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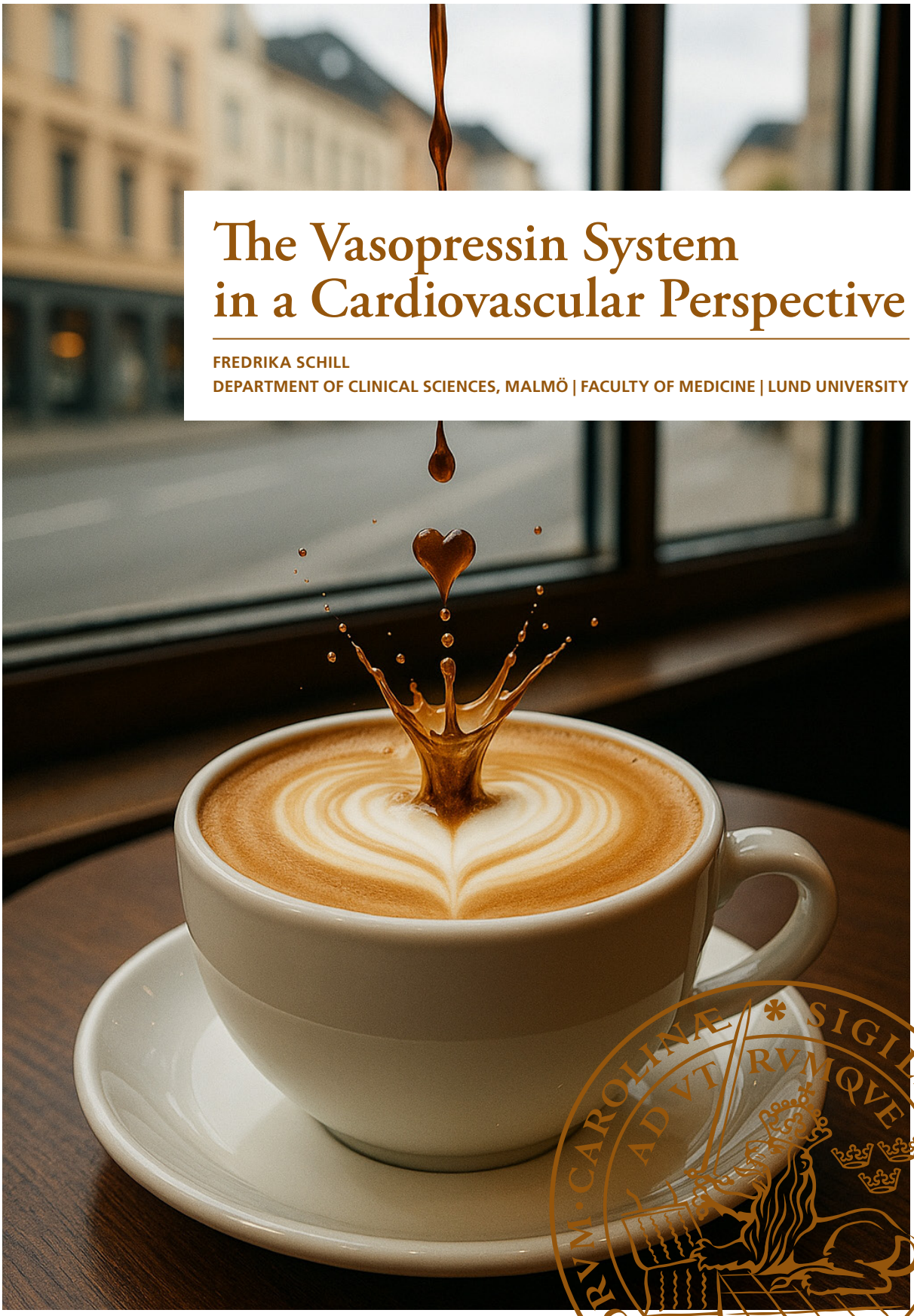

The Vasopressin System in a Cardiovascular Perspective

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract:

The aim of this thesis was to explore the relationship between the vasopressin surrogate marker copeptin and different physiological systems, with a special focus on cardiovascular disease. Copeptin concentration was investigated in relation to hematopoietic markers, the cardiovascular surrogate markers coronary calcium score (CACS) and pulse wave velocity (PWV) as well as to future risk of heart failure development. Coffee intake, as a lifestyle modification, was also explored in relation to copeptin, using both an epidemiological and an experimental design.

In **paper I** we investigated the predictive value of copeptin concentration on future heart failure development in 5297 participants of the Malmö Preventive Project. We found that individuals in the highest quartile of copeptin concentration had a significantly higher risk of heart failure development compared to the lowest quartile after, in median, 11 years of follow-up.

In **paper II** we analysed the association between copeptin concentration and high CACS (>100) and PWV (>10 m/s) in 5303 individuals from the Swedish cardiopulmonary bioimage study (SCAPIS). The top tertile of copeptin was, compared with reference tertile 1, significantly associated with both high CACS and high PWV after adjustment for cardiovascular risk factors.

In **paper III** we explored the association between copeptin concentration and hematopoietic markers in 5312 participants from SCAPIS. Increasing copeptin tertile was significantly associated with increasing erythrocyte count, red blood cell distribution width, erythrocyte volume fraction, hemoglobin, leukocytes and neutrophils after adjustment for relevant confounders. Increasing copeptin tertile was, however, not associated with change in mean corpuscular volume, lymphocyte or thrombocyte count.

In **paper IV** we first analysed the association between coffee intake and copeptin concentration among 3270 participants from the Malmö Offspring Study. Increasing coffee intake was significantly associated with decreasing copeptin concentration, after adjustment for relevant confounders and total fluid intake. Secondly, copeptin concentration was measured in 26 participants of an experimental cohort where coffee intake was increased acutely. We found that acute ingestion of coffee intake by 4 deciliters significantly lowered copeptin concentration.

Key words: Vasopressin, copeptin, heart failure, cardiovascular disease, arterioscleroisis, atherosclerosis, hematopoesis, inflammation, coffee intake, fluid intake, population cohorts, experimental cohort.

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MADE IN SWEDEN

To Caspian, Elian and the inexhaustible curiosity.

Table of Contents

Abstract

The aim of this thesis was to explore the relationship between the vasopressin surrogate marker copeptin and different physiological systems, with a special focus on cardiovascular disease. Copeptin concentration was investigated in relation to hematopoietic markers, the cardiovascular surrogate markers coronary calcium score (CACS) and pulse wave velocity (PWV) as well as future risk of heart failure development. Coffee intake as a lifestyle modification was also explored in relation to copeptin, using both an epidemiological and an experimental setting.

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Populärvetenskaplig sammanfattning

Denna avhandling har för avsikt att utforska hormonet vasopressin och dess möjliga inblandning i flera olika kroppsliga funktioner, framförallt hjärt/kärlsystemet. Vasopressin, även känt som antidiuretiskt hormon, utsöndras från en körtel i hjärnan som kallas hypofysen. Detta sker i första hand när kroppen behöver spara på vatten. En minskad mängd vatten i kroppen kan bero på lågt vattenintag, större vätskeförluster (svettning, diarré, kräkningar) eller en stor blödning. I sådana situationer är det av största vikt att spara på vatten vilket sker framförallt genom att minska urinproduktionen. Vasopressin minskar njurarnas urinproduktion och stimulerar också törst för att återställa kroppsvattenbalansen.

Forskning har visat att vasopressin har ett stort antal olika andra effekter i kroppen. Vasopressin kan till exempel dra ihop blodkärl, stimulera till levring av blod och påverka blodsockernivåerna med mera. Vasopressinkoncentrationen i blodet mäts bäst genom analys av en markör som utsöndras samtidigt och i samma mängd som vasopressin, copeptin. Det är visat att höga nivåer av copeptin oftare ses hos människor med olika sjukdomstillstånd. Bland annat har högre nivåer av copeptin visat sig öka risken för diabetes, kranskärlssjukdom och ökad dödlighet i hjärt-kärlsjukdom. Människor med högre koncentrationer av copeptin har också oftare njursvikt och övervikt. Experimentella och genetiska studier pekar mot att det finns ett orsakssamband mellan högt vasopressin och utveckling av diabetes. Det är därför viktigt att undersöka sambandet mellan vasopressin och andra sjukdomstillstånd ytterligare samt att, om möjligt, lära sig mer om eventuella bakomliggande sjukdomsmekanismer. Detta framförallt eftersom det går att sänka vasopressinnivåerna på ett enkelt sätt; genom att dricka vatten.

I vår första studie ville vi ta reda på om personer med högre koncentrationer av copeptin i blodet har en ökad risk för hjärtsvikt. Vi undersökte detta bland deltagare i en stor befolkningsstudie på mer än 5000 personer. Det visade sig att den fjärdedelen av studiedeltagarna med högst koncentration av copeptin i blodet hade en dubblerad risk för hjärtsvikt 11 år senare, jämfört med den fjärdedelen med lägst koncentration.

I vår andra studie undersökte vi om personer med högre copeptinkoncentration hade en högre förekomst av åderförkalkning mätt med skiktröntgen av hjärtats kranskärls kalkinnehåll (s.k. coronary calcium score) och kärlstyvhet i de större artärerna (s.k. pulse wave velocity). Bland drygt 5000 deltagare i en annan befolkningsstudie såg vi att den tredjedel av deltagarna med högst copeptin också oftare hade högt kalkinnehåll i hjärtats kranskärl och dessutom oftare hög kärlstyvhet.

I vår tredje studie utforskade vi sambanden mellan copeptin och olika blodkroppar. Vi kunde se att ökande copeptinkoncentration hade ett samband med ökande antal röda och vita blodkroppar. Vi kunde däremot inte se något samband med antalet blodplättar.

I vår sista studie undersökte vi om kaffedrickande är relaterat till copeptinnivåer i blodet. Vi ville studera detta eftersom man tror sig veta att kaffe kan öka urinmängderna och, precis som vasopressin, därmed kanske kan påverka vätskebalansen i kroppen. Dessutom har tidigare studier visat att kaffe kan påverka risk för diabetes och hjärtkärlsjukdom, alltså sjukdomstillstånd som vasopressin också har kopplats till. Bland över 3000 deltagare i en befolkningsstudie såg vi att de som drack mest kaffe över tid hade lägre koncentrationer av copeptin jämfört med de som drack mindre kaffe. Med en experimentell studiedesign undersökte vi också 27 personer som fick dricka 4 dL kaffe mycket snabbt. Vi kunde se att deltagarnas copeptinnivåer sjönk nästan direkt efter kaffeintaget och därefter höll sig på en lägre nivå i flera timmar. Vi såg dessutom en liten sänkning av copeptinnivåerna när deltagarna drack en ytterst liten mängd (10 ml) vatten.

Sammantaget har vi med dessa studier visat att ökade vasopressinnvåer, mätt genom copeptin, verkar vara kopplat till ökad risk för framtida hjärtsvikt och har ett samband med åderförkalkning. Vi har också sett att ökad copeptinconcentration hänger ihop med högre antal röda och vita blodkroppar. Vi har till sist också visat att de individer som konsumerar mest kaffe i befolkningen har lägre copeptinnivåer samt att copeptinkoncentrationen kan sänkas snabbt genom att dricka kaffe.

List of Papers

This thesis is based on the following original papers, referred to in the text by their Roman numerals. They are appended at the end of the thesis.

Paper I

Schill F, Timpka S, Nilsson P M, Melander O, Enhörning S. Copeptin as a predictive marker of incident heart failure. ESC Heart Failure. 2021 Aug;8(4):3180-3188.

Paper II

Schill F, Persson M, Engström G, Melander O, Enhörning S. Copeptin as a marker of atherosclerosis and arteriosclerosis. Atherosclerosis. 2021 Dec;338:64-68.

Paper III

Schill F, Engström G, Melander O, Timpka S, Enhörning S. The possible role of the vasopressin system in hematopoiesis. Scientific Reports. 2024 Mar 1;14(1):5085.

Paper IV

Schill F, Timpka S, Hellstrand S, Melander O, Enhörning S. Coffee Intake and the Vasopressin System : an epidemiological and experimental study. Endocrine Connections. 2025 Sep 5;14(9):e250100.

Abbreviations

AC	Adenylyl cyclase
ACTH	Adrenocorticotropic hormone
APKD	Autosomal dominant polycystic kidney disease
AQP2	Aquaporine type 2
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
BMI	Body mass index
CACS	Coronary calcium score
cAMP	Cyclic adenosine monophosphate
CI	Confidence interval
CKD	Chronic kidney disease
C-f	Carotid-femoral
CT	Computed tomography
DAG	Diacylglycerol
eGFR	Estimated glomerular filtration rate
EVEREST	Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study
EVF	Erythrocyte volume fraction
Gq/Gs	G-protein subunits
G1	Generation 1
Hb	Hemoglobin
HbA1C	Beta-N-1-deoxy fructosyl hemoglobin
HDL	High density lipoprotein cholesterol
HPA	Hypothalamic-pituitary-adrenal
hsCRP	High sensitivity c-reactive protein
ICD	International Classification of Diseases
IP3	Inositol 1,4,5-triphosphate
L	Liter
LDL	Low density lipoprotein cholesterol

Ln	Natural logarithm
MCV	Mean corpuscular volume
MOS	Malmö Offspring Study
MPP	Malmö Preventive Project
MPP Re-exam	Malmö Preventive Project Re-examination Study
MRproANP	Mid-regional pro-atrial natriuretic peptide
PKC	Protein kinase C
PLC	Phospholipase C
PVN	Paraventricular nucleus
PWV	Pulse wave velocity
RAAS	Renin-aldosterone-angiotensin system
RDW-SD	Red blood cell distribution width standard deviation
SCAPIS	Swedish cardiopulmonary bioimage study
SD	Standard deviation
SIADH	Syndrome of Inappropriate Antidiuretic Hormone
SNS	Sympathetic nervous system
TRACE	Time-Resolved Amplified Cryptate Emission Technology
vWF	Von Willebrand factor
V1aR	Vasopressin receptor 1a
V1bR	Vasopressin receptor 1b
V2R	Vasopressin receptor 2

Introduction

Homeostasis is a self-regulating process by which a living organism can maintain internal stability while adjusting to changing external conditions (1). The human body has numerous delicate systems to achieve homeostasis, where the endocrine system plays an essential part. In 1895, Oliver and Schäfer took an important step towards the discovery and understanding of the hormone vasopressin as a central regulator of cardiovascular homeostasis. They demonstrated that extracts of the pituitary gland rapidly increased blood pressure when injected intravenously into different animal species (2). That vasopressin also possessed antidiuretic effects was later discovered by separate experiments by Farini (3) and Vongraven (4) in 1913. It then took over 40 years until Vignaud (5) and Acher (6) isolated the peptide and better could describe its structure and functions. The discoveries of vasopressin eventually contributed to Vignaud winning the Nobel Prize in Chemistry 1955.

Physiology of the vasopressin system

Synthesis and central regulation

Antidiuretic hormone, arginine vasopressin (AVP) or simply vasopressin, is a cleavage product of the prohormone prepro-AVP which is predominantly synthesized by magnocellular neurosecretory cells in the supraoptic nucleus and the paraventricular nucleus (PVN) of the hypothalamus. After synthesis, prepro-AVP is further transported along axons down to the posterior pituitary gland for storage. During this transport, the prohormone is cleaved into active vasopressin, neurophysin II and copeptin. Neurophysins are carrier proteins for vasopressin whereas the function of copeptin is not fully understood but suggested to play a role in the folding and maturation of prepro-AVP (7, 8). Axons from the PVN also project to other areas of the brain such as the eminentia mediana, limbic system, amygdala and spinal cord (9). Upon activation of osmoreceptive neurons located in the organum vasculosum of the lamina terminalis and the subfornical organ, the posterior pituitary release vasopressin (10). Vasopressin release is stimulated in proportion to the increase in plasma osmolality, along with the sensation of thirst. A plasma osmolality of around 280 mosmol/L suppresses vasopressin release completely (11), but the osmolality threshold of vasopressin release may vary depending on certain conditions such as pregnancy (12) .

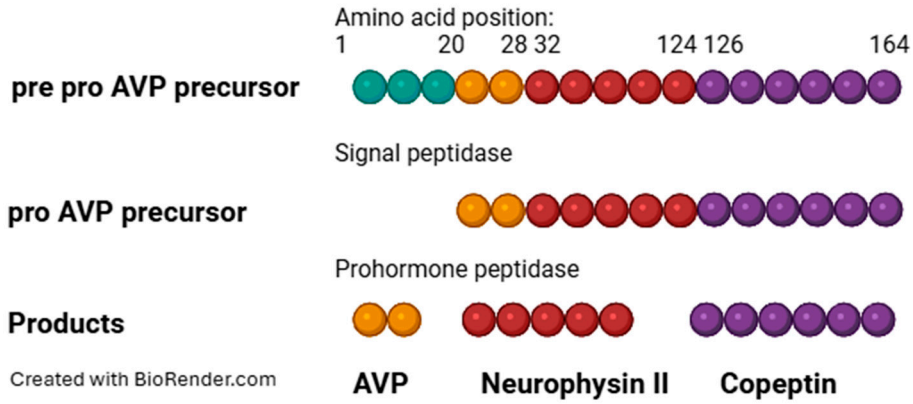


Figure 1. Structure of prepro-AVP and its cleavage products; AVP, neurophysin II and copeptin. Prepro-AVP is encoded by a gene in the short arm of chromosome 20 (20p13). After synthesis in the magnocellular neurons in the supraoptic nucleus and paraventricular nucleus of the hypothalamus, prepro-AVP is cleaved by signal peptidase and further by prohormone peptidase into AVP, neurophysin II and copeptin. The enzymal cleavage is processed during axonal transport to the posterior pituitary. AVP contains nine amino acids whereas copeptin contains 39 amino acids (13).

Vasopressin release can also be stimulated by a decrease in systemic blood pressure detected by baroreceptors in the aortic arch and carotid sinuses, which in turn project to hypothalamic neurons by the vagus nerve. The pathways of vasopressin stimuli and release are illustrated in Figure 2.

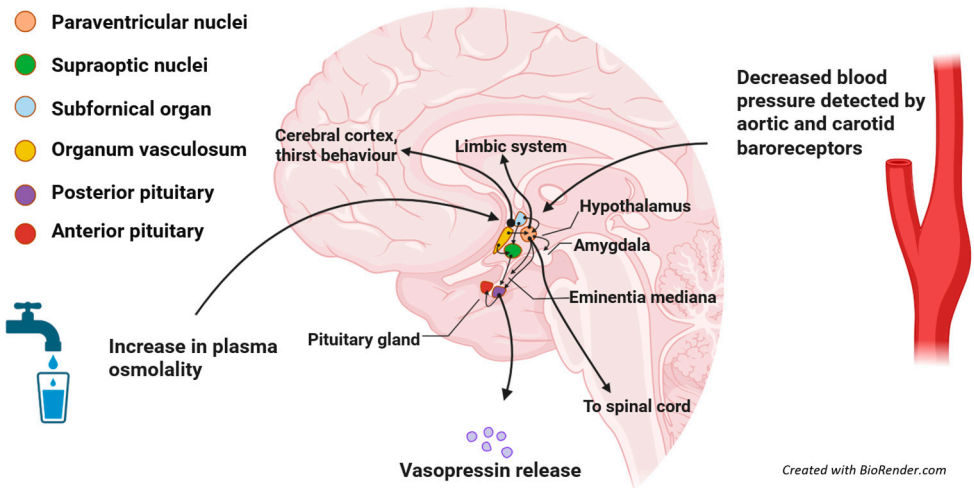


Figure 2. Synthesis and release of vasopressin.

Receptors and their effects

The main function of vasopressin is to maintain a stable plasma osmolality and blood volume/pressure by regulation of renal water reuptake and vascular tonus. This is achieved by effects mediated by renal and vascular vasopressin receptors. The vasopressin receptors have, however, also been found in various other peripheral tissues and organs throughout the body, illustrating the pleiotropic effects of vasopressin (see Table 1). Vasopressin also acts as a neurotransmitter with central regulating effects on emotions and behavior as well as the autonomic nervous system including stress response (Figure 2) (9, 14, 15).

Table 1. Distribution and function of vasopressin receptors.

	Location	Effects
V1aR	Vascular smooth muscle cells	Vasoconstriction
	Myocardial cells	Myocardial hypertrophy
	Brain	Temperature regulation, cognitive functions, emotional response, social behavior
	Thrombocytes	Thrombocyte aggregation
	Hepatocytes	Glycogenolysis, gluconeogenesis
	Myometrium	Uterine contraction
	Adrenal cortex	Glucocorticoid release
	Adipose tissue	Regulation of adipocyte differentiation
	Osteoblasts/osteoclasts	Bone remodeling
V1bR	Anterior pituitary	Corticotropin, growth hormone and prolactin secretion
	Adrenal medulla	Catecholamine release
	Brain	Stress adaptation, cognitive functions, regulation of social behavior
	Pancreas	Glucagon release
	Adipose tissue	Regulation of adipocyte differentiation
V2R	Basolateral membrane of renal collecting duct	Insertion of aquaporin-2 channels
	Vascular endothelium	Release of vWF and factor VIII
	Alveolar epithelial cells	Immunomodulation
	Osteoblasts/osteoclasts	Bone remodeling

Abbreviations: V1aR; vasopressin receptor 1a, V1bR; vasopressin receptor 1b, V2R; vasopressin receptor 2, vWF; von Willebrand factor.
References: (16, 17, 20-24).

There are three known vasopressin receptors: the vasopressin receptor 1a (V1aR), the vasopressin receptor 1b (V1bR) and the vasopressin receptor 2 (V2R). All of these vasopressin receptors are cell membrane bound G-protein-linked receptors. The different receptor effect pathways are schematically illustrated in Figure 3. The V1aR and V1bR exert their effects through activation of phospholipases C, D and A2, protein kinase C and an increase in intracellular calcium (16). The V1aR mediates vasoconstriction and thrombocyte aggregation in the vessels and glycogenolysis and gluconeogenesis in the liver (17, 18). The V1bR is found in the

anterior pituitary gland where it modulates adrenocorticotropic hormone (ACTH) release during stress response. The V1bR is also found in, for example, the adrenal medulla and the pancreas where it affects catecholamine and glucagon release (7). The V2R is best known for its renal effect stimulating water reuptake, but the receptor is also present in, for example, vascular endothelium, osteoblasts, osteoclasts and alveolar epithelium. The V2R uses another signaling pathway than the V1aR and V1bR, acting through cyclic adenosine monophosphate (cAMP) and protein kinase A as second messengers. In the basolateral membrane of the renal collecting duct, V2R signaling results in recruitment of aquaporin type 2 channels which in turn increase water permeability and reuptake (19). The V2R also mediates sodium chloride reabsorption in the renal thick ascending limb (20).

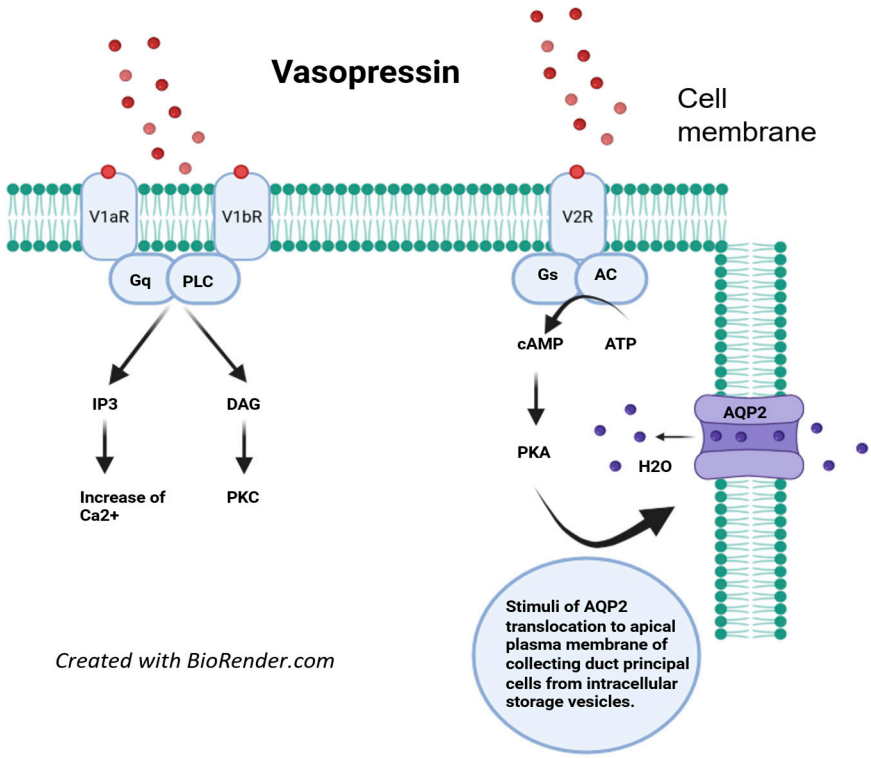


Figure 3. Vasopressin receptor signaling pathways.
Abbrevations: V1aR; vasopressin 1a receptor, V1bR; vasopressin 1b receptor, V2R; vasopressin 2 receptor, Gq/Gs; G-protein subunits, AC; adenylyl cyclase, PLC; phospholipase C, IP3; inositol 1,4,5-triphosphate, DAG; diacylglycerol, PKC; protein kinase C, cAMP; cyclic adenosine monophosphate, ATP; adenosine triphosphate, AQP2; aquaporine type 2.

Measurement of vasopressin by the surrogate marker copeptin

The analysis of vasopressin concentration is complicated and unreliable due to its small peptide size (21). Instead, it has been shown that measurement of the precursor protein cleavage product, copeptin, serves as an accurate estimate of vasopressin concentration. Copeptin, constituting the c-terminal part of prepro-AVP, is released in equal quantities as vasopressin and therefore correlates well with vasopressin concentration in plasma.

Copeptin can be analysed reliably by immunoassays, with different assays compared and described in detail previously (21-23). The methods sufficient for routine clinical use is a sandwich immunoluminometric assay and the further developed automated immunofluorescent analysis performed on B·R·A·H·M·S KRYPTOR compact PLUS (Thermo Fisher).

In the automated analysis, two fluorescent tracers, one "donor" and one "acceptor", linked to antibodies are brought together to form an immunocomplex with the antigen (copeptin). Non-radiating energy is then transferred from donor to acceptor. This results in an intensified fluorescent signal being emitted from the immunocomplex with a time delay which can be measured. The signal is proportional to the antigen (copeptin) concentration (24, 25). This technique is referred to as Time-Resolved Amplified Cryptate Emission Technology (TRACE).

Like vasopressin, copeptin is considered to undergo renal clearance from plasma, why glomerular filtration rate correlates with plasma copeptin concentration (26-28). Under stable conditions, copeptin concentration ranges between 1 – 12 pmol/L (29). Men tend to have higher plasma copeptin concentration than women, whereas the concentration is largely unaffected by age (28, 30).

The specific cardiovascular effects of vasopressin and its possible importance in disease

Blood pressure regulation

Even though vasopressin was first discovered for its vasoconstrictive effects, the importance of the vasopressin system in the regulation of blood pressure is still debated. It has been shown that the concentration of vasopressin needed to induce vasoconstriction greatly exceeds the concentration needed to affect osmolality. The baroreflex activity induced by vasopressin stimulus also entails a reflexive decrease in heart rate that counteracts blood pressure rise. Further, vasopressin is known to have vasodilatory effects in certain vascular beds. Under stable conditions, the

vasopressin system is therefore thought to play a minor role in the regulation of blood pressure (7). However, vasopressin is a major regulator of vascular tonus when cardiovascular homeostasis is compromised by for example hemorrhage, hypovolemia or vasoplegic chock (7, 31). Under these circumstances, vasopressin exerts vasoconstrictive effects through direct vasoconstriction of arterioles, potentiates the effects of norepinephrine and angiotensin II, and antagonizes the vasodilatory effects of nitric oxide and atrial natriuretic peptides (31).

It is debated whether vasopressin is of importance in the development of hypertension (32-36). Several cross-sectional studies have shown positive associations between copeptin concentration and hypertension (37). However, so far, a role of vasopressin in development of hypertension has been easier to show in animal models than in studies on humans (38, 39).

Myocardial effects

The effects of vasopressin stimuli on the myocardium and coronary vessels are complex. Vasopressin is shown to induce both vasoconstriction and vasodilation of coronary arteries. It has been postulated that the opposite effects may be explained by the oxygen tension status, with vasoconstrictive effects during conditions with sufficient oxygen supply, and vasodilation during hypoxic states (31). The inotropic effects of vasopressin are also under debate. While vasopressin in animal studies has been shown to increase left ventricular end-systolic pressure and to decrease stroke volume, cardiac output and inotropy, other animal studies have shown positive inotropic effects, often with lower doses of vasopressin. It is thought that the net effect of vasopressin on myocardial function is dependent on a complex interplay between oxygen supply, the concentration of vasopressin and the balance of effects on coronary vascular tonus and myocardium. Beyond its acute effects, vasopressin is thought to promote cardiomyocyte hypertrophy through effects on myocyte protein synthesis mediated by the V1aR (31). The vasopressin induced cardiomyocyte hypertrophy has been proposed to play a role in the cardiac remodeling process seen in heart failure (40-42).

Vasopressin and its involvement in other neuroendocrine systems

Neuroendocrine activation is a key feature in several cardiovascular conditions, such as hypertension and heart failure. An overactive renin-aldosterone-angiotensin system (RAAS) and activation of the sympathetic nervous system (SNS) is essential in both hypertension and heart failure development, leading to a vicious circle of water and sodium retention and vasoconstriction (43). In addition, all of these systems interact with the vasopressin system. Vasopressin is known to induce renin and aldosterone release (7, 44), and vasopressin release can in turn be stimulated by angiotensin II, illustrating its involvement in the RAAS. Vasopressin can also

contribute to catecholamine release as part of the activation of SNS. As described above, vasopressin is released from the hypothalamus directly into the median eminence with further effects on the anterior pituitary gland. Here, V1bR mediates ACTH release which in turn induces corticosteroid release by the adrenal gland. Vasopressin can also directly mediate corticosteroid release from the adrenal cortex. The vasopressin stimulus of ACTH and corticosteroids is part of the response to stress, along-side with the ACTH and cortisol release activated by corticotropin releasing hormone.Thus, the vasopressin-mediated ACTH release could be looked upon as being part of the hypothalamic-pituitary-adrenal (HPA) axis. The cardiovascular effects of an activated SNS, RAAS and HPA axis, and their importance in disease, have been described in detail previously (45-50).

The role of vasopressin in inflammation

Inflammation has been repeatedly demonstrated to be a cardiovascular risk factor. Individuals suffering from chronic inflammatory diseases are known to have an increased risk of several cardiovascular conditions such as myocardial infarction, heart failure and stroke (51). Cardiovascular disease is commonly a result of arteriosclerosis. Arteriosclerosis of the smaller arteries, atherosclerosis, is an inflammatory process characterized by endothelial dysfunction which results in increased permeability to lipoproteins and their accumulation, leukocyte recruitment and differentiation, and thrombocyte activation (52). The endothelial dysfunction is probably an important contributor to the risk of cardiovascular disease development in inflammatory states, but certainly not the only one. Dyslipidaemia, diabetes mellitus and life-style parameters are other factors interacting with the inflammatory process in the development of cardiovascular disease (53).

The vasopressin system is involved in inflammation through several complex pathways. Various inflammatory cytokines, for example interferon-gamma, interleukin 1 and interleukin 6, have been shown to correlate with vasopressin release (54, 55). Further, vasopressin receptors are found on human mononuclear cells where they affect cell migration as well as cytokine and antibody release (54). Vasopressin can also enhance T helper 1 cell action, lymphocyte response and macrophage phagocytosis (54, 55). These actions indicate that vasopressin acts as a pro-inflammatory hormone, contributing to the worsening of the inflammatory process. Some studies have, however, shown that vasopressin can downregulate the immune response. In one animal experiment, the inflammatory response in rat lung tissue was decreased after being exposed to vasopressin, and the immunomodulatory effect was inhibited by vasopressin antagonists (54). In another study, even though vasopressin could stimulate the migration of leucocytes, the leukocyte migratory response was decreased when the cells were pre-incubated with vasopressin (55).

Vasopressin is also involved in inflammatory response through interactions with the HPA axis. As mentioned, vasopressin stimulates corticosteroid release both through ACTH secretion and through direct stimulation of the adrenal cortex. Glucocorticosteroids are well known immunosuppressants, even though pro-inflammatory actions are also seen with complex net effects on inflammation (56) .

Vasopressin and hemostasis

Vasopressin derivates have been used for the treatment of von Willebrand's disease and mild haemophilia for decades. The derivates stimulate von Willebrand factor (vWF), factor VIII and tissue plasminogen activator release from secretory granules in vascular endothelial cells. These factors are essential in hemostasis, binding thrombocytes to endothelial cells and initiating the coagulation cascade. Vasopressin has also been shown to directly activate thrombocytes (57). Vasopressin derivates can therefore be used in the treatment of bleeding due to thrombocyte dysfunction which can occur with the use of for example aspirin (54). Thrombocyte activation and aggregation are key features of the atherosclerotic process and specifically important in acute coronary syndromes and stroke (58). Anti-thrombocyte therapy is therefore a cornerstone in the treatment of these diseases. Vasopressin receptors are present also on the thrombocytes. However, whether these receptors are involved in hemostasis, or which other effects they may exert, is poorly understood (59, 60).

Vasopressin and erythropoiesis

An over-activated erythropoiesis, erythrocytosis, has known links to cardiovascular disease, morbidity and mortality (61-64). Both higher blood viscosity resulting from erythrocytosis and functional alterations of the erythrocytes have been proposed as underlying mechanisms for these links (62, 65). It is, however, not clarified whether an increased concentration of erythrocytes within normal limits is correlated with increased cardiovascular risk (64).

Like many hematopoietic cells, erythrocytes also possess vasopressin receptors. Since the vasopressin response is important in the counteraction of blood loss, eg. through vasoconstriction and fluid retention, it has been proposed that vasopressin could be of importance also in erythropoiesis. In one study, Mayer et al. found that the V1aR expressed on mouse hematopoietic stem cells play an important role in the regulation of erythropoiesis. They also found that all three vasopressin receptors are expressed on human hematopoietic stem cells and that individuals lacking vasopressin, i.e. suffering from central diabetes insipidus, were prone to anemia (66). Although contradicting results exist, there are thus implications of an involvement of the vasopressin system in erythropoiesis (67, 68).

Vasopressin and diabetes mellitus

Diabetes mellitus as a cardiovascular risk factor is well established (69). As mentioned, vasopressin receptors are found both in the pancreas and liver. Through these, vasopressin is proposed to be of importance in glucose control. Acting through the V1aR, vasopressin can stimulate glycogenolysis and gluconeogenesis in the liver and glucagon secretion in the pancreas (70, 71). Glucagon is known to have an important role in diabetes development (72). The before mentioned vasopressin stimulation of ACTH release has been reported to be resistant to glucocorticoid feedback. Since glucocorticoids have known effects on glucose regulation, this could also be of relevance for diabetes development (73, 74).

Vasopressin is known to be elevated among patients with poorly controlled diabetes mellitus, and in healthy individuals vasopressin infusion leads to increased blood glucose levels (75, 76). Further, copeptin has been shown to predict new onset diabetes mellitus independently of established risk factors, including fasting glucose and insulin (77). Although exact mechanisms are to be clarified, one Mendelian randomization study have shown evidence of a causal link between vasopressin and hyperglycemia in men (78). Animal and human interventional data also points at improved glucose regulation with decreasing vasopressin and copeptin concentration (79, 80).

Vasopressin and renal disease

Although the water resorptive effect of the renal V2R is best recognised, all three vasopressin receptors are found in the kidney (81). The functions of the renal V1aR and V1bR are however complex and not fully understood (82). It has been proposed that vasopressin receptors can be of importance in the development of renal failure. Animal studies have shown that chronic infusion of a V2R-agonist contributes to proteinuria and renal function decline (83, 84). In humans, experiments have shown that V2R-agonists increase urinary albumin excretion (84). Inversely, low circulating vasopressin levels due to increased water intake, or decreased vasopressin effect due to V1aR/V2R antagonism, improves kidney function in rats (85, 86). In population-based studies, elevated copeptin independently predicts development of both estimated glomerular filtration rate (eGFR) decline, chronic kidney disease (CKD) and other specified kidney diseases (87-89). Vasopressin is also thought to be mechanistically involved in the progression of autosomal dominant polycystic kidney disease (ADPKD) (90). The vasopressin inhibitor, Tolvaptan, has shown beneficial results on glomerular filtration rate decline in ADPKD and is approved as treatment in this specific condition (91).

CKD is a substantial cardiovascular risk factor. Cardiovascular mortality accounts for around 40 to 50% of deaths among patients with advances CKD and half of CKD patients in stage 4-5 have cardiovascular disease (92). Several mechanisms are relevant for the development of cardiovascular disease in CKD, but this is however

not within the scope of this thesis and are therefore not covered in detail. Briefly, endocrine and enzymal activation along with release of inflammatory cytokines are associated with kidney injury and insufficiency. Both these mediators, as well as the hemodynamic consequences of the renal function decline per se, contribute to vascular and cardiac damage and further negative effects for the kidney (92).

Behavioural aspects of vasopressin – fluid intake

Low water intake can cause dehydration with potentially deleterious physiological effects, and chronic underhydration has been linked to cardiovascular disease (93, 94). Also, among non-dehydrated individuals, an increased water intake seems to be associated with better cardiometabolic outcomes. For example, high water intake has been associated with a favourable metabolic profile and decreased diabetes risk (95-97). One meta-analysis of five cohort studies has reported a significant inverse association between total water intake and risk of cardiovascular mortality (98). Induction of inflammation, increased blood viscosity, coagulation factors and sodium concentration as well as alterations of the vasopressin system have been proposed as possible underlying mechanisms to these associations.

Following an osmotic vasopressin stimulus, a sufficient volume of water needs to be reabsorbed or ingested to restore plasma osmolality and supress vasopressin secretion. There are, however, evidence that oral and gut reflexes to drinking can also be of importance in the suppression of vasopressin secretion. Previous studies have shown that plasma vasopressin falls quickly following drinking, even before any osmolality change can be detected (99). It has been shown that vasopressin neurons respond to water intake by rapidly decreasing their activity, and that this system involves activation of the lamina terminalis (100). Activation of both oropharyngeal and gut receptors have been proposed as a signaling pathway in this mechanism (101). Not only the volume of ingested water per day, but also drinking pattern, is therefore another rather understudied aspect of how vasopressin concentration can be regulated. Additionally, even if a plasma osmolality above 285 mOsm/kg is said to stimulate thirst, many other factors – such as hormonal influences, psychological factors and age – affects the extent to which an individual increases fluid intake by osmoresceptor stimuli (102, 103). It is therefore of interest to further investigate how lifestyle factors and drinking habits affect vasopressin concentration and possibly also cardiovascular risk.

Coffee consumption and vasopressin

In this thesis, we focus on one behavioral aspect that could possibly affect copeptin concentration: the drinking of coffee. Coffee consumption is a daily habit of many, often leading to both an extra intake of fluid and a repetitive drinking pattern of smaller fluid quantities regularly every day. The impact of coffee consumption on a

variety of different diseases, including cardiovascular disease, has been largely investigated mostly in epidemiological studies. Although findings are somewhat conflicting, coffee intake seems to be generally safe with indications of beneficial associations with cardiovascular diseases in some studies (104-110).

Coffee is also said to have a diuretic effect which most often is attributed to the effects of caffeine on renal adenosine receptors (111). Previous studies have also proposed an effect of caffeine on vasopressin activity mediated through cyclic adenosine monophosphate (cAMP) (112). Vasopressin exerts its renal effects through the V2R, which in turn transmits intracellular signals via cAMP. Caffeine is thought to slow down the degradation of cAMP, which would in turn amplify the effect of vasopressin (112). An increased vasopressin effect stimulated through this pathway could then be speculated to, in the long term, lower vasopressin concentration through negative feed-back mechanisms (113). Regardless of the exact mechanism, both vasopressin and coffee intake have effects on body fluid balance. In addition, they are both associated with several aspects of cardiovascular disease. These common traits have thus motivated the investigation of links between coffee intake and the vasopressin system in this thesis.

The importance of the vasopressin system in cardiovascular disease

The numerous proposed and proven cardiovascular effects of vasopressin have led to speculations regarding its involvement in cardiovascular disease. Epidemiologically, increased vasopressin concentration has been linked to several cardiovascular diseases and outcomes. For example, increased vasopressin, measured by copeptin, predict coronary artery disease and cardiovascular mortality (114). Copeptin has also been shown to be of prognostic value in the evaluation of patients with myocardial infarction and heart failure (115, 116). The evidence of a mechanistic effect of vasopressin on disease development is, however, less investigated.

A low fluid intake could be said to stimulate a state of “chronically” increased vasopressin concentration. This type of state could probably also be affected by other lifestyle or behavioral patterns, such as intake of coffee. Theoretically, the conservation of water, vasoconstrictive effects and myocardial remodeling induced by vasopressin stimulus could be speculated to contribute to heart failure development. Further, the involvement of vasopressin inflammatory pathways along with its known effects on glucose regulation could be proposed to accelerate the atherosclerotic process. So far, these mechanisms have not been proven. The involvement of vasopressin in hemostasis and erythropoiesis and, in turn, their possible cardiovascular effects are other speculative links between vasopressin and cardiovascular disease. The physiological effects of vasopressin and the theoretical consequences in disease are illustrated in Figure 4.

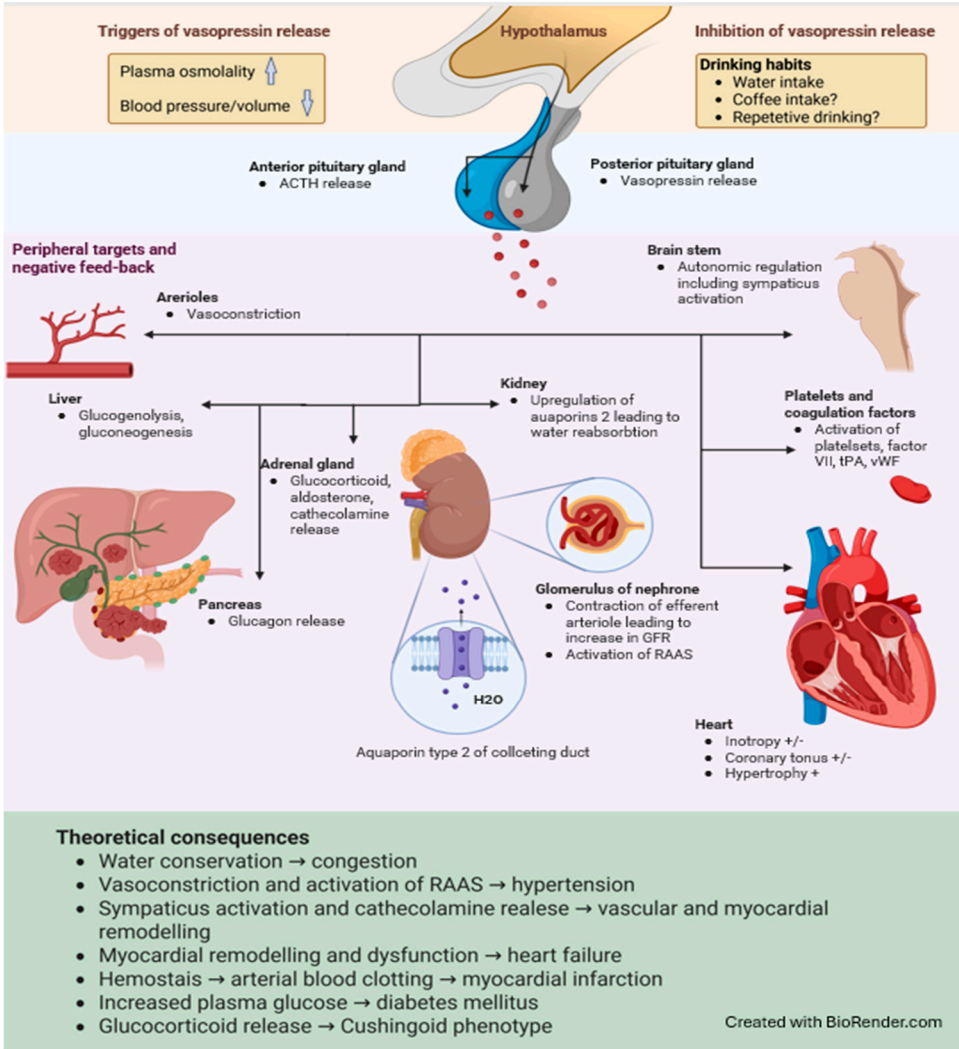


Figure 4. Physiological effects of vasopressin and the theoretical consequences in disease. Vasopressin is synthesized in the hypothalamus and released from the posterior pituitary gland in response to increase in plasma osmolality and/or decrease in blood volume (10). Vasopressin is involved in autonomic regulation and sympaticus activation (117). Vasopressin stimulates reabsorption of water through renal aquaporins type 2. Vasopressin also stimulates ACTH release, vasoconstriction of arterioles, glycogenolysis and gluconeogenesis in the liver, glucocorticoid and aldosterone release from the adrenal gland and glucagon secretion from the pancreas (7). Vasopressin activates hemostasias through effects on thrombocytes and coagulation factors (54). Myocardial vasopressin receptors (V1aR) can induce myocardial hypertrophy and has both positive and negative effects on inotropy and coronary arterial tonus (31, 41).

Preventive aspects – modification of the vasopressin system

As previously discussed, several neuroendocrine systems, such as the RAAS and SNS, are highly involved in cardiovascular disease, and the inhibition of these systems are corner stones in the treatment of for example hypertension and heart failure. If the vasopressin system could be identified as an additional modifiable system causally linked to cardiovascular risk and/or disease, this could have important health benefits.

Vasopressin inhibitors, so called vaptans, are used for treatment of several medical conditions, however not proven to be effective in cardiovascular diseases. The V2R antagonist, Tolvaptan, has been evaluated in the EVEREST (Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study) study to investigate possible beneficial effects on heart failure with signs and symptoms of volume overload. The study failed to show any statistically significant favorable or unfavorable effects on overall survival or the combined endpoint of cardiovascular mortality or heart failure hospitalization (118). Smaller studies have found possible beneficial effects of dual V1aR and V2R antagonists in heart failure (119), and other studies are ongoing (120, 121). However, the V2R antagonist Tolvaptan is only approved by the American Food and Drug Administration for treatment of hyponatremia linked to either heart failure or Syndrome of Inappropriate Antidiuretic Hormone (SIADH) and for the treatment of kidney function decline in adults at risk of rapidly progressing ADPKD. The intravenous dual V1aR/V2R antagonist Conivaptan is approved for the treatment of hyponatremia in hospitalized patients with euvolemic and hypervolemic hyponatremia. In Europe, Tolvaptan is only used for treatment of SIADH and ADPKD, although off-label use in heart failure related hyponatremia may exist. So far, there are no studies examining vasopressin antagonists in other cardiovascular conditions than heart failure.

Since several studies indicate an activated vasopressin system years before development of disease, it could be of interest to target this system for disease prevention. A low water intake is an important determinant of high vasopressin concentration, whereas vasopressin secretion can be easily reduced by a high water intake (122). Because of the known links between vasopressin and cardiovascular and metabolic risk factors, it can be hypothesized that an increased water intake can be beneficial as a cardiovascular prevention method through the suppression of vasopressin secretion. Since increased fluid intake is an easy and low-cost intervention, even small positive effects on cardiovascular risk would, if found, be of importance.

It has previously been shown that the most prominent water-induced vasopressin reduction is observed in individuals who are low drinkers with consequently low urine volume, high urine osmolality and high plasma vasopressin (122). Among rats genetically prone to obesity, it has been shown that vasopressin suppression, by an increased water intake, can reverse insulin resistance and hepatic fat accumulation

(122). In an ongoing trial on humans (H2O Metabolism Trial, NCT03422848) the potential of drinking water to reduce cardiometabolic risk in habitually low-drinking adults with high vasopressin/copeptin concentration is investigated. The results from the pilot study to this larger trial has shown that 1.5L of increased water intake per day for 6 weeks significantly lowered copeptin concentration as well as fasting plasma glucose (79). Further studies on whether an increased fluid intake can affect cardiovascular risk through interactions with the vasopressin system is warranted.

Rationale

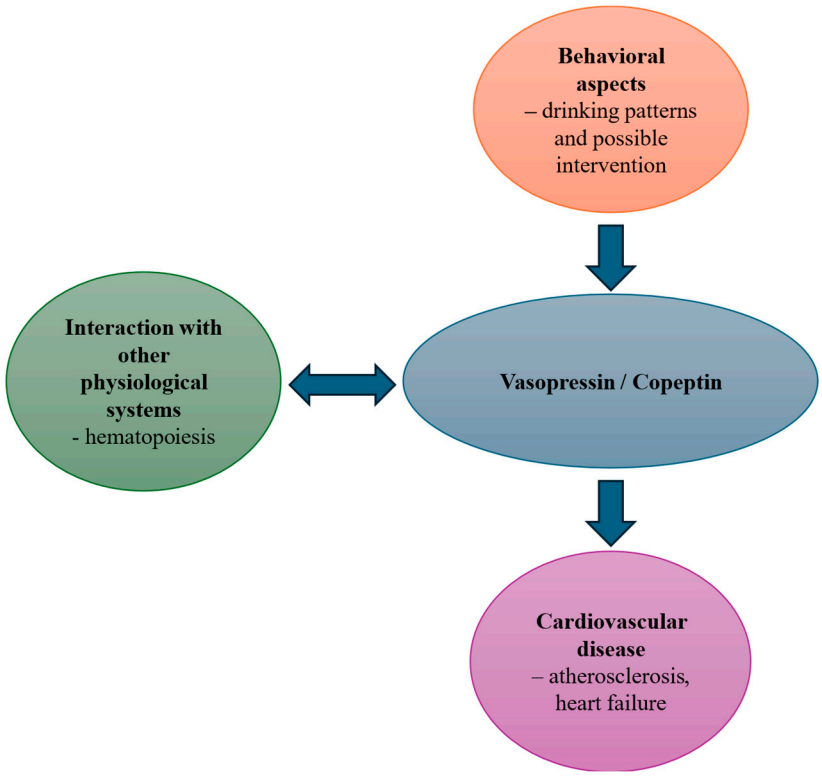


Figure 5. The different angles of the vasopressin system approached in this thesis. Behavioral aspects with focus on coffee intake. Interactions with other physiological systems with possible relevance in cardiovascular disease, such as hematopoiesis. Links with cardiovascular diseases such as coronary atherosclerosis and heart failure.

The purpose of this thesis was to explore the vasopressin system in a cardiovascular perspective. We wanted to learn more about which cardiovascular diseases that are linked to the vasopressin system. More specifically, we explored its links to heart failure and arteriosclerosis.

In an attempt to understand possible mechanisms underlying the already established links with cardiovascular disease, we also sought to find new possible aspects of the

vasopressin system in human physiology. Since vasopressin is known to be involved in hemostasias and has been linked to inflammation and erythropoiesis, we hypothesized that the vasopressin system could play a role in hematopoiesis. This was of particular interest since several types of hematopoietic cells are known to be involved in the arteriosclerotic process.

Vasopressin secretion has been shown to be modifiable by altered fluid intake, and the widely consumed beverage coffee has, just like vasopressin, been shown to associate with metabolic and cardiovascular diseases. We therefore aimed to explore the possible effects of coffee consumption on vasopressin concentration.

Aims

Paper I

To investigate the possible predictive roll of copeptin on heart failure development.

Paper II

To evaluate the association between copeptin and objective measures of arteriosclerosis and coronary atherosclerosis.

Paper III

To explore the possible associations between the concentration of copeptin and several hematopoietic cells.

Paper IV

To understand whether copeptin concentration is affected by coffee intake.

Methods

Author's contribution to the papers

Paper I-IV

- Conceptualisation: The author contributed to the idea and design of the papers, with major contributions from supervisors.
- Statistical Analysis: The author performed the statistical analyses.
- Interpretation: The author interpreted the results, in discussion with supervisors and co-authors.
- Writing/publishing: All drafts have been written by the author and revised after input from supervisors and co-authors. The publication process was independently managed by the author. The author has also been the corresponding author of all manuscripts.
- The author did not contribute to the gathering of data in the cohorts or in the experimental study.

Study designs

Table 2. Overview of the four papers.

	Paper I	Paper II	Paper III	Paper IV
Design	Prospective observational cohort study.	Cross-sectional observational cohort study.	Cross-sectional observational cohort study.	Cross-sectional observational cohort study and crossover random order trial.
Study population	MPP re-exam cohort	SCAPIS cohort	SCAPIS cohort	MOS cohort Experimental study group
Sample	N = 5297	N = 5303	N = 5312	N = 3270 (MOS) N = 26 (Coffee trial)
Primary outcome	Hazard ratio between copeptin quartile at baseline and incident heart failure.	Association between copeptin tertile and high CACS (>100) and high PWV (>10 m/s).	Association between copeptin tertile and hematopoietic markers.	MOS: Association between coffee intake (tertile) and copeptin concentration. Coffee trial: Copeptin concentration change from baseline.
Statistical analyses	Cox-regression analysis. Kaplan-Meier estimates.	Multivariate logistic regression analysis.	Multivariate linear regression analysis.	Multivariate linear regression analysis. Wilcoxon paired test.

Abbreviations: MPP; Malmö Preventive Project, SCAPIS; the Swedish CArdioPulmonary bioimage Study, MOS; Malmö Offspring Study, CACS; coronary calcium score, PWV; pulse wave velocity.

Cohorts

All papers in this thesis consist of analyses of data from three large observational population-based cohorts: the Malmö Preventive Project (MPP) Re-examination Study, the Swedish CArdiopulmonary bioimage Study (SCAPIS) and the Malmö Offspring Study (MOS). These three cohorts are described in detail below. The fourth paper also entails analysis of data from an experimental study described at the end of the methods section.

Malmö Preventive Project (MPP)

MPP is a Swedish population-based prospective cohort started in 1974. The aim of the cohort was to examine a large strata of the adult population living in the city of Malmö, Sweden, in order to find high-risk individuals for preventive intervention on cardiovascular risk factors, alcohol abuse, impaired glucose tolerance and breast cancer. 30587 adults (21911 male and 8676 female) participated in the screening programme between 1974 and 1992. The participants were at this point between 32 and 51 years of age and the attendance rate was 71.2%. Participants were screened for risk factors of interest by filling out a self-administered questionnaire and through a physical examination, a panel of laboratory tests, a glucose intolerance test, and a mammography for women. The study also included an intervention program aimed at treating incidentally found risk factors or diseases. During the time of inclusion of participants, it was emphasized that a control group with non-participants, without access to the screening or intervention program, would be useful. Therefore, from this point, only every second age cohort was invited, leaving every other age cohort as a control group (123). However, neither data from the baseline investigation, nor data from the control group, have been analysed since only the MPP Re-examination data has been used in this thesis.

MPP Re-examination Study (MPP re-exam)

All participants of MPP who were alive and still residing in Malmö between 2002 and 2006 were at this point invited for a re-examination in which 18238 individuals participated (participation rate 72%). At the re-examination, the participants were between 53 and 81 years old and cardiovascular risk factors were reassessed. All participants were given verbal and written information of the study and signed an informed consent form before entering the study. Participants were also given information of the results of the examinations. If new-onset diseases were discovered (type 2 diabetes mellitus, dyslipidaemia or hypertension) the participants were offered an appointment with a physician if they did not have a personal family physician.

The Ethics Committee of Lund University, Sweden, approved the Malmö Preventive Project Re-examination Study (registration number 85/2004). The study complied with the Declaration of Helsinki (124).

Examinations

All participants underwent a physical examination. Blood pressure was measured using an oscillometric device twice after 10 min of rest in a supine position, together with pulse rate. Height (meters) and weight (kilograms) were measured in light indoor clothing without shoes. Waist and hip circumference (centimetres) were measured.

Questionnaire

The participants self-assessed their health and described their medical history and medications in a questionnaire. Cigarette smoking was assessed in the questionnaire, with current cigarette smoking defined as any use within the past year.

Laboratory analyses

Blood samples were taken for the analysis of fasting plasma glucose, serum total cholesterol, serum triglycerides and serum high density lipoprotein cholesterol (HDL) (Beckman Coulter LX20, Beckman Coulter Inc., Brea, USA). Low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald formula (125). If the fasting plasma glucose measured at the first visit was elevated (≥ 7.0 mmol/L), new blood samples were drawn on the second visit. Plasma samples were taken in the complete re-investigation sample and frozen to – 80°C for later analyses. In paper I of this thesis, these samples were used for later analysis of copeptin (n=5410) and midregional pro-atrial natriuretic peptide (MRproANP) (n=5415) by using commercially available immunoassays (B.R.A.H.M.S AG, Hennigsdorf, Germany). The plasma samples selected for analysis of copeptin and MRproANP were randomly chosen with the only exclusion criterion being prior participation in the other large population-based prospective cohort study from Malmö, called the Malmö Diet and Cancer study (126).

The Swedish CArdioPulmonary bioImage Study (SCAPIS)

SCAPIS is a multi-site observational cohort study organised in collaboration between six Swedish universities. In this cohort, the cardiopulmonary health of individuals aged 50–64 years, residing in the six respective cities (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå and Uppsala) was investigated. Between 2013 and 2018, a random selection of 30154 men and women aged 50-64 years were recruited from the Swedish population register. The overall participation rate was 50%. The participants completed a health questionnaire with 140 questions

relating to the research aims. Further, participants underwent examinations including anthropometry, electrocardiography, spirometry, blood pressure measurement, ankle-brachial index measurement and registration of physical activity. Spirometry was performed. Imaging of the carotids, coronary arteries, chest and abdomen were performed by different imaging modalities. In the computed tomography (CT) of the chest, coronary calcium score (CACS) was calculated. A venous blood sample (fasting) and urine sample was collected and stored for later analysis. All centres analysed total cholesterol, HDL, triglycerides, calculated LDL, plasma glucose, beta-N-1-deoxy fructosyl Hb (HbA1c), high-sensitivity C-reactive protein and creatinine as minimum, with the possibility of optional analyses.

A total of 6251 individuals were examined at the Malmö site. Each regional site was allowed to include optional examinations that would not interfere with or affect the core examinations (127). In SCAPIS Malmö hematopoietic markers were measured (Hb, red blood cell distribution width standard deviation (RDW-SD), mean corpuscular volume (MCV), erythrocyte volume fraction (EVF), erythrocytes, leukocytes, lymphocytes, neutrophils, thrombocytes) by using the certified central clinical laboratory at the Skåne University Hospital in Malmö, Sweden. RDW-SD was measured using a Sysmex XN-10 counter (www.sysmex.com) and was calculated as the width (femtoliters, fL) of the erythrocyte distribution curve at the relative height of 20% above the baseline. Copeptin was analysed from biobanked plasma by using a KRYPTOR Compact Plus device and commercially available chemiluminescence sandwich immunoassay copeptin ProAVP kit with coated tubes (BRAHMS Copeptin proAVP KRYPTOR; TermoFisher Scientifc). Carotid-femoral pulse wave velocity (C-f PWV) measurement was included in the baseline examination for participants examined in Malmö.

Examination specifications

Weight was measured with participants in light clothing, using calibrated scales. Blood pressure was measured in the brachial artery of both arms after 5 min of supine rest and calculated as the average of two stable measurements. If plasma glucose was ≥7.0 mmol/L, a repeated sample was taken at the second visit to establish a potential diagnosis of diabetes. Prevalent diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/L, HbA1C ≥48 mmol/mol or use of antidiabetic medication. The calcium content in each coronary artery, visualized by CT (Somatom Definition Flash, Siemens Medical Solution, Forchheim, Germany) was measured and summed to produce a total CACS according to the scoring system previously described by Agatston et al. (128). C-f PWV was measured with Sphygmocor Xcel (Atcor Medical, Australia) in supine position after 5 min of rest with cuffs on the upper left arm and on the right thigh approximately 10–20 cm below the groin. The carotid-femoral distances were measured from the femoral pulse to the upper edge of the thigh cuff and from the carotid pulse to the upper edge

of the thigh cuff. A carotid tonometer was then used simultaneously with the leg cuff to capture blood pressure waveforms at the carotid and femoral sites. Two measurements of c-f PWV were then obtained. If the difference between the measurements differed more than 0.5 m/s, a third measurement was performed. The mean value of the measurements was used in further analysis.

The multicentre study was ethically approved by the Umeå University Ethical Review Board whereas the present study was approved by the Lund University Ethical Review Board (registration number 2018-979). All participants provided written informed consent.

Malmö Offspring Study (MOS)

MOS is a population-based cohort with baseline examinations performed between the years 2013-2021. The aim of MOS was to investigate risk factors of importance for family traits of chronic disease by linking risk factor levels in parents with outcomes in their offspring. Genetic-, epigenetic- and circulating biomarkers were collected along with gut and oral microbiota, vascular imaging, family history, medical history, cognitive function, diet and other lifestyle factors as well as social aspects. The study participants of MOS consisted of invited adult children and grandchildren to participants in the Malmö Diet and Cancer Study Cardiovascular Cohort, which is a random subpopulation of the Malmö Diet and Cancer Study (126). The Malmö Diet and Cancer Studyis a population-based cohort performed between 1991 and 1996 investigating the relationship between a diet rich in fat and low in fiber, and several different cancer forms. 28098 middle-aged individuals participated and formed the generation 1 (G1) in MOS. To investigate the offspring of G1, children and grandchildren to index individuals in G1 were recruited using public register information from the Swedish Tax Agency. After identification of these potential participants, invitations were sent by mail and were followed up by phone calls. All individuals were given written and oral information before signing an informed consent.

Ethical approval for MOS was obtained from the Regional Ethics committee in Lund (registration number 2012/594). The study was performed in line with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and other relevant guidelines.

Examinations

Anthropometrics and resting blood pressure were measured in a standardized manner. Fasting plasma samples were drawn to analyse lipids (triglycerides, HDL, LDL), fasting glucose and creatinine at the Department of Clinical Chemistry, Malmö. Additional plasma samples were frozen and stored in − 80 °C in a local biobank for later analysis of copeptin which was analysed by a KRYPTOR Compact

Plus device and commercially available chemiluminescence sandwich immunoassay copeptin ProAVP kit with coated tubes (Thermo Scientifc BRAHMS Copeptin proAVP KRYPTOR).

Lifestyle parameters were assessed in an extensive web-based questionnaire. Dietary intake was assessed through a validated web-based 4-day dietary record designed by the Swedish National Food Agency ("Riksmaten 2010") in which all consumed foods and beverages were noted during 4 consecutive days starting the day after the inclusion visit.

Experimental cohort (paper IV)

Between 2011 and 2016, 39 individuals participated in a study investigating the effects of both extra fluid (water) intake as well as an increased coffee intake. The participants were aged 20–70 years without any prior known health conditions and were found through advertisements in local press, advertisements directed toward staff at Lund University, and telephone contacts with the participants of two previous population-based cohort studies performed in Malmö (the Malmö Diet and Cancer Study and MPP). The recruitment to the coffee experiment was, however, prematurely stopped due to frequent gastrointestinal side effects, and therefore only 27 participants completed the coffee procedure.

The study was approved by the regional ethics committee in Lund (registration number 2010/740). Written informed consent was obtained from all subjects. The trial was registered at ClinicalTrials.gov (*Water and Coffee Intervention in Humans*, registration number: NCT06165185, URL: Study Details | Water and Coffee Intervention in Humans | ClinicalTrials.gov).

Examination specifications

Each participant underwent three acute intervention procedures: 1 L of water, 4 dL of coffee, or 10 mL of water (control). The results from the water intervention have already been described and published previously (129). Each participant underwent the procedures in random order, and each procedure was separated with a washout period of three weeks. In this study, we analysed the coffee and control procedures.

The coffee ingested during the coffee experiment comprised commercial instant coffee powder (Nestlé Nescafé instant coffee). For each deciliter of coffee, 7.5 mL (~1.75 g) of coffee powder was mixed with 1 dL of tap water.

Upon arriving to the clinical research unit, fasting sampling for urine osmolality, plasma osmolality, plasma sodium, plasma potassium, and plasma copeptin was performed. Thereafter, the participants ingested 4 dL of coffee (coffee procedure)

or 10 mL of water (control procedure) for a maximum of 20 min. Afterward, repeated blood sampling for plasma copeptin analysis was performed every 30 min for 4 hours.

Definitions of variables

Paper I

Incident heart failure was defined as the primary diagnosis of heart failure according to the Swedish National Inpatient Register, the Swedish Hospital-based outpatient care register or the Cause-of-death Register according to its International Classification of Diseases, ninth revision (ICD-9) code 428 or International Classification of Diseases, tenth revision (ICD-10) code I11.0 and I50. Myocardial infarction related heart failure was defined as a diagnosis of heart failure on the same day or after a non-fatal acute myocardial infarction. Non-myocardial infarction related heart failure was defined as a diagnosis of heart failure without a previous diagnosis of myocardial infarction, or a heart failure diagnosis captured at least 1 day before the diagnosis of a myocardial infarction. Myocardial infarction was defined based on ICD-9 code 410 or the ICD-10 code I21.

Diabetes mellitus was defined as a fasting plasma glucose ≥7.0 mmol/L, a self-reported physician diagnosis of diabetes, or use of antidiabetic medication. Furthermore, prevalent diabetes cases were captured by using six different national and regional diabetes registers (the Malmö HbA1c register, the nationwide Swedish National Diabetes Register, the regional Diabetes 2000 register of the Scania region, the Swedish National Inpatient Register, the Swedish Hospital-based outpatient care register and the Cause-of-death Register). Cigarette smoking was assessed by a self-administered questionnaire, with current cigarette smoking defined as any use within the past year.

Paper II

High c-f PWV and CACS were defined as a value above 10 m/s and 100 respectively. For c-f PWV this cut-off is a well-established marker of arterial stiffness and increased risk for cardiovascular events (130). For CACS, a value of >100 was considered a reasonable cut-off since it has shown to identify individuals with a moderately increased risk of coronary atherosclerosis and cardiovascular events, even if different cut-offs have been used in other studies and clinically (131).

Prevalent hypertension was defined as having a brachial systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 or use of antihypertensive medication.

Prevalent diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/L, HbA1C ≥48 mmol/mol or use of antidiabetic medication at inclusion.

Paper III

Hypertension was defined as having a brachial systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 or use of antihypertensive medication on inclusion. Smoking was defined as answering yes to a question on active smoking in a questionnaire at inclusion. Prevalent diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/L, HbA1C ≥48 mmol/mol or use of antidiabetic medication. Anemia was classified as having a Hb concentration of less than 130 grams (g)/ liter (L) for men and 120 g/L for women according to the World Health Organisation's definition.

Leisure time physical activity the last 12 months was collected through questionnaires. In the questionnaire, the participants were asked how much they moved and exerted themselves physically during their leisure time. The answers were graded in a four-grade scale ranging from self-reported sedentary lifestyle to regular intense physical activity according to the Saltin-Grimby Physical Activity Level Scale (132).

Paper IV

Total coffee intake included all types of coffee (filtered, instant, boiled, espresso etc.) and was based on self-registered cups converted into mean intake in g per day. Drinking water intake included self-registered intake of tap and mineral water and was calculated as a mean in g per day. Total fluid intake included water in beverages such as coffee and food moisture and was calculated as mean intake in g per day.

Current smoking was defined as answering yes to the question "do you smoke" in the web-based questionnaire.

Statistical methods

Statistical analysis was performed using SPSS statistical software (version 25.0 and 29; SPSS, Chicago, Illinois, USA) for calculations in all papers. A two-sided P value of < 0.05 was considered significant in all analyses.

Paper I

Copeptin was transformed using the natural logarithm (ln) because of skewed distribution to the right.

Multivariate Cox-regression analysis was performed for the relationship between increasing copeptin concentration and incident heart failure, incident myocardial infarction related heart failure and non-myocardial infarction related heart failure. Both the standard deviation (SD) of log-transformed copeptin and sex-specific quartiles of copeptin were used, depending on the model. The analyses were adjusted for the conventional cardiovascular risk factors age, sex, systolic blood pressure, diabetes mellitus, body mass index (BMI), antihypertensive therapy, smoking, LDL and HDL.

Kaplan-meier plots were constructed to illustrate cumulative incident rates of heart failure in different quartiles of baseline copeptin levels.

The association between copeptin and heart failure depending on diabetes status was analysed using an interaction term between prevalent diabetes and copeptin on incident heart failure.

The correlation coefficient between copeptin an MRproANP was assessed by using Pearson correlation.

Multicollinearity was investigated by using a variance inflation factor.

Paper II

Copeptin was transformed using the ln and was then divided into sex-specific tertiles.

PWV and CACS were examined as categorical variables defined as “high” if being over a defined threshold (CACS >100 and PWV >10 m/s).

Multivariable logistic regression models were used to assess the associations between increasing copeptin tertile and high CACS and PWV, respectively.

All models were adjusted for age and sex and in the next step additionally adjusted for prevalent hypertension, prevalent diabetes mellitus, BMI, smoking status, HDL, triglycerides, creatinine and high sensitivity c-reactive protein (hsCRP).

Paper III

Sex-specific tertiles of the ln of copeptin concentrations were used throughout.

Mean values of the hematopoietic markers per tertile of copeptin were compared and analysed by an ANOVA test.

Linear regression analyses were used to assess the association between increasing sex-specific copeptin tertile and levels of erythrocytes, Hb, EVF, RDW-SD, MCV, leukocytes, neutrophils, lymphocytes and thrombocytes.

Linear associations were also analysed between SD increment in logarithmically transformed copeptin concentration and the hematopoietic markers.

All models were adjusted for age and sex, and in the next step additionally adjusted for BMI, current smoking, prevalent diabetes, hypertension, creatinine and leisure time physical activity.

Sensitivity analyses were madein which linear regression analyses investigating the association between increasing copeptin tertile and hematopoietic markers in strata of very low (<3) or higher (>/=3) hsCRP as well as in non-anemic participants were performed.

Paper IV

The study consisted of one epidemiological and one experimental part.

Analysis of epidemiological data

Coffee consumption, drinking water intake and total fluid intake was divided into sex-specific tertiles. The ln of continuous copeptin was used for all analyses.

The association between coffee intake and copeptin was tested in a linear regression analysis with copeptin as the dependent variable. The association was tested with adjustment for age and sex, and then additionally for creatinine, HDL, LDL, triglycerides, glucose, systolic blood pressure, BMI and smoking, and finally additionally for drinking water intake and total fluid intake.

The association between coffee intake and plasma copeptin was also analysed in strata (sex specific tertiles) of drinking water intake as well as total fluid intake.

Data from all linear regression analyses were expressed as unit change (Beta (95% confidence intervals)) in the dependent variable per tertile increase in coffee intake.

Analysis of experimental data

Median (25^{th}-75^{th} percentile) plasma copeptin concentrations at different times (30, 60, 90, 120, 150, 180, 210, 240 minutes) post coffee load or control procedure was compared with the median baseline concentration.

The change from baseline was tested by Wilcoxon paired test.

Ethical considerations

The analyses made in all four papers are based on previously collected data, the author has not been involved in the collection of the data. All research has been conducted in accordance with the Declaration of Helsinki, and all participants were provided written informed consent. All participants were thoroughly informed about of the potential of future research projects on the collected information, including not prespecified research questions and biomarkers. All study protocols have been approved by the regional Ethics Committee of Lund University (registration numbers noted in the cohort description sections above). The experimental trial described in paper IV was registered at ClinicalTrials.gov.

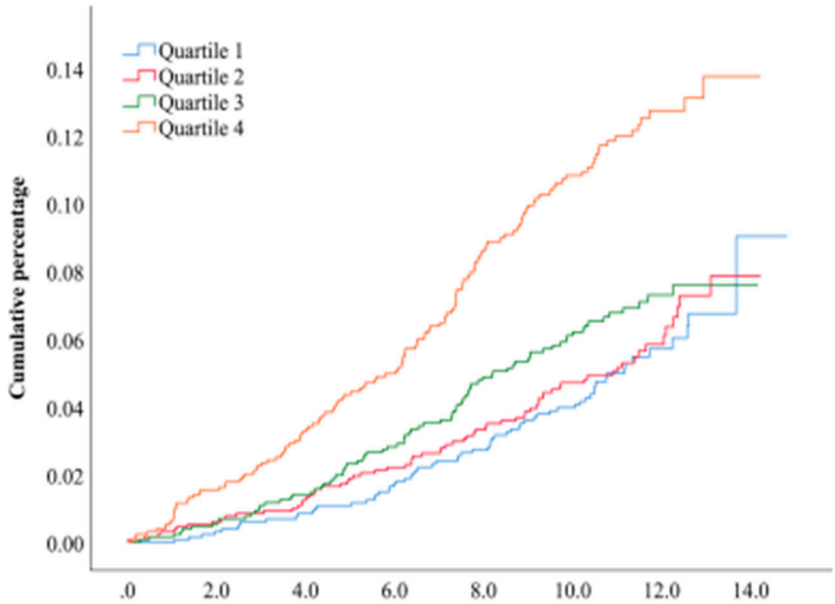
Summary of results

Paper I

Data from 5297 participants was analysed, after excluding 23 participants with incomplete data and 90 participants with heart failure at baseline. Participants were predominantly males with a mean age of70 years. The median follow-up time was 11.1 years (8.9–12.1, 25th–75th percentile).

In total, 350 incident heart failure events occurred during follow-up. Of these, 99 heart failure events were classified as myocardial infarction related and 251 as non-myocardial infarction related. Cumulative incident rates of heart failure in different quartiles of baseline copeptin levels are illustrated in Kaplan-Meier curves (Figures 6-8), with the highest incident rates of heart failure in the highest quartile of copeptin for all categories (any heart failure, myocardial infarction-related heart failure, and non-myocardial infarction -related heart failure). The difference between curves was tested with log-rank test with P values of <0.001 for all.

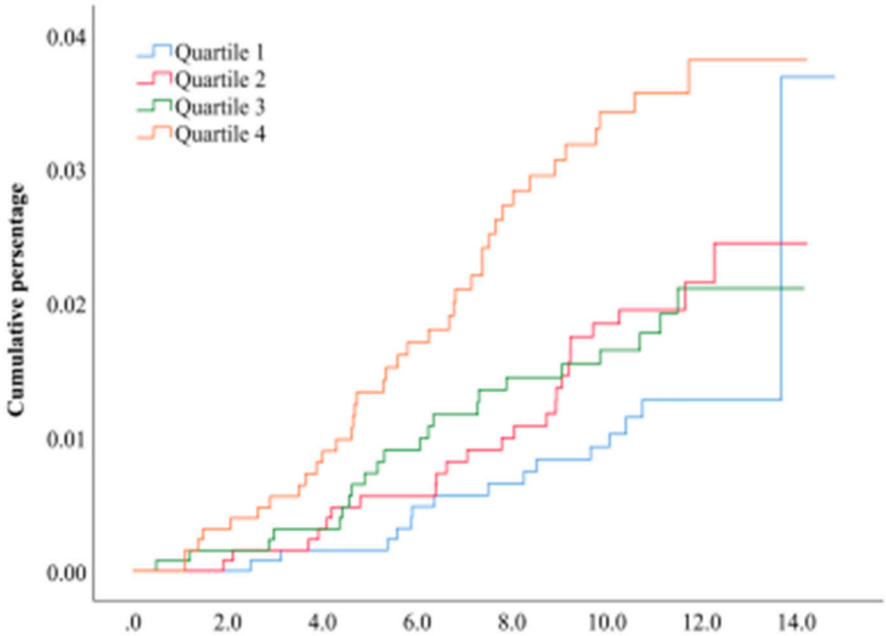
Individuals free from heart failure at baseline and belonging to the top quartile of copeptin had, after multivariate adjustment for conventional risk factors (age, sex, systolic blood pressure, diabetes mellitus, BMI, antihypertensive therapy, smoking, LDL and HDL), a significantly increased risk of developing heart failure when compared with the reference quartile 1 in Cox regression analysis. The risk was increased by 63% for any heart failure, by 100% for myocardial infarction related heart failure and by 47% for non-myocardial infarction related heart failure. Comparable results were found when assessing the risk of heart failure per SD increase in ln copeptin, also after additional adjustment for eGFR and for ln MRproANP as well as for both men and women separately.



Number at risk

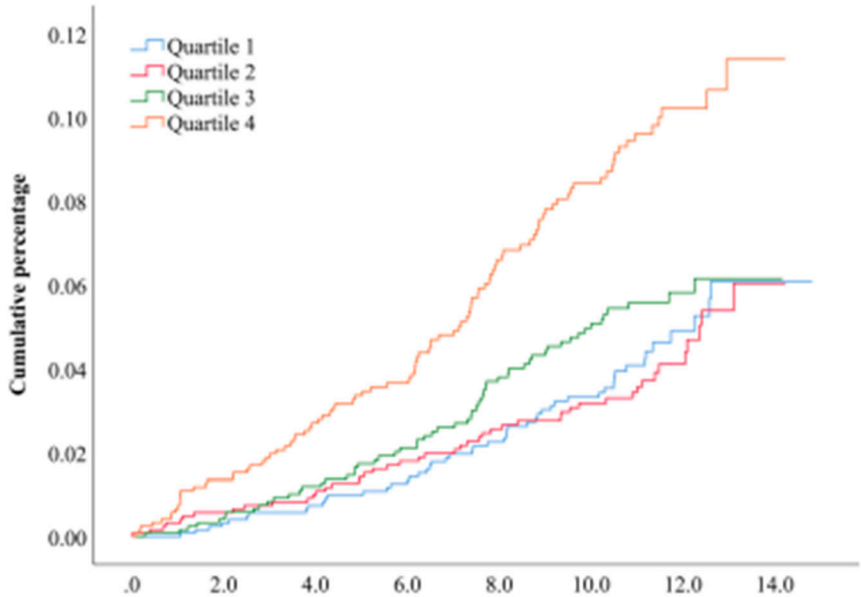
Quartile 1	1328	1306	1264	1202	1122	1035	323	25
Quartile 2	1323	1290	1239	1167	1096	1002	365	29
Quartile 3	1327	1284	1204	1129	1049	952	349	22
Quartile 4	1319	1245	1158	1036	897	784	308	16

Figure 6. Kaplan-Meier event rates for heart failure according to quartiles of baseline copeptin levels.



Number at risk								
Quartile 1	1328	1306	1264	1202	1122	1035	323	25
Quartile 2	1323	1290	1239	1167	1096	1002	365	29
Quartile 3	1327	1284	1204	1129	1049	952	349	22
Quartile 4	1319	1245	1158	1036	897	784	308	16

Figure 7. Kaplan-Meier event rates for heart failure classified as myocardial infarction related heart failure according to quartiles of baseline copeptin levels.



Number at risk

Quartile 1	1257	1229	1181	1115	1029	944	284	23
Quartile 2	1244	1198	1144	1065	992	914	336	27
Quartile 3	1248	1189	1102	1026	948	859	304	17
Quartile 4	1216	1131	1049	942	809	707	272	15

Figure 8. Kaplan-Meier event rates for heart failure classified as non-myocardial infarction related heart failure according to quartiles of baseline copeptin levels.

Paper II

Complete data on fasting plasma copeptin, PWV and CACS was available in 5303 individuals. The mean age was 57.5 years and 46.7% were men. In general, individuals with high CACS and PWV had a worse cardiovascular risk profile with higher prevalence of diabetes mellitus and smoking and higher blood pressure. The median copeptin concentration was higher within the groups of high CACS and high PWV as compared to the groups of lower CACS and PWV.

Increasing tertile of copeptin was significantly associated with both high CACS and high PWV, after adjustment for age and gender (Table 2). The associations remained significant after adjustment of additional cardiovascular risk factors (hypertension, diabetes mellitus, BMI, smoking status, HDL, triglycerides, creatinine and hsCRP) (Table 2).

Paper III

Complete data was available in 5312 individuals, excluding individuals with incomplete data on copeptin or other covariates (n=939). Mean age of the included participants was 57.5 years, and 46.7% were men.

Mean values of erythrocytes, RDW, EVF, Hb, leukocytes and neutrophils increased with increasing copeptin tertile, whereas there were no significant differences in mean value of MCV, thrombocytes or lymphocytes between tertiles of copeptin.

In linear regression analyses, increasing copeptin tertile was significantly and positively associated with all hematopoietic markers except MCV and lymphocytes after adjustment for age and sex. In the multivariate adjusted analyses, significant associations were found between increasing copeptin tertile and increasing erythrocytes, RDW, EVF, Hb, leukocytes, and neutrophils, respectively, after adjustment for age, sex, BMI, current smoking, prevalent diabetes, hypertension, creatinine and physical activity, whereas the association was not significant for MCV, lymphocytes and thrombocytes (Table 3).

Consistent results were found in the linear regression analyses when SD increase of logarithmically transformed copeptin was used instead of copeptin tertiles, with the exception of a significant association between MCV and copeptin in the fully adjusted model.

The same results were found when analysing the subgroup of non-anemic participants. Within groups of very low (< 3) or higher (≥ 3) hsCRP concentration, the results remained in both groups, except that copeptin did not remain significantly associated with leukocytes among individuals with higher hsCRP.

Table 2. High coronary calcium score (>100) and high pulse wave velocity (>10 m/s) in tertiles of copeptin.

	Copeptin tertile 1	Copeptin tertile 2	Copeptin tertile 3	*p* trend
Copeptin, pmol/L[a]				
Males	4.14 (1.43–5.42)	7.03 (5.44–8.91)	12.54 (8.93–456.7)	
Females	2.83 (0.65–3.44)	4.08 (3.45–5.07)	6.96 (5.08–407.4)	
High coronary calcium score	N = 1800	N = 1783	N = 1720	
Adjustment[b]		1.159 (0.943–1.426)	1.449 (1.185–1.772)	<0.001
Adjustment[c]		1.081 (0.874–1.335)	1.264 (1.024–1.559)	0.027
High pulse wave velocity	N = 1800	N = 1783	N = 1720	
Adjustment[b]		1.227 (0.951–1.583)	1.538 (1.203-1.967	0.001
Adjustment[c]		1.198 (0.918–1.563)	1.392 (1.070–1.809)	0.014

BMI, body mass index; TG, triglycerides; HDL, high density lipoproteins; hsCRP, high sensitive c-reactive protein.

[a] Expressed as median (minimum – maximum).

[b] Adjusted for age and gender.

[c] Adjusted for age, gender, BMI, TG, HDL, prevalent diabetes mellitus, hypertension, smoking, creatinine, hsCRP.

Table 3. Hematopoietic markers associated with increasing tertile of copeptin concentration (n = 5312) in linear regression analysis. Data expressed as unit (95% confidence interval) change in outcome variable per tertile increase in copeptin concentration. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, current smoking, prevalent diabetes, hypertension, creatinine physical activity. Abbreviations: RDW-SD, red cell distribution width standard deviation; EVF, erythrocyte volume fraction; MCV, mean corpuscular volume.

	Beta (95% CI)	*P**
Erythrocytes, 10^6/µl		
Model 1	0.058 (0.046 – 0.070)	<0.001
Model 2	0.039 (0.026 – 0.051)	<0.001
RDW-SD, fL		
Model 1	0.202 (0.102 – 0.302)	<0.001
Model 2	0.200 (0.099 – 0.302)	<0.001
EVF, %		
Model 1	0.502 (0.413 – 0.592)	<0.001
Model 2	0.383 (0.292 – 0.474)	<0.001
Hemoglobin, g/L		
Model 1	1.406 (1.096 – 1.716)	<0.001
Model 2	0.979 (0.664 – 1.293)	<0.001
MCV, fL		
Model 1	-0.020 (-0.159 – 0.119)	0.777
Model 2	0.106 (-0.035 – 0.246)	0.140
Leukocytes, count/µl		
Model 1	235 (169 – 301)	<0.001
Model 2	121 (56 – 186)	<0.001
Lymphocytes, count/µl		
Model 1	36 (-3.8 – 76)	0.076
Model 2	9.0 (-31 – 49)	0.663
Neutrophils, count/µl		
Model 1	174 (136 – 212)	<0.001
Model 2	99 (62 – 137)	<0.001
Thrombocytes, count/µl		
Model 1	2059 (125 – 3994)	0.037
Model 2	1400 (585 – 3386)	0.167

Paper IV

Results from the observational study – the Malmö Offspring Study

Complete data was available in 3270 participants and used for further analysis. Mean age was 43 years and 46.7 % were men. Individuals in the highest coffee tertile had a coffee intake of in median 575 ml per day and were older, more often men and smokers with a higher BMI, LDL and blood pressure (systolic and diastolic) compared with the lowest tertile. Participants in the highest tertile of coffee intake also had a greater drinking water intake and total fluid intake and lower copeptin concentration compared to the lowest coffee tertile. The lowest coffee tertile constituted mostly of zero coffee consumers.

We found a significant association between increasing sex-specific coffee intake tertile and decreasing copeptin concentration. The significance withheld after further adjustment for age, sex, creatinine, HDL, LDL, triglycerides, glucose, systolic blood pressure, BMI and smoking, as well as after additional adjustment for either drinking water intake or fluid intake (Table 4). When stratifying the analysis on drinking water intake (sex-specific tertiles) and total fluid intake (sex-specific tertiles) respectively, a significant association between coffee intake and copeptin was found only in the two lowest tertiles of drinking water intake and the lowest tertile of total fluid intake (i.e. among individuals with the lowest habitual fluid intake) in a model adjusted for age and sex.

Results from the experimental study

85% of the participants were women and the mean age was 60 years. Median copeptin concentration was slightly higher at the start of the coffee experiment compared to the control procedure (4.88 and 3.76 picomol/liter (pmol/L) respectively).

The mean copeptin concentrations after acute ingestion of 4 dL of coffee decreased significantly within 30 minutes after coffee intake and remained significantly lower than baseline throughout the 4-hour measurement period ($P<0.001$ for all). The lowest copeptin concentration was observed after 150 minutes with a median reduction from baseline of 27% (3.58 pmol/L) (Figure 9).

During the control procedure (ingestion of 10 ml of water), copeptin decreased to a minimum of, in median, 3.38 (2.80, 5.10) after 120 minutes. The copeptin change at 120, 150 and 180 minutes was statistically significant ($p = 0.006$, 0.028 and 0.036 respectively), whereas the change was non-significant at the other time points (Figure 10).

Table 4. Association between coffee intake and copeptin concentration in the Malmö Offspring Study (n=3270).

	Per coffee tertile increase (beta (CI))*	P	Coffee tertile 2 vs 1 (beta (CI))†	P	Coffee tertile 3 vs 1 (beta (CI))‡	P
Model 1	−0.10 (−0.13–−0.07)	<0.001	−0.10 (−0.16–−0.04)	<0.001	−0.19 (−0.25–−0.14)	<0.001
Model 2	−0.09 (−0.11–−0.06)	<0.001	−0.09 (−0.14–−0.035)	0.001	−0.17 (−0.23–−0.12)	<0.001
Model 3	−0.08 (−0.11–−0.05)	<0.001	−0.09 (−0.14–0.03)	0.003	−0.16 (−0.22–−0.11)	<0.001
Model 4	−0.07 (−0.10–−0.04)	<0.001	−0.08 (−0.14–−0.03)	0.004	−0.14 (−0.20–−0.08)	<0.001
Model 5	−0.03 (−0.06–−0.001)	0.045	−0.06 (−0.11–−0.0053)	0.03	−0.06 (−0.12–−0.0102)	0.042

*Data expressed as unit change in ln-transformed copeptin per tertile increase in coffee intake. †Data expressed as unit change in ln-transformed copeptin if belonging to coffee tertile 2 instead of coffee tertile 1. ‡Data expressed as unit change in ln-transformed copeptin if belonging to coffee tertile 3 instead of coffee tertile 1.

Models:

Model 1: crude.

Model 2: adjusted for age and gender.

Model 3: adjusted for age, sex, creatinine, HDL, LDL, triglycerides, glucose, systolic blood pressure, BMI, and smoking.

Model 4: adjusted for age, sex, creatinine, HDL, LDL, triglycerides, glucose, systolic blood pressure, BMI, smoking, and drinking water intake.

Model 5: adjusted for age, sex, creatinine, HDL, LDL, triglycerides, glucose, systolic blood pressure, BMI, smoking, and total fluid intake.

n = 3,133 for models 3–5.

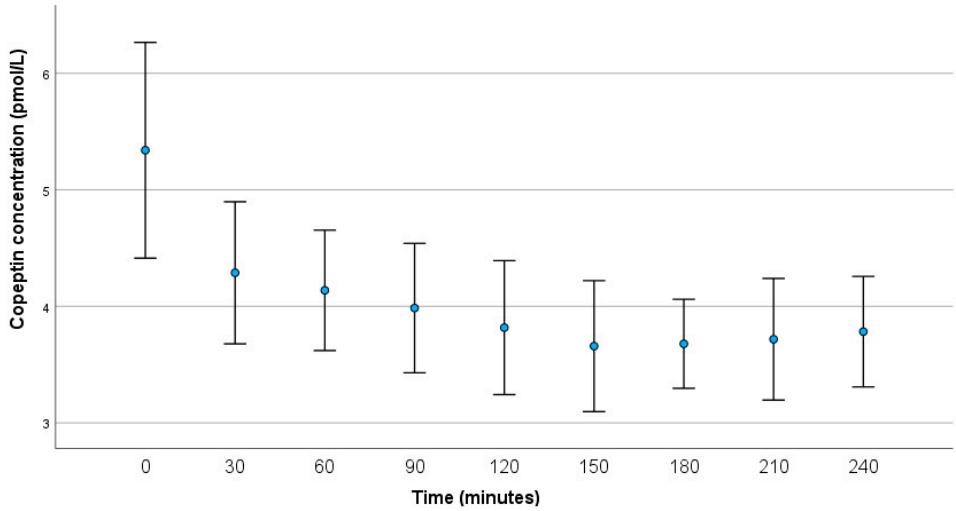


Figure 9. Effects of acute coffee load on plasma copeptin. Plasma copeptin concentration measured at baseline and minutes after intake of 4 dL of coffee (n=26). Bars illustrating mean and 95% confidence interval. At baseline ("0") median plasma copeptin concentration was 4.88 (25^{th}, 75^{th} percentile 3.40-7.26) pmol/L. The lowest plasma copeptin concentration was seen after 150 minutes with a median copeptin of 3.58 (25^{th}, 75^{th} percentile 2.58-4.36) pmol/L. Copeptin concentration was significantly lower than baseline at all time points (P<0.001 for all).

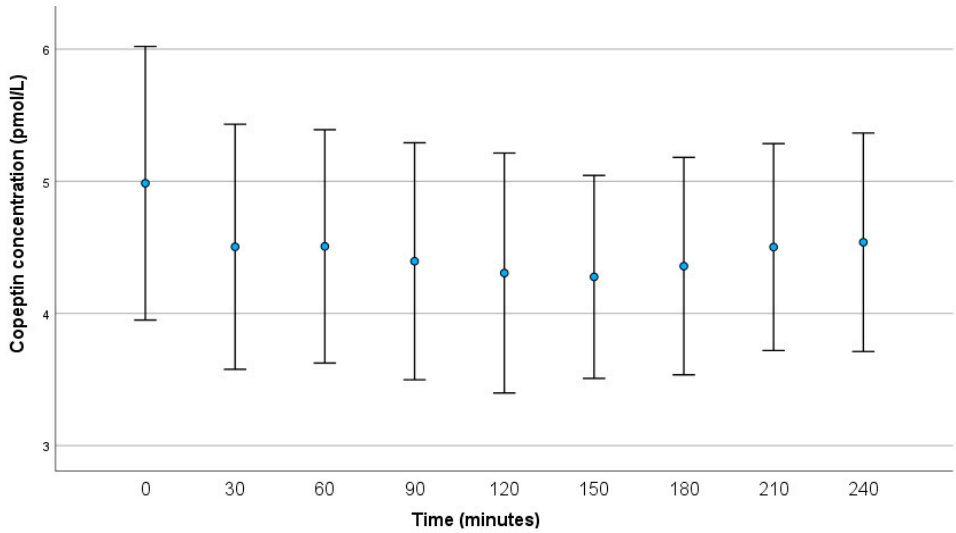


Figure 10. Effects of acute intake of 10 ml of water on plasma copeptin (n=26). Bars illustrating mean and 95% confidence interval. At baseline ("0") median plasma copeptin concentration was 3.76 (25^{th}, 75^{th} percentile 3.06-7.31) pmol/L. The lowest plasma copeptin concentration was seen after 120 minutes with a median copeptin of 3.38 (25^{th}, 75^{th} percentile 2.80-5.10) pmol/L. Copeptin concentration was significantly lower than baseline at 120, 150 and 180 minutes (p = 0.006, 0.028 and 0.036 respectively), and non-significantly lower at the other time points.

Discussion

In this thesis, we have explored several aspects of the vasopressin system of relevance for cardiovascular risk and disease. In the first two papers, the cardiovascular condition heart failure and cardiovascular risk markers (CACS, PWV) were investigated for their association with elevated copeptin concentration. In the third paper, a relatively new aspect of the vasopressin system – its association with hematopoietic markers – was examined. Finally, the impact of the lifestyle factor coffee drinking was evaluated for its association with copeptin concentration and release.

As presented in the introduction section, copeptin is established as a marker for several cardiovascular risk factors, but it has also shown to be able to predict disease (77, 114, 133). Speculations regarding the causal link between the vasopressin system and several of these conditions are ongoing. Both medical treatments and preventive options targeting the vasopressin system is also under investigation (79, 121, 129). A deeper understanding of the vasopressin system is needed to further investigate whether intervention on this system could be relevant for cardiovascular health.

Copeptin and heart failure

In paper I of this thesis, we have confirmed an association between an upregulated vasopressin system and heart failure. It has been known for decades that the vasopressin/copeptin concentration is increased in heart failure (134, 135) and that copeptin concentration is a good prognostic marker in decompensated heart failure with or without myocardial infarction (136-139). This have led to before mentioned speculations regarding the involvement of vasopressin in heart failure development (41), and have motivated clinical trials directed at vasopressin antagonism (42, 118). Our study has shown that copeptin concentrations corresponding to the top 25% of the population is associated with a significantly increased risk of future heart failure diagnosis. The relationship was seen after adjustment for several possible confounding factors and among both those developing myocardial infarction related heart failure and non-myocardial infarction related heart failure. These results again put light on the possible importance of the vasopressin system in heart failure. Apart from possibly being mechanistically involved in the pathophysiology of heart failure, our results also propose that copeptin could be of value in the evaluation of future risk of heart failure development. Since heart failure is a growing global health issue afflicted with high morbidity and mortality rates (140), the

identification of individuals at risk is of great importance in order to enable timely preventive care and treatment.

Copeptin, arteriosclerosis and atherosclerosis

The leading cause of cardiovascular disease is arteriosclerosis and atherosclerosis. In paper II of this thesis, we have illustrated the association between increased copeptin concentration and markers of arteriosclerosis and atherosclerosis measured as CACS and PWV. Individuals belonging to the tertile with highest copeptin concentration had significantly higher CACS and PWV, defined as >100 and >10 m/s respectively, compared with individuals belonging to the lowest tertile. This adds to the already established knowledge of copeptin as a marker of several cardiovascular conditions, but also specifies copeptin as a marker of coronary atherosclerosis as well as arteriosclerosis of the greater arteries. Even if the cross-sectional design prevents speculations regarding causal links between the vasopressin system and arteriosclerosis, the study proposes that copeptin is of value in the evaluation of arteriosclerotic load. As with heart failure, risk markers that can be used to identify arteriosclerosis is essential in the further evaluation and prevention of cardiovascular disease.

Copeptin and hematopoietic markers

In paper III of this thesis, we explored the possible role of vasopressin in hematopoiesis, which is a relatively recently discovered area of vasopressin physiology. It is known that vasopressin receptors are present on several hematopoietic cells, but their functions are, in most cases, unknown. Several studies have also proposed importance of the vasopressin system in inflammation and a role as a regulator of leucocyte action. Further, vasopressin is known to be important in primary hemostasis through the action on thrombocytes and release of coagulation factors. Lastly, some studies point at a possible role of vasopressin in erythropoiesis. We have explored these links further epidemiologically by looking at possible associations between an increment of copeptin and changes in hematopoietic markers. Significant associations were found between copeptin and both leukocyte count and erythrocyte count as well as other erythropoietic markers also after adjustment for several possible confounding factors. The evidence of an association between copeptin and thrombocyte count was, however, low after the multivariate adjustment. Even though this study does not reveal the underlying causes of these relationships, we look upon the results as a first step towards understanding the vasopressin system in relation to hematopoiesis.

Even though not discussed in the published scientific paper, an interesting aspect of the links between the vasopressin system and hematopoiesis is the previously demonstrated relevance of hematopoiesis in cardiovascular diseases. As described in the introduction of this thesis, several hematopoietic markers have been linked to

cardiovascular risk and disease (64, 141), but the underlying mechanisms of these associations are complex and debated. It is also possible that hematopoietic cells are only markers of disease rather than causally linked to the pathogenesis. The results of paper III make it, however, possible to speculate around an involvement of the vasopressin system in the regulation of both leukocytes and erythrocytes. The vasopressin system could, hypothetically, be proposed as a link between these hematopoietic markers and cardiovascular disease. Thus, we regard paper III as hypothesis generating and are hopeful that research on the relevance of the vasopressin system in hematopoiesis will continue.

Copeptin and coffee intake

In paper IV of this thesis, we have discovered new links between coffee intake and copeptin concentration. In the MOS cohort, we found that higher coffee intake was associated with lower copeptin concentration, independent of factors commonly associated with both copeptin concentration and coffee intake. This association appeared to be driven by the individuals with the lowest total fluid and/or drinking water intake. Since this study did not investigate the different compounds of coffee, we still do not know whether the association is explained by extra fluid intake, by components of the coffee itself or other unknown factors. Further, as discussed in the introduction of this thesis, it has been proposed that small quantities of fluid intake can lower vasopressin levels even before changes in plasma osmolality can be detected. It is therefore possible that a habitual, repetitive pattern of coffee drinking is the most important underlying factor behind a decreased copeptin concentration among many coffee drinkers. This theory is supported by the fact that copeptin concentration was significantly reduced both after ingestion of 4 dL of coffee and after ingestion of 10 ml of water. Furthermore, our results contribute to the complexity of the debate concerning the proposed link between coffee drinking and a decreased risk of cardiovascular disease reported in several studies (106). One could speculate that coffee intake lowers the cardiovascular risk through the suppression of vasopressin. This would be in line with research pointing at an increased fluid intake as a possible life style adaptations for cardiometabolic prevention (79, 129). Even though any effect of coffee intake on cardiovascular disease risk would be expected to be a small contributor to the overall risk, the understanding of possible pathophysiological mechanisms is of great importance for development of prevention and treatment strategies. Much more research in the area is warranted to further develop these types of hypotheses.

In summary, this thesis aspires to bring forward the knowledge of the vasopressin system in a cardiovascular perspective. The four included papers have covered various aspects of vasopressin physiology; from a cardiovascular risk marker and predictor of heart failure to its involvement in other physiological systems (hematopoiesis) and behavioral patterns (coffee intake).

Limitations

All four papers included in this thesis have analysed data derived from Swedish population-based cohorts. Paper IV also entails an additional experimental part. As with all epidemiological research, the risk of selection bias is important, as it may result in study population characteristics which differ from the background population it aims to study, making it non-representative. It is usually hard to determine whether the population participating in a cohort study is different from the non-participants and in which way. However, previous studies report that participants in population-based cohorts are more likely to be female, to have a higher social status, healthier lifestyles and better subjective health than nonparticipants (142).

The MPP re-exam cohort was used in paper I. Even though participation rate was high (72 %) in the MPP re-exam, a comparison between participants and non-participants have shown differences in several aspects of social and demographic characteristics as well as in total mortality and cause-specific mortality (123). Of importance is, however, that the initial MPP study included an interventional program with follow-up and treatment of e.g. hypertension, diabetes and suspected breast cancer, which could have influenced the differences in mortality rates. Further, no analysis of differences between participants and non-participants has been made for the MPP re-exam. In the SCAPIS study, the participation rate was 50% which was lower than in the MPP re-exam. In the analysis of the SCAPIS data, participants had a higher individual socioeconomic status and lived in more affluent neighborhoods than non-participants (143). Since lower socioeconomic status is a known risk factor for mortality and morbidity, this could influence the results derived from SCAPIS data (144). In MOS, the participation rate was 47%, but comparisons between non-participants and participants have not yet been performed. The MOS cohort consists of an already selected population (children/grandchildren) derived from the Malmö Diet and Cancer Study. In the Malmö Diet and Cancer Study there are probable population selection differences (145). Thus, it is likely that there are differences in participation characteristics also in the MOS cohort. Taken together, previous data indicate that non-participants of the cohorts used in this thesis constitute of individuals with characteristics associated with a higher cardiovascular risk, which would thus limit the generalizability of the results of this thesis to a healthier Swedish population. There is, however, so far nothing indicating that the vasopressin system as a cardiovascular

risk factor would be less relevant among a population with a higher cardiovascular risk. Rather, copeptin has been shown to serve as a more potent cardiometabolic risk marker in a group of Iraqi born Swedes with a lower socioeconomic profile compared with a group of Swedes that were born in Sweden (146). Thus, it is more likely that a selection bias towards a healthier population in all cohorts would tend to bias the results toward the null. However, unknown factors influenced by selection bias could of course be of relevance for our results.

Specific study limitations

Paper I

The diagnoses of heart failure were retrieved by using three nation-wide registers (the Swedish National Inpatient Register, the Swedish Hospital-based outpatient care register and the Cause-of-death Register). Only a primary diagnosis of heart failure was used since this have shown higher validity than using secondary diagnoses (147). Heart failure diagnoses in primary health care were not retrieved by the registers, and non-diagnosed patients were obviously also miss-classified in the study. This probably led to an underestimation of the heart failure incidence. If a diagnosis of heart failure in an early stage was missed at the time of inclusion, there is a risk that a high copeptin value was rather a marker of heart failure in an early stage than a predictor of future disease development. We argue that this does not exclude copeptin as a predictive marker for future development of clinically recognized heart failure. Finally, information on type and/or severity of heart failure was not available, making the predictive value of copeptin in this aspect less precise.

Paper II

The cross-sectional design of this study prevents conclusions regarding a causal relationship between the vasopressin system and arteriosclerosis/atherosclerosis. Further, a substantial number of individuals were excluded from the analysis due to lack of data, even though this group seemed to be comparable with the individuals included in the analysis regarding baseline characteristics. The cut-offs used for PWV (>10 m/s) and CACS (>100) is not equal to established arteriosclerosis or coronary atherosclerosis and many individuals with high CACS or PWV never develop cardiovascular disease. Copeptin as a marker of high CACS or PWV could be labeled a “surrogate marker of a surrogate marker”, which in turn could be argued to be imprecise in the evaluation of cardiovascular risk. Also, for CACS, the cut-off at >100 was chosen by the authors as a reasonable balance between sensitivity and specificity for identifying cardiovascular risk. However, other cut-offs, which could

be argued to be more relevant, have been used in other studies. To better evaluate the actual value of copeptin as a marker of arteriosclerosis and coronary atherosclerosis, studies on copeptin and proven disease development would be more reliable.

Paper III

The cross-sectional design of paper III makes speculations regarding the importance of the vasopressin system in hematopoiesis complicated. The possible mechanisms discussed in this thesis are however still relevant for generating new hypotheses and stimulating further research. Other limiting factors are the lack of qualitative analyses of leukocyte and thrombocyte function, which would have deepened the understanding and discussion further. Lastly, a moderate number of participants were excluded from analyses due to lack of data. However, they were comparable to the individuals included in the analyses in most of the baseline characteristic parameters.

Paper IV

In the fourth paper, the intent with the two methodologies used was to compensate for the weakness seen with cross-sectional cohort studies. However, the number of individuals included in our coffee experiment was small, predominantly females and lacked younger participants. Furthermore, only approximately two thirds of the intended participants did eventually start the intervention, since it was prematurely terminated as a result of gastrointestinal side effects. On the other hand, the results from the MOS analyses, which were much more robust in number of participants and details on baseline characteristics, support the findings from the experimental study. The MOS analyses were, however, cross-sectional and excluded a moderate number of participants due to lack of data. Neither, details on which types of coffee that were ingested were documented. Further, as with all research on dietary patterns, recall bias and psychological factors affecting honest reporting have possibly affected the results. Finally, as discussed in detail in paper IV, from our data it cannot be clarified whether the associations between coffee intake and copeptin are effects driven by components within the coffee, are linked to the extra intake of fluid, associated with repetitive drinking or related to other unknown factors.

Conclusion

This thesis has investigated the links between vasopressin, measured by copeptin, and several aspects of relevance for cardiovascular disease. The four papers aim to contribute to the further understanding of the pathophysiology linked to the vasopressin system and the importance of copeptin as a cardiovascular risk marker.

From the results of this thesis, it can be concluded that among healthy middle-aged individuals in three different Swedish population-based cohorts:

- Copeptin independently predicts new onset heart failure in median 11 years before diagnosis.
- Copeptin is independently associated with CACS and PWV, i.e. markers of atherosclerosis and arteriosclerosis.
- Copeptin is positively associated with increasing leukocyte count and several markers of erythropoiesis including erythrocyte count.
- Increasing coffee intake is associated with decrement of copeptin concentration, and in a small experimental study copeptin concentration is lowered by acute ingestion of coffee.

Future perspectives

Cardiovascular disease is an emerging global concern where new risk evaluation tools, preventive strategies as well as treatments are essential. Since copeptin concentration has been shown to correlate with several aspects of cardiometabolic disease, an interesting aspect of copeptin is its use in cardiovascular risk assessment. As preventive strategies are best aimed at individuals at high risk for disease, it is of great value to identify high risk individuals with good precision. Many cardiovascular risk scores are available, mostly including imprecise cardiovascular risk factors such as age, gender and blood pressure. Risk estimates are therefore broad and hard to apply on a specific individual. An integration of more specific cardiovascular risk factors to refine these risk scores would therefore be of value. One interesting approach could be to evaluate the incorporation of copeptin concentration in cardiovascular risk prediction scores. In order to do so, the predictive value of copeptin needs to be evaluated for every specific cardiovascular condition and validated in multiple prospective studies.

The results from this thesis, and other research, indicates that the vasopressin system is upregulated in cardiovascular disease. Future studies investigating whether the inhibition/lowering of vasopressin or blocking of certain vasopressin receptors is beneficial for cardiovascular risk is therefore highly warranted. This question is partly approached by the ongoing Hydration To Optimize Metabolism Trial. Another way to further investigate the effect of counteracting the vasopressin system is through studies on vasopressin antagonists. The EVEREST trial did not find any benefit of single V2R-antagoism on patients with established heart failure (118). It has been discussed that these neutral results were related to the compensatory rise in circulating vasopressin seen with V2R antagonism leading to an enhanced activation of the V1aR. Through its effects on the cardiomyocytes and blood vessels, V1aR stimulation could hypothetically have induced structural changes and myocardial hypertrophy counteracting the positive effects of the V2R antagonism (41, 42). The intravenous dual V2R/V1aR antagonist Conivaptan has therefore been studied in heart failure with positive results on diuresis and hemodynamics (119). Because of the interactions with P450 isoenzyme, the oral version has been withdrawn from further development (148). In rat models, pharmacological blockade of the vasopressin system with the V2/V1aR inhibitor BAY1753011 has been studied with positive results, both on the diuretic effect, obtained without the RAAS activation commonly seen with diuretics, as well as on

profibrotic markers on the cardiomyocytes (149). Despite some positive findings, new research on vasopressin antagonists in heart failure, as well as in other cardiovascular conditions, are lacking. Further studies evaluating multiple receptor vasopressin antagonists or lifestyle treatments lowering vasopressin secretion such as increased fluid intake as a preventing strategy or as treatment for patient groups with established cardiovascular disease and high copeptin concentration would therefore be of great interest. In established heart failure with signs of volume overload, an increased fluid intake is, however, not beneficial.

As mentioned, vasopressin is a hormone with pleiotropic effects, many of which are poorly understood. In paper III, we found cross-sectional associations between copeptin concentration and markers of erythropoiesis and leucocyte count. The specific erythropoietic effect of vasopressin has been investigated by Mayer et al who found evidence of an erythropoietic effect of vasopressin in rat models (66). The results led to an application of patent by the same author for the development of a method to stimulate erythropoiesis by stimulating the V1bR. However, to date no trial in this field seems to be ongoing. The evaluation of a possible stimulating effect of vasopressin analogues on erythropoiesis would contribute to the understanding of the vasopressin system and could also potentially lead to development of new treatment methods for anemia.

There are several ways to further investigate the physiology of the vasopressin system and its regulation in relation to drinking habits. In paper IV, we propose that a repetitive drinking pattern could result in a lower copeptin concentration. We investigate the effects of coffee intake on copeptin concentration but from the available data we cannot conclude if any specific component of coffee possibly have copeptin lowering abilities. These questions may be answered with another coffee intervention design, including multiple interventions with different liquids containing different volumes of water and specific compounds of coffee.

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