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COPEPTIN IN CARDIOVASCULAR DISEASE AND DYSGLYCEMIA

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For Dad and Mom

Vits er þörf,
þeim er víða ratar;
dælt er heima hvat;
at augabragði verðr,
sá er ekki kann
ok með snotrum sitr.

He hath need of his wits who wanders wide,
aught simple will serve at home;
but a gazing-stock is the fool who sits
mid the wise, and nothing knows.

- *Hávamál*

Copeptin in cardiovascular disease and dysglycemia

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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CONTENTS

ABSTRACT

Background The impaired prognosis in patients with cardiovascular disease (CVD) and dysglycemia is not fully explained by traditional risk markers, among them hyperglycemia and hyperlipidemia. Understanding the developmental mechanism of CVD and identifying potential biomarkers are important parts in attempts to reduce cardiovascular mortality and morbidity in people with and without dysglyemia. The overall aim was to study biomarkers, in particular copeptin, in hopes of shedding light on the reasons for this association. Copeptin, a marker of vasopressin release, has been suggested to be involved in both the development of CVD and dysglycaemia.

Aims. The general aims were to evaluate copeptin levels in relation to CVD and dysglycemia by studying:
1. the association between copeptin and Insulin-like Growth Factor Binding Protein-1 (IGBFP-1) and the development of levels over time in patients with acute myocardial infarction (MI) and type 2 diabetes mellitus (T2DM) **(Study I)**
2. the copeptin levels and their prognostic importance in patients with acute MI and newly detected glucose abnormalities **(Study II)**
3. the copeptin levels beyond the acute phase of MI, whether they differ between known and unknown MI and to explore the prognostic implications of copeptin in relation to markers of stress and heart failure **(Study III)**
4. whether copeptin is associated with early manifestations of atherosclerosis and the prognostic impact of copeptin in individuals without previous MI **(Study IV)**

Acute MI and T2DM. Copeptin and IGFBP-1 were analyzed in patients with acute MI and known T2DM (median age 70 years; men 68%), measured at hospital admission (n=393) and discharge (n=309) and three months later (n=288). The primary outcome was cardiovascular events (CVE) after 2.5 years of follow up. The copeptin-levels were 21.8 pmol/L (median) at admission, 8.5 pmol/L at discharge and 8.4 pmol/L three months later. IGFBP-1 increased over time. Copeptin and IGFBP-1 correlated with each other at all time points. Copeptin, not IGBFP-1, remained a predictor for CVE at all time points in adjusted Cox-regression analysis.

Acute MI and newly discovered glucose abnormalities. Copeptin was analyzed in patients (n=166) with acute MI without previously known glucose abnormalities (median age 64 years; 70% men) and in age and gender matched controls (n=168). Based on an oral glucose tolerance test the participants were classified as having normal (NGT) or abnormal glucose tolerance (AGT). The primary outcome was total mortality. The copeptin levels were higher in patients (median 10.5 pmol/L) than for controls (5.9 pmol/L; $p<0.01$). Patients with AGT had higher copeptin levels than those with NGT ($p<0.01$). Copeptin was associated with increased mortality in unadjusted Cox-regression analyses.

Elderly individuals with previous MI. Copeptin, cortisol and NT-proBNP were analyzed in 926 participants in the observational ICELAND MI-study (median age 76 years; 49% men). A total of 246 had a previous MI whereof 91 were recognized (RMI) and 155 previously unknown (UMI) but discovered with cardiac magnetic resonance imaging. The primary outcome was CVE during 9.1 years of follow up. The copeptin levels were higher in individuals with previous MI independent of whether it was previously known or not. Copeptin correlated with evening cortisol and NT-proBNP. Copeptin was associated with CVE and total mortality after adjusting for cortisol and NT-proBNP separately. Copeptin continued to associate with total mortality in the final model (including copeptin, copeptin measured in the morning and evening, NT-proBNP, age, sex, serum creatinine and previous heart failure). Copeptin was not associated with heart failure or MI.

Elderly individuals and atherosclerosis. Copeptin and coronary artery calcium (CAC) score were analyzed in 677 participants without MI from the ICELAND MI-study. The Agatston method was used to measure CAC score by means of computed tomography. The CAC score was divided into four categories: CAC score 0 (no visible plaque), 1-99 (mild), 100-399 (moderate) and ≥400 (extensive plaque burden). The primary outcome was CVE during a median of 9.1 years follow up. Individuals with CAC score 1-399 had similar copeptin levels as those with a CAC score 0 while participants with CAC ≥400 had significantly higher copeptin levels. Copeptin was not associated with CVE but with total mortality in unadjusted analysis and after adjustments for CAC score but not after adjusting for sex and age. The event rates were significantly higher for participants with high CAC score, irrespective of copeptin level.

Conclusion. Copeptin was elevated in patients with acute MI, especially in those with newly detected glucose abnormalities. The levels remain elevated in the post-MI phase independent of dysglycemia. The relationship between copeptin and IGFBP-1 during the acute phase of MI persisted in the post-MI phase in patients with T2DM. Copeptin correlated with stress and heart failure markers, but this did not fully explain the association with total mortality. Individuals with high CAC scores had high copeptin levels, but the prognosis was influenced by other factors. In summary, the results support the theory that copeptin should be seen as an expression of general disease and subsequent poor prognosis, and not as a specific marker for CV disease or dysglycemia.

SAMMANFATTNING

Bakgrund. Hjärt-kärlsjukdom och dysglykemi är en vanlig kombination, som är förenad med en ofördelaktig prognos. Detta förklaras inte till fullo av traditionella riskfaktorer så som hyperglykemi och hyperlipidemi. Förbättrad insikt om mekanismer bakom utvecklingen av hjärt-kärlsjukdom i kombination med identifikation av nya, tänkbara biomarkörer för aterosklerosutvecking är en förutsättning för att minska kardiovaskulär död- och sjuklighet hos personer med och utan dysglykemi. Huvudsyftet var att studera biomarkörer, särskilt copeptin, i hopp om att belysa faktorer bakom detta samband. Copeptin, en markör för frisättning av vasopressin, har föreslagits vara involverad i både utvecklingen av hjärt-kärlsjukdom och dysglykemi.

Mål. Att granska copeptin i relation till hjärt-kärlsjukdom och dysglykemi genom att studera:
1. om det tidigare beskrivna sambandet mellan copeptin och Insulin-like Growth Factor Binding Protein-1 (IGBFP-1) kvarstår vid sjukhusutskrivningen och tre månader därefter hos patienter med akut hjärtinfarkt och känd typ 2 diabetes mellitus (T2DM), samt att studera utvecklingen av biomarkörnivåerna över tid **(Studie I)**
2. copeptinnivåerna samt att undersöka dess prognostiska betydelse hos patienter med akut hjärtinfarkt och nyupptäckt glukosstörning **(Studie II)**
3. copeptinnivåerna hos individer i stabil fas efter en hjärtinfarkt och om dessa skiljer sig ifall infarkten var tidigare känd eller ej samt att undersöka copeptins prognostiska förmåga i förhållande till markörer av stress och hjärtsviktmarkörer **(Studie III)**
4. huruvida copeptin är associerat med olika grader av ateroskleros **(Studie IV)** och copeptins prognostiska betydelse hos deltagare utan hjärtinfarkt.

Akut hjärtinfarkt och typ 2 diabetes. Copeptin och IGFBP-1 analyserades hos patienter med akut hjärtinfarkt och känd T2DM (medianålder 70 år; män 68%), mätt vid tidpunkterna för ankomst till sjukhus (n=393) och utskrivningen (n=309) samt tre månader senare (n=288). Det primära utfallsmåttet var hjärt-kärlhändelser, under 2.5 års uppföljning. Copeptin-nivåerna var 21.8 pmol/L (median) vid ankomst, 8.5 pmol/L vid utskrivning, och 8.4 pmol/L efter tre månader. IGFBP-1 ökade över tid. Copeptin och IGFBP-1 korrelerade med varandra vid alla tillfällen. I en justerad Cox-regressionsanalys kvarstod associationen mellan copeptin, men inte IGFBP-1, och kardiovaskulär händelse vid alla tillfällen.

Akut hjärtinfarkt och nyupptäckta glukosstörningar. Copeptin analyserades hos patienter (n=166) med akut hjärtinfarkt utan kända glukosstörningar (medianålder 64 år; 70% män) samt hos friska kontroller (n=168). Med hjälp av ett oralt glukostoleranstest delades deltagarna in i två grupper med normal (NGT) respektive onormal glukostolerans (AGT). Det primära utfallsmåttet var dödlighet. Copeptinnivåerna var högre hos patienter (median 10.5 pmol/L) än kontroller (5.9 pmol/L; $p<0.01$). Patienter med AGT hade högre copeptinnivåer än de med NGT ($p<0.01$). Copeptin var associerat med ökad dödlighet i ojusterade Cox-regressionanalyser, men dessa samband kvarstod inte efter justeringar.

Äldre individer med tidigare hjärtinfarkt. Copeptin, kortisol och NT-proBNP analyserades hos 926 deltagare i den observationella ICELAND MI-studien (medianålder 76 år; 49% män). Totalt hade 246 individer tidigare hjärtinfarkt. Av dessa var 91 kända sedan tidigare medan 155 upptäcktes i samband med MR undersökning av hjärtat. Primära utfallsmåttet var hjärt-kärlhändelser under 9.1 års uppföljning. Copeptinivåerna var högre hos individer med tidigare hjärtinfarkt jämfört med de utan samt skilde sig inte mellan de med tidigare känd eller okänd hjärtinfarkt. Copeptin korrelerade med kortisol uppmätt på kvällen och NT-proBNP. Copeptin var associerat med hjärt-kärlhändelser efter justering för kortisol och NT-proBNP separat. Associationen mellan copeptin och total dödlighet kvarstod i den slutliga modellen. Copeptin var inte associerat med hjärtinfarkt eller hjärtsvikt.

Äldre individer och ateroskleros. Copeptin och Coronary Artery Calcium (CAC) score analyserades hos 677 deltagare utan tidigare hjärtinfarkt i ICELAND MI-kohorten. Agatson-metoden användes för at mäta CAC score med hjälp av skiktröntgen. CAC score delades in i fyra kategorier: 0 (ingen synlig plack); 1-99; 100-399; ≥400 (omfattande plackbörda). Det primära utfallsmåttet var hjärt-kärlhändelser under 9.1 års uppföljning. Individer med CAC score <400 hade liknande copeptinnivåer medan deltagare med CAC score ≥400 hade signifikant högre copeptinnivåer. Copeptin var inte associerat med hjärt-kärlhändelser. Copeptin var associerat med total dödlighet i ojusterad analys och efter justeringar för CAC score, men inte efter justering för kön och ålder. Antalet händelseser var signifikant högre för deltagare med hög CAC score, oavsett copeptinnivå.

Slutsatser. Copeptin var förhöjt vid akut hjärtinfarkt, framförallt hos individer med nyupptäckta glukosstörningar. Nivåerna förblev förhöjda i efterförloppet. Associationen mellan vasopressin och IGFBP-1 hos patienter med typ 2 diabetes under den akuta fasen av hjärtinfarkt kvarstod även i efterförloppet. Copeptin korrelerade med markörer för stress och hjärtsvikt. Detta förklarade dock inte fullständigt sambandet med total dödlighet. Individer med höga CAC score hade höga copeptinnivåer, men prognosen påverkades av andra faktorer. Sammantaget talar de aktuella fynden för att copeptin bör ses som ett uttryck för allmän sjukdom och efterföljande dålig prognos däremot inte som en specifik markör för kardiovaskulär sjukdom eller dysglykemi.

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ACS	Acute coronary Syndrome
AGES	Age, Gene/Environment Susceptibility
AMI	Acute Myocardial Infarction
CABG	Coronary Artery Bypass Grafting
CAC	Coronary Artery Calcium
CAD	Coronary Artery Disease
Copeptin	C-terminal pro vasopressin
CVD	Cardio Vascular Disease
CVE	Cardio Vascular Event
DIGAMI 2	The Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction 2
IFG	Impaired Fasting Glucose
GAMI	Glucose Tolerance in Patients with Acute Myocardial Infarction
HbA1c	Glycated Hemoglobin A1c
HR	Hazard Ratio
ICELAND MI	Imaging Cardiac Evaluation to Locate Areas of Necrosis and Detect MI
IFG	Impaired Fasting Glucose
IGF-1	Insulin Growth Factor-1
IGFBP-1	Insulin-like Growth Factor Binding Protein-1
IGT	Impaired Glucose Tolerance
IHD	Ischemic Heart Disease
MI	Myocardial Infarction
NT-proBNP	N-terminal prohormone brain natriuretic peptide
OGTT	Oral Glucose Tolerance Test
RMI	Recognized Myocardial Infarction
T2DM	Type 2 Diabetes Mellitus
UMI	Unknown Myocardial Infarction
WHO	World Health Organization

LIST OF ORIGINAL PAPERS

The thesis is based on the following studies, which are referred to by their Roman numerals.

I. Smaradottir MI, Catrina S-B, Brismar K, Norhammar A, Gyberg V, Mellbin LG.

Copeptin and insulin-like growth factor binding protein-1 during follow-up after an acute myocardial infarction in patients with type 2 diabetes: A report from the Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction 2 cohort

Diab Vasc Dis Res. 2019 Jan;16(1):22-27
doi: 10.1177/1479164118804451

II. Smaradottir MI, Ritsinger V, Gyberg V, Norhammar A, Näsman P, Mellbin LG.

Copeptin in patients with acute myocardial infarction and newly detected glucose abnormalities - A marker of increased stress susceptibility? A report from the Glucose in Acute Myocardial Infarction cohort

Diab Vasc Dis Res. 2017 Mar;14(2):69-76
doi: 10.1177/1479164116664490

III. Smaradottir, MI, Andersen K, Gudnason V, Näsman P, Rydén L, Mellbin LG.

Copeptin is associated with mortality in elderly people

Eur J Clin Invest. 2021 Feb 11:e13516
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IV. Smaradottir, MI, Andersen K, Gudnason V, Näsman P, Rydén L, Mellbin LG.

Copeptin is related to coronary atherosclerotic plaque burden in elderly people

Manuscript

INTRODUCTION

CARDIOVASCULAR AND CORONARY ARTERY DISEASE

Cardiovascular Disease (CVD) is still the main cause of death globally. Six conditions are attributable to over 95% of these deaths: coronary artery disease (CAD), atrial fibrillation, cardiomyopathy, hypertensive heart disease (resulting in heart failure), rheumatic heart disease and stroke (1). CAD, the main contributor to the burden of CVD, is mostly caused by atherosclerosis and has different manifestations as further outlined below (2). The World Health Organization estimated that 17.9 million people died from CVD in 2019 representing 32% of overall worldwide deaths (3). In the European Union (EU) 36% of all mortality, approximately 1.8 million, were linked to CVD causes in 2017 (4). The somewhat higher proportion of CVD deaths in the EU compared to global CVD death rates is probably due to the predominance of intermediate to high income countries in the EU, where deaths due to lower respiratory tract infections and diarrheal diseases are uncommon (5). In addition to consequences for the individual the economic burden of CVD is high. As an example the annual EU cost for health care of CVD is estimated to €210 billion (4).

During the last 20 years there has been a decreasing mortality in CVD in the western world due a combination of preventive efforts and better treatment including revascularization procedures and improved pharmacological tools (1, 6). In contrast, the number of people living with CVD increases due to a combination of improved survival, an aging population and a population growth in middle- to low-income countries (1, 7, 8).
INTERHEART, a case-control study including 15 152 cases with a first acute myocardial infarction (AMI) and 14 820 age- and gender-matched controls from 52 countries showed that 90% of the population attributable risk of AMI in men and 94% in women were explained by nine modifiable risk factors: abdominal obesity, diabetes, hypertension, increased blood lipids, lack of regular physical activity, a poor psychosocial environment, smoking, too low consumption of fruits and vegetables and too much alcohol (9). Self-reported diabetes was one of the strongest risk factors both in men and women.

The atherosclerotic process
Atherosclerosis is a chronic inflammatory condition of the arterial wall involving both the innate and adaptive immune system. It begins with dysfunction of the monolayer of endothelial cells and eventually leading to the buildup of plaques in the inner layer of the arteries, the intima. In response to endothelial injury, e.g. sheer stress and inflammation, the endothelial cells secrete cytokines and adhesion molecules from the injured area thereby attracting monocytes. Monocytes attach and migrate through the vascular intima. Once through they differentiate into macrophages that further release cytokines. The compromised vascular barrier eventually leads to infiltration and retention of low density lipoprotein (LDL), one of the most atherogenic lipoproteins, in the intima (10). Subsequently the retained LDL becomes modified, e.g. by oxidation and the macrophages engulf and accumulate the oxidized LDL and form foam cells (11). This process activates inflammatory signaling pathways, further promoting immune cell recruitment and LDL modification (oxidation). Smooth muscle cells migrate from the medial layer to the intima where they proliferate and produce extracellular matrix and are also able to take up oxidized LD and differentiate into foam cells (12). When the foam cells have become saturated by oxidized LDL, apoptosis occurs and the content is

released into the matrix. This accumulation of modified LDL particles, foam cell formation, macrophage and smooth muscle cell apoptosis in combination with ineffective clearance of dead cells, causes an increase in the inflammatory response, eventually resulting in the formation of a central necrotic lipid core, covered by a cap of fibrous tissue just below the endothelial layer (10, 13). This fibrous cap may become thin (thin-cap fibroatheroma) and susceptible to rupture (14, 15) exposing its thrombogenic interior to the blood, activating the formation of a thrombus (luminal thrombosis), the eventual complication of atherosclerosis. Deposits of calcium occur through all these steps of plaque formation, and is thought to start as microcalcification (not visual by computed tomography (CT) (16)) in the intima as vesicles are released as macrophages and small muscle cells die in the intima, mediating mineralization (17, 18). These microcalcifications fuse with disease progression, eventually forming plates of calcium deposits within the necrotic core material and the extracellular matrix (Figure 1)(19, 20).

The atherosclerotic process is thought to be significantly influenced by risk factors mentioned above including hyperlipidemia, hypertension, smoking and diabetes (21). The exact mechanisms are not fully understood but current evidence suggest that hyperlipidemia and hyperglycemia are capable of disrupting the homeostasis of a normally functioning endothelial layer, leading to decreased production and bioavailability of nitric oxide (NO), causing endothelial dysfunction that eventually leads to the initiating the atherosclerotic process (22, 23). Atherosclerosis can be asymptomatic or associated with symptoms and can result in different clinical manifestations as outlined below.

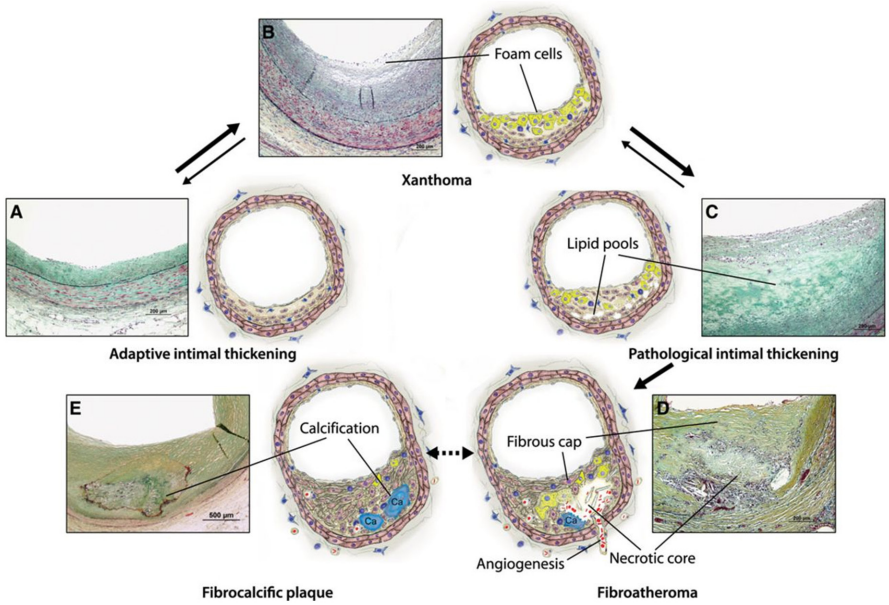

Figure 1. Mechanism of plaque formation. Adapted Bentzon et al. (24) by permission of Wolter's Kluwer Health.

Coronary artery calcification

The coronary artery calcification (CAC) can be detected and quantified by means of a noninvasive imaging technique – a multidetector row computed tomography (CT). The amount of CAC observed on CT correlates histologically with the total plaque burden (25). An area is calcified if the density is >130 Hounsfield-units (HU) in at least three adjacent pixels (area ≥1mm^2). The most commonly used tool to quantify the severity of the calcification is the Agatston method that uses the weighted sum of CAC multiplied by density weighting factor related to the maximum CT attenuation within a given calcified lesion. The resulting unit is named the CAC score (26).

$$\text{Agatston score}_{(\text{lesion})} = \text{Area} \times \text{Density weighting factor}$$

Density weighting factor 1: 130-199 HU, 2: 200-299 HU, 3: 300-399 HU, 4: ≥400 HU

$$\text{Agatston score}_{(\text{total})} = \sum \text{Agatston score}_{(\text{lesion})}$$

The CAC score does not mirror the plaque vulnerability but it reflects the coronary artery plaque burden i.e. it represents an expression for the extent of the CAD (27). A standardized way to describe different degrees of the plaque burden is to express the CAC score as belonging to one of four categories: 0: no calcification visible (no identifiable calcified plaque); 1-99 CAC score (minimal-mild plaque); 100-399 CAC score (moderate plaque); ≥400 CAC score (extensive atherosclerotic plaque). These categories indicate a very low, low-moderate, moderate-high and very high risk for CAD (28, 29). Of note is that although the presence of CAC demonstrates the presence of coronary atherosclerosis the calcification does not correlate with the narrowing of the lumen, as the specificity of a stenosis of ≥50% is only 50% (30).

CAC score has become a clinically available cardiovascular (CV) risk assessment tool (31). The 2019 American College of Cardiology (ACC)/American Heart Association (AHA) guidelines on the Assessment of Cardiovascular Risk stated that measuring CAC score with CT should be considered when there are uncertainties in primary prevention intervention i.e. whether cholesterol lowering drugs should be used (32). Its usefulness is, however, less well established by evidence. The European Society of Cardiology guidelines on CVD prevention in clinical practice from 2021 underline that measuring CAC score should be considered in asymptomatic individuals at moderate risk for cardiovascular events (CVE) for primary prevention (33).

Clinical manifestations of coronary artery disease

As mentioned above, atherosclerosis can lead to CAD, which in turn may present itself in different ways. CAD may be asymptomatic during long periods of time. As atherosclerotic plaques grow they may protrude into the arterial lumen and cause narrowing, thereby diminishing blood flow in the affected artery. Stable CAD is characterized as episodes of reversible mismatch between myocardial demand/supply of oxygen and nutrients causing ischemia or hypoxia inducible by stressors such as exercise and emotions (34). These episodes usually cause symptoms in the form of chest pain or chest discomfort i.e. angina pectoris.

Acute and life-threatening manifestations of coronary atherosclerosis, an acute coronary syndrome (ACS), include unstable angina, myocardial infarction (MI) and sudden cardiac death. MI is subdivided into non-ST elevation MI (NSTEMI), and ST-elevation MI (STEMI) based on their manifestations on the electrocardiogram (ECG). An ACS is usually caused by a ruptured plaque or plaque erosion triggering the development of an acute thrombosis that leads to an abrupt and critical reduction in blood flow (35). The subsequent thrombotic obstruction it is typically incomplete in unstable angina and NSTEMI, however, complete in STEMI (36). The symptoms and clinical definitions of MI are further outlined below. The most severe form of CAD is when it is presented as sudden cardiac death.

Myocardial infarction

The universal definition of MI was updated in 2018 by the joint task force of ESC and ACC, AHA, and the World Health Federation as the presence of acute myocardial injury in the setting of evidence of acute myocardial ischemia, recognized by abnormal cardiac biomarkers (rising/falling of cardiac troponin values (cTn) values) (37). MI is classified into various subtypes based on pathological, clinical, prognostic and treatment differences. Type 1 MI is caused by an acute plaque disruption (rupture or erosion), which can cause a thrombotic occlusion of the coronary artery. Type 2 MI is a MI secondary to myocardial ischemia due to imbalance between oxygen supply and demand (e.g. coronary embolism, anemia, coronary artery spasm, arrhythmias, hyper- or hypotension). It may e.g. be produced by severe hypotension/shock with or without the presence of CAD. Type 3 MI includes conditions causing symptoms of myocardial ischemia together with new ECG changes or ventricular fibrillation, in patients suffering cardiac death before blood samples of biomarkers are obtained, or before an elevation of biomarkers has had time to develop. Types 4 a-c are MI related to percutaneous coronary interventions and Type 5 MI to coronary artery bypass grafting (37).

The clinical criteria for Type 1 MI is the rising and/or falling pattern of cTn values in the setting of signs of acute myocardial ischemia including at least one of the following: new onset ischemic ECG changes or the development of new pathological Q-waves; imaging evidence of new loss of viable myocardium/new regional wall abnormal mobility; or a coronary thrombus visualized by coronary angiography or autopsy and/or symptoms of acute myocardial ischemia (37). In the presence of classical symptoms, often a combination of chest, mandibular, upper extremity or gastrointestinal discomfort, the MI is usually recognized by the patient and/or the health care providers. However, patients may also be asymptomatic or suffer atypical symptoms such as palpitations, breathing problems, sweating, lightheadedness and upset stomach. These manifestations may sometimes be misinterpreted as of musculoskeletal, gastrointestinal or respiratory origin. A MI can thus remain undetected if these signs are misinterpreted. Such MI is called a silent or unrecognized MI (UMI). It is not discovered until the myocardial damage becomes visible by means of different imaging modalities. This may be an ECG where UMI is defined as new pathologic Q waves or various imaging techniques including echocardiography, nuclear imaging or cardiac magnetic resonance (CMR). CMR is the most sensitive method detecting MI with a sensitivity of 83% and a specificity of 86% (38), thereby disclosing a higher number of UMI than those diagnosed by means of an ECG (39, 40). The size of myocardial scars detected by late gadolinium enhancement by CMR does not seem to change over time making the detection possibility of UMI scars reliable (41).

UMI was first described in 1912 by James B. Herrick, who grouped patients with coronary obstructions seen during autopsy according to their clinical manifestations. He reported on a couple of patients with "little or no pain" before they died (42). In 1984 the Framingham Study revealed that 28% and 35% of all MI (diagnosed by means of ECG) in men respective women were UMI. This type of MI was more common in elderly men, a pattern not replicated in women in whom the proportion between UMI and recognized MI (RMI) did not vary much with age. Furthermore, it was noted that the prognosis of the patients with UMI was similar to that in patients with RMI (43). A compatible proportion of ECG recognized UMI was reported in the observational Reykjavik study and once again it was reported that patients with UMI had similar prognosis as those with as RMI (44, 45). The diagnosis of UMI in the Framingham, Reykjavik and subsequently other similar studies (46, 47) were based on ECG changes. This makes it likely that the true proportion of UMI was underestimated since it is well known that ECG changes may disappear over time (48). In a substudy from the Reykjavik cohort including 936 elderly Icelandic individuals (median age 76; 48% male), who were investigated with both ECG and CMR, the prevalence of CMR detected UMI was 17% (n= 157) while the corresponding prevalence by means of ECG was 5% (n=46; $p<0.001$) (49). In addition, the prevalence of CMR detected UMI was higher than that of RMI (17% vs. 9.7%). The mortality event rate was similar when CMR detected UMI was compared to RMI ($p = 0.40$). The prevalence of UMI was higher in patients with diabetes (49) in particular in the presence of albuminuria (50). The reason is not fully understood, but it may be secondary to autonomic neuropathy, involving the pain perception pathway from the heart (51).

DYSGLYCEMIA

Diabetes, a heterogeneous group of metabolic disorders characterized by persistent hyperglycemia, is estimated to be the fourth leading cause of disability worldwide (52). In 2019 the estimated global prevalence in the adult population between the age of 20 and 79 years was 9.3% corresponding to 463 million cases. This proportion is predicted to increase to 10.9% (700 million) by 2045 (53). In total about 727 billion US dollars of the global health expenditure are invested in diabetes and its complications, a sum expected to increase to 776 billion by 2045 if the expected development is not counteracted (54).

The main categories of diabetes are type 1 diabetes mellitus, type 2 diabetes mellitus (T2DM), gestational diabetes mellitus, and other specific types such as maturity onset diabetes of the young and secondary diabetes. The majority (90%) of patients with diabetes have T2DM, and this condition is, together with IGT, the focus of the present thesis. T2DM is characterized by hyperglycemia induced by peripheral insulin resistance in combination with deficient β-cell insulin production and secretion (60, 61). Individuals with T2DM often lack symptoms of hyperglycemia and may remain undiagnosed for years (62, 63), and during this period of time several complications may develop. Chronic hyperglycemia is associated with dysfunction of small vessels, i.e. microvascular complications such as retinopathy, nephropathy and neuropathy. In addition, there is an increased risk for macrovascular complications comprising CV, cerebrovascular and peripheral artery disease manifestations (64). In addition to hyperglycemia, patients with T2DM often have other risk factors for vascular complications.

The most important are hypertension (prevalence in T2DM %) (65, 66), and dyslipidemia (prevalence in T2DM 70%) (67). Accordingly, T2DM is a multifactorial disease in need of multifactorial management (68).

The term dysglycemia is often used to describe the different categories of diabetes as outlined below as well as preceding conditions all characterized by various degrees of elevated glucose levels. Intermediate hyperglycemia, or pre-diabetes, comprises impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) both early steps in the development from normoglycemia to T2DM. The yearly conversion rate from pre-diabetes to T2DM is about 5-10% (56, 57). IFG reflects distorted hepatic glucose output (58), while IGT mainly is due to inadequate glucose uptake secondary to insulin resistance and/or reduced capacity to produce insulin (59). In this thesis, the term dysglycemia includes IFG, IGT and T2DM.

Etiology of type 2 diabetes

The exact etiology of T2DM is not known. A genetic predisposition is of considerable importance as well as environmental and behavioral factors that result in physical inactivity and overweight/obesity, thus increases insulin resistance likely due to the release of free fatty acids and cytokines from the adipose tissue (69, 70). The onset of hyperglycemia can trigger both β-cell dysfunction and insulin resistance. In response, the pancreatic β-cells compensate by increasing their mass as well as function inducing hyperinsulinemia, which during some time results in adequate glucose control (71). However, by time the β-cells fail due to exhaustion which leads to the development of hyperglycemia and subsequent diabetes (71).

Diagnostic tests and classification of dysglycemia

The diagnostic criteria for diabetes were first published in 1965 by the World Health Organization (WHO) (72). They were subsequently updated and the current criteria as issued by the WHO and the American Diabetes Association (ADA) are outlined in Table 1 (73). Three methods, fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), and glycated hemoglobin A1c (HbA1c) are recommended for the diagnosis of diabetes. Two positive tests are required to establish the diagnosis unless the patient has classical symptoms of hyperglycemia (e.g. polyuria and polydipsia) and a random plasma glucose ≥11.1 mmol/L. The glycemic threshold for diagnosing diabetes is based on the cut-off point above which the retinopathy starts to increase (55, 74). The risk for macrovascular complications is not accounted for in the current diagnostic criteria but seems to start already below the cut off for diabetes (75). The implication is that the risk for CVD is already increased in patients with IGT and that a first manifestation of diabetes may be a CVE (76).

WHO encourages the measurement of the fasting plasma glucose (FPG) as well as the plasma glucose levels two hours after an ingestion of 75 g of glucose (2hPG) derived from an OGTT to be performed even in the absence of overt fasting hyperglycemia in people at high risk for diabetes (77). The use of HbA1c, reflecting the average plasma glucose levels the last 8-12 weeks, has been recommended as a diagnostic test with a cut point for diagnosing diabetes of 48 mmol/mol (6.5%). A problem is that values <48 mmol/mol (6.5%) do not exclude dysglycemia (78). Interestingly, it is only the 2hPG that predicts CVE in patients with CAD without previous T2DM (79).

The diagnostic criteria for prediabetes including cut-off levels are presented in Table 1. They are similar for WHO and ADA as regards IGT but differ for IFG since ADA recommends a lower threshold (5.6 mmol/L vs. 6.1 mmol/L) (Table 1). This level was introduced 2003 to enable a better identification of people at risk for T2DM and CVD. Due to lack of evidence of benefits regarding a reduction of progression to T2DM and of CVE WHO has neither adapted this lowering of fasting glucose nor the concept of a high-risk HbA1c (Table 1) (76).

Table 1. A summary of recommendations for diagnostic criteria for diabetes and prediabetes from WHO 2006, 2011 and 2019 as well as ADA 2019.

Glucometabolic state	WHO	ADA
High-risk HbA1c[3]	–	39-47 mmol/mol (5.7-6.4%)
Impaired fasting glucose		
Fasting plasma glucose[1]	6.1-6.9 mmol/L	5.6-6.9 mmol/L
2 hour plasma glucose[2]	<7.8 mmol/L (if measured)	<7.8 mmol/L (if measured)
Impaired glucose tolerance		
Fasting plasma glucose[1]	<7.0 mmol/L	<7.0 mmol/L
2 hour plasma glucose[2]	7.8-11.0 mmol/L	7.8-11.0mmol/L
Diabetes		
Fasting plasma glucose[1]	≥7.0 mmol/L	≥7.0 mmol/L
or 2 hour plasma glucose[2]	≥11.1 mmol/	≥11.0 mmol/L
$HbA1_C$[3]	≥ 48 mmol/mol (6.5%)	≥ 48 mmol/mol (6.5%)
Random blood glucose[4]	≥11.1 mmol/L	≥11.1 mmol/L

[1] Minimum 8 hours fasting, [2] Venous plasma glucose measured after ingestion of 75g oral glucose load dissolved in 250 ml water. [3] IFCC (DCCT), [4] Along with hyperglycemic symptoms, such as polydipsia, polyuria and polyphagia.

DYSGLYCEMIA AND CARDIOVASCULAR DISEASE

The association between diabetes and diseases of the heart has been known for over 100 years. In 1883 Vergely recognized a frequent association between diabetes and angina pectoris. He was so intrigued by this that he recommended the examination of the urine in patients with angina pectoris (80). In the middle of the 20th century several large epidemiological studies showed that CVD was frequent in patients with diabetes, and furthermore that the survival rate after an AMI was more dismal than for those without diabetes (81-83). It was speculated that diabetes might accelerate and increase the extent of atherosclerotic changes in arteries (83).

People with T2DM have a two to four times increased risk of CVD (84-86). T2DM has indeed been considered to be a CAD equivalent (87) in terms of CVD risk. This enhanced risk is already present in the presence of prediabetes (88) and may accordingly be seen as linked to dysglycemia. The unfavorable prognosis increases in fact almost linearly with increasing levels of plasma glucose. The proportion of AMI in patients with diabetes mellitus has declined during the last decades (89) together with an improved prognosis as shown e.g. by data from the Swedish National Diabetes Register. It is, however, still increased (90) as demonstrated in the Swedish Coronary Care register where the one year mortality after AMI is almost two times higher in those with T2DM (91).

The mechanisms behind the predisposition to develop atherosclerotic complications in patients with T2DM is not fully understood. Hyperglycemia, dyslipidemia and insulin resistance are all important links by inducing both structural and functional changes in the arterial wall as well as inducing a prothrombotic state. In the presence of dysglycemia the bioavailability and biological activity of NO is reduced leading to endothelial dysfunction and vascular remodeling (92). Furthermore, hyperglycemia activates four signaling mechanisms mainly in endothelial cells: protein kinase C, the hexosamine and polyol pathway fluxes and increases the advanced glycation end production formation (93, 94). These pathways eventually lead to overproduction of reactive oxygen species (ROS), that further decreases the NO availability. The ROS accumulation activates transcription factors promoting the expression of adhesion molecules and cytokines that compromises vascular function i.e. further increasing endothelial dysfunction and stimulate oxidative stress and vascular inflammation (95). Hyperglycemia furthermore increases the availability of plasminogen activator inhibitor-1, fibrinogen, factors VII and X thereby contributing to a thrombogenic environment (96) and vasoconstriction via upregulation of endothelin-1 along with hyperinsulinemia (97).

Although hyperglycemia is the hallmark of T2DM (98, 99) randomized trials in patients with T2DM, aiming at tight glycemic control by means of various glucose-lowering drugs alone or in combination, have been inconclusive as regards the possibility to lower the increased CV risk. The UK Prospective Diabetes Study (UKPDS), which explored improved blood glucose control achieved by means of sulphonylureas or insulin, did not reveal any significant impact on CV outcomes apart from in a subgroup of overweight patients with T2DM treated with metformin (100, 101). Likewise, the Veterans Affairs Diabetes Trial (VADT), and the Action in Diabetes and Vascular Disease:Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trials failed in their attempts to improve the CV prognosis by means of strict glycemic control (102) while the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was stopped prematurely due to an increased CV mortality in patients randomized to intensive glucose-lowering, targeting a HbA1c <6.0% (42 mmol/mol) compared to standard therapy aiming at a HbA1c between 7.0-7.9% (53-63 mmol/mol) (103, 104). The potential explanation for these failures is that other factors than hyperglycemia in itself are important contributors to the dismal prognosis. Recent cardiovascular outcome trials (CVOTs) using several glucagon like peptide 1 receptor agonists and sodium glucose transporter-2 inhibitors revealed that these drugs can improve CV prognosis (105, 106). The cardioprotective effects seem not only to be related to glucose lowering but also to a beneficial impact on other CV risk factors, as well as potential direct effects on the CV system (107). Accordingly, hyperglycemia in patients with T2DM should probably be seen as a marker of underlying pathophysiological processes, as described above, which contributes but is not the only causal factor. This indicates a more complex relationship between dysglycemia and CVD, expanding the interest for other connecting pathways such as those discussed in this thesis.

VASOPRESSIN

Although recognized since 50 years that extracts of the pituitary gland, presumably vasopressin, elevated the blood pressure of dogs (109) it was not until 1951 Turner and colleagues first isolated vasopressin, also known as the antidiuretic hormone (108). During the following years Vincent du Vigneaud, an American biochemist, succeeded to chemically synthesize vasopressin (and oxytocin), for which he in 1955 was awarded the Nobel prize in Chemistry by the following reason: ”For his work on biochemically important sulphur compounds, especially for the first synthesis of a polypeptide hormone”(110).

Vasopressin is a small neuropeptide containing nine amino acids in a ring structure. It is synthesized as the precursor prepro-vasopressin by magnocellular neurosecretory cells (Figure 2). These cells originate from the supraoptic (primarily) and paraventricular nuclei of the hypothalamus and project through the pituitary stalk to axon terminals in pars nervosa of the posterior pituitary. After the synthetization of prepro-vasopressin in the hypothalamus, the pre-part of the protein is removed and a vasopressin precursor migrates along the neural axons, where it undergoes additional processing and subsequently is stored in neurosecretory vesicles in the axon terminals of the magnocellular cells as the final hormonal products: vasopressin, copeptin and neurophysin II (vasopressin carrier protein). Upon proper stimuli, vasopressin, accompanied by copeptin and neurophysin II, are secreted into the systemic circulation through the cavernous sinus and superior vena cava (111).

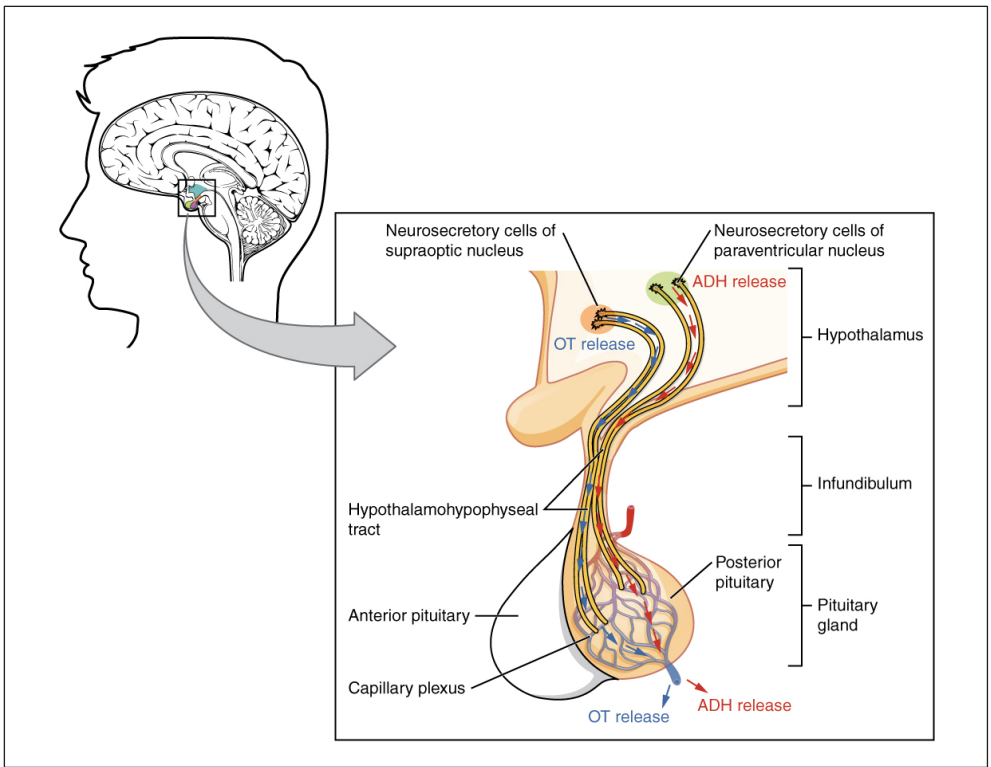

Figure 2. Vasopressin production in the pituitary gland and release from hypothalamus. Reproduced by OpenStax College – Anatomy & Physiology, Connexions with permission Web site. http://cnx.org/content/col11496/1.6/, Jun 19, 2013., CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=30148142

The hormone vasopressin plays an essential role in osmoregulation and hemodynamic control due to vasoconstriction and water retention. Its release is controlled by osmotic and non-osmotic pathways (112) with hypothalamic osmoreceptors as the main regulators responding to increased plasma osmolality (113). Nonosmotic stimuli via baroreceptors in the left atrium, carotid sinus and aortic arch can also cause vasopressin release. This occurs if the neuronal output from the baroreceptors decreases due to low blood pressure, inducing a release of vasopressin from the hypothalamus (114, 115).

Circulating vasopressin acts via three guanine nucleotide protein-coupled receptors (116) located on different tissues and with specific functions (Table 2). The effects following V1a and V1b-receptor activations are mediated by calcium signals while the effects of the V2-receptor activations are mediated by cyclic AMP.

Table 2. The locations and effects of the three described receptors of vasopressin.

Receptor	Location	Function
V1a	Vascular smooth cells	Vasoconstriction
	Platelets	Platelet aggregation
	Renal vasculature	Reduces medullary blood flow
	Liver	Glycogenolysis
	Brain	Cortisol synthesis and secretion
	Myocytes	Hypertrophy
V1b	Brain	ACTH secretion
	Pancreas	Augments glucagon and insulin release
V2	Kidney collecting duct	Increased urine concentration by aquaporin 2 recruitment
	Endothelial cells	Release of von Willebrand factor and Factor VIII

The V1a- receptors are mainly located on vascular smooth muscle cells where vasopressin activation results in arterial vasoconstriction (117). V1a-receptors are also expressed on platelets and activation may cause platelet aggregation and subsequent thrombosis (114, 115). Furthermore, V1a-receptors are expressed in the kidney (118, 119) and in the liver where they are suggested to stimulate metabolic pathways including glycogenolysis and gluconeogenesis (120). Finally, V1a-receptors are found on the myocytes where they exert hypertrophic effects by increasing protein synthesis and activation of cardiac fibrinoblasts (121, 122).

The V1b-receptors are located in the anterior pituitary gland, mediating secretion of adrenocorticotrophic hormone (ACTH) release resulting in subsequent catecholamine release from the anterior pituitary gland as well as the adrenal gland (123). Moreover, they are expressed on the pancreatic islet cells (124) promoting the release of insulin and glucagon (125-127) further indicating that vasopressin has a complex role in glucose homeostasis (128-130).

The most well-known effect of the "antidiuretic" vasopressin is regulation of the reabsorption of water via the V2- receptors (131), mainly located in the collecting ducts of the kidneys. Activation causes redistribution and insertion of aquaporin 2-rich vesicles in the luminal membrane of the cells. The aquaporin 2 are vasopressin dependent water channels which regulate water permeability and allow an increase in water reabsorption when the osmotic driving force is present. Via the V2-receptors vasopressin also stimulates sodium reabsorption. This mechanism, explains why insufficiency or absence of vasopressin, a condition called diabetes insipidus, is characterized by hyponatremia, along with polyuria and polydipsia. Finally, the V2-receptors are found in the endothelium where vasopressin seems to increase the release of von Willebrand factor (vWF) and Factor VIII (132, 133).

There are two ways of reducing vasopressin activation: 1) increased fluid intake, which lowers plasma osmolality and therefore has a negative feedback on vasopressin secretion (134); 2) vasopressin receptor antagonism by means of pharmacological agents. Vaptans constitutes a class of drugs that act by blocking the action of vasopressin at its receptors with varying selectivity. Terlipressin is a selective V1a-receptor agonist. It is used in patients with esophageal varices and a hepatorenal syndrome as it reduces the portal venous blood flow (135). Furthermore, selective V2-receptor antagonists (e.g. Tolvaptan) are used to treat polycystic kidney disease, and syndrome of inappropriate antidiuretic hormone secretion, with aquaresis and increased plasma osmolality as the mechanism of action (136, 137).
In contrast, desmopressin, a synthetic analogue of 8-arginine vasopressin with V2-receptor selective actions, increases vasopressin activation and thereby water absorption, which leads to increased antidiuretic effects. It is used for the treatment of central diabetes insipidus, a rare disease caused by partial/complete deficiency of vasopressin (138).

The first vasopressin radioimmunoassays were developed in 1973 by Robertsons's group from United States of America (USA) (139). Vasopressin has a short biological half-life in the circulation (about 10-35 minutes) (140). It is rapidly metabolized and cleared by the hepatic vasopressinases (141, 142) and renal clearance (143). In addition, it is unstable ex vivo and >90% is bound to platelets which makes it difficult to measure (139, 144).

COPEPTIN

Considering the problems with direct measurements of vasopressin alternative methods have been developed. Copeptin, the C-terminal part of pre-provasopressin (Figure 3) has emerged as a surrogate marker for vasopressin release. It was discovered and characterized as a 39-amino acid glycopeptide in the 1970s (145, 146), but not babtized to copeptin until 1986, by B Levy and colleagues (147). Copeptin is secreted in equimolar amount to vasopressin and it is more stable in the circulation and easier to measure (148). It does not seem to have any biological function on its own although it has been hypothesized that it contributes to the 3D folding of the vasopressin precursor as it ascends down the neuronal axon before it is released into the blood stream (149). It is not known how copeptin is cleared from the body, but it has been speculated that there is at least a partial renal clearance since copeptin has been identified in urine (150).

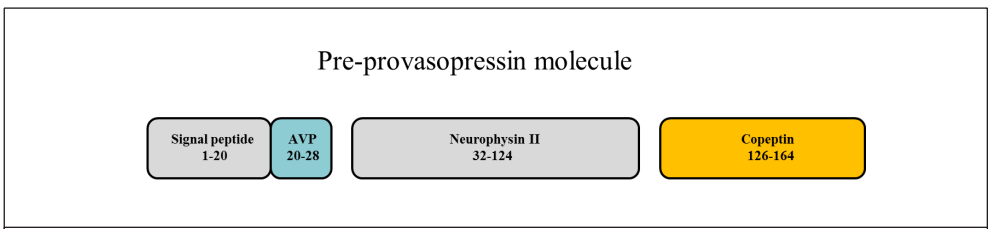

Figure 3. Overview of pre-provasopressin molecule. The numbers represent amino acids.

Copeptin measurements

The assessment of copeptin does not require any complex pre-analytical steps. It can be measured manually or with fully automated assays (151). Moreover, unlike vasopressin, copeptin measurements only require small amount of plasma (50µL compared to 400µL for vasopressin). These factors make analysis of copeptin as a suited alternative to routine measurements of vasopressin.

Several copeptin assays are available whereof two of sufficient quality to be accepted for clinical use within the EU (150). Alternate assays, available in USA and China "for research only", lack technical and clinical validation (129). The original manual sandwich immunoluminometric assay (LIA), the B.R.A.H.M.S. CT-proAVP LIA, was originally described by Morgenthaler and colleagues (152). It is based on a one-step assay with coated tube technology with one antibody bound to tubes (polystyrene), and another labeled for chemiluminescence detection. The lower detection limit is 0.4 pmol/l, and the functional assay sensitivity (FAS) <1 pmol/L (152). This assay has been succeeded by a fully automated immunofluorescent assay, the B.R.A.H.M.S. KRYPTOR Compact Plus. It uses another technology, called Time Resolved Amplified Cryptate Emission. Instead of washing/ separation steps to eliminate background noise it lengthens the light from the right signal. The analytical detection limit with this method is 0.7 pmol/L, and FAS <1.08 pmol/L. The incubation time is only 14 minutes compared to the 2 hours for CT-proAVP LIA.

Copeptin in healthy populations and various diseases

The median copeptin level, which is uninfluenced by age, ranges between 3.8 to 6.0 pmol/L in healthy cohorts (152-155), but with higher levels in men than women (156). The levels are influenced by renal function with increasing levels in chronic and end stage kidney disease compared to normal kidney function (157, 158). Copeptin levels correlate strongly with osmotic changes. Thus, fluid intake in healthy cohorts causes a rapid decline while the levels increase during thirst (150).

Copeptin is a promising prognostic marker in several medical conditions causing high stress levels, e.g. acute MI. In 101 critically ill patients copeptin values increased significantly with disease severity. Patients with sepsis had a median copeptin level of 50.0 (interquartile range (IQR): 8.5-268) pmol/L), those with severe sepsis had 73.6 (IQR 15.3-317) pmol/L), while the highest levels were seen in patients with septic shock, 171.5 (IQR: 35.1-504) pmol/L (159). Increasing copeptin levels have also been related to the severity and outcome of lower respiratory tract infections. Of 545 patients admitted to an emergency department with symptoms of lower respiratory tract infection, those with community acquired pneumonia had significantly higher copeptin levels than those with lower respiratory tract infections such as acute exacerbations of chronic obstructive pulmonary disease, and acute bronchitis (30.5 [IQR: 18.2-58.9] pmol/L vs. 13.8 [6.2-25.9] pmol/L, $p<0.001$) (154). In this patient material, the copeptin levels were significantly higher in those who died (70.0 [28.8-149.0] pmol/L vs. 24.3 [10.8-43.8] pmol/L, $p<0.001$). Nickel et al. proposed that copeptin is a potential marker for biomarker based risk prediction in elderly individuals presenting at an emergency department with various nonspecific complaints, such as "not feeling well", as copeptin was significantly higher in non-survivors than survivors (160, 161).

Copeptin has also been studied in the context of different manifestion of CVDs as further outlined below and then in particular with a focus on risk prediction. De Marchis et al. reported that copeptin predicted functional outcome and mortality after three months in patients with ischemic stroke. The median copeptin level at admission was 14.2 (IQR: 5.9-46.5) pmol/L (162). Furthermore, copeptin predicted recurrent vascular events in patients with transient ischemic attacks or stroke (163).

Copeptin and coronary artery disease
That copeptin levels are increased in patients with MI was initially described in 2007 by Khan et al. in the Leicester Acute Myocardial Infarction Peptide (LAMP) study (155). Copeptin levels were highest at the time of hospital admission for MI (day 1 vs. day 2 -5; $p<0.01$) reaching a plateau after three to five days and with higher values exceeding those in healthy controls. In 2009 Reichlin et al. showed that copeptin measured in 487 patients with symptoms indicating AMI increased the sensitivity and specificity of Troponin T (TnT) to exclude AMI (164). The ability of copeptin to rule out AMI, when used together with TnT, has subsequently been confirmed (165-167). This is reflected in the European Society of Cardiology Guidelines for the management of acute coronary syndromes in patients without persistent ST-segment elevation (168), which recommends copeptin as an additional biomarker, in combination with troponin, for the early rule-out of MI when sensitive or high-sensitivity cardiac troponin assays are unavailable. Furthermore, copeptin has shown to be a significant predictor for CVE, and mortality after AMI (155, 169).

The reason for the elevated copeptin levels during AMI is not fully explained, but considering that vasopressin is a stress hormone, it may reflect endogenous stress (170) as outlined for other acute diseases. Vasopressin may also have detrimental effects on the myocardium, eventually leading to heart failure, a hypothesis supported by the fact that elevated copeptin levels after AMI has been related to an increased risk of heart failure (171, 172). Elevated copeptin levels are indeed seen both in acute and chronic heart failure (173-175), and it has been suggested that vasopressin is involved in the pathophysiology of heart failure besides an activation of the sympathetic nervous system and the renin-angiotensin aldosterone axis (165-167). Chronic activation of the vasopressin signaling secondary to low cardiac output via stimulation of the non-osmotic pathway and subsequent activation of the V1a-receptors in the myocytes is thought to contribute to left ventricular remodeling. Furthermore, copeptin predicts mortality and re-hospitalization due to heart failure in patients with chronic heart failure (148). Molvin et al showed that in 286 patients hospitalized with newly diagnosed heart failure or exacerbated heart failure, both elevated copeptin and NT-proBNP levels were associated to higher mortality at discharge (176). It was, however, only NT-proBNP that was associated with re-hospitalization due to cardiac causes, indicating that the development of HF induces an imbalanced neurohormonal compensatory response, which is not fully understood.

Vasopressin may also be directly involved in the pathophysiology of CAD. As described above there are vasopressin receptors in e.g. the endothelium. Accordingly, vasopressin may facilitate thrombosis via activation of platelet receptors, as well as the release of important proteins important for homeostasis (von Willebrand factor and Factor VIII) (132, 133). It is therefore of interest to further study copeptin in more stable phases of atherosclerosis both in people with and without previous MI.

Copeptin and dysglycemia
Activation of the vasopressin system, measured as copeptin, has been related to several glucometabolic conditions. An association has been reported between copeptin levels and insulin resistance, obesity as well as the metabolic syndrome in observational studies (177-181). Increased copeptin levels have also been associated with an increased risk of diabetes. In a report based on the Malmö Diet and Cancer Study (MDC; a 10-year prospective case-control study) by Enhörning et al. (181) participants in the top quartile of copeptin levels had a 2- to 3-fold excess risk of developing diabetes during 12.6 years of follow-up compared with those in the lowest quartile.

In a substudy from the second Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) trial copeptin, measured in patients with T2DM at hospital admission due to an AMI, correlated with insulin like growth factor binding protein-1 (IGFBP-1), one of six binding proteins for insulin growth factor 1(IGF-1). It was also shown that copeptin was the stronger predictor of CVE, and since it seemed to explain the prognostic impact of IGFBP-1 in patients with T2DM and AMI, it was suggested that the IGF-1 axis may be activated via the vasopressin system (169). This suggested that copeptin may be a pathogenetic factor of interest to address to improve outcome in such patients. Since both vasopressin and IGFBP-1 levels are influenced by stress it is, however, important to study whether the findings in the acute phase of a MI remain in less stressful states to further explain their actual role (182).

INSULIN LIKE GROWTH FACTOR BINDING PROTEIN-1

The IGFBP-1 modulates the availability and activity of IGF signaling as the binding prolongs the half-life of IGF and prevents the activation of receptor signaling. Low levels of IGFBP-1 are related to increased risk of T2DM in a general population and possibly also to increased CV risk (183). During the development of T2DM the IGFBP-1 concentrations decreases indicating increased hepatic insulin resistance. In contrast, high levels of IGFBP-1 in AMI patients and T2DM have been related to increased CV mortality and morbidity (184). This is potentially caused by a decreased insulin production due to beta-cell dysfunction. Low IGF levels have been related to the development of T2DM and to acute MI.

IGFBP-1 is mainly produced by the liver. The production is up-regulated in response to pro-inflammatory cytokines, physiological stress and down-regulated by inhibitory effects of insulin (185). Other factors may influence the IGFBP-1 and IGF-1 axis. A connection between the IGF and the vasopressin hormonal systems has been suggested. In a study of 14 patients with diabetes insipidus the IGFBP-1 levels increased when the vasopressin analogue desmopressin was infused, suggesting a pathophysiological relation between the two hormonal system (186). Furthermore, high levels of IGFBP-1 was associated with increased all-cause mortality in patients with HF whether caused by an ischemic event or not (187) and has been related to the development of HF in elderly people (188).

CORTISOL

Cortisol is a glucocorticoid hormone synthesized in the adrenal cortex and an important part of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a central part of the neuroendocrine response system and cortisol is considered an important stress hormone. In this context stress is a physiological condition when something disrupts the homeostasis balance of the body, e.g. during an AMI (189). A stressor initiates the release of corticotroponin-releasing hormone (CRH) through brain stem and limbic pathways from the hypothalamus and once in the pituitary gland, CRH stimulates the secretion of adrenocorticotropic hormone (ACTH), which in turn stimulates the synthesis of cortisol from the adrenal glands (190). Cortisol plays an important role in stress response as it contributes to energy supply by potentiating gluconeogenesis via glucagon-stimulation, thereby contributing to increased glucagon output (191). This could explain the increased cortisol levels described in T2DM (192), and the metabolic syndrome (193).

A potential interplay between vasopressin and cortisol has been described. For example vasopressin correlates well with cortisol levels in patients with different degrees of acute stress (170). Further studies are of interest to explore the relation between these two stress markers.

NT-PROBNP

Pre-pro B-type natriuretic peptide (BNP) precursor is synthesized and secreted mainly by ventricular cardiomyocytes in response to increased mechanical load and wall distension (194). Pre-proBNP is then processed to the active BNP fragment and the inactive N-terminal pro B-type natriuretic peptide (NT-proBNP). BNP decreases the cardiac preload and afterload by relaxing smooth muscles causing a reduction of the systemic vascular resistance and central venous pressure in combination with an increased natriuresis. The value of these biomarkers is mainly explored and used in relation to heart failure. Similar to the relation between vasopressin and copeptin, BNP and NT-proBNP are released in equimolar amounts where NT-proBNP has a longer half-life and in addition is more stable in room temperature than BNP (195). Hence, NT-proBNP has become a commonly used biomarker in patients with heart failure, important both for diagnostic purposes and risk stratification (196). Since it has been proposed that vasopressin is involved in the pathophysiology of heart failure it is of interest to study vasopressin in relation to NT-proBNP (197-199).

AIMS

The overall aim was to study biomarkers of prognostic importance, in particular copeptin, in dysglycemic patients with CVD. The overall hypothesis was that copeptin could be a pathophysiological factor for the development of CVD, as well as dysglycemia.
The specific aims in the different parts of the thesis were:

Study I
To study if the previously observed predictive value of copeptin and IGFBP-1, at the time for hospital admission for an acute coronary syndrome, remains when measured at hospital discharge and three months thereafter.

Study II
To characterize copeptin levels and to explore their prognostic importance in patients with acute myocardial infarction with and without newly detected glucose abnormalities.

Study III
To evaluate whether the previously observed association between copeptin and myocardial infarction extends beyond the acute phase of the disease, whether copeptin differs between known and unknown myocardial infarction as well as to evaluate the prognostic information of copeptin and explore whether it is associated with markers of stress and/or heart failure

Study IV
To assess whether copeptin is associated with the different degrees of coronary atherosclerosis expressed as the coronary artery calcium score and the prognostic impact of copeptin in participants without previous myocardial infarction.

MATERIAL AND METHODS

STUDY POPULATIONS IN SUMMARY

This thesis comprises data from four studies originating from three different study populations as summarized in Table 3.

Table 3. An overview of the four studies on which the cohorts of this thesis are based.

Study	I	II	III	IV
Data source Participants (no)	DIGAMI 2 1253	GAMI 322	ICELAND MI 926	ICELAND MI 926
Time of data collection	1998-2003	1998-2002	i. 2002-2006 ii. 2004-2007	
Design	Randomized controlled trial	Case-control study	Cohort study	
Present participants (no)	393 At discharge 309 After 3 months 288	322	926	677
Median follow-up (years)	2.5	Patients 11.6 Controls 10.4	9.1	9.1
Outcomes	1.CVE[1] 2. a. CV mortality b. Non-fatal MI/stroke	1.Total mortality 2. CV mortality 3. Major CVE[2]	1. CVE[3] 2. Total mortality 3. Heart failure 4. MI	1. CVE[3] 2. Total mortality

i. Random recruitment, ii. All eligible and willing participants with T2DM.[1] CV mortality and non-fatal MI or stroke, [2] AMI, stroke, severe heart failure or CV death, [3] CV mortality, stroke, MI, PCI or CABG.

DATA SOURCES AND STUDY POPULATIONS

Study I The Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction 2 (DIGAMI 2)

The DIGAMI 2 was a prospective randomized, multicentre, open trial with blinded evaluation comparing three different glucose lowering management protocols in patients with T2DM and suspected AMI recruited January 1998 to May 2003 at 44 centres in Denmark, Finland, the Netherlands, Norway, Sweden, and the United Kingdom (200). A total of 1253 patients (mean age 68 years; 67% males) were randomized to three groups 1) 24-hour insulin-glucose infusion followed by subcutaneous insulin-based long-term glucose control (group 1; n=474), 2) 24-hour insulin-glucose infusion followed by standard glucose control (group 2; n=473) and 3) glycemic management according to local practice (group 3; n=306). The median follow-up was 2.5 (interquartile range 1.03 – 3.00) years. No patient was lost to follow up. The objective was to compare mortality and morbidity difference between the groups. An independent committee unaware of group allocation adjudicated all events. Since mortality and morbidity did not differ significantly between the three groups they have been merged into one epidemiological cohort for the purpose of the present study. A total of 575 patients from all three original groups participated in a pre-planned biochemical program with repeated blood sampling at 3, 6, 9 and 12 months.

The patient population in **Study I** comprised participants in the biochemical program in whom both copeptin and insulin-like growth factor binding protein-1 (IGFBP-1) measurements were available at the time of hospital admission (n=393), at hospital discharge (n=309) and 3 months thereafter (n=288) (Figure 4). Data from samples at hospital admission have been reported previously (169). Thus, **Study I** is a follow up of the original report with the intention to explore whether the findings persisted during the post MI period.

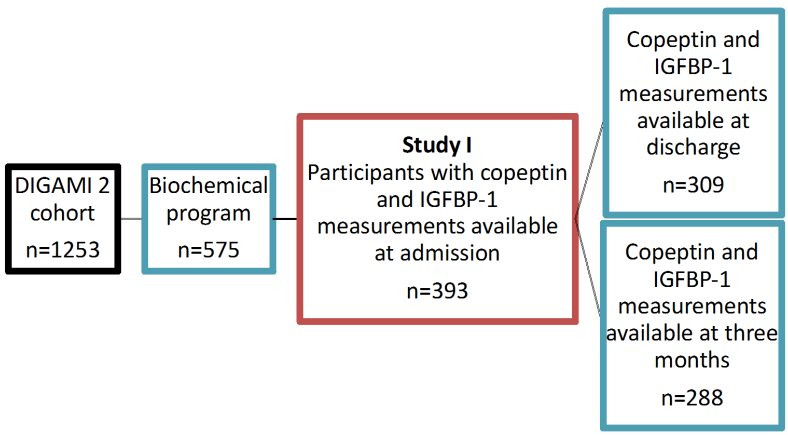

Figure 4. Flowchart reflecting the study participants in **Study I**.

Study II Glucose Tolerance in Patients with Acute Myocardial Infarction (GAMI)
GAMI was a prospective observational study, which recruited patients admitted to two Swedish coronary care units for acute myocardial infarction November 1998 to December 2000. All patients were ≤80 years, free from previously known T2DM and with a baseline capillary blood glucose <11.1 mmol/L and serum creatinine <200 μmol/L. Blood glucose concentrations were analysed as soon as possible after admission and all patients were planned for a standardized OGTT. A total of 181 participants were enrolled, of whom 168 were characterized before hospital discharge, by means of the OGTT, as having either normal glucose tolerance (NGT), or abnormal glucose tolerance (AGT; impaired glucose tolerance (IGT) or T2DM). Sex- and age matched controls (n=185), without previously known diabetes or CVD apart from hypertension, were recruited from the general Swedish population January 2001 to July 2002. The patients and controls were followed for CVE (AMI, stroke, severe heart failure, CV mortality), and total mortality until December 31, 2011 i.e. during a median of 11.6 years for patients and 10.4 years for controls. Information on CVE were obtained from outpatient and hospital records if needed complemented by a telephone interview with the participant or a close relative. Mortality causes were categorized by two experienced physicians according to available hospital records and to the ICD-10 codes on the death certificates obtained from the Swedish National Death Registry. The reasons were subsequently categorized as CV (death from MI/aortic dissection/stroke/sudden death), cancer or other reasons. One patient and one control were lost to follow up (201, 202).

Study II comprised patients with stored blood samples available for copeptin measurement taken the morning following admission (n=166) and from baseline for the controls (n=168). In this subset glucose categorization was available in 154 patients at discharge (NGT=48, AGT=106) and in all controls (NGT=109, AGT=59).

Study III and IV – The Imaging Cardiac Evaluation to Locate Areas of Necrosis and Detect MI (ICELAND MI)

The Imaging Cardiac Evaluation to Locate Areas of Necrosis and Detect MI (ICELAND MI), is a substudy of the Age, Gene/Environment Susceptibility (AGES)-Reykjavík study (n=5764) (203). The AGES-Reykjavík included survivors from the original Icelandic Reykjavík study, a randomly selected population-based cohort of Icelandic people born between 1907 and 1935 and living in the greater Reykjavik area. They were followed since 1967. The participants in the ICELAND MI study were enrolled from January 2004 to January 2007. The endpoints, CVE, heart failure and total mortality, were based on a national mortality register with authentication by means of all death certificates and hospital records. The median time of follow up was 9.1 years ending February 28, 2010 for heart failure and December 31, 2014 for the other events. Study participants were examined by means of a questionnaire, blood sampling, blood pressure recordings, ECG and cardiac magnetic resonance (CMR) at three occasions (49, 203).

Of the 936 participants in the ICELAND MI study 248 had a previous MI either supported by hospital/surveillance records and defined as a recognized MI (RMI, n=91), or a "clinically silent" MI based on the CMR findings and defined as an unrecognized MI (UMI, n=157).
Study III included the 926 of the 936 participants in ICELAND MI, who had blood samples available for copeptin analysis. Samples from ten participants were excluded from further analysis due to insufficient amounts of blood or the formation of blood clots (Figure 5).
Study IV included individuals from the original ICELAND MI cohort without a MI in whom copeptin and CAC measurements were available (49). Eleven participants without MI were excluded due to lacking copeptin analyses (n=8) or CAC scores (n=3) (Figure 5).

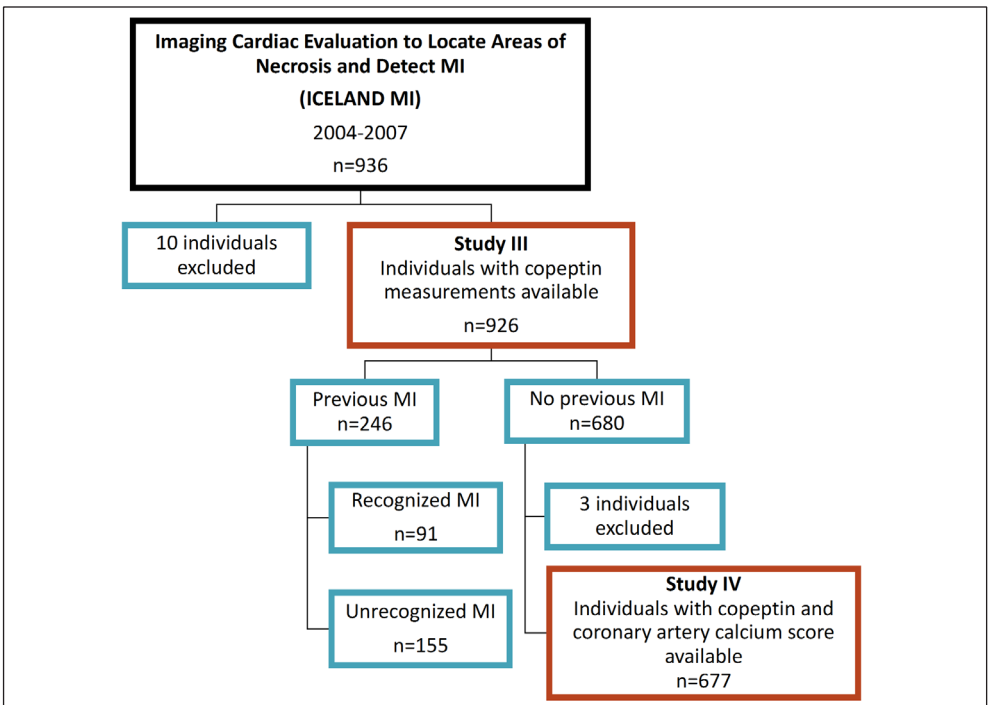

Figure 5. Flowchart reflecting the study participants in **Studies III** and **IV**.

DEFINITIONS

Myocardial infarction
In **Study I-II** patients had an AMI in the presence of typical symptoms (chest pain lasting at least 15 minutes during the last 24 hours) and/or new Q-waves and/or ST-segment changes in two or more leads. They were included in the presence of a final diagnosis of MI according to the joint consensus of the European Society of Cardiology (ESC) and ACC (204).

In **Study III-IV** participants were classified as having a RMI if they had a history of MI supported by hospital or surveillance records (49). An UMI was defined as myocardial damage disclosed by the CMR as a late gadolinium enhancement in the sub-endocardial myocardium in the absence of any hospital or surveillance records indicating MI.

Type 2 diabetes mellitus
In **Study I** T2DM was defined as patients already diagnosed and receiving glucose lowering treatment, whether it was diet, tablets or insulin, as well as those with an admission blood glucose >11.0 mmol/L according to criteria established 1979 by the National Diabetes Group (205).

In **Study II**, T2DM and IGT were defined according to the 1998 WHO classification (206). T2DM was diagnosed if the fasting venous plasma glucose was >7 mmol/L or the 2-h post glucose was >11 mmol/L. IGT was considered present if the fasting plasma venous glucose was < 7.0 mmol/L and the 2-h post glucose load ≥7.8 mmol/L and ≤11 mmol/L.

In **Study III-IV** individuals were considered to have T2DM in the presence of a positive case history, a fasting glucose >7.0 mmol/L or if they were prescribed glucose lowering drugs at the baseline visit (49).

Quantification of coronary artery calcium
The observed coronary calcifications were quantified by means of the Agatston method and presented as a CAC score (26). CAC score was categorized into four standard categories in Study IV: 0: zero calcification visible (no identifiable plaque), 1-99 CAC score (minimal-mild plaque), 100-399 CAC score (moderate plaque), ≥400+ CAC score (extensive atherosclerotic plaque), indicating a very low, low-moderate, moderate-high to very high risk for CVD (28, 207).

LABORATORY ANALYSES

Copeptin
In **Study I** copeptin was analysed using a sandwich immunofluorescence assay (LUMI test CT-proAVO, B.R.A.H.M.S. AG, Hennigsdorf/Berlin, Germany) in plasma samples obtained at the time for hospital admission (before the initiation of glucose lowering strategies) and at the time for hospital discharge and three months thereafter. All specimens were stored at -70°C at the Karolinska University Hospital pending analysis. The lower detective limit was 0.4 pmol/L and the functional assay sensitivity (<20% interassay coefficient of variation) <1 pmol/L (148). In **Studies II-IV** copeptin was analysed locally at the Cardiology Unit, Department of Medicine, Karolinska Institutet, Solna, Sweden by means of a fully automated, immunofluorescent assay (B.R.A.H.M.S. KRYPTOR Compact Plus, Hennigsdorf, Germany). The analytical detection limit was 0.7 pmol/L and the functional assay sensitivity <1.08

pmol/L (148). In **Study II** copeptin was analysed in fasting plasma samples taken on the day after hospital admission and stored at -70°C at the Karolinska University Hospital pending analysis. In **Studies III-IV** copeptin was analysed in fasting blood samples collected from 2002 to 2006 and stored at -70°C at the Icelandic Heart Association in Kópavogur, Iceland until shipped with courier in dry ice to Sweden.

IGFBP-1
IGFBP-1 in **Study I** was measured in samples obtained at the same time points as described for the copeptin measurements. The analysis was performed with a radioimmunoassay as described by Póvoa et al. (208) with a sensitivity of 3 μg/L and intra-assay and interassay coefficients of variation of 3% and 10% respectively. The samples were stored at -70°C at the Karolinska University Hospital prior to analysis.

Cortisol
Two cortisol saliva samples were collected in **Study III** with the Salivette device (Sarsedt, Rommelsdorf, Germany). The first sample was taken by the participant right before going to sleep the evening before the clinic visit and the latter was collected 45 min after waking up (209). The samples were analyzed with a time-resolved immunoassay with fluorescence detection with intra-assay variability <10% and inter-assay variability <12% (210) with a lower detection limit was 0.43 nmol/L for a 50 μL sample.

NT-proBNP
NT-proBNP was measured using a fully automated Cobas e411 analyzer utilizing Immunoassay, the sandwich principle (Roche Diagnostics, Mannheim, Germany).

Oral glucose tolerance test
In **Study II** a standardized OGTT (75g glucose dissolved in 200ml water) was performed on the day of hospital discharge (day 4 or 5) for patients and at the inclusion visit for controls. Blood glucose was measured in capillary whole blood one and two hours after the glucose load with a HemoCue® photometer (HemoCue® AB, Ängleholm, Sweden) with coefficient variation ≤3.5%.

Blood glucose and HbA1c
In **Study I** a random blood glucose was acquired as soon as possible after hospital admission while a fasting blood glucose was obtained on the day of hospital discharge and three months later. In **Study II** a random blood glucose was measured as soon as possible after arrival to the coronary care unit for patients while a fasting blood glucose was obtained each morning until discharge. Blood glucose was taken in controls after an overnight fast. HbA1c (reported as MonoS) was analysed for **Studies I-II** at a central laboratory (Department of Laboratory Medicine, Malmö General Hospital, Sweden) by high-performance liquid chromatography from whole capillary blood applied on filter paper with an upper normal limit of 5.3% and a coefficient variation of <3% (Boehringer Mannheim Scandinavian AB, Bromma, Sweden)(211).

In **Studies III-IV** blood glucose and HbA1c were analysed from fasting blood samples collected 2002 to 2006 in the laboratory of the Icelandic Heart Association in Kópavogur, Iceland (49, 203).

OUTCOMES

In **Study I** the primary outcome was a CVE defined as first of CV mortality, non-fatal MI, or stroke while each of the individual events served as secondary endpoints (204).
In **Study II** the primary outcome was total mortality. CV mortality defined as death from AMI, aortic dissection, stroke, or sudden death without any obvious reason served as the secondary outcome and a third outcome was a major CVE defined as the first of MI, stroke, severe congestive heart failure or CV mortality. In **Studies III-IV** the primary outcome was a CVE defined as first of CV death, stroke, MI, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG). The secondary outcome in **Studies III-IV** was total mortality. In **Study III** a third outcome was heart failure and a fourth MI.

STATISTICAL ANALYSIS

All analyses in **Studies I-IV** were performed by means of SAS software (SAS version 9.3, 9.4; SAS Institute, Cary, North Carolina, USA). A two-tailed p-value of <0.05 was accepted as statistically significant.

Descriptive statistics
Continuous variables are presented as median and interquartile ranges (IQRs), and categorical data as numbers and percentages unless otherwise stated. Fischer's exact test was used to explore whether there were any non-random associations between dichotomous variables (**Studies I-IV**). Wilcoxon two-sample test was used to assess differences in continuous variables of the baseline characteristics (**Studies I-IV**). Associations between continuous variables were assessed by means of Spearman rank correlation test (**Study I-IV**). Kruskal-Wallis test was used to explore potential differences in copeptin levels in the AGT and NGT subgroups of patients and controls (**Study II**), copeptin values between groups and subgroups (**Study III**), and to estimate differences between the classes of CAC scores as regards copeptin values (**Study IV**). In the subsets of participants stratified by those with and without events Wilcoxon two-sample test was used to assess differences in copeptin levels (**Study II-III**). This test was furthermore used to explore differences in CAC score stratified by dichotomous variables, as well as the difference in CAC score between participants with and without an event (**Study IV**). Hettmansperger and McKean linear model aligned rank test was applied to adjust copeptin levels for differences in age when comparing levels between individuals with and without AGT (212) (**Study II**).

Survival analysis
The relationship between one standard deviation (SD) increase of copeptin and outcomes in **Studies I-IV** was assessed using Cox's proportional hazard regression analysis and presented as hazard ratio (HR) and 95% confidence intervals (95% CI). In **Study I** the SDs for copeptin and IGFBP-1 were calculated separately and in **Study II** SD was calculated separately for patients and controls. In **Study III** SD was calculated separately for copeptin, morning cortisol, evening cortisol and NT-proBNP. In **Study IV** SD was calculated separately for copeptin and CAC score. Due to skewed distributions copeptin (Studies I-IV) and IGFBP-1 (**Study II**) were log transformed prior to analysis.

In **Study I** each of the predefined time points: hospital admission, hospital discharge and three months thereafter, was used as baseline for the subsequent prognostic evaluations.

Two univariable models were applied, one for each biomarker. Furthermore, two multiple analyses, the first including both biomarkers and the final including copeptin as well as the potential confounders age, creatinine clearance, and heart failure. These variables were chosen since they were predictors of outcome in the DIGAMI 2 trial. They were also used in the previous report on copeptin and IGFBP-1 at admission for MI (169, 200).

In **Study II** copeptin was analysed in univariable models for patients (AGT & NGT) and controls (AGT & NGT) subsequently adjusted for the following variables one at a time: age, admission creatinine (only available in patients), gender, previous congestive heart failure, previous MI, BMI and HbA1c. These variables were selected based on the results in the previous report on copeptin in the DIGAMI 2 cohort (169) and based on clinical experience. Further analyses were not performed due to the limited number of events.

In **Study III** different models were performed. The first models were univariable analysis separate for copeptin, morning cortisol, evening cortisol and NT-proBNP. The next step was a multiple model where copeptin was adjusted for morning cortisol, evening cortisol and NT-proBNP respectively. The last was a stepwise model including copeptin, morning cortisol, evening cortisol, NT-proBNP, age, sex, serum creatinine and previous heart failure. These variables were selected based on results from the Wilcoxon and the Spearman analysis in **Study III** as well as available literature (152, 155, 169).

In **Study IV** copeptin was first analysed in an univariable model, where after two multiple models were applied. The first included copeptin and CAC score, and the final model included copeptin, CAC score, age and sex. These variables were selected based on results from the Wilcoxon and Spearman analyses in **Study IV** as well as previous reports (169, 213, 214). Kaplan-Meier survival curves were constructed in **Study IV** to illustrate time trends in CVE, and total mortality based on the four previously mentioned CAC classes (0, 1-99, 100-399, and ≥400) in combination with the copeptin levels (< or ≥ the median of 6.4 pmol/L). As the prognosis was similar in the three first CAC classes and significantly worse for those with CAC ≥400, the CAC score was dichotomized in two categories: <400 and ≥400 and presented as such. Log rank test for trend was used to estimate differences in survival distributions of the event samples.

ETHICAL CONSIDERATIONS

All studies were performed according to guidelines for good clinical practice and the recommendations of the Declaration of Helsinki (215). Local ethic review boards approved the DIGAMI 2 (**Study I**; 96-164) protocol while the ethical committee at Karolinska Institutet approved the GAMI (**Study II**; 98-039/99-406) protocol. The National Bioethics Committee of Iceland approved the AGES-Reykjavik study and the ICELAND MI substudy (**Study III-IV**; VSN:00-063-V6/00-063-V6+3/14-00-35). All patients and participants have provided written, informed consent prior to enrolment.

RESULTS

BASELINE CHARACTERISTICS

Important baseline participant characteristics for **Studies I-IV** are presented in Table 4.

Table 4. Baseline characteristics for **Studies I-IV**. Continuous data are presented as median and interquartile ranges (IQR), and categorical data as numbers and percentages.

Study	**I**	**II** Patients vs. Controls	**III**	**IV**
Number of individuals	393	166 vs. 168	926	677
Demographics				
Age (years)	70.0 (60.8-77.0)	63.5 (57.0-71.0) vs. 64.0 (57.5-72.0)	76.0 (72.0-81.0)	76.0 (72.0-80.0)
Female (%)	124 (32)	50 (30) vs. 53 (32)	477 (52)	387 (57)
Smokers, n (%)	84 (22)	58 (35) vs. 21 (13)	105 (11.3)	72 (10.6)
BMI, kg/m^2	28 (25-31)	26.3 (23.7-29.4) vs. 25.9 (23.6-28.4)	27.3 (24.6-20.0)	27.3 (24.5-29.9)
Previous disorders				
Myocardial infarction, n (%)	148 (38)	34 (20) vs. 0 (0)	246 (27)	0 (0)
Hypertension, n (%)	215 (55)	56 (34) vs. 26 (16)	770 (83)	549 (80)
Heart failure, n (%)	78 (20)	13 (8) vs. 0 (0)	25 (3)	8 (6)
Diabetes according to definitions for each study, n (%)	393 (100)	0 (0) vs. 0 (0)	331 (36)	222 (33)
Hypercholesterolemia, n (%)	135 (34)	24 (5) vs. 14 (8)	537 (58)	361 (53)
Biochemical characteristics				
Blood glucose (mmol/L)	11.8 (9.2-14.9)	6.2 (5.6-7.4) vs. 5.0 (4.6-5.4)	5.7 (5.3-7.0)	5.7 (5.3-6.6)
HbA1c (%)	7.1 (6.2-8.3)	4.9 (4.6-5.3) vs. 4.6 (4.3-5.0)	5.7 (5.4-6.1)	5.7 (5.4-6.0)
Total cholesterol (mmol/L)	5.0 (4.2-5.8)	6.0 (5.2-6.8) vs. NA	5.4 (4.6-6.3)	5.6 (4.8-6.3)
Creatinine µmol/L	92 (78-110)	91 (82-102) vs. NA	85 (73-100)	84 (71-97)
Copeptin (pmol/L)	21.8 (9.7-50.1)	10.5 (6.8-18.6) vs. 5.9 (3.6-9.6)	6.8 (4.1-11.7)	6.4 (3.9-10.8)

NA: Not available.

STUDY I

Copeptin and IGFBP-1 levels

In the cohort of 393 patients with T2DM admitted to hospital for AMI the median copeptin levels were 21.8 pmol/L (IQR: 9.7-50.1 pmol/L) at admission, 8.5 pmol/L (IQR: 4.80-16.1 pmol/L) at discharge and 8.4 pmol/L (IQR: 4.5-15.2 pmol/L) three months thereafter (Figure 6). The copeptin levels differed significantly between the time for hospital admission and discharge and between admission and three months later, however, not between discharge and after three months.

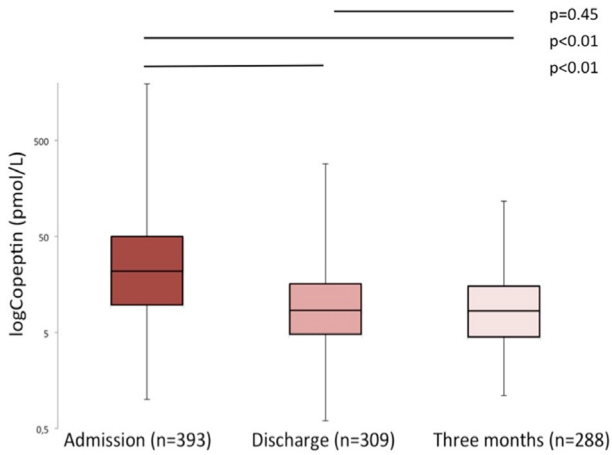

Figure 6. The median copeptin levels at admission for AMI, at discharge and after three months. Boxes span the 25th-75th percentile and whiskers outline minimum and maximum levels. Wilcoxon singed-rank test was used to evaluate difference over time (p-values).

The IGFBP-1 levels were lowest at the time for hospital admission (median 23.0 µg/L; IQR: 12.0-51.0 µg/L) and increased significantly to a median of 33.0 µg/L (IQR: 18.0-52.0 µg/L) at discharge and 36.0 µg/L (IQR: 18.0-56.0 µg/L) after three months (Figure 7).

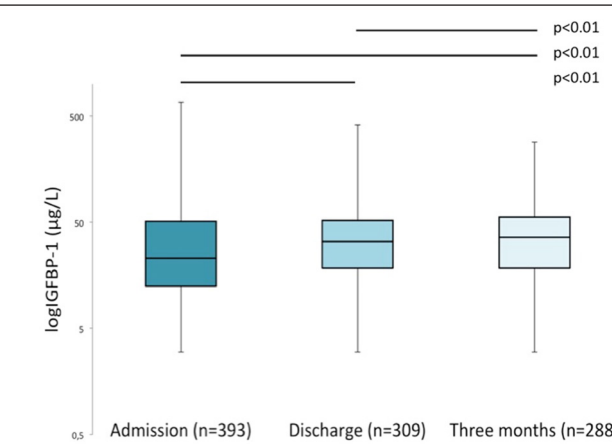

Figure 7. IGFBP-1 levels at admission for MI, at discharge and three months thereafter. Boxes span the 25th-75th percentile, with median depicted and whiskers outline minimum and maximum levels. Wilcoxon signed-rank test was used to evaluate difference over time (p-values).

There was a significant correlation between copeptin and IGFBP-1 at all time-points: admission r=0.53 (p<0.001), discharge r=0.31 (p=<0.001) and after three months r=0.14 (p=0.02) (Figure 8 a-c).

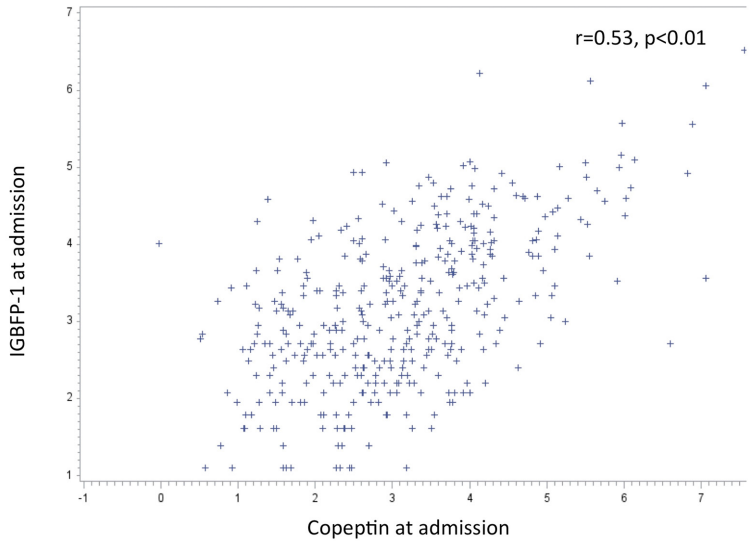

Figure 8a. The correlation between copeptin and IGFBP-1 (logarithmic scale) at admission for acute myocardial infarction in patients with type 2 diabetes mellitus.

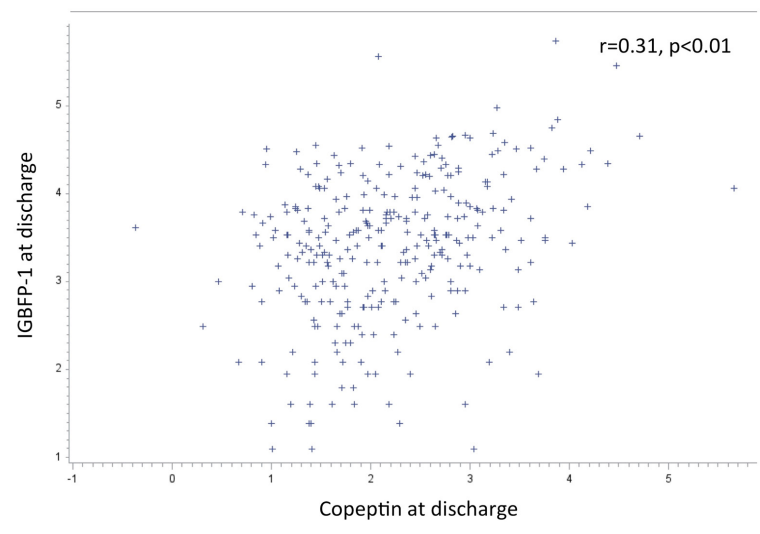

Figure 8b. The correlation between copeptin and IGFBP-1 (logarithmic scale) at discharge for acute myocardial infarction in patients with type 2 diabetes mellitus.

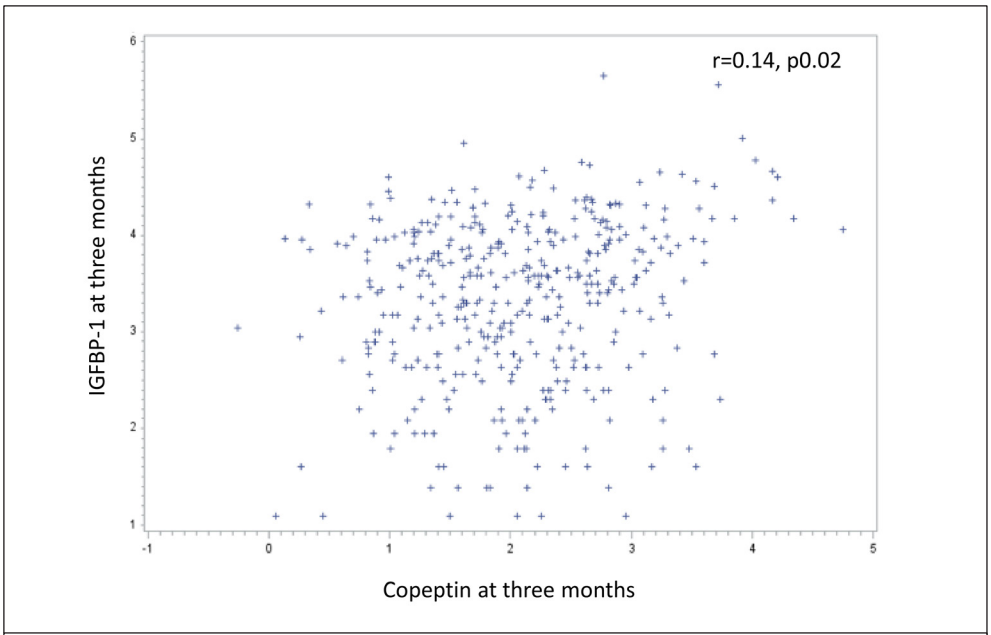

Figure 8c. The correlation between copeptin and IGFBP-1 (logarithmic scale) at three months after hospitalization for acute myocardial infarction in patients with type 2 diabetes mellitus.

Copeptin, IGFBP-1 and their prognostic capabilities

During a median follow-up time of 2.5 years (IQR: 1.1-3.0) 95 (24.2%) patients died, 77 (19.6%) from CV causes while 55 patients (15.0%) had a non-fatal re-infarction and 25 (6.4%) a non-fatal stroke.

In the unadjusted analyses copeptin predicted all events at each of the three time points (Table 5) while IGFBP-1 predicted CVE and CV mortality at admission and at discharge. In a multiple model containing both biomarkers, copeptin remained associated with all events at all time-points apart from non-fatal re-infarction and stroke when measured at hospital discharge while IGFBP-1 predicted CVE at discharge and CV mortality at admission and discharge.

In the final multiple model comprising both biomarkers, age, creatinine clearance and heart failure, copeptin remained as an independent predictor of CVE at all time points and of CV mortality at admission and non-fatal re-infarction or stroke at admission and after three months.

Table 5. Unadjusted and adjusted predictive ability of copeptin and IGFBP-1 assessed by Cox proportional hazard regression at hospital admission and discharge and after three months in patient with acute myocardial infarction and T2DM.

Cardiovascular events			**Admission**	**Discharge**	**Three months**
Univariable unadjusted	Copeptin	n	138	99	75
		HR (95% CI)	1.59 (1.41-1.81)	1.76 (1.44-2.15)	2.14 (1.63-2.80)
		p	<0.01	0.01	<0.01
	IGFBP-1	n	138	102	77
		HR (95%CI)	1.49 (1.26-1.77)	1.72 (1.32-2.24)	1.28 (0.95-1.73)
		p	<0.01	<0.01	NS (0.11)
Multiple model[1] copeptin and IGFBP-1	Copeptin	n	138	99	75
		HR (95% CI)	1.53 (1.31-1.78)	1.61 (1.29-2.00)	2.13 (1.60-2.83)
		p	<0.01	<0.01	<0.01
	IGFBP-1	n	138	99	75
		HR (95% CI)	1.10 (0.90-1.34)	1.36 (1.04-1.80)	1.01 (0.75-1.36)
		p	NS (0.35)	0.03	NS (0.95)
Multiple model[2]	Copeptin	n	129	96	73
		HR (95% CI)	1.34 (1.15-1.56)	1.37 (1.08-1.73)	1.75 (1.30-2.34)
		p	<0.01	0.01	<0.01
Cardiovascular mortality			**Admission**	**Discharge**	**Three months**
Univariable unadjusted	Copeptin	n	77	48	30
		HR (95% CI)	1.81 (1.54-2.14)	1.97 (1.49-2.61)	2.21 (1.42-3.42)
		p	<0.01	<0.01	<0.01
	IGFBP-1	n	77	51	30
		HR (95% CI)	1.99 (1.57-2.51)	2.23 (1.49-3.33)	1.56 (0.94-2.61)
		p	<0.01	<0.01	NS (0.09)
Multiple model[1]	Copeptin	n	77	48	30
		HR (95% CI)	1.56 (1.27-1.92)	1.71 (1.26-2.32)	2.07 (1.31-3.29)
		p	<0.01	<0.01	<0.01
	IGFBP-1	n	77	48	30
		HR (95% CI)	1.41 (1.06-1.86)	1.67 (1.09-2.55)	1.20 (0.73-1.97)
		p	0.02	0.02	NS (0.48)
Multiple model[2]	Copeptin	n	70	46	30
		HR (95% CI)	1.43 (1.16-1.77)	1.28 (0.91-1.80)	1.48 (0.92-2.37)
		p	<0.01	NS (0.15)	NS (0.10)
Non-Fatal Myocardial Infarction or Stroke			**Admission**	**Discharge**	**Three months**
Univariable unadjusted	Copeptin	n	77	64	58
		HR (95% CI)	1.35 (1.13-1.61)	1.41 (1.08-1.85)	1.98 (1.46-2.69)
		p	<0.01	0.01	<0.01
	IGFBP-1	n	77	66	60
		HR (95% CI)	1.11 (0.88-1.39)	1.60 (1.16-2.20)	1.20 (0.86-1.67)
		p	NS (0.37)	<0.01	NS (0.30)
Multiple model[1]	Copeptin	n	77	64	58
		HR (95% CI)	1.43 (1.16-1.77)	1.28 (0.96-1.71)	1.99 (1.44-2.76)
		p	<0.01	NS (0.09)	<0.01
	IGFBP-1	n	77	64	58
		HR (95% CI)	0.87 (0.67-1.14)	1.37 (0.98-1.93)	0.98 (0.70-1.36)
		p	NS (0.32)	NS (0.07)	NS (0.89)
Multiple model[2]	Copeptin	n	74	63	56
		HR (95% CI)	1.25 (1.02-1.54)	1.22 (0.90-1.66)	1.81 (1.29-2.53)
		p	0.03	NS (0.20)	<0.01

[1] Including copeptin and IGFBP-1, [2] Adjusted for heart failure, age and creatinine clearance, NS = not significant (p≥0.05).

STUDY II

Copeptin levels

Copeptin levels were measured in 166 patients with AMI without previously known T2DM and in 168 control subjects matched for age and gender. The median copeptin level was significantly higher in patients (10.5 pmol/L) than among controls (5.9 pmol/L; p<0.01). Patients with AGT had significantly higher levels (12.2 pmol/L) than those with NGT (7.9 pmol/L; p<0.01). No significant difference was seen in copeptin levels of controls with AGT vs. NGT (6.5 vs. 5.6 pmol/L; p=0.16) (Figure 9).

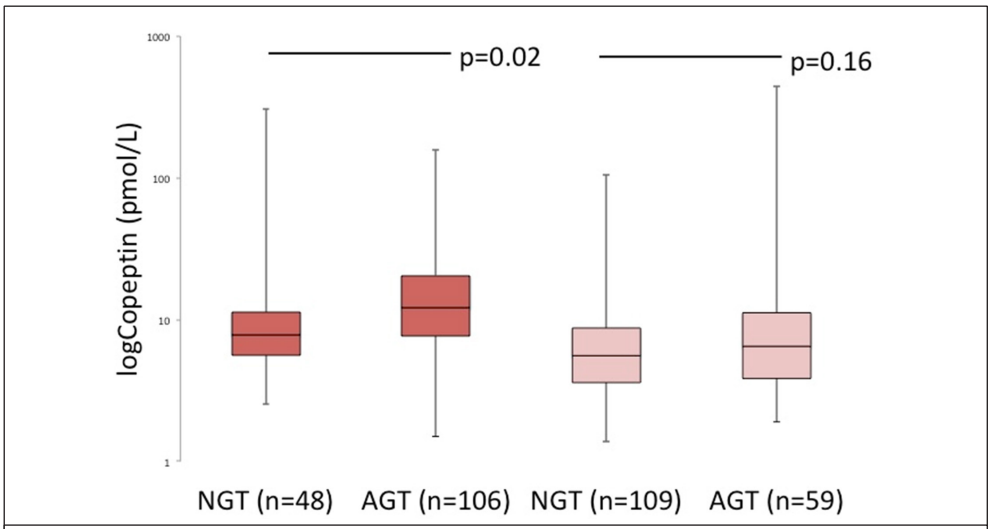

Figure 9. The median copeptin levels (pmol/L) in the subset of patients (red boxes, n=154) and controls (pink boxes, n=168) with known glucose categorization (defined by an OGTT). Boxes span the 25th-75th percentile and whiskers outline minimum and maximum levels, p-value = Kruskal-Wallis test.

Prognostic capabilities of copeptin

The median time of follow up was 11.6 years for patients and 10.4 years for controls during which 59 patients and 24 controls died whereof 33 respective 10 from CV mortality. A total of 72 patients had a major CVE (the first of MI, stroke, severe congestive heart failure or CV mortality) and those with an event had higher copeptin levels than those who did not (total mortality: 15.4 pmol/L (IQR: 7.6-25.1 pmol/L) vs. 9.0 pmol/L (IQR: 0.3-15.4 pmol/L), p<0.01).

In the unadjusted Cox's proportional hazard regression analysis an increase of copeptin by one SD in the patient cohort predicted total death, CV death and major CVE (Figure 10). In the adjusted models copeptin was significantly associated with total death and CV mortality among patients after adjusting for each of the following variables alone: admission creatinine, gender, HbA1c, and previous congestive heart failure, but failed as a predictor after single adjustments for age, BMI, and previous AMI. Copeptin remained a significant predictor of major CVE after adjusting for gender and HbA1c but not after other factors. Further analyses were not performed due to the limited number of events (patients n=72, controls n=26). In the control cohort copeptin predicted major CVE in the unadjusted analysis but not after adjusting for gender and BMI. Copeptin did not predict total or CV mortality in unadjusted analyses (Figure 10).

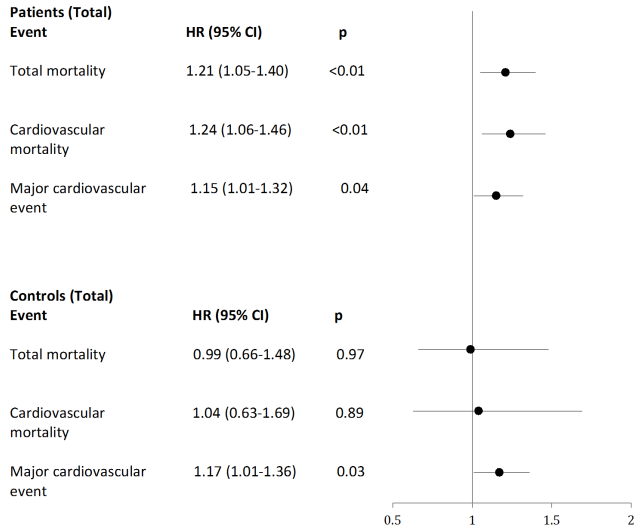

Figure 10. Unadjusted predictive ability of copeptin in the total patient and control cohorts assessed by Cox proportional hazard regression (increase in one standard deviation). Presented as Hazard Ratio (HR) and 95% Confidence Intervals (95% CI).

STUDY III

Copeptin levels

Copeptin levels were determined in a cohort of 926 elderly Icelandic individuals (mean age 76.0 years). The median copeptin level for the total cohort was 6.8 pmol/L. The levels were significantly higher in participants with previous MI than among those without (8.9 vs. 6.4 pmol/L, p<0.01; Figure 11). There was no significant difference between those with RMI and UMI (9.2 vs. 8.8 pmol/L; p=0.68). Participants with T2DM had higher median copeptin levels than those without in the total cohort (8.0 pmol/L vs. 6.3 pmol/L; p<0.01). The pattern also existed in the group without previous MI (7.8 pmol/L vs. 5.7 pmol/L; p<0.01), but not in those with MI (Figure 11).

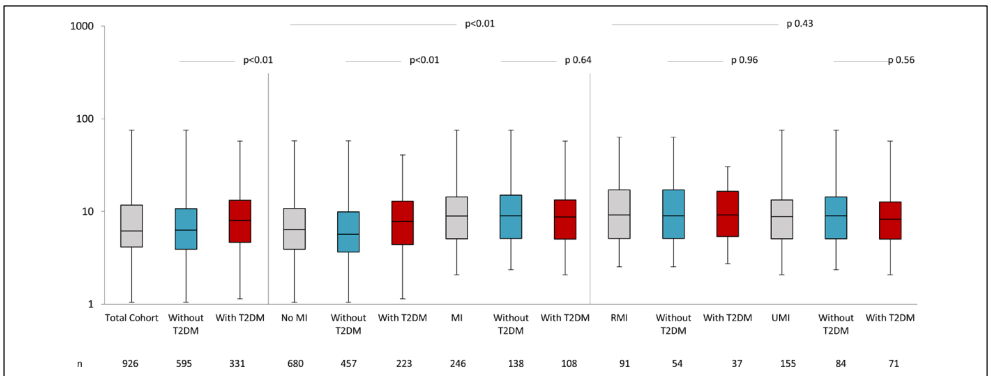

Figure 11. The median Copeptin levels (pmol/L) for all participants with copeptin available (n=926) as well as for the subgroups without MI (n=680), with previous MI (n=246), RMI (n=91) and UMI (n=155). Each group is further divided based on the diagnosis of T2DM or not. Boxes span the 25th-75th percentile and whiskers outline minimum and maximum levels, p-values = Kruskal-Wallis test.

Cortisol and NT-proBNP levels
The median morning cortisol and evening cortisol level were 17.4 µg/dL and 2.3 µg/dL respectively for the total cohort. The morning cortisol levels did not differ between those with and without previous MI, but evening cortisol levels were higher for participants with previous MI (2.6 vs. 2.1 µg/dL, $p<0.01$). There was no significant difference in either morning or evening cortisol levels for those with RMI and UMI.

The median NT-proBNP was 141.4 pg/mL in the total cohort. The NT-proBNP was significantly higher for participants with previous MI than for those without (228.5 pg/mL vs. 122.4 pg/ml, $p<0.01$). There was no significant difference in NT-proBNP levels in the subgroups RMI and UMI ($p=0.17$).

Correlation of biomarkers
In the total cohort copeptin did not correlate with morning cortisol ($r=-0.01$, $p=0.85$) however copeptin correlated with evening cortisol ($r=0.11$, $p<0.01$). This correlation was also seen in those without a previous MI ($r=0.10$, $p=0.02$).
Copeptin correlated with NT-proBNP only in the total cohort ($r=0.07$, $p=0.04$).

Prognostic capabilities of copeptin
During the follow up period (median 9.1 years) 392 participants had a CVE (cardiovascular death/stroke/MI/PCI/CABG), 368 died, while 82 were diagnosed with heart failure and 119 suffered a re-infarction/or a first MI (Table 6).

In unadjusted Cox's proportional hazard regression analysis copeptin was significantly associated with CVE in the total cohort, but not in the groups with or without previous MI. Copeptin remained associated with CVE after adjustments for morning and evening cortisol separately. In the model comprising copeptin and NT-proBNP, both biomarkers remained significantly associated with CVE (Table 6). In the final stepwise model only NT-proBNP continued to associate with CVE.

In unadjusted analyses copeptin was significantly associated with total mortality in the total cohort, as well as for those with and without previous MI (Table 6). Copeptin continued to associate with total mortality in the total cohort as well as for those with and without previous MI after separate adjustments for morning cortisol, evening cortisol and NT-proBNP. Copeptin remained associated with total mortality in the final stepwise model for the total cohort and for the group with previous MI.

None of the studied biomarkers (copeptin, morning cortisol, evening cortisol, NT-proBNP) were associated with heart failure in unadjusted analyses (Table 6).

In unadjusted analyses copeptin was associated with recurrent MI in the total cohort and also after separate adjustments for morning and evening cortisol levels as well as NT-proBNP. Copeptin did not remain significantly associated with MI in the stepwise model.

Table 6. Unadjusted and adjusted predictive ability of log copeptin (increase of one standard deviation) assessed by Cox proportional hazard regression analyses and presented as hazard ratio (HR) and 95% confidence intervals (CI).

	Total cohort		**No myocardial infarction**		**Myocardial infarction**	
Cardiovascular events						
n=392	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Copeptin*	1.16 (1.05-1.29)	<0.01	1.06 (0.93-1.20)	0.42	1.10 (0.94-1.29)	0.22
Morning cortisol*	0.99 (0.89-1.10)	0.87	1.04 (0.93-1.16)	0.49	0.84 (0.67-1.05)	0.12
Evening cortisol*	0.99 (0.90-1.09)	0.87	1.01 (0.90-1.13)	0.91	0.92 (0.78-1.09)	0.34
NT-proBNP*	1.29 (1.22-1.37)	<0.01	1.32 (1.21-1.44)	<0.01	1.22 (1.09-1.36)	<0.01
Stepwise model[1]	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
	1) NT-proBNP 1.24 (1.17-1.32)	<0.01	1) NT-proBNP 1.31 (1.19-1.45)	<0.01	1) NT-proBNP 1.22 (1.10-1.36)	<0.01
Total mortality						
n=368	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Copeptin*	1.31 (1.18-1.45)	<0.01	1.21 (1.06-1.38)	<0.01	1.32 (1.10-1.57)	<0.01
Morning cortisol*	1.01 (0.92-1.11)	0.82	1.04 (0.93-1.15)	0.53	0.96 (0.80-1.12)	0.68
Evening cortisol*	1.07 (0.94-1.11)	0.69	1.02 (0.92-1.14)	0.65	1.02 (0.87-1.19)	0.83
NT-proBNP*	1.34 (1.27-1.41)	<0.01	1.40 (1.29-1.51)	<0.01	1.31 (1.18-1.45)	<0.01
Stepwise model[1]	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
	2) NT-proBNP 1.23 (1.16-1.31)	<0.01	2) NT-proBNP 1.37 (1.24-1.51)	<0.01	2) Copeptin 1.49 (1.23-1.79)	<0.01
	3) Copeptin 1.21 (1.08-1.35)	<0.01				
Heart failure						
n=82	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Copeptin*	1.04 (0.85-1.27)	0.72	1.05 (0.81-1.38)	0.71	1.02 (0.75-1.38)	0.92
Morning cortisol*	0.93 (0.36-2.40)	0.88	0.47 (0.06-3.63)	0.47	1.07 (0.72-1.58)	0.76
Evening cortisol*	1.09 (0.40-2.97)	0.87	1.27 (0.18-8.80)	0.81	1.00 (0.64-1.58)	0.99
NTproBNP*	1.02 (0.88-1.19)	0.77	1.01 (0.83-1.23)	0.93	1.05 (0.77-1.41)	0.77
Stepwise model[1]	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
	No effects met the 0.05 level for entry into the model		No effects met the 0.05 level for entry into the model		No effects met the 0.05 level for entry into the model	
Myocardial infarction						
n=119	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Copeptin*	1.29 (1.08-1.55)	<0.01	1.20 (0.93-1.54)	0.17	1.15 (0.89-1.50)	0.28
Morning cortisol*	1.04 (0.92-1.19)	0.52	1.10 (0.95-1.26)	0.20	0.92 (0.67-1.26)	0.62
Evening cortisol*	1.06 (0.97-1.17)	0.22	1.11 (1.00-1.23)	0.06	0.73 (0.46-1.16)	0.18
NTproBNP*	1.27 (1.14-1.40)	<0.01	1.20 (0.97-1.48)	0.09	1.21 (1.01-1.44)	0.04
Stepwise model[1]	**HR (95% CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
	1) NTproBNP 1.19 (1.06-1.33)	<0.01	3) Evening cortisol 1.12 (1.01-1.26)	0.04		

* Univariable. [1]Including: copeptin, morning cortisol, evening cortisol, NT-proBNP, age, sex, serum creatinine, and previous heart failure (only presenting significant results of the three biomarkers).

STUDY IV

Copeptin and Coronary artery calcium score

Study IV comprised 677 participants from the ICELAND MI cohort who according to CMR were free from previously or newly detected MI, and in whom CAC score information and copeptin levels were available. The median follow-up was 9.1 years during which 229 participants had a CVE and 232 died.

The median copeptin level was 6.4 pmol/L and the median CAC score was 227.0 in the total cohort. The median copeptin levels gradually increased by increasing CAC category (Figure 12) and copeptin was significantly higher for participants with CAC score ≥400 compared to those with score of 0. This was also reflected in the total cohort as a significant correlation between copeptin and CAC score when studied as continuous variables (r=0.12, p<0.01).
Participants with T2DM (n=222) had a significantly higher copeptin levels and CAC score when compared to those without T2DM (7.8 vs. 5.7 pmol/L; p<0.01 respective 306.5 vs 203.7; p=0.03).

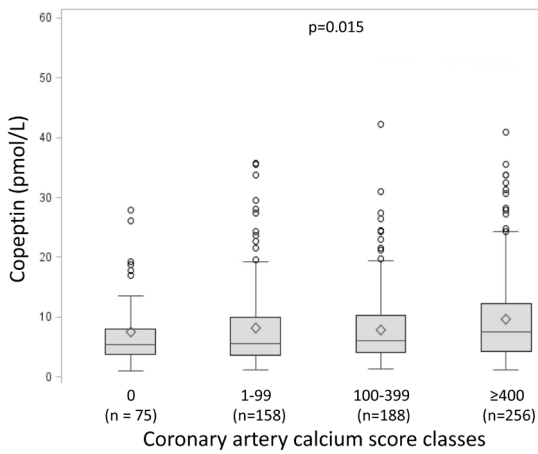

Figure 12. Distribution of copeptin in the different CAC classes. P-value represent the difference in copeptin levels between CAC-classes (ANOVA).

Copeptin, coronary artery calcium score and their prognostic capabilities

In unadjusted Cox's proportional hazard regression analyses in the total cohort Copeptin was associated with total mortality but not CVE. CAC score was associated with both events. In a model including both copeptin and CAC score, copeptin remained significantly associated with total mortality. When further tested in the final multiple model (model 2), the HR was attenuated and not statistically significant (Table 7).

Table 7. Unadjusted and adjusted predictive ability of copeptin in the total cohort, assessed by Cox proportional hazard regression analyses and presented as hazard ratio (HR) and 95% confidence intervals (CI).

Total participants	**Cardiovascular events** n=229		**Total mortality** n=232	
	HR (95% CI)	p	HR (95% CI)	p
Copeptin*	1.06 (0.93-1.21)	0.365	1.22 (1.07-1.39)	0.003
Copeptin [1]	1.01 (0.89-1.15)	0.900	1.17 (1.03-1.34)	0.018
Copeptin [2]	0.98 (0.85-1.12)	0.736	1.09 (0.95-1.25)	0.218

* Univariable. [1] Model 1: Adjusted for CAC score, [2] Model 2: Adjusted for CAC score, age and female sex.

As outlined in Figure 13, the CV prognosis was similar in those with a CAC score <400 and in those with CAC score ≥400 regardless of the copeptin level. Similar findings were seen for total mortality (Figure 14).

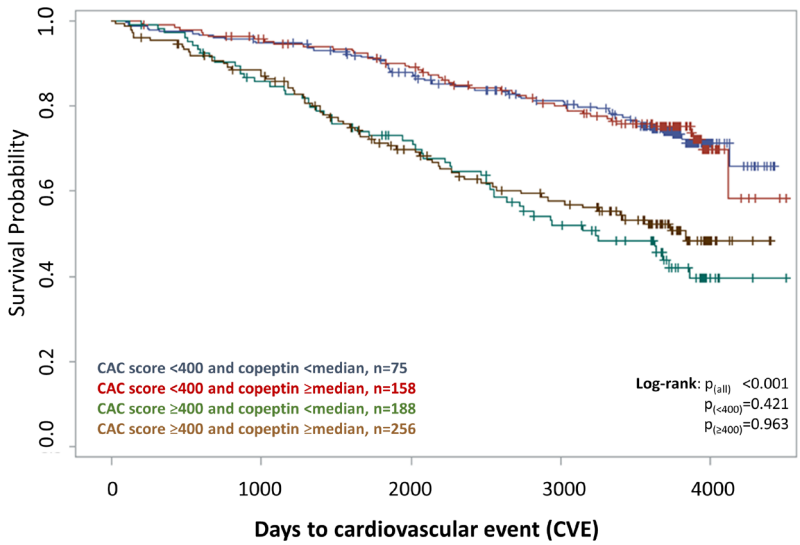

Figure 13. Kaplan Meier survival plot for coronary artery calcium score </≥400, stratified based on copeptin level </≥ median, p-value = Log rank test between all classes (p_{all}), for those <400 ($p_{<400}$), and ≥400 (and $p_{\geq 400}$), respectively.

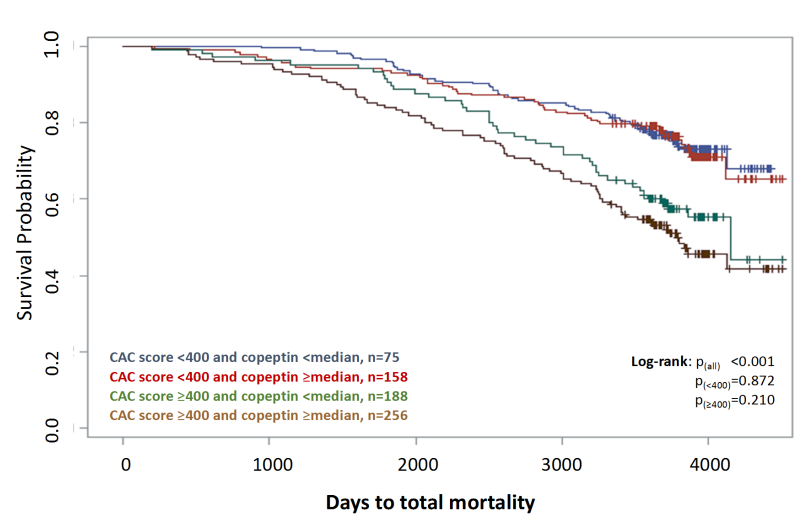

Figure 14. Kaplan Meier survival plot for coronary artery calcium score </≥400, stratified based on copeptin level </≥ median, p-value = Log rank test between all classes (p_{all}), for those <400 ($p_{<400}$), and ≥400 (and $p_{\geq 400}$), respectively.

GENERAL DISCUSSION

Although CVD prognosis has improved in general, individuals with CVD and dysglycemia have a more dismal prognosis compared to those without glucose perturbations. This is not fully explained by the well-known risk markers such as hyperglycemia and hyperlipidemia. Identification of new biomarkers and elucidating their roles are an important step in an attempt to understand the reasons for the discrepant prognosis between CAD patients with and without dysglycemia. An increased understanding may generate new therapeutic with the potential to decrease the enhanced CV mortality and morbidity in people with and without dysglycemia. This thesis studied the hypothesis that copeptin, a marker for vasopressin, is involved in the development of CVD, as well as dysglycaemia, a hypothesis that could not be confirmed. It rather seemed as if copeptin is a marker of general disease.

COPEPTIN AND CORONARY ARTERY DISEASE

The immediate increase and swift decrease in copeptin levels in the context of AMI has made copeptin a clinically interesting marker to rule out AMI. In an observational study copeptin measured in patients immediately upon admission for chest pain added valuable information to the conventional estimation of cardiac troponin T (cTnT), a marker of cardiac necrosis, by improving the sensitivity and the negative predictive value of cTnT alone (164). Whether the activation of vasopressin, measured as increase in copeptin level is related to the pathophysiology of CVD as a mediator, eventually leading to AMI and complications such as heart failure, or if it is a rather a confounder, marker of stress has been debated and is further studied in this thesis.

Vasopressin activation, measured as copeptin, in the acute phase of a MI was determined in two different cohorts. In **Study I** the highest median copeptin level, 21.8 pmol/L, was seen at admission (169), with a rapid decrease to 8.5 pmol/L at the time for hospital discharge four to five days after admission, however remaining elevated compared to what has been reported in healthy individuals (median 3.8-6.0 pmol/L) (152-155). These findings are in line with results from the LAMP study (patients n=980; and controls n=700) (155). A sub cohort in the LAMP study comprising of 132 patients with AMI had daily blood samples during the first five days. The copeptin levels were significantly higher day one vs. days two to five ($p<0.001$), quickly reaching a plateau at day three to five (155). The copeptin levels measured in the plateau remained higher than values in the control cohort (7.0 vs. 3.8 pmol/L, $p<0.001$). A difference between **Study I** and the LAMP study is that all patients in **Study I** had T2DM compared to about one fifth in the LAMP study. This may have impacted the copeptin levels.

In **Study II**, the copeptin elevation was indeed more prominent for patients diagnosed with AGT during the hospital stay than among those with NGT. Hence, the presence of dysglycemia may have implications for the vasopressin response and seems to be associated with higher copeptin levels. The copeptin levels in patients participating in **Study II** was 10.5 pmol/L, which is lower than among those in **Study I** (21.8 pmol/L). A possible explanation may be different stages of dysglycemia. All patients in **Study I** had T2DM, a condition that was an exclusion criterion in **Study II** in which 31% had NGT and the remainder newly detected AGT based on an OGTT. Another possibility is the different timing of copeptin measurements. As already mentioned, copeptin was measured at hospital admission in **Study I** while samples

were taken the morning following admission in **Study II.** Moreover, the differences between the patient cohorts should be taken into consideration. The patients in **Study I** were older, had higher BMI and a more frequent history of hypertension and heart failure.

The main stimulus for vasopressin release is an increase in plasma osmolality and/or a decrease in circulating volume acting via baroreceptors, and copeptin is known to respond quickly to changes in plasma osmolality and fluid status (216, 217). Hence, a potential explanation for the copeptin elevation during AMI, as in **Study I** and **II,** could be a reflection of the vasopressin activation secondary to the hemodynamic shift, e.g. decrease in blood pressure and increase in left ventricular end-diastolic pressure that may be induced by an occlusion of a coronary artery, thereby triggering the baroreceptors (218). **Study III** extended the findings in **Study I** by showing that copeptin is elevated in individuals with a previous MI, i.e. in a more stable phase suggesting a persistent activation of vasopressin. A hypothesis, proposed by Kaplan et al. in 2010, is that the copeptin elevation reflects the chronic burden of stress even after the resolution of the acute stressor (219). This may possibly relate to underlying stress secondary to myocardial remodeling induced by myocardial damage. This prolonged activation of vasopressin may eventually result in further damage, such as heart failure during the recovery process, further explored in thesis.
The activation of the vasopressin receptors, as described in the introduction, may have detrimental effects on the heart and the CV system. Vasopressin may have pro-thrombotic effects by increasing release of Factor VII and von Willebrand factor (vWF) from endothelial cells via activation of endothelial V2-receptor, which may be particularly harmful in patients with AMI. Moreover, vasopressin activation has several actions that may contribute to the post-MI remodeling. Increased protein synthesis of myocytes via the activation of the V1a-receptors, may lead to left ventricular hypertrophy (220), and afterload can increase due to vasoconstriction via V1a-receptor activation on vascular smooth muscle cells. Activation of V2-receptors, can furthermore increase the blood volume, thus leading to increased risk of heart failure. Indeed, in a study by Kelly et al. increased copeptin levels measured at day three to five post-AMI correlated inversely with left ventricular ejection fraction and remodeling five months later (171). In addition, a study including 54 patients with STEMI investigated the relationship between copeptin and the MI size. Copeptin measured at two days after onset of symptoms was associated with a larger acute and chronic infarct size, as well as a more compromised myocardial function and increased remodeling four months later as determined by CMR (221).

Study III revealed that the copeptin levels were higher than in healthy cohorts (3.8 to 6.0 pmol/L) (152-155) regardless of whether the MI was previously known or not. This indicates a similar degree of vasopressin activation in UMI and RMI, a finding supported by their similar prognosis (49). Age could also play a role as **Study III** only includes elderly individuals. The impact of age on copeptin has, however, not been fully established. To try to elucidate whether the increased levels of copeptin in patients with CAD may be related to heart failure and myocardial dysfunction, copeptin was studied in relation to the heart failure marker NT-proBNP in **Study III**. Copeptin significantly correlated with NT-proBNP, but remained associated with CVE, MI and total mortality after adjustments for NT-proBNP. Furthermore, copeptin did not predict heart failure which suggests that other factors, e.g. increased stress, diabetes mellitus, and not direct CV causes may contribute to the impaired prognosis in individuals with increased copeptin.

Taken together the results from **Study III** suggest that the reasons for copeptin elevation are multiple and complex, and that it may be an expression for general disease or frailty. In this context, general disease could be thought of as an accumulation of different underlying comorbidities as well as a combination of different risk factors making the afflicted person more susceptible and vulnerable to further illness and a compromised prognosis. In the light of this assumption the copeptin increase during AMI seen in **Study I** and **II** might be a response to the acute endogenous stress of the MI itself, and the increase in copeptin and the association to prognosis may then be an expression of general disease rather than directly related to the cardiac condition. This agrees with the fact that vasopressin is not only considered an antidiuretic but also a stress hormone (219) involved in the HPA-axis. Vasopressin is suggested to affect the HPA-axis by potentiating the CRH action at the pituitary level resulting in increased adrenocorticotropic hormone (ACTH) secretion via V1b-receptor that in turn increases cortisol release that escapes the negative feedback loop (222, 223) thereby increasing the production of cortisol from the adrenal glands. Indeed, copeptin elevation has been reported in several acute non-cardiac diseases, such as sepsis (224), stroke (225) and pneumonia (224).

To further explore the possible relation of vasopressin to stress, copeptin was explored in relation to cortisol in **Study III**, which included measurements of morning and evening cortisol. This revealed a significant correlation between copeptin and evening measurements. It was, however, only copeptin that remained significantly associated with CVE and total mortality following adjustments for cortisol, indicating that the prognostic implications of copeptin are only partly explained by stress.

Whether the increase in vasopressin and the effects of the different receptors translates into worse prognosis was studied in different patient materials. In **Study I** copeptin, measured at admission for an AMI, predicted the composite CVE (CV mortality and non-fatal MI or stroke) during a median of 2.5 years of follow-up, and likewise when measured at the time of hospital discharge and three months thereafter. This supports the findings in the LAMP study, where copeptin predicted heart failure and total mortality during 60 days of follow up after AMI (155). Furthermore, copeptin measured three days after admission predicted total mortality and CVE in 244 patients with signs and symptoms of heart failure after AMI (172). In **Study II,** recruiting patients with AMI, copeptin was only significantly associated with a major CVE, as well as the other endpoints, in unadjusted analyses. This may be a result of lack of power due to a relatively low number of events despite an extensive period of follow-up (median 11.6 years). The discrepant findings may, however, also relate to different inclusion criteria. All patients in **Study I** had established T2DM, while known diabetes was an exclusion criteria in **Study II.** The potential impact of dysglycemia is further discussed below.

That the predictive capability of copeptin is complex gains support by previous studies that demonstrate that copeptin predict short and long term mortality and morbidity in chronic obstructive pulmonary disease (226) and traumatic brain injury (227) as well as one year mortality after stroke (228). In a prospective, multicenter observational cohort study, including 984 patients (age 56 years; males 67%) with non-cardiac causes of chest pain including musculoskeletal, anxiety and gastroesophageal reflux diseases, lower-airway infections, or non-cardiac but unknown etiologies copeptin was elevated (>13 pmol/L) in 22% of the

population. Elevated copeptin levels were associated with an almost threefold increased risk of dying compared to copeptin levels <13 pmol/L during a follow up of median 2.1 year (229). The elevation and the prognostic capacity of copeptin in these non-cardiac acute conditions imply that copeptin rather constitutes a non-specific marker for general disease. This assumption gains support by **Study III** since copeptin predicted CVE, total mortality and myocardial infarction in unadjusted analysis in the total cohort and in participants with MI although it, in this more stable setting of CAD, only remained as a predictor for total mortality after adjustments.

COPEPTIN AND ATHEROSCLEROSIS

In **Study IV** copeptin was studied in relation to different atherosclerotic plaque burdens as mirrored by different CAC scores in an elderly cohort free from previously known and unknown MI. Although the results from **Studies II-III** indicated that copeptin is reflecting a general vulnerability rather than being a direct pathophysiological participant in the mechanism of CAD, there are several mechanisms of vasopressin activation that may be involved with the atherosclerotic process. Such as, the V2-receptor is involved in the release of the von Willebrand factor and Factor VIII, as well as the NO production (230). Accordingly, dysregulation could potentially lead to the development of endothelial dysfunction. **Study IV** offered an opportunity to further elucidate the role and impact of vasopressin in this context, i.e. copeptin in relation to CAC score, a marker of the atherosclerotic process.

Study IV showed that individuals without MI (RMI and UMI) and with a CAC score ≥400 (implying an extensive plaque burden) had significantly higher copeptin levels than those with a lower plaque burden, who in fact had similar copeptin levels as those with CAC score 0 (indicating no signs of plaques). The correlation between copeptin and CAC score was, however, weak. An unsubstantial or lack of association between atherosclerosis and copeptin was also seen when copeptin was studied regarding intima-media thickness, another measurement of early atherosclerosis. In the population-based cohort study KORA (Cooperative Health Research in the Region of Augsburg, southern Germany) F4 study, copeptin was measured in 1596 individuals (median age 56.6 years, males 48.9%, previous MI/stroke 4.6%), and studied in relation to the intima-media thickness of the common carotid artery measured with ultrasound. The copeptin levels were not associated with increased intima-media thickness after adjustments for gender and age. The conclusion was that copeptin, a mirror of vasopressin activation, did not exert proatherogenic effects (231).

In unadjusted analyses increasing copeptin was a predictor for total mortality in **Study IV,** but this did not remain in the final model where the CAC score was a strong predictor for both total mortality and CVE, which is in agreement with previous studies were high CAC score (≥400) were associated with a dismal CV prognosis in diverse clinical settings (25, 232, 233). CAC score, a useful tool when estimating the CV risk (234), has been suggested to represent the extent of CAD (235). The fact that CAC was a stronger predictor for CVE and although copeptin levels were elevated in participants with extensive plaque burden, copeptin was not significantly associated with the event, supports the belief that it is not the vasopressin activation but the severity of CAD that is essential for the prognostic effect. Taken together, this favors the opinion that the elevation of copeptin seen with the more extensive plaque burden reflect the general state of disease rather than serve as an indicator that vasopressin has a pathophysiological role in development of the atherosclerosis.

COPEPTIN AND DYSGLYCEMIA

Vasopressin elevation in diabetes was first described in 1979 (236), and has since been suggested to play a role in dysglycemia. As already underlined, high copeptin levels were seen in patients with established T2DM at admission for AMI and three months later (**Study I).** The median admission level was high compared with what has been reported in mixed cohorts with AMI. This was supported by **Study II** in which patients with AMI and newly diagnosed AGT had significantly higher copeptin levels than those with NGT. When looking into details on the dysglycemic status in the LAMP-study there was a similar pattern, i.e. patients with diabetes (type 1 and 2, 22% of the total cohort) had significantly higher copeptin levels than those without diabetes (8.9 vs. 6.5 pmol/L, <0.005) (155). These findings were confirmed in an international multicenter study recruiting 370 patients with diabetes (type 1 and 2) along with 1621 without admitted to the emergency department with symptoms suggestive of AMI. Copeptin levels measured at admission were higher in patients with compared to those without diabetes (10.4 vs. 6.2 pmol/L: $p<0.01$) irrespective of the underlying cause of chest pain (237). This suggests that the increase in copeptin during AMI, or perhaps CAD in general, is not only related to the CAD in patients with diabetes. Diabetes in itself seems to be associated with higher copeptin levels, and hence more vasopressin activation, which may mirror a higher sensitivity to stress.

In the presence of diabetes, the previously described potentially detrimental effects of vasopressin (e.g. increased pre- and afterload and hypertrophy) may be particularly harmful, not at least since diabetes in itself is related to endothelial dysfunction and a pro-thrombotic state which may result in a more extensive myocardial damage. It may also be that patients with T2DM are more susceptible to stress triggered by AMI, as they already have an underlying chronic disease resulting in increased vasopressin activation during the acute phase of an AMI. As an indication copeptin measured in patients with AMI and AGT in **Study II** was a significant predictor for CVE in unadjusted analyses, but not for patients with NGT. The vasopressin activation during AMI may also have a negative impact on the blood glucose since vasopressin may have direct effects on the glucose metabolism via various actions as outlined in Figure 15.

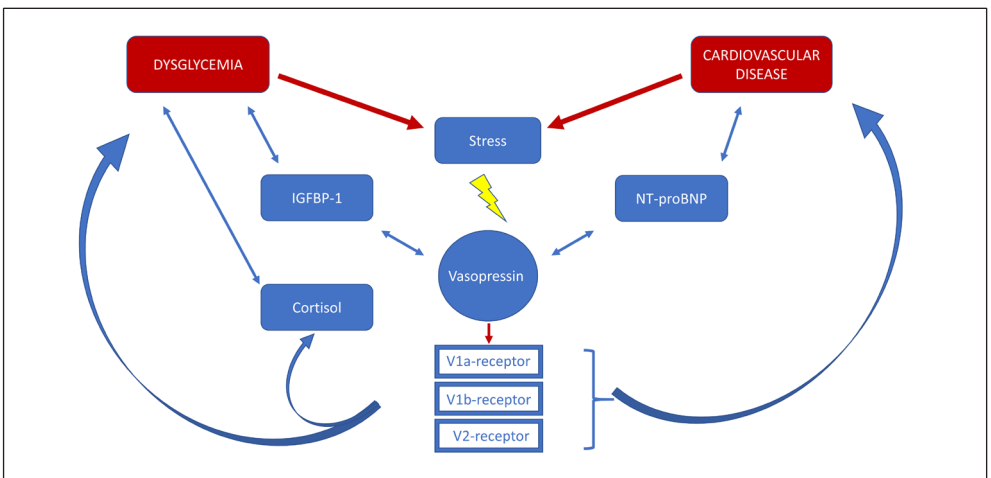

Figure 15. A potential link between vasopressin, dysglycemia and cardiovascular disease.

This may in turn be associated with an impaired prognosis (238). Further support for this assumption is gained from a report from the Swedish population-based prospective Malmö Diet and Cancer (MDC)-Cardiovascular Cohort. In this middle-aged cohort copeptin predicted a composite endpoint including CAD, heart failure and mortality after adjustment for conventional risk factors in participants with but not in those without diabetes (224). In addition, in the prospective observational British Regional Heart Study, a trial including older English men with a mean follow up of 13 years, elevated copeptin levels at baseline were associated with an increased risk of stroke and CVD mortality in men with (n=428), however, not in those without (n=3108) diabetes (239). This could mean that the predictive impact of copeptin in CVD, is driven by co-morbidity, e.g. diabetes.

Several observational studies reported on higher levels of copeptin in individuals with T2DM. In the previously mentioned MDC study Enhörning et al. studied participants with diabetes at baseline and noted that they had significantly higher copeptin levels compared to those with normal fasting glucose (6.9 vs 5.1 pmol/L; $p<0.001$) (181). Similar findings, significantly higher copeptin levels (9.4 pmol/L vs. 4.1 pmol/L; $p<0.001$) were seen in a Chinese cohort study in which copeptin was measured in 306 patients with long-standing T2DM (median duration 11 years) and compared to 200 age- and gender matched healthy controls (240). Furthermore, in a multicenter, community based study (n=2490) baseline copeptin in the high quartile was associated with increased risk to develop the metabolic syndrome (177). Copeptin at baseline has also been shown to be an independent risk factor for the development of diabetes in population based studies (181, 241-243), and increasing copeptin levels has been associated to increased insulin-levels, insulin resistance (180, 242, 244) and to genotypes linked to an elevated risk of hyperglycemia (244). Whether vasopressin activation, measured as copeptin, in the context of T2DM reflects the increased endogenous stress following the metabolic disorder or if it is a pathophysiological factor in the development of T2DM remains to be established.

In **Study I** copeptin remained elevated in a presumably less stressful state three months after the AMI. Since all patients in this study had T2DM it is not possible to answer the question whether this continued elevation of copeptin was due to dysglycemia or if it only related to the recent MI. In this perspective, **Study III** adds important information since there was no significant difference in copeptin levels between patients with a previous MI whether they had T2DM or not (9.0 vs. 8.7 pmol/L). **Study I** indicates that the impact of T2DM in relation to vasopressin in patients with already established CAD is more pronounced during situations of acute stress. However, in patients without established CAD the situation may be different (**Studies II** and **III)**. Increased copeptin has been associated with T2DM, and it might be that the presence of vasopressin accentuates the impact of dysglycemia, or vice versa.

The present thesis indicates that copeptin may be seen as a marker of general stress or illness. Interestingly the presence of T2DM in patients with CAD seems to have less importance for prognosis than the CAD per se, although it may increase the susceptibility to stress in acute settings.

There are several possible mechanisms for how vasopressin activation may be directly or indirectly involved in dysglycemia. As mentioned in the introduction, the V1a-receptor and V1b-receptors may be involved in the glucose homeostasis, thus long-term vasopressin

activation may disrupt this homeostasis, leading to increased glucose production and disturbed insulin/glucagon secretion (245, 246, 127, 181). In experimental studies, mice lacking the V1a-receptor have increased vasopressin, impaired glucose tolerance and increased insulin resistance (247). Furthermore, mice with V1b-receptor deficiency have lower blood glucose and increased insulin sensitivity (248), showing how distortions in vasopressin regulation potentially may lead to the development of T2DM. Another possible explanation could be through the HPA-axis, as hypercortisolism due to elevated vasopressin levels eventually may lead to dysglycemia (249, 250). Activation of the HPA-axis could furthermore be the reason for the elevation during AMI as a reaction to acute stress. Other possible mechanisms for this potential association between copeptin and T2DM has been suggested. Vasopressin is a hemodynamic hormone, regulating the body water balance, and recently an interventional study showed that increasing water intake over six weeks in healthy individuals resulted in a decrease in the copeptin levels (251). Observational studies have proposed that too low water consumption could be a factor in the development of T2DM (252).

The studies included in the present thesis are of epidemiological and observational nature. They do therefore not allow an interpretation of the exact mechanisms behind the association between an increase in copeptin and T2DM. **Study I** does, however, add information on the possible interaction between vasopressin and another hormonal system, the IGF-axis. Perturbations in the IGF-axis has been associated with a higher risk of developing dysglycemia. In patients with diabetes insipidus, desmopressin infusion, a vasopressin analogue, increased IGFBP-1 levels suggesting a relationship between vasopressin and the IGF-axis (186). This was supported by a previous report from the DIGAMI 2 cohort, that is the same as in **Study I** where copeptin measured during admission of AMI in patients with T2DM was associated with IGFBP-1 levels. **Study I** expanded these findings by showing that the association between copeptin and IGFBP-1 persisted until three months after the AMI, and in addition that copeptin was a stronger predictor of CVE at all time points. This implies that vasopressin may influence the IGF-axis (169), and that the complex activation of vasopressin may have a previously unknown activation via the IGF-axis. These observations are of great interest to further investigate.

STRENGTHS

This thesis was based on data from four study cohorts comprising data from three observational studies and one randomized control trial. The four studies have several strengths.

Study I included patients from multiple countries and patients were carefully categorized and researched. Another strength is the availability of blood samples from discharge and at a three month visit after an AMI, making it possible to measure biomarkers during the aftermath of the acute event. This brings important information about the behavior of the biomarkers copeptin and IGFBP-1 in a more stable state, as well as a possibility to explore their prognostic significance.

Study II had a long follow up period and almost none of the participants were lost to follow up**.** The OGTT-test was used to categorize both patients and controls regarding their glycemic state, making it possible to study the impact of newly diagnosed AGT. This offered an opportunity to explore and compare copeptin in a cohort including participants with pre-

diabetes as well as those without previously known dysglycemia. Another strength was the presence of a control cohort. This is important in biomarker studies since it offers an opportunity to compare levels, and minimizes confounding related to age-and gender.

Studies III and **IV** are based on well-defined and thoroughly followed cohorts. All participants were characterized during three visits to the Icelandic Heart Association and blood analyses were performed according to a standardized quality protocol and with the use of internal control samples. Imaging examinations were performed on the second visit during which the participants underwent CMR imaging, CT and ultrasound. An independent reading center interpreted all images, and 5-19% of them were re-evaluated by external experts. Furthermore, individuals with UMI were included. This offered a unique opportunity to explore copeptin levels in this manifestation of MI, something that to the best of my knowledge has not been done before. The study did also include two other biomarkers, cortisol and NT-proBNP, allowing an investigation of copeptin in relation to well-known markers of stress (cortisol) and heart failure (NT-proBNP).
Study IV included data from CT imaging with quantification of coronary artery calcium. This gave an opportunity to exclude individuals with established MI, and explore the copeptin levels with regard to plaque burden and thereby focusing on earlier stages of CAD.

LIMITATIONS

The epidemiological character of **Studies I-IV** precludes conclusions on causality but provides results which are hypothesis generating. As in all observational studies there is a possibility for selection bias, play of chance and confounders which all may lead to erroneous conclusions. We cannot conclude whether copeptin is a mediator or a confounder, but the overall results indicate that it is rather a confounder. Elevated copeptin is a marker of increased vulnerability but may also be a mediator. Young adults were not represented in these populations and therefore the generalization to the such groups as various ethnic groups is undermined.

Study I was based on a biochemical-substudy from the DIGAMI 2 (169). Not all participants from the original study were included as not all centers participating had the ability to partake in the biochemical program. However, the baseline characteristics have reported to be similar for participants in the biochemical-substudy as for those who did not participate (184). Another limitation to **Study I** was that not all patients in the biochemical substudy with copeptin and IGFBP-1 values at admission have values from both discharge and at the three months visit. This was due to logistics reasons including unwillingness to participate, to far between measurements, death, and unacceptable storage of sample. This resulted in some difference in baseline characteristics, first and foremost that the cohort with three months values available were somewhat younger.
Study II had a long follow up but there was a relatively small sample size limiting the statistical interpretation. **Studies I** and **II** were based on patient material from the 1990s to early 2000s. Both treatment for CAD and T2DM has evolved since then.

In **Study III** the CMR was not performed on the same day as the biomarker measurements. Blood samples were taken at the first study visit while the imaging was performed at the second. This could lead in some discrepancy, as a stressful event could have happened in the meantime causing in increased vasopressin activation, resulting in incompatible blood and

imaging results. The same applies to **Study IV** which is based on results from CT, that was not performed on the same day as the copeptin measurements. Hence different factors which may be difficult to control may contribute to a variation of copeptin release. In **Study III** cortisol measurements were collected only at one occasion, thus increasing the possibility of error. Furthermore, the follow up of heart failure was shorter than for the other outcomes in **Study III**, and the relatively low number of events could explain the lack of association between copeptin and heart failure as seen in previous studies.

In **Study IV** the range of the CAC score in the categories 0, 1-99, 100-399 and ≥400, was chosen to categorize the severity of plaque burden. This was based on general agreement, but other categorizations have been used making it challenging to compare results between studies.
Participants in **Studies III** and **IV** had a high median age (76 years), the results might thus not be applicable to younger age groups. **Studies III** and **IV** were furthermore based on cohorts that included a high number of participants with T2DM, and thus the results may not be applicable to the general population. The study populations included individuals with newly diagnosed T2DM defined as one fasting plasma glucose >7.0 mmol/L, but to make a diagnosis according to the WHO two measurements are needed.

ETHICAL REFLECTIONS

This thesis is based on epidemiological biomarker studies derived from previously published observational as well as randomized clinical studies. The studies were approved by the regional ethical committees and were conducted in agreement with the Declaration of Helsinki. All study participants were informed about the studies, and had given oral or written consent to the initial study as well as to further biomarker sub-studies. This was without specific biomarkers defined at the time of initial study, thus given consent for saving their blood samples in a biobank for future research. All personal data and information was de-identified and gathered by a third party thus ensuring privacy. Blood samples were stored and categorized by using barcodes to respect the privacy of the study participants, making the risk of tracking the patient minimal as the decipher key is kept under lock.

FUTURE PERSPECTIVE

The patient cohorts in this thesis were individuals with or at risk of developing CVD and/or dysglycemia. It was not possible to explore why copeptin continued to stay elevated after a MI as the thesis was based on observational studies. One may speculate that the elevation may be due increased endogenous stress, possibly in addition to a hemodynamic shift. It would be interesting to follow up on this in future studies in which frequent measurements of copeptin together with cortisol, troponin and NT-proBNP during and several months after an AMI as well as in a matched control group.

Current knowledge indicates that there is a relation between copeptin and different manifestations of dysglycemia, but further research is needed to clarify whether vasopressin plays a role in glucose homeostasis, or if copeptin elevation in people with dysglycemia is due to increased chronic stress following the metabolic disease. This thesis raises the question whether it is dysglycemia as such that is the endogenous stress factor that, possibly via the IGF-axis, triggers vasopressin release. Such activation may possibly aggravate the

dysglycemia further via its receptors, increasing the risk for T2DM. This in an interesting hypothesis in need of further evaluation. Future studies may include experimental studies e.g. by including two groups of mice with established diabetes, of which one group with the gene/genes for the IGF-pathway knocked out. The mice would then be kept on high-glucose diet and followed with copeptin measurements to see if there is a difference in vasopressin activation between the groups. Another interesting question and possible aspect to study in the future is whether there is an association between copeptin and the newer glucose lowering medication for T2DM, as many has shown to be cardioprotective in the previously mentioned CVOTs (105, 106) but the exact reasons for this is not fully understood.

A possibility in biomarker studies is that it may generate ideas on therapeutic treatment. In this context vasopressin receptor antagonists, vaptans, should be taken into considerations. Vaptans block the action of vasopressin with varying selectivity. Vaptans have attracted attention as a potential heart failure therapy since they increase urinary output causing body weight reduction without influencing serum electrolytes (253). The role of vaptans in heart failure is, however, suggested as minimal and their ability to affect-long term morbidity and mortality has so far been similar to that of placebo when tested in a clinical trial (254).
This thesis concludes that copeptin should be seen as a marker for general disease. It is therefore of interest to study vaptans in relation to stress and hopefully reduce the harm following the vasopressin action. As vasopressin acts on three different types of receptors (V1a, V1b and V2), where the activation of all could potentially be harmful, one may explore the effect by the use of unselective vaptans. This could be done in randomized trials, including two groups of patients admitted to the hospital due to a stressful event (e.g. infection, MI, etc.) where the first cohort receives unselective vaptans and the second placebo. Copeptin would then be measured before and after administration of vaptans. The hope is to decrease general endogenous stress level, and thus reduce total mortality.

This thesis indicates that the elevated copeptin levels reflect an underlying general disease and that it not is specifically related to CVD. Just as troponin is informative of heart damage, copeptin should perhaps be seen as a risk predictor of poor outcome. The increased understanding of vasopressin, via measuring copeptin, and its relation to acute and chronic stress is of interest as it may potentially generate new therapeutic possibilities where the goal is to reduce mortality and morbidity. Finally, one should also consider the accessibility as well as the cost-effectiveness of measuring copeptin. As of now copeptin is almost only used in research and seldomly in a clinical setting. The use of copeptin does not make economic sense until its clinical usefulness has been verified.

CONCLUSION

Based on the results of the studies of this thesis it can be concluded that:

1. Copeptin levels, which are elevated in patients with AMI, decreases quickly during the hospital stay, but remains elevated in the post-MI phase, regardless whether the MI was previously recognized or not.

2. There seems to be an association between elevated levels of copeptin and diabetes with the implication that the elevation most likely reflects underlying stress. This may relate to dysglycemia even if other factors may contribute. There is a potential complementary pathway from the activation of vasopressin to T2DM via the IGF-axis.

3. Copeptin correlated weakly with biomarkers of heart failure (NT-proBNP), and stress (cortisol). Copeptin continued to associate with CVE and total mortality after separate adjustments for cortisol and NT-proBNP and remained significantly associated with total mortality in a stepwise model. This indicates that heart failure and/or stress cannot fully explain the prognostic implications of copeptin.

4. Copeptin gradually increased with increasing degrees of coronary atherosclerotic plaque burden in individuals without previous MI. Independent of CAC score, copeptin was associated with total mortality, a relation that disappeared after adjustments for age and sex suggesting that elevated copeptin is an expression for a general state of disease rather than an indicator of a pathophysiological role of vasopressin in the development of CAD.

5. Together the results support the theory that copeptin should be seen as an expression of general disease and subsequent poor prognosis, and not a specific marker for CV disease or dysglycemia.

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