Chapter 12

General discussion and future perspectives
Over the years various effects of vasopressin have been discovered, ranging from its well-known role in water homeostasis to many others, such as vasocostriction, activation of platelet aggregation, increasing glucocorticoid activity, stimulating renal acid excretion, and altering blood glucose levels by stimulating glycogenolysis and insulin secretion.\(^1\) The clinical significance of some of these effects is not immediately apparent. These effects are initiated by a family of vasopressin dependent receptors: the V1, V2 and V3 receptors.\(^3\) Gaining insight in the significance of these effects may be especially relevant when vasopressin concentrations are chronically elevated due to iatrogenic intervention, as is the case when a V2 receptor antagonist is prescribed.

In order to study the effects of vasopressin, measurement of its plasma concentration is needed. Unfortunately, the assay to measure vasopressin is time-consuming and requires a particular expertise, and is therefore not easily implemented in clinical practice.\(^4\) As alternative, measurement of copeptin was introduced as surrogate marker.\(^4\) Copeptin and vasopressin are split from a common precursor peptide and released in equal amounts from the pituitary gland into the circulation. The general aim of this thesis was to improve the interpretation of copeptin as marker of vasopressin concentration and to use copeptin for studying the various effects of vasopressin throughout the body.

**General discussion of this thesis**

The first part of this thesis, measurement of copeptin as marker for vasopressin, was dedicated towards gaining a better understanding of the correct interpretation of copeptin. Upon its introduction as surrogate marker, copeptin was said to have a superior ex-vivo stability compared to vasopressin.\(^5\) If true, this is an important benefit of a biomarker measured in large biobank cohorts. However, this claim was not substantiated by the necessary data.

Therefore, in Chapter 3 of this thesis, the effects of various pre-analytical conditions on copeptin concentrations were compared to vasopressin, aiming to investigate the ex-vivo stability of both peptides. We found that vasopressin concentrations decreased significantly at higher relative centrifugal forces, whereas copeptin concentrations were not affected. This can be attributed to vasopressin binding to the V1 receptor that is present on platelets, whereas there was no indication for such an effect for copeptin.\(^7\) Next, the effects of short-term storage of whole blood and plasma samples at room temperature or refrigerator temperature were studied, to investigate the effect of a delay in sample processing before centrifugation or long term storage. Copeptin proved to have a superior stability as it was stable up to 24 hours under all conditions. Finally, the effects of long-term storage and of repeated freezing and thawing, conditions relevant for biobank studies, were investigated. When stored at -80 °C or -150 °C both copeptin and vasopressin were stable up to four months, whereas storage at -20 °C caused vasopressin concentration to decrease after 1 month. Vasopressin concentration decreased after four freeze-thaw cycles, whereas copeptin concentration was not affected by repeated freezing and thawing. Thus, Chapter 3 demonstrates that in studies where copeptin and vasopressin are measured and compared directly, it is important to take note of the pre-analytical sample handling protocol that is required for the correct measurement of vasopressin. If the necessary requirements are not met, conclusions could be made based on ex-vivo rather than in-vivo differences. Copeptin requires a less stringent protocol, rendering measurement of this surrogate marker an attractive alternative to study vasopressin activity.

Copeptin has been measured in various populations, ranging from healthy persons to patients with different types of kidney disease.\(^5\) As stated, copeptin is measured as reflection of vasopressin concentration because both are released by the pituitary gland in a 1:1 ratio. However, a simultaneous release does not guarantee that these peptides can be measured interchangeably. For copeptin to reflect vasopressin correctly, it should mirror its plasma concentration from minute to minute. For this to be true, not only production rates, but also volume of distribution and in particular clearance rates of both peptides should be comparable. Both vasopressin and copeptin are small peptides, made up of nine and 37 amino acids, respectively, with molecular weights of 1 and 5 kilo Dalton. Their size suggests that elimination will depend on kidney function, given that peptides of these sizes are usually filtered by the glomerulus and in this way cleared from the circulation.\(^9\) A decrease in kidney function may therefore result in higher plasma concentrations of copeptin and vasopressin. It is not known whether clearance of copeptin and vasopressin are affected to the same extent by an impaired kidney function. Therefore, the question remained how well copeptin reflects vasopressin concentration if measured in patients with kidney disease. Although several studies have investigated the relationship between vasopressin and copeptin during states of water deprivation or excess, not many have studied their relationship in the context of impaired kidney function.\(^20\) The only study on this topic was hindered by the fact that it included samples that had been in cold-storage for a considerable time before measurement of copeptin and vasopressin, and without optimal pre-analytical processing of blood samples for vasopressin measurement, thereby potentially affecting the results.\(^22\) Therefore, in Chapter 4 we studied the association between kidney function and copeptin, vasopressin and the copeptin/vasopressin ratio in a cohort of 127 participants with kidney disease. In these patients kidney function ranged from >90 mL/min/1.73m\(^2\) to zero, meaning patients on hemodialysis without residual kidney function. In this cohort, both copeptin as well as vasopressin concentrations were higher in patients with a lower kidney function. This could be the result of an increased release on the central level, as reaction to a decreased concentrating capacity of the diseased kidneys. Alternatively, it could be a reflection of a decreased clearance of copeptin and vasopressin from the circulation. The copeptin/vasopressin ratio was similar in all subjects with a kidney function above 30 mL/min/1.73m\(^2\). In subjects with a kidney function below this threshold, copeptin concentrations were raised in comparison to the vasopressin concentrations, thus resulting in a higher ratio. This could point towards a lower clearance of copeptin in comparison to vasopressin indicating that the contribution of the kidneys to the clearance of copeptin is larger than for vasopressin. Thus, these data suggest that
copeptin might be used as a reflection of vasopressin release in a large proportion of the population. Nonetheless, there may be an effect of kidney function on the relationship, that should not be disregarded.

To shed light on the possible influence of kidney disease on copeptin concentration, in Chapter 5, the clearance mechanism of copeptin was studied in Wistar rats. By infusion of radioactive labeled copeptin and repeated blood withdrawals, the total body clearance, urinary clearance, plasma half-life and tissue deposition of copeptin was calculated from a two-phase distribution model. Both healthy rats and rats with a decreased kidney function were included to study the effect of kidney failure on these parameters. From these experiments, we concluded that the total body clearance of copeptin was approximately 0.36 mL/min/100g body weight in the healthy animals. In the animals with an impaired kidney function, the clearance rate was considerably lower, namely 0.25 mL/min/100g body weight. This suggests that the kidneys play a significant role in the clearance of copeptin. Our data indicate that this is not due to urinary clearance, for the amount of copeptin found in urine was negligible. Of note, after sacrifice, copeptin was predominantly found in the kidney, and not in other organs such as liver, long or heart. Apparently copeptin was eliminated from the circulation via glomerular filtration, where after it was reabsorbed by the renal tubuli and metabolized by tubular enzymes. To further strengthen the evidence for a primary role for the kidneys in the clearance of copeptin, copeptin was added to perfusate in an isolated perfused kidney. In this set-up, it was also noted that copeptin was cleared from the circulating fluid, thereby confirming the role of the kidney. Comparing these data with data of vasopressin described in literature, the total body clearance rate of copeptin is approximately 10 times lower than that of vasopressin, with a corresponding plasma half-life of approximately an half hour versus only a few minutes. With respect to clearance rate and half-life data for copeptin, clearance was more or less similar to those of creatinine. These data, together with the results presented in chapters 4 and 5, suggest that copeptin clearance is affected by impaired kidney function, whereas vasopressin clearance is predominantly by extrarenal clearance, most likely attributed to the effects of endopeptidases present on the vascular wall. These differences should be kept in mind when interpreting plasma values of copeptin as surrogate for vasopressin in patients with kidney disease.

The next three chapters formed the second part of this thesis and concerned the main effect of vasopressin: antidiuresis. Copeptin is measured as surrogate marker for vasopressin’s antidiuretic activity in the context of research, but it is only seldom used in clinical practice. We examined the potential of copeptin as aid for patient care. To start, Chapter 6 presented an example of the use of copeptin in clinical practice to come to a diagnosis. In this case report, the development of symptomatic hyponatremia after a colonoscopy is described. This form of hyponatremia is referred to as ‘bowel prep’ hyponatremia, because it is the result of the laxative that is used in preparation for this examination. Central to the pathophysiology of this clinical entity is an inadequate increase in plasma vasopressin in combination with an oral water overload. Measurement of a high copeptin concentration illustrated this.

Next, Chapter 7 was written as comment on an article that describes the use of copeptin to determine the cause of the polyuria-polydipsia syndrome. These authors determined cutoffs for copeptin concentrations, before and after an arginine stimulation test, to differentiate between central diabetes insipidus, nephrogenic diabetes insipidus and psychogenic polydipsia. Given that these entities are hard to differentiate with use of the current diagnostic possibilities, this novel approach may be a great aid in clinical practice. However, while determining these copeptin cutoffs, the effect of kidney disease on the plasma copeptin concentration was not taken into account. This novel study with ingenious design thereby also demonstrates the pitfalls of interpreting copeptin correctly.

In addition to serving as diagnostic marker, copeptin has also been studied as prognostic marker for the rate of progression of kidney disease, to complement existing markers such as creatinine based estimation of the glomerular filtration rate. In most kidney diseases, not only the glomerular filtration rate is affected by the disease, but also other functions, such as the ability of the kidneys to concentrate urine. In response to this damage, plasma vasopressin and copeptin concentrations increase. Alternatively, the urine concentrating capacity can be estimated by calculating the urine-to-plasma urea ratio. Urea plays a pivotal role in concentrating urine. The concentration gradient in the medulla that is necessary to draw water from the tubular lumen back to the circulation is built by cycling of urea through the kidneys. Therefore, it was hypothesized that the concentrating capacity of the kidneys can be represented by the ratio between the concentration of urea in urine and its plasma concentration. In contrast to copeptin, measurement of urea is available in most hospitals, making the urine-to-plasma urea ratio an attractive alternative to copeptin as risk marker for disease progression. In Chapter 8, we studied the urine-to-plasma urea ratio in one disease population in particular, namely autosomal dominant polycystic kidney disease (ADPKD). In this disease, the growth of multiple cysts causes the structural integrity of the kidneys to be damaged, thereby decreasing the urine concentrating capacity of the kidneys. This damage is already present early in the disease. We hypothesized that the extent of early damage, e.g. the extent to which the urine concentrating capacity is impaired, might predict future kidney function decline. First, we showed that in 28 ADPKD patients the actual urine concentrating capacity measured with a prolonged water deprivation test was associated with future kidney function decline in the following 6 years. Next, the urine-to-plasma urea ratio was validated as representation of the concentrating capacity of the kidneys. Thereafter, we showed that the urine-to-plasma urea ratio was associated with kidney function decline over the course of four years in a cohort of 583 ADPKD patients, even when adjusted for other disease predictors such as age, baseline estimated glomerular filtration rate, total kidney volume and type of DNA mutation.

Thus, the chapters of the second part of this thesis investigated how studying the antidiuretic effect of vasopressin, either by measurement of copeptin or by assessment of the concentrating capacity of the kidney, can be of aid in clinical practice.

Finally, in the last part of this thesis, additional effects of vasopressin beyond its antidiuretic effect were investigated in patients with increased plasma vasopressin.
concentrations due to an iatrogenic intervention: ADPKD patients who used the selective vasopressin V2 receptor antagonist tolvaptan to halt disease progression. This treatment induces a state of nephrogenic diabetes insipidus and consequently, via feed-back mechanisms, a chronic elevation of plasma vasopressin. Due to the high selectivity of tolvaptan, which targets the V2 receptor only, the V1 and V3 receptors are activated more than usual.

First, in Chapter 9, the effect of a chronic high vasopressin concentration on the hypothalamic-pituitary-adrenal (HPA) axis was studied. In preclinical studies, via binding to the V3 receptor, vasopressin potentiates the effect of corticotrophin-releasing hormone on the adenohypophysis, thereby increasing adrenocorticotropic hormone (ACTH) release.36–38 ACTH stimulates the adrenal gland to produce cortisol. Furthermore, vasopressin has also been shown to stimulate adrenal cells directly via the V1 receptor, thus also increasing cortisol production.37,38 This activation of the HPA axis may be of clinical relevance, in particular in patients with kidney disease, because chronically elevated cortisol levels may add to their cardiovascular burden. To study this, urine and plasma glucocorticoids of 27 ADPKD patients on and off treatment with tolvaptan were assessed and their baseline values were compared to those of 81 age- and sex-matched healthy controls and 27 age-, sex, and kidney function matched IgA nephropathy patients. Three weeks of treatment with tolvaptan increased copeptin concentration more than three-fold. However, there was no clinically significant activation of the HPA axis due to treatment. We observed only an increase in the cortisone excretion, the inactive counterpart of cortisol. In contrast, biologically active cortisol itself and also total glucocorticoid excretion, which includes breakdown products, had not changed. In fact, there was even an indication for a trend towards a lower glucocorticoid production during tolvaptan treatment. When comparing healthy subjects with patients with a decreased kidney function, a decreased excretion of both cortisol and cortisone was noted, while the overall glucocorticoid excretion remained similar. These kidney disease patients also had higher copeptin levels compared to the healthy controls, but again no activation of the HPA axis was observed. Together, these data indicate that the role of vasopressin in determining the release of glucocorticoids is limited. Next, in Chapter 10, the effect on the acid-base homeostasis was studied. We hypothesized that use of tolvaptan would result in an increase in bicarbonate reabsorption in the collecting ducts of the kidneys, via activation of the V1 receptors present on the alpha-type intercalated cells.39 In this small scale clinical study, 31 patients who started tolvaptan treatment in our center were included. Venous blood gasses were drawn before start of therapy and after the highest tolerable dose of tolvaptan was reached. Tolvaptan was started at a dosing schedule of 45 mg in the morning and 15 mg in the evening, to be increased to 60/30 mg after one month and to 90/30 mg the month thereafter if tolerated. Treatment induced a significant increase in plasma copeptin concentration. When comparing the venous blood gasses after this titration period to the baseline values, in contrast to our hypothesis, we found no change in serum bicarbonate. On the contrary, we found evidence for a change towards a more acidic state, accompanied by an increased pCO₂. Confirmation of the validity of this observation can be found in pre-clinical studies, that suggest that V1 activation decreases the respiratory rate.40–42 However, these findings need to be corroborated by prospective, longer term studies, which might include additional measures for renal acid-base handling, such as urinary citrate excretion and urinary ammonium excretion.

Finally, in Chapter 11, the effect of an increase in vasopressin concentration on blood pressure was the main subject of interest. Given that many physicians associate vasopressin with vasoconstriction, there is some concern that prolonged treatment with tolvaptan could have a detrimental effect on the blood pressure. To address this, we performed a post-hoc study of the TEMPO 3:4 trial. This is a multicenter placebo-controlled randomized controlled trial in which 1445 ADPKD patients received either tolvaptan or placebo for a period of three years to assess the efficacy of tolvaptan to halt disease progression. We evaluated the course of blood pressure over time during the trial period, comparing both study arms after short- and long term tolvaptan use. We took into account that patients were treated with antihypertensives and that those regimens could be changed according to good clinical practice. Our analyses did not show an acute effect of tolvaptan compared to placebo on blood pressure after a 3 week titration period, even though use of tolvaptan increased copeptin concentration considerably. Over the duration of the study, we observed a gradually developing decrease in blood pressure between both study arms in favor of the tolvaptan group. This difference was not explained by changes in the antihypertensive regimen of these patients. We hypothesize that it may be explained by a beneficial effect of tolvaptan on disease progression and thereby attenuation of secondary effects of kidney disease such as hypertension.

From these last three chapters we conclude that the other effects of vasopressin, although prominent in vitro, are not as straightforward in vivo. In contrast to the role of vasopressin in anti-diuresis, its effects on other physiological processes may be down-regulated by other regulatory mechanisms.

Future perspectives

Copeptin can be an interesting addition to the clinician’s toolbox. In the field of endocrinology, most progress to implement copeptin in clinical practice has been made. Prospective studies have evaluated the use of copeptin in the diagnostic work-up of patients with the polyuria-polydipsia syndrome to determine the underlying cause.28–43 In the field of nephrology, copeptin has been studied as biomarker to predict future kidney function decline.16,27,30,45 Interest for copeptin as prognostic marker has been strengthened because of the novelty of what this marker potentially represents: an easy to measure marker of the urine concentrating capacity of the kidneys. Thus, copeptin may have added value compared to other biomarkers that mostly represent glomerular filtration rate. By combining the information from biomarkers representing glomerular function, e.g. creatinine based estimated of the glomerular filtration rate, and urine
concentrating capacity, such as copeptin, one can strive towards a more comprehensive assessment of overall kidney function. A few steps have still to be taken before copeptin can be used to differentiate between fast or slowly progressive kidney disease in clinical practice. Cutoff values need to be established and prospectively validated. When designing these studies, one should consider using a standardized method of blood collection to increase the prognostic value of copeptin. It seems reasonable to suggest that these samples should be obtained after an overnight fast, thus presenting the urine concentrating capacity of the kidneys with a water preservation challenge. Furthermore, the results from the first part of this thesis indicate that kidney function should be taken into consideration for when copeptin is used as surrogate marker of vasopressin.

In addition to copeptin, the urine concentrating capacity can also be evaluated by calculation of the urine-to-plasma urea ratio. If the plasma copeptin concentration is regarded as representation of the central stimulus for water conservation, then the urine-to-plasma urea ratio can be seen as representation of the kidney’s ability to respond to that stimulus. When the kidneys are not able to achieve an osmolar gradient in the medulla due to an underlying disease despite an adequate vasopressin stimulus, the urine-to-plasma urea ratio will be affected. This theoretical difference in the interpretation of both biomarkers for urine concentrating capacity merits further study.

There is another reason that vasopressin, and measurement of copeptin, have gained the interest of the research community. More and more evidence has become available that an increased vasopressin concentration does not only reflect an impaired kidney function, but that this hormone also has a contributory role to determine the rate of kidney disease progression. There is one kidney disease in particular, in which the detrimental role of vasopressin is well-established. Vasopressin is central in the pathophysiology of ADPKD. By binding to the V2 receptor on the tubular cells, vasopressin induces cAMP generation that in turn leads to all sorts of intracellular processes that cause cell proliferation and fluid secretion and thus to cyst growth and thereby to acceleration of disease progression. This insight has led to the development of the first treatment to halt disease progression: the V2 receptor antagonist tolvaptan. Tolvaptan has been shown to postpone the need for kidney function replacement therapy by several years if started at a young age. As kidney function replacement therapy in the form of dialysis or kidney transplantation are both associated with significant morbidity, mortality and a negative impact on quality of life, tolvaptan provides an undeniable benefit for ADPKD patients. Unfortunately, tolvaptan also has a significant impact on quality of life due to its antagonistic properties, it induces considerable polyuria and nocturia, causing thirst, inconvenience during daily activities and sleep deprivation in some cases. Therefore, before treatment is started, the expected benefit should be carefully assessed. Not all ADPKD patients will reach end-stage kidney disease. Other markers such as copeptin or the urine-to-plasma urea ratio may be added to these decision trees in the future. In addition to selecting patients who are likely to have a rapidly progressive phenotype, estimation of the effectiveness of treatment could also be of aid when treatment benefits are weighted against side effects. Treatment effectiveness can in some cases be measured by a biomarker change prompted by treatment initiation. In chronic kidney disease, the biomarker used most often for this purpose is urine albumin excretion. This may also be true for ADPKD. However, high levels of albuminuria are not a primary feature of ADPKD and may therefore not be applicable to all patients. Given that tolvaptan’s primary effect is reduction of the urine concentrating capacity via V2 antagonism, its effect may be measured through markers that represent the antidiuretic capacity. There, copeptin measurement may again come to use. This option has been studied in a post-hoc study of the TEMPO 3:4 trial, which demonstrated that a change in plasma copeptin is a fairly good marker for treatment effectiveness. To validate use of copeptin for this purpose, a next step would be to prospectively decide which patients to treat based on their initial copeptin response and compare kidney outcomes over time.

Tolvaptan has an incremental positive effect on kidney function decline over time. It is therefore advised to treat ADPKD patients for a long period. More subtle, insidious off-target effects of chronically elevated vasopressin concentrations should therefore be evaluated carefully. These off-target treatment effects might arise from increased V1 and V3 receptor activation, evoked by a strong increase in plasma vasopressin concentrations. Although there have been studies that followed patients on tolvaptan for a number of years, none have addressed these concerns specifically. In this thesis, three potential detrimental effects have been assessed, namely increased activity of HPA axis, imbalance in the acid-base homeostasis via increased reabsorption of bicarbonate and an increase in blood pressure. Our findings are reassuring, because they did not demonstrate overt negative effects of vasopressin acting on these physiological systems. However, it might be debated whether our studies were sensitive enough to detect a disturbance in homeostasis. For instance, we did not test these systems by imposing a stressor on them, thereby not assessing their capacity for adjustments in challenging circumstances. Furthermore, in these studies we only investigated short term effects, which were all indicative for a positive long term outcome, but requires formal confirmation. Future studies should evaluate these concerns and may address other V1 and V3 receptor mediated effects that were not part of this thesis, such as a change in glucose metabolism or an increased risk for anxiety and depression.
Conclusion

This thesis draws attention to the opportunities that copeptin provides for clinical practice. Measurement of copeptin can give new insights into the clinical relevance of the numerous effects of vasopressin, most importantly the antidiuretic effect. As marker of the urine concentrating capacity of the kidneys, copeptin represents a function of the kidney that is not often measured, although vital for homeostasis. Integrating copeptin measurement into clinical practice may therefore result in a more complete evaluation of kidney function, which could be an important advancement of the assessment of disease severity and progression. The studies described in this thesis may have helped to achieve these goals.

References


