among generally healthy persons are substantially lower than the copeptin concentrations found as part of the endocrine stress response seen in critically ill and severely stressed patients [2-8,28,29].

4.4. Copeptin and abnormalities in glucose and insulin metabolism

So, which physiological or pathophysiological mechanisms may link increased activity of the AVP system, as reflected by higher plasma copeptin concentrations, to abnormalities in glucose and insulin metabolism? In fact, there could be more than one mechanism (the role of AVP-stimulated increased pituitary-adrenal axis activity is discussed in section 4.5).

Several AVP-receptors have been identified (V1A, V1B and V2) [8,30], and in *in vitro* experiments and animal models, AVP has been shown to increase insulin and glucagon secretion, depending on glucose levels, and to increase hepatic glycogenolysis and gluconeogenesis [8,30]. In normal male volunteers [31], an intravenous infusion of AVP did not affect serum insulin concentrations or glucose disposal but resulted in higher plasma glucose concentrations through an increase in glucose appearance [31]. Plasma glucagon concentrations also increased significantly during the AVP infusion, and the authors of the paper therefore speculated that the acute hyperglycaemic effect of AVP could be primarily mediated by stimulation of glucagon release [31]. That there could be a connection between chronic higher activity of the AVP system and higher circulating glucagon concentrations has recently gained support by the finding that fasting plasma copeptin concentrations are significantly associated with fasting plasma glucagon concentrations in generally healthy obese men and men of normal weight [32].

Many studies have found that copeptin is significantly associated with hyperinsulinemia and IR, and in this study, the principal copeptin-related metabolic abnormality was higher plasma insulin concentrations and higher IR and not higher plasma glucose concentrations, thus at odds with the acute AVP intravenous infusion study [31]. Nevertheless, in the chronic state, higher plasma glucose concentrations will lead to higher plasma insulin concentrations, and, as described by Shanik *et al*., based on studies in experimental animals and specific clinical conditions in humans, for example insulinomas and hypothalamic obesity, hyperinsulinemia has been shown to play a role in initiating,

sustaining or expanding IR [33]. Further, our data also suggest that the link between increased activity of the AVP system and IR could involve low physical fitness, a very strong predictor of IR [34,35]. Accordingly, the strength of the association between copeptin and HOMA2-IR was substantially reduced when adjustment was made for fitness. The nature of the relationship between increased activity of the AVP system and low physical fitness is unclear. Nevertheless, aerobic exercise training may decrease fasting plasma copeptin concentrations [36].

Finally, with respect to the principal copeptin-related metabolic abnormality found in this study, in 1988, Reaven proposed that IR with compensatory hyperinsulinemia was an important cause of several metabolic abnormalities, such as glucose intolerance, hypertriglyceridemia, low HDL-C levels and high BP [9]. In the present study, plasma copeptin concentrations were associated with IR (and hyperinsulinemia), but not with the other metabolic factors related to IR, when adjustments were made for hyperinsulinemia or IR. This finding suggests to us that IR could be the primary driver of the AVP-system related metabolic abnormalities observed in many studies in middle-aged and elderly populations [2-8].

4.5. Copeptin, mental stress and the pituitary-adrenal axis

In this study, a higher depression score, which we used as an estimate of mental stress [23], was significantly associated with higher copeptin concentrations among the young adults (no measures of mental status were available among the adolescents). Further, adjusted for plasma copeptin concentrations, the strength of the association between depression score and IR diminished. This suggests that AVP could be a mediating factor between mental stress and IR and its associated metabolic components [14-16]. The notion that mental stress leads to higher circulating copeptin concentrations is supported by the medical literature, which also suggests the AVP-stimulated increased pituitary-adrenal axis activity could be involved [8]. In one study, 25 medical students were tested immediately prior to a written examination and after its conclusion [10] Comparing before and after, the students had higher heart rate, reported a higher stress level, and had higher serum concentrations of copeptin and cortisol after the study [10]. In another study, 20 healthy adults underwent the Trier Social Stress Test [11]. During the test, both serum concentrations of copeptin and cortisol increased, and feelings of tension and avoidance were particularly associated with greater increases in serum copeptin concentrations [11]. In a further study, induction of panic symptoms in 30 healthy male human subjects by intravenous cholecystokinin-tetra peptide, a substance well known to induce panic symptoms and activate the pituitary-adrenal axis activity, resulted in substantial increases in circulating concentrations of copeptin and cortisol [37]. Further, children exposed to maltreatment, such as physical abuse, neglect, emotional maltreatment and sexual abuse, and individuals with suicidal behaviour have higher circulating copeptin concentrations [10,13]. Finally, although depressed ambulatory patients, participating in an exercise study, had similar plasma copeptin concentrations to controls [36], among individuals attempting suicide, circulating copeptin concentrations, measured within the first hour of patient admission, were strongly correlated with both depression scores (r=0.63, P<0.001) [13] and anxiety scores (r=0.76, P<0.001) [13].

With respect to copeptin and increased pituitary-adrenal axis activity, many studies, although not all [11], have found circulating copeptin concentrations to be associated

(r>0.40) with either higher 24-hr urine cortisol excretion or higher circulating cortisol concentrations [12,19,29,32]. This has led to speculations that the associations between copeptin and IR and the metabolic factors related to IR could be the result of AVP-stimulated increased pituitary-adrenal axis activity leading to hypercortisolaemia and subsequently to cortisol-related metabolic abnormalities, including glucose intolerance and IR [4,8,17,38].

4.6. Adolescents versus young adults

Among our adolescents, we did not find any of the previously reported associations between copeptin and IR and the metabolic factors related to IR. Nevertheless, other studies have found associations between copeptin and the metabolic factors related to IR in children and adolescents [18-20], but the associations found have overall been less consistent compared with the studies in adults [2-8]. We have no obvious explanation for this age-related discrepancy, but it is important in this connection that the well described negative association between insulin and IR and the natriuretic peptide system could not be found among our adolescents, either [39], and that others have also found that age modulates the association of copeptin with IR [40]. Accordingly, in a family-based cross-sectional study, the association between copeptin and IR could only be found among the middle-aged and elderly but not among the younger adults [40]. We speculated that metabolic dysfunction could take some time to develop.

4.7. Strengths and limitations

The strengths of this study are its large sample size (although the participation rate was modest) and its community-based nature. It is a limitation that we do not have any measurements of kidney function and plasma sodium and plasma or urine cortisol as well as level of psychological stress in the adolescents and length of fasting. Further it is a limitation that our study is cross-sectional and therefore we cannot draw any conclusion regarding causality.

4.8. Conclusions

In this study, we found that age, based on different results in adolescents versus young adults, and psychological stress, as determined by an increased depression score, appear to influence the association between copeptin and IR.

Declarations of interest

None.

Acknowledgement

The European Youth Heart Study obtained funding from the Danish Council for Strategic Research, the Danish Heart Foundation and the Danish Health Fund. The funding sources was not involved in the study.

References

1. Christ-Crain M, Fenske W. Copeptin in the diagnosis of vasopressin-dependent disorders of fluid homeostasis. *Nat Rev Endocrinol* 2016; **12**:168-176.
2. Saleem U, Khaleghi M, Morgenthaler NG, Bergmann A, Struck J, Mosley TH Jr, Kullo IJ. Plasma carboxy-terminal provasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. *J Clin Endocrinol Metab* 2009; **94**:2558-2564.
3. 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**:E1065-1072.
4. Enhörning S, Bankir L, Bouby N, Struck J, Hedblad B, Persson M, Morgenthaler NG, Nilsson PM, Melander O. Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmö Diet and Cancer Study cardiovascular cohort. *Int J Obes (Lond)* 2013; **37**:598-603.
5. Asferg CL, Andersen UB, Linneberg A, Goetze JP, Jeppesen JL. Copeptin, a surrogate marker for arginine vasopressin secretion, is associated with higher glucose and insulin concentrations but not higher blood pressure in obese men. *Diabet Med* 2014; **31**:728-732.
6. Wannamethee SG, Welsh P, Papacosta O, Lennon L, Whincup PH, Sattar N. Copeptin, insulin resistance, and risk of incident diabetes in older men. *J Clin Endocrinol Metab* 2015; **100**:3332-3329.
7. Roussel R, El Boustany R, Bouby N, Potier L, Fumeron F, Mohammedi K, Balkau B, Tichet J, Bankir L, Marre M, Velho G. Plasma copeptin, AVP gene variants, and incidence of type 2 diabetes in a cohort from the community. *J Clin Endocrinol Metab* 2016; **101**:2432-2439.
8. Melander O. Vasopressin, from regulator to disease predictor for diabetes and cardiometabolic risk. *Ann Nutr Metab* 2016; **68 Suppl 2**:24-28.
9. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**:1595-1607.
10. Coelho R, Levandowski ML, Mansur RB, da Cunha GR, Asevedo E, Zugman A, Salum GA, Gadelha A, Pan PM, Rizzo LB, Manfro G, Mari JJ, Rohde LA, Miguel EC, Bressan RA, Brietzke E, Grassi-Oliveira R. Serum copeptin in children exposed to maltreatment. *Psychiatry Clin Neurosci* 2016; **70**:434-441.
11. Urwyler SA, Schuetz P, Sailer C, Christ-Crain M. Copeptin as a stress marker prior and after a written examination--the CoEXAM study. *Stress* 2015; **18**:134-137.
12. Siegenthaler J, Walti C, Urwyler SA, Schuetz P, Christ-Crain M. Copeptin concentrations during psychological stress: the PsyCo study. *Eur J Endocrinol* 2014; **171**:737-742.
13. Atescelik M, Yilmaz M, Korkmaz S, Goktekin MC, Gurger M, Ilhan N. The relationship between ghrelin and copeptin levels, and anxiety and depression levels in suicide attempts. *Clin Psychopharmacol Neurosci* 2017; **15**:256-260

14. Bergmann N, Gyntelberg F, Faber J. The appraisal of chronic stress and the development of the metabolic syndrome: a systematic review of prospective cohort studies. *Endocr Connect* 2014; **3**:R55-80.
15. Vancampfort D, Correll CU, Wampers M, Sienaert P, Mitchell AJ, De Herdt A, Probst M, Scheewe TW, De Hert M. Metabolic syndrome and metabolic abnormalities in patients with major depressive disorder: a meta-analysis of prevalences and moderating variables. *Psychol Med* 2014; **44**:2017-2028.
16. Yan YX, Xiao HB, Wang SS, Zhao J, He Y, Wang W, Dong J. Investigation of the relationship between chronic stress and insulin resistance in a chinese population. *J Epidemiol* 2016; **26**:355-360.
17. Pasquali R. The hypothalamic-pituitary-adrenal axis and sex hormones in chronic stress and obesity: pathophysiological and clinical aspects. *Ann N Y Acad Sci* 2012; **1264**:20-35.
18. Tenderenda-Banasiuk E, Wasilewska A, Filonowicz R, Jakubowska U, Waszkiewicz-Stojda M. Serum copeptin levels in adolescents with primary hypertension. *Pediatr Nephrol* 2014; **29**:423-429.
19. Rothermel J, Kulle A, Holterhus PM, Toschke C, Lass N, Reinehr T. Copeptin in obese children and adolescents: relationships to body mass index, cortisol and gender. *Clin Endocrinol* 2016; **85**:868-873.
20. Schiel R, Perenthaler TJ, Steveling A, Stein G. Plasma copeptin in children and adolescents with type 1 diabetes mellitus in comparison to healthy controls. *Diabetes Res Clin Pract* 2016; **118**:156-161.
21. Riddoch C, Edwards D, Page A, Froberg K, Anderssen SA, Wedderkopp N, Brage S, Cooper AR, Sardinha LB, Harro M, Klasson-Heggebø L, van Mechelen W, Boreham C, Ekelund U, Andersen LB, The European Youth Heart Study Team. The European Youth Heart Study–cardiovascular disease risk factors in children: rationale, aims, design and validation of methods. *J Phys Act Health* 2005; **2**:115–129.
22. Ried-Larsen M, Grøntved A, Østergaard L, Cooper AR, Froberg K, Andersen LB, Møller NC. Associations between bicycling and carotid arterial stiffness in adolescents: The European Youth Hearts Study. *Scand J Med Sci Sports* 2015; **25**:661-669.
23. Klakk H, Kristensen PL, Andersen LB, Froberg K, Moller NC, Grøntved A. Symptoms of depression in young adulthood is associated with unfavorable clinical- and behavioral cardiovascular disease risk factors. *Prev Med Rep* 2018; **11**:209-125.
24. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998; **21**:2191-2192.
25. Goharian TS, Gimsing AN, Goetze JP, Faber J, Andersen LB, Grøntved A, Jeppesen J. Mid-regional pro-atrial natriuretic peptide and blood pressure in adolescents: effect of gender and pubertal stage. *Blood Press* 2015; **24**:347-352.
26. Kinser PA, Goehler LE, Taylor AG. How might yoga help depression? A neurobiological perspective. *Explore (NY)* 2012; **8**:118-126.
27. van Gastel MD, Meijer E, Scheven LE, Struck J, Bakker SJ, Gansevoort RT. Modifiable factors associated with copeptin concentration: a general population cohort. *Am J Kidney Dis* 2015; **65**:719-727.

28. Lin C, Wang N, Shen ZP, Zhao ZY. Plasma copeptin concentration and outcome after pediatric traumatic brain injury. *Peptides* 2013; **42**:43-47.
29. Katan M, Morgenthaler N, Widmer I, Puder JJ, König C, Müller B, Christ-Crain M. Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. *Neuro Endocrinol Lett* 2008; **29**:341-346.
30. Mavani GP, DeVita MV, Michelis MF. A review of the nonpressor and nonantidiuretic actions of the hormone vasopressin. *Front Med (Lausanne)* 2015; **Mar 24, 2, 19 doi**: 10.3389/fmed.2015.00019.
31. Spruce BA, McCulloch AJ, Burd J, Orskov H, Heaton A, Baylis PH, Alberti KG. The effect of vasopressin infusion on glucose metabolism in man. *Clin Endocrinol (Oxf)* 1985; **22**:463-468.
32. Lundegaard Asferg C, Bjørn Andersen U, Linneberg A, Goetze JP, Holst JJ, Jeppesen JL. Copeptin, a surrogate marker for arginine vasopressin secretion, is positively associated with glucagon. *Diabet Med* 2018 **Sep 22**. doi: 10.1111/dme.13820. [Epub ahead of print]
33. Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care* 2008; **31 Suppl 2**:S262-268.
34. Rosenthal M, Haskell WL, Solomon R, Widstrom A, Reaven GM. Demonstration of a relationship between level of physical training and insulin-stimulated glucose utilization in normal humans. *Diabetes* 1983; **32**:408-411.
35. Bogardus C, Lillioja S, Mott D, Reaven GR, Kashiwagi A, Foley JE. Relationship between obesity and maximal insulin-stimulated glucose uptake in vivo and in vitro in Pima Indians. *J Clin Invest* 1984; **73**:800-805.
36. Krogh J, Gøtze JP, Jørgensen MB, Kristensen LØ, Kistorp C, Nordentoft M. Copeptin during rest and exercise in major depression. *J Affect Disord* 2013; **151**:284-290.
37. Demiralay C, Agorastos A, Yassouridis A, Jahn H, Wiedemann K, Kellner M. Copeptin - A potential endocrine surrogate marker of CCK-4-induced panic symptoms? *Psychoneuroendocrinology* 2017; **76**:14-18.
38. Di Dalmazi G, Pagotto U, Pasquali R, Vicennati V. Glucocorticoids and type 2 diabetes: from physiology to pathology. *J Nutr Metab* 2012;2012:525093. doi: 10.1155/2012/525093.
39. Goharian TS, Goetze JP, Faber J, Andersen LB, Grøntved A, Jeppesen JL. Associations of proatrial natriuretic peptide with components of the metabolic syndrome in adolescents and young adults from the general population. *Am J Hypertens* 2017; **30**:561-568.
40. Canivell S, Mohaupt M, Ackermann D, Pruijm M, Guessous I, Ehret G, Escher G, Pechère-Bertschi A, Vogt B, Devuyst O, Burnier M, Martin PY, Ponte B, Bochud M. Copeptin and insulin resistance: effect modification by age and 11 β-HSD2 activity in a population-based study. J Endocrinol Invest 2018; **41**:799-808.

Table 1. General characteristics of the study population stratified by age group

Variables	Adolescents (n=340-351)	Young Adults (n=612-617)	*P*-value
Demographic			
Female (%)	55.8	52.4	0.30
Age (years)	15.7±0.39	24.3±2.97	<0.001
Occasional or regular smoking (%)	26.9	27.4	0.51
Frequency of alcohol intake once/month or more often (%)	63.8	78.5	<0.001
Anthropometric			
Height (cm)	171.1±8.5	173.7±9.6	<0.001
Weight (kg)	60.2 (54.8-66.7)	71.0 (62.3-82.2)	<0.001
BMI (kg/m^2)	20.3 (18.8-22.5)	23.5 (21.4-26.1)	<0.001
Waist circumference (cm)	71.0 (67.5-75.5)	78.8 (72.5-86.0)	<0.001
Hemodynamic			
Systolic BP (mmHg)	112.8±11.1	115.0±10.4	<0.002
Diastolic BP (mmHg)	61.7±6.4	69.7±8.0	<0.001
Lipid			
Total cholesterol (mmol/L)	4.0±0.7	4.6±0.8	<0,001
LDL cholesterol (mmol/L)	2.3±0.6	2.7±0.8	<0.001
HDL cholesterol (mmol/L)	1.3±0.3	1.4±0.4	<0.001
Triglycerides (mmol/L)	0.86 (0.6-1.1)	1.08 (0.8-1.4)	<0.001
Metabolic			
Glucose (mmol/L)	4.9±0.5	5.0±0.4	<0.001
Insulin (pmol/L)	48.4 (36.1-63.3)	39.1 (28.5-56.1)	<0.001

HOMA2-IR (units)	0.9 (0.7-1.2)	0.7 (0.5-1.0)	<0.001
Copeptin (pmol/L)	6.97 (4.7-9.8)	5.49 (3.7-7.9)	<0.001
Tanner stages			
Post pubertal[a] (%)	86.3	-	-

Data are presented as mean ± standard deviation (SD) for normally distributed data or as median (interquartile range) for non-normally distributed variables. Categorical variables are presented as frequency percent. Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA2-IR, homeostasis model assessment 2 of insulin resistance. [a]Tanner stage 4 and 5.

Table 2. Linear regression-based associations of plasma natural logarithm of copeptin with variables of interest stratified by age group

Variables	Adolescent *B* (95% CIs) (n=314-319)	*P*	Young adults *B* (95% CIs) (n=584-587)	*P*	*P* for age-group by copeptin interaction
Body mass index (kg/m^2)	-0.04 (-0.06 to -0.01)	<0.014	0.02 (-0.01 to 0.04)	0.21	<0.002
Waist circumference (cm)	-0.02 (-0.04 to 0.002)	0.72	0.01 (-0.005 to 0.032)	0.14	<0.004
Systolic blood pressure (mmHg)	-2.08 (-4.27 to 0.11)	0.062	0.43 (-0.99 to 1.84)	0.55	<0.037
Diastolic blood pressure (mmHg)	-0.15 (-1.50 to 1.20)	0.83	1.59 (0.40 to 2.79)	<0.010	<0.019
Total cholesterol (mmol/L)	-0.04 (-0.19 to 0.11)	0.59	0.09 (-0.03 to 0.21)	0.16	0.58
Low-density lipoprotein cholesterol (mmol/L)	0.003 (-0.05 to 0.06)	0.90	0.03 (-0.02 to 0.07)	0.24	0.76
High-density lipoprotein cholesterol (mmol/L)	-0.02 (-0.08 to 0.05)	0.65	-0.02 (-0.07 to 0.02)	0.33	0.18
Ln triglycerides (mmol/L)	-0.04 (-0.14 to 0.05)	0.38	0.09 (0.02 to 0.15)	<0.011	<0.024
Glucose (mmol/L)	-0.03 (-0.13 to 0.07)	0.56	0.10 (0.03 to 0.16)	<0.004	<0.030
Ln insulin (pmol/L)	-0.01 (-0.12 to 0.09)	0.80	0.19 (0.11 to 0.26)	<0.001	<0.007
Ln HOMA2-IR (units)	-0.01 (-0.12 to 0.09)	0.78	0.19 (0.11 to 0.27)	<0.001	<0.006

Data are expressed as unstandardized regression coefficients (*B)* with 95% confidence intervals (CIs). All data in the adolescent group are adjusted for age, gender and Tanner stages. All data in the young adult group are adjusted for age and gender. The interaction analysis included adjustment for sex. Abbreviations: HOMA2-IR, homeostasis model assessment 2 of insulin resistance; Ln, natural logarithm.

Table 3. Relationship between degree of insulin resistance (HOMA2-IR) and psychological stress (depression score) with successive adjustments for candidate mediators

Outcome variable	Depression score	Ln Copeptin	Fitness
	***B* (95% CIs)**	***B* (95% CIs)**	***B* (95% CI)**
Ln HOMA2-IR, units	0.0092 (0.0033 to 0.0150)	-	-
(n = 576)	P<0.002		
Ln HOMA2-IR, units	0.0080 (0.0022 to 0.0137)	0.159 (0.079 to 0.239)	-
(n = 576)	P<0.007	P<0.001	
Ln HOMA2-IR, units	0.0016 (-0.0042 to 0.0075)	0.095 (0.020 to 0.171)	-0.0295 (-0.0349 to -0.0240)
(n = 539)	P=0.58	P<0.013	<0.001

Data are presented as unstandardized regression coefficients (*B*) with 95% confidence intervals (CIs). All models are adjusted for age, sex, smoking status and alcohol intake. Abbreviations: HOMA2-IR, homeostasis model assessment 2 of insulin resistance; Ln, natural logarithm.