Osmotic Diuresis in the Treatment of Hyponatremia

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Dean

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2 CONTRIBUTION OF THE MD-PHD STUDENT

Manuscript 1: An Increase in Plasma Sodium Levels is Associated With an Increase in Osteoblast Function in Outpatients with Chronic SIAD

- Samples selection* and shipment
- Elaboration of a statistical analysis plan and statistical analysis with R
- Manuscript writing**
- Submission to a scientific journal
- Oral presentation of the results at the American Congress of Endocrinology (ENDO2023, Chicago (USA))
- Poster presentation of the results at the European Congress of Endocrinology (ECE2023, Istanbul (TUR)) and at the Swiss Congress of Endocrinology (SGED2022, Bern (CH))

The original idea and planning of the analysis was done by the other first author PD Dr. Julie Refardt.
*Sample selection was done by JR and SM.
**All coauthors reviewed and edited the first draft of the manuscript and its revisions.

Manuscript 2: Prevalence of Admission Hyponatremia in Diabetic Patients Treated With and Without an SGLT2-Inhibitor - a Cross-Sectional Study

- Study original idea and design
- Writing of the study protocol
- Approval by the Ethic Committee
- Coordination of data extraction by the IT Department of the University Hospital of Basel
- Elaboration of a statistical analysis plan
- Data cleaning and statistical analysis with R*
- Manuscript writing**
- Submission to a scientific journal
- Poster presentation of the results at the Swiss Congress of Endocrinology (SGED2021, Bern (CH))

*Dr. Deborah Vogt helped coding the loops necessary for data cleaning.
**All coauthors reviewed the first draft of the manuscript

Manuscript 3: Effect of Protein Supplementation on Plasma Sodium Levels and Urinary Urea Excretion in Patients with Chronic SIAD – a Monocentric Open-Label Proof-of-Concept Study – the TREASURE Study

- Elaboration of the study design*
- Writing of the study protocol*
- Approval by the Ethic Committee*
- Trial registration*
- On-site study planning*
- On-site study conduct*
    - Screening
    - Recruitment
    - Study visits
    - Logistics
    - Safety Reporting

- Initial statistical analysis**
- Manuscript writing***
- Submission to a scientific journal*
- Oral presentation of the results at the European Congress of Endocrinology (ECE2023, Istanbul (TUR))
- Poster presentation of the results at the American Congress of Endocrinology (ENDO2023, Chicago (USA)) and at the German Congress of Endocrinology (DGE 2023, Baden-Baden (GER)

*Equally contributing with the other first author Dr. Cihan Atila. SM and CA elaborated the study design with Prof. Mirjam Christ-Crain and then shared equally all study tasks marked with a star.
**SM performed the initial statistical analysis necessary for abstract submission. CA performed the complete final statistical analysis for the manuscript.
***SM wrote the first draft of the whole introduction, parts of the method and the whole conclusion sections. CA wrote the first draft of parts of the methods and the whole results section. All authors reviewed and edited the first draft of the manuscript.

Other relevant tasks during the MD-PhD

EMPOWER Study (NCT04447911), coordinating investigator

- Writing of the study protocol*
- Authorization of competent authorities (ethic committee, Swissmedic)*
- On-site study planning*
- On-site study conduct*
    - Screening
    - Recruitment
    - Study visits
    - Logistics
    - Study Reporting
- Coordinating investigator on-site for Basel and for all the other study centers*
- Preparation and successful pass of a routine Swissmedic inspection**

*Under the supervision of PD Dr. Julie Refardt and Prof. Mirjam Christ-Crain.
**In collaboration with Cemile Bathelt and under the supervision of PD Dr. Julie Refardt and Prof. Mirjam Christ-Crain.

SANDx Study (NCT03202667)

- Review of the final manuscript
- Oral presentation of the results at the American Congress of Endocrinology (ENDO2022, Atlanta (USA))
- Oral presentation of the results at the Swiss Congress of Endocrinology (SGED2022, Bern (CH))

PROPHECY Study (NCT05542056), investigator

- Original study idea
- Elaboration of the study design*
- On-site study conduct**
    - Recruitment**

- Study visits**

*In collaboration with the coordinating investigator Dr. Julia Beck
**Under the supervision of the coordinating investigator Dr. Julia Beck

HIT Study (NCT03557957), investigator

- On-site study conduct*
  - Screening*
  - Recruitment*
  - Study visits*

*under the supervision the coordinating investigator Dr. Laura Potasso and principal investigator PD Dr. Julie Refardt.

BONA Study, investigator

- On-site study conduct*
  - Screening
  - Recruitment
  - Study visits

*under the supervision of the coordinating investigator Dr. Laura Potasso

3 SUMMARY OF THE PROJECT

BACKGROUND Hyponatremia is the most common electrolyte disturbance and is associated with increased mortality and morbidity, including an increased risk for osteoporosis. Preclinical studies suggest an hyponatremia-mediated osteoclasts activation, whereas clinical studies rather suggest osteoblasts downregulation, therefore the effect of hyponatremia correction on bone metabolism needs to be studied further. The most common cause of euvolemic hyponatremia and the main etiology addressed in this thesis is the syndrome of inappropriate antidiuresis (SIAD). The different treatment options target the pathophysiological free water excess and include restricting fluid intake and/or increasing renal water excretion, either with AVP receptor antagonists or with osmotic diuresis. The latter can be achieved with sodium/glucose cotransporter 2 (SGLT2) inhibitors that induce glucosuria or with oral urea. Our group showed in two double-blind placebo controlled trials that SGLT2 inhibitors increased sodium levels in SIAD. Whether their chronic use prevents hyponatremia on hospital admission remained unknown. Animal data suggest that the effect of urea could be achieved with a large quantity of dietary protein but whether this concept was applicable to humans was unknown.

OBJECTIVES This MD-PhD had 3 objectives; first, to investigate the effect of hyponatremia correction on bone metabolism; second, to investigate the potential of SGLT2 inhibitors in preventing hyponatremia on admission; and third, to investigate the therapeutic potential of a high-protein supplementation in outpatients with chronic SIAD as compared to oral urea.

METHODS Manuscript 1 is a preplanned secondary analysis of a randomized, double-blind, placebo-controlled, crossover trial investigating the effect of an increase in sodium levels on serum bone markers in chronic SIAD. Manuscript 2 is a retrospective cross-sectional study aiming to compare the prevalence of hyponatremia on hospital admission in patients with diabetes mellitus type 2 (T2DM) treated with SGLT2 inhibitors as compared to matched T2DM control patients without SGLT2 inhibitors. Manuscript 3 is a proof-of-concept interventional controlled study investigating the effect of 90 g protein supplementation per day in the form of protein powder for 7 days, as compared to 30 g oral urea per day for 7 days, on plasma sodium levels of outpatients with chronic SIAD.

RESULTS First, an increase in plasma sodium levels in outpatients with hyponatremia due to chronic SIAD, even when mild, was associated with an increase in bone formation mirrored by an increase in a surrogate marker of osteoblast function. This was independent of empagliflozin treatment. Second, despite their effect in overt SIAD, SGLT2 inhibitors were not associated with a reduced hyponatremia prevalence in patients with T2DM. Third, a one-week high-protein supplementation increased plasma sodium levels in patients with chronic SIAD through protein-induced ureagenesis. The effects were achieved without additional fluid restriction and comparable to a one-week oral urea intake.

CONCLUSION Increasing sodium levels in chronic SIAD stimulates osteoblasts but the effect on bone mineral density needs further research. In chronic SIAD, plasma sodium can be increased with glucose-induced osmotic diuresis with an SGLT2 inhibitor or with urea-induced osmotic diuresis using a high-protein supplementation or oral urea. However, chronic treatment of T2DM with SGLT2 inhibitors does not prevent hyponatremia on hospital admission. Whether a long-term protein-rich diet is effective for SIAD and whether SGLT2 inhibitors are effective for hyponatremia causes other than SIAD should be investigated in future studies.

4 ZUSAMMENFASSUNG DES PROJEKTES

HINTERGRUND Die Hyponatriämie ist die häufigste Elektrolytstörung und ist assoziiert mit einer erhöhten Mortalität und Morbidität, inklusive eines erhöhten Risikos für Osteoporose. Präklinische Studien suggerieren, dass bei einer Hyponatriämie die Osteoklasten aktiviert werden, in klinischen Studien zeigt sich jedoch eher eine Inhibierung der Osteoblasten. Die Auswirkungen der Hyponatriämie auf den Knochenmetabolismus muss deshalb weiter erforscht werden. Die häufigste Ätiologie einer euvolämen Hyponatriämie und die in dieser These am meisten thematisierte Ursache ist das Syndrom der inadäquaten Antidiurese (SIAD). Die Therapie hat zum Ziel, den pathologischen Überschuss an freiem Wasser zu reduzieren. Mögliche Behandlungsoptionen beinhalten Flüssigkeitsrestriktion und/oder Förderung der renalen Ausscheidung von freiem Wasser, erreicht durch die Anwendung von Vasopressin-Antagonisten oder osmotische Diurese. Eine osmotische Diurese kann erreicht werden durch die Gabe von einem Natrium/Glukose-Kotransporter-2-Hemmer (SGLT2-Hemmer), welcher eine Glukosurie induziert, oder von Harnstoffpulver. Unsere Forschungsgruppe zeigte in zwei doppelblinden, Placebo-kontrollierten Studien, dass SGLT2-Hemmer den Natriumspiegel bei einem SIAD erhöhen. Nicht bekannt war, ob die chronische Anwendung der Entwicklung einer Hyponatriämie in Patienten mit Diabetes mellitus Typ 2 vorbeugen kann. Daten aus Tieren suggerieren, dass der Effekt von Harnstoff auch mit einer grossen Menge an Nahrungsprotein erreicht werden kann, ob dies auch im Menschen gilt war jedoch unklar.

ZIELE Diese MD-PhD These hatte 3 Ziele. 1) Die Auswirkung der Hyponatriämiekorrektur mit einem SGLT2-Hemmer auf den Knochenmetabolismus zu untersuchen. 2) Das Potenzial von SGLT2-Hemmern zu untersuchen, einer Hyponatriämie bei Spitaleintritt vorzubeugen und 3) Der Vergleich des therapeutischen Potenzials von einem proteinreichen Nahrungsergänzungsmittel und Harnstoffpulver in ambulanten Patienten mit chronischem SIAD.

METHODEN Manuskript 1 ist eine Sekundäranalyse einer randomisierten, doppelblinden, Placebo-kontrollierten, Crossover-Studie, welche die Auswirkungen eines Natriumanstiegs auf Knochenmarker im Serum untersucht. Manuskript 2 ist eine retrospektive Querschnittstudie, welche die Prävalenz von Hyponatriämie bei Spitaleintritt zwischen Typ 2 Diabetikern mit und ohne SGLT2-Hemmer vergleicht. Manuskript 3 ist eine interventionelle kontrollierte Proof-of-Concept-Studie, welche die Auswirkungen einer Proteinsupplementierung (90g/d über 7 Tage) auf den Natriumspiegel von ambulanten Patienten mit chronischem SIAD untersucht und vergleicht mit der Einnahme von Harnstoffpulver (30g/d über 7 Tage).

ERGEBNISSE Bei ambulanten Patienten mit einer chronischen SIAD-bedingten Hyponatriämie war ein Natriumanstieg assoziiert mit einer Zunahme der Knochenbildung durch eine erhöhte Aktivität der Osteoblasten. Dieser Effekt war unabhängig von der Behandlung mit Empagliflozin. Trotz ihres Effektes bei einem SIAD waren SGLT2-Hemmer nicht mit einer reduzierten Prävalenz von Hyponatriämie in Typ 2 Diabetikern assoziiert. Eine einwöchige proteinreiche Supplementierung erhöhte den Natriumspiegel in Patienten mit einem chronischen SIAD durch proteinbedingte Produktion von Harnstoff. Der Effekt wurde ohne zusätzliche Flüssigkeitsrestriktion erzielt und war mit jenem einer einwöchigen Harnstoffeinnahme vergleichbar.

ZUSAMMENFASSUNG Ein Natriumanstieg bei chronischem SIAD stimuliert die Osteoblasten, die Wirkung auf die Knochendichte muss allerdings noch untersucht werden. Eine Erhöhung des Plasmanatriums bei einem chronischen SIAD erfolgt durch osmotische Diurese, entweder glucoseabhängig (erzeugt durch einen SGLT2-Hemmer) oder harnstoffvermittelt (wahlweise erzeugt durch Proteinsupplementierung oder Harnstoffgabe). Die chronische Behandlung von Typ 2 Diabetikern mit SGLT2-Hemmern beugt dem Auftreten einer Hyponatriämie bei Spitaleintritt jedoch nicht vor. Ob eine langfristige proteinreiche Diät effektiv ist, und ob SGLT2-Hemmer für andere Ursachen der Hyponatriämie als das SIAD wirksam sind, sollte weiter erforscht werden.

5 RÉSUMÉ DU PROJET

CONTEXTE L'hyponatrémie est le trouble électrolytique le plus fréquent et est associé à des taux accrus de mortalité et de morbidité, notamment un risque augmenté d'ostéoporose. Les études précliniques suggèrent que l'hyponatrémie active les ostéoclastes alors que les études cliniques suggèrent plutôt une inhibition des ostéoblastes, c'est pourquoi l'effet de la correction de l'hyponatrémie sur le métabolisme osseux doit être étudié d'avantage. La cause la plus fréquente de l'hyponatrémie euvolémique et l'étiologie principalement adressée dans cette thèse, est le syndrome de l'antidiurèse inappropriée (SIAD). Les différentes options thérapeutiques ont pour cible l'excès pathologique d'eau libre et incluent la restriction hydrique et/ou l'augmentation de l'excrétion d'eau par les reins, soit avec des inhibiteurs des récepteurs de l'arginine vasopressine ou par une diurèse osmotique. Cette dernière peut être induite avec des inhibiteurs du sodium/glucose cotransporteur 2 (SGLT2) induisant une glucosurie, ou de la poudre d'urée. Notre groupe a montré dans 2 essais à double insu contrôlés par placebo que les inhibiteurs du SGLT2 augmentent les taux de sodium les patients avec un SIAD. Cependant, l'effet de leur utilisation chronique sur la natrémie lors d'admissions hospitalières était inconnu. Des études chez l'animal suggèrent que l'effet de l'urée pourrait être atteint avec de larges quantités de protéines alimentaires, mais aucune donnée chez l'humain n'était disponible.

OBJECTIFS Ce MD-PhD avait 3 objectifs; le premier, d'investiguer l'effet de la correction de l'hyponatrémie avec un inhibiteur du SGLT2 sur le métabolisme osseux; le second, d'investiguer le potentiel des inhibiteurs du SGLT2 à prévenir l'hyponatrémie lors d'admission hospitalière de patients avec un diabète de type 2; et le troisième, d'investiguer le potentiel thérapeutique d'une supplémentation riche en protéine chez des patients ambulatoires avec un SIAD chronique, comparé à de la poudre d'urée.

METHODES Le manuscrit 1 est une analyse secondaire préplanifiée d'une étude croisée, randomisée, à double insu, contrôlée par placebo, examinant l'effet d'une augmentation de la natrémie sur les marqueurs sériques osseux de patients avec un SIAD chronique. Le manuscrit 2 est une étude rétrospective transversale ayant but de comparer la prévalence de l'hyponatrémie lors d'admission hospitalière des diabétiques de type 2 recevant un inhibiteur du SGLT2 avec celle des diabétiques de type 2 sans ces médicaments. Le manuscrit 3 est une étude interventionnelle contrôlée de validation de concept examinant l'effet de 90 g de supplémentation protéinée par jour sous forme de poudre de protéine durant 7 jours, comparés à 30 g de poudre d'urée par jour durant 7 jours, sur les taux de sodium sanguin de patients ambulatoires avec un SIAD chronique.

RESULTAT Une augmentation du sodium sanguin chez des patients ambulatoires avec un SIAD chronique, même léger, a conduit à une augmentation de la formation osseuse, par l'augmentation d'un marqueur des ostéoblastes. Cela indépendamment du traitement d'empagliflozine. Malgré leurs effets sur le SIAD déclaré, les inhibiteurs du SGLT2 ne semblent pas réduire la prévalence de l'hyponatrémie lors d'admissions hospitalières de patients avec un diabéte de type 2. Une semaine de supplémentation riche en protéine a augmenté le taux de sodium sanguin chez des patients atteints de SIAD chronique en augmentant la production d'urée. Ces effets ont été atteints sans restriction hydrique additionnelle et sont comparables aux effets obtenus après une semaine de prise de poudre d'urée.

CONCLUSION L'augmentation du sodium chez les patients avec un SIAD chronique stimule les ostéoblastes mais son effet sur la densité osseuse reste inconnu et doit encore être investigué. Le sodium sanguin de patients avec un SIAD peut être augmenté grâce à une diurèse

osmotique, soit médiée par le glucose en utilisant un inhibiteur du SGLT2, soit médiée par de l'urée en utilisant une supplémentation riche en protéine ou de la poudre d'urée. Cependant, un traitement chronique du diabète de type 2 avec un inhibiteur du SLGT2 n'empêche pas le développement d'une l'hyponatrémie lors d'une admission hospitalière. L'effet d'un régime riche en protéine sur le long terme pour le SIAD ainsi que l'effet des inhibiteurs du SGLT2 pour des causes d''hyponatrémie autres que le SIAD doivent être examinés dans des études futures.

6 ABBREVIATIONS

AIC	Akaike Information Criterion
ACE	Angiotensin Converting Enzyme
ACTH	AdrenoCorticoTropic Hormone
ALAT	ALanine AminoTransferase
ARB	Angiotensin II Receptor Blocker
ARNI	Angiotensin Receptor/Neprilysin Inhibitor
ASAT	ASpartate AminoTransferase
AVP	Arginine VasoPressin
AVP2R	Arginine VasoPressin Receptor 2
AQP2	AQuaPorin 2
ATT	Average Treatment effect in the Treated
BMI	Body Mass Index
BPM	Beat Per Minute
CI	Confidence Interval
CNS	Central Nervous System
COVID	COronaVIrus Disease
CSW	Cerebral Salt Wasting
CTX	C-Terminal cross-linking telopeptide
DDP4	DipeptiDyl Peptidase-4
EABV	Effective Arterial Blood Volume
eCDF	empirical Cumulative Distribution Function
ECF	ExtraCellular Fluid
EFWC	Electrolyte Free Water Clearance
EKNZ	EthikKommission Nordwest- und Zentralschweiz
EP	Prostaglandin E_2 receptor
FDA	american Food and Drug Administration
FE	Fractional Excretion
FR	Fluid Restriction
FWC	Free Water Clearance
(e)GFR	estimated Glomerular Filtration Rate
GLP	Glucagon-Like Peptide
HFpEF	Heart Failure with preserved Ejection Fraction
HFrEF	Heart Failure with reduced Ejection Fraction
ICD	International statistical Classification of Diseases and related health problems
ICF	IntraCellular Fluid
ICT	Information and Communication Technologies
ICU	Intensive Care Unit
IL	InterLeukin
ISE	Ion Selective Electrode
IQR	InterQuartile Range
MELD	Model for End-stage Liver Disease
NCC	Thiazide-sensitive NaCl Cotransporter
NCT	National Clinical Trial
ODS	Osmotic Demyelination Syndrome
P1NP	Procollagen type 1 N-terminal Propeptide
PGE2	Prostaglandin E2
PGT	Prostaglandin Transporter
PV	Predictive Value

RAAS	Renin Angiotensin Aldosterone System
RCT	Randomized Controlled Trial
SAH	SubArachnoid Hemorrhage
SIAD	Syndrome of Inappropriate AntiDiuresis
SID	Strong Ions Difference
SGLT2	Sodium/GLucose co-Transporter 2
SMD	Standardized Mean Difference
SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SON	SupraOptic Nucleus
SSRI	Selective Serotonin Reuptake Inhibitors.
T2DM	Type 2 Diabetes Mellitus
TAH	Thiazide Associated Hyponatremia
TUG	Timed Up and Go test
USA	United States of America
UT	Urea transporter
UTI	Urinary Tract Infection
VAS	Visual Analog Scale

7 STATE OF THE ART

Hyponatremia, defined as a plasma sodium concentration below 135 mmol/l, is the most common electrolyte imbalance in clinical practice[1]. Hyponatremia is not a disease per se but the expression of a disturbed salt and water homeostasis (*Sections 7.1* and *7.2*) that can result from a variety of aetiologies, frequently combined, making its diagnosis and management a medical challenge (*Section 7.4*). Hyponatremia is thus a very heterogenous disorder[2], usually classified based on its duration, its biochemical characteristics, its symptoms severity and volume status[3]. Acute hyponatremia can be severely symptomatic and is considered as a medical emergency, whereas manifestations of chronic hyponatremia are more subtle and include gait instability[4-6], falls[4], osteoporosis[7-9], fractures[8,9] and cognitive impairment[4,5] (*Section 7.3*). In addition, hyponatremia is recognized as a marker of poor prognosis in multiple diseases, but a causative role has not been clearly demonstrated to date[10-18]. Importantly, there is increasing evidence that correcting hyponatremia could improve clinical outcome[4,6,19-29]. Treatment of hypovolemic hyponatremia and acute severely symptomatic hyponatremia is well established. By contrast, most patients with chronic euvolemic or hypervolemic hyponatremia leave the hospital still hyponatremic[30], therefore further research is warranted to ameliorate patients management (*Section 7.5*).

7.1 PHYSIOLOGY OF SODIUM AND WATER HOMEOSTASIS

Sodium plays an important role in vital homeostatic requirements such as cell volume, organ perfusion and neurotransmission. Its homeostasis is extremely complex and still incompletely understood[31]. It is present in large amount in plasma, bones, skin, muscles and brain[32,33]. Two percent of sodium is in the intracellular fluid (ICF) and 98% is in the extracellular fluid (ECF), distributed among the fluid, the collagen matrix and the glycosaminoglycan gel of the triphasic interstitium[31]. In addition, sodium is the most common cation in the blood and the main determinant of plasma osmolality[34].

Equation 1: Plasma osmolality

$$P_{Osm} = 2\,x\,P_{Na} + P_{Urea} + P_{Glucose}$$

Water is mainly ingested orally and to a lesser extent generated by metabolic reactions[31]. Water can cross the cell membrane and its distribution across compartments is driven by osmotic active solutes (so-called effective “osmoles”) that create *tonicity* (= effective osmolality) of plasma and other compartments, which again is mainly driven by sodium concentration under physiological conditions. Plasma urea does contribute to osmolality but not to tonicity because of its nearly free transcellular movement.

Equation 2: Plasma tonicity

$$P_{Ton} = 2\,x\,P_{Na} + P_{Glucose}$$

Plasma and ECF tonicities are equal and any deviation is restored by the free water permeability of the cell membrane[35].

Sodium sequestration in certain compartments and sodium and water renal handling aim to keep plasma tonicity and organ perfusion constant despite high variations in dietary salt and water intake. Natriuretic peptides, the renin angiotensin aldosterone system (RAAS), the pituitary neuropeptide arginine vasopressin (AVP) and the kidneys as their target organs, are thereby key parts of the regulating machinery. ECF volume variation is mainly corrected by adapting renal sodium handling whereas deviations of osmolality are mainly restored by adjusting renal water handling and thirst[35]. Already minimal variations in osmolality (1-2%) are sensed by central osmoreceptors in the organum vasculosum of the lamina terminalis[36]. These neurons are free from the blood-brain barrier and mechanically activated by cell shrinkage when the surrounding plasma tonicity increases[36]. They activate thirst centers and magnocellular neurons of the supraoptic and paraventricular hypothalamic nuclei that project their axons to the neurohypophysis[36]. AVP is synthesized by these neurons as a preprohormone and is then released by the neurohypophysis together with its cleaved byproducts neurophysin II and copeptin[36]. In contrast, a decrease in plasma osmolality suppresses AVP secretion[35]. AVP endorses its role of antidiuretic hormone by activating its renal V2 receptor (AVP2R) and thereby increasing the expression of water channels aquaporin 2 (AQP2) in the principal cells of the connecting tubules and collecting ducts[36]. Changes in effective arterial blood volume (EABV) and osmolality regulation are partially intertwined, because the body should be able to prioritize volume preservation over tonicity[37]. Decrease in EABV lowers the osmotic threshold for AVP secretion, on the one hand via baroreceptors activation in the left atrium, aorta and carotid sinus[38],on the other hand via angiotensin II which has been shown to increase osmosensitivity of magnocellular neurosecretory cells of supraoptic neurons in vitro[39].

7.2 PATHOPHYSIOLOGY OF HYPONATREMIA

Determinants of the plasma sodium concentration are summarized by the Edelman's equation[40], which highlights the deciding influence of body water on plasma sodium concentration.

Equation 3: Determinants of plasma sodium concentration (Edelman's equation)[40]

$$P_{Na} = \frac{exchangeable\ Na^+ \ + exchangeable\ \ K^+}{Total\ body\ water}$$

Hyponatremia hence predominantly results from a relative total body water excess that cannot be excreted, rather than from a sodium depletion. Water excretion by the kidneys represents the main component of water output and depends on AVP activity, glomerular filtration rate (GFR) and solute intake[36]. Consequently, an excessive water intake or a change in any of these

parameters can cause hyponatremia. Isolated immoderate hydration is very rarely causative alone, because healthy kidneys can bring down urine osmolality to 50-100 mOsm/l and thus excrete up to 18l of maximal diluted urine per day upon a normal dietary solute intake[36]. If water intake exceeds the maximal excretable urine volume determined by solute intake, as seen in the "tea and toast" syndrome and beer potomania, hyponatremia develops[41]. Way more commonly, renal free water excretion is impaired, either due to reduced GFR, decreased dietary solute intake or most frequently increased AVP secretion or sensitivity[34,36]. Non-osmotic increased AVP activity can either be triggered by a cortisol deficiency, a low EABV or being inappropriate both in regards to plasma osmolality and hemodynamics as seen in the syndrome of inappropriate antidiuresis (SIAD)[35,36].

As mentioned in the previous section, reduced plasma sodium concentration often implies reduced tonicity. Acute changes in plasma tonicity induce osmotic water flow from the ECF to the ICF and subsequent cell swelling that can have serious consequences, especially for brain cells whose expansion is impeded by the skull rigidity. Intracranial pressure would rise and lead to the clinical manifestation of acute severe hyponatremia, i.e., headache, nausea, vomiting, seizure, obtundation, coma and in case of brain herniation, cardiac arrest[3]. In contrast, if sodium and tonicity slowly drop over at least 48 hours, cell volume can be maintained constant thanks to the allostatic extrusion of intracellular ions and organic osmolytes[42]. It has been hypothesized that the following adaptations contribute to the burdens associated with chronic hyponatremia (*Section 7.3*)[42]. First, brain cells put in a hyponatremic environment extrude the main excitatory neurotransmitter glutamate[42,43]. Imbalance in the glutamatergic system is associated with cognitive disorders and impaired neuronal plasticity[44]. Second, cardiomyocytes extrude taurine to preserve atria and ventricle filling[42,45] and animal studies report cardiomyopathy upon taurine deficiency[42,46,47]. Finally independently of cell volume variation, low sodium levels activate osteoclasts[48] and make human bone marrow-derived mesenchymal stromal cells more prone to differentiate to adipocytes rather than to osteoblasts[49].

7.3 Burdens and Clinical Manifestations of Chronic Hyponatremia

7.3.1 Epidemiology and Significance for Public Health

Computing the exact hyponatremia prevalence is hampered by the diverging design and inconsequential cut-offs in the available studies[1]. Nevertheless, hyponatremia is considered as the most common electrolyte disturbance encountered in clinical practice with an estimated prevalence of 5% in the community[50,51], 5-15% on hospital admission[52-54] and 20-40% during hospitalization[53,55-57]. Importantly, hyponatremia only rarely appears in the diagnoses list[57], which captures the lack of awareness among physicians. Hyponatremia is associated with an increased

risk for rehospitalization[58-61], longer hospital stays[60-64], transfer to the intensive care unit (ICU)[54,61], discharge to care facilities[60,65] and therefore not surprisingly with supplemental costs[61,66].

7.3.2 Clinical Significance of Hyponatremia and Its Correction

Hyponatremia is associated with increased mortality in a broad spectrum of diseases[67], in hospitalized patients[59,67], in primary care setting[68] and in the general population[69,70]. Uncertainty remains regarding the exact relevance of hyponatremia severity[58] and etiology[60,71-73]. Systematic reviews and metanalyses suggest a reduction of all-cause mortality through hyponatremia improvement[21,74]. However, based on current evidence it remains unclear, whether hyponatremia is a marker of a more progressive disease, or whether it also causally contributes to increased mortality. A large ongoing RCT should soon shed light on this matter[75].

On account of the brain adaptation in chronic hyponatremia (*Section 7.2*) and the accumulating clinical evidence, it seems coherent that chronic hyponatremia impacts cognitive functions. For instance, a Belgian study investigated changes in reaction time in 8 hyponatremic patients with chronic SIAD before and after reaching normonatremia, and in 8 treated normonatremic SIAD patients before and after treatment discontinuation that led to hyponatremia recurrence[4]. Response latency was increased by 74 milliseconds when patients were hyponatremic, independently of whether hyponatremia occurred first[4]. In comparison, this latency was increased by 25 milliseconds in normonatremic controls in whom blood alcohol concentration was raised to 0.6 g/l[4].

Chronic hyponatremia is associated with an increased risk for falling in the community[76], on hospital admission[4,77,78] and during hospitalization[79-81]. A population-based prospective study found that a drop in sodium from 135 mmol/l to 130 mmol/l increased fall risk to the same extent as aging from 60 to 73 years old[76]. Furthermore, the Belgian study mentioned in the previous paragraph found that patients with chronic hyponatremia displayed more variability in center of gravity when hyponatremic while walking in tandem on a platform[4]. While stabilization was observed after hyponatremia correction in these studies[4,5], other works could not demonstrate an effect of sodium improvement on gait or balance[26,29,82].

Even more unfavourably, due to their propensity for falling, hyponatremic patients are at increased risk for osteoporosis and fractures[83]. Bones are rich in sodium with 234 mmol per kilogram bone, of which 15-45% is exchangeable[32]. Therefore, it is evolutionarily plausible that hyponatremia leads to bone destruction in order to mobilize sodium[42]. As mentioned in *Section 7.2*, an hyponatremic environment activates osteoclasts and impairs osteoblasts differentiation[49]. Furthermore, histological bone loss[7,84] and reduced bone sodium content[84] could be demonstrated in rats made hyponatremic. In humans, acute hyponatremia development reduced[85] osteoblasts activity and correcting hyponatremia in hospitalized patients increased[86]

osteoblasts activity. One randomized double-blind, placebo-controlled study in outpatients with chronic euvolemic and hypervolemic hyponatremia investigated the effect of a 3-week treatment with tolvaptan and observed a reduction in bone resorption resulting in a trend towards osteoblasts activation and osteoclasts inhibition[20]. Overall, there is a need to better characterize the effects of hyponatremia correction on bone metabolism, which was the objective of Manuscript 1.

In view of the accumulating evidence, hyponatremia should not be considered as clinically silent. However, the heterogeneity conferred by study designs and hyponatremia itself hamper the distinction between direct causative implication and confounding by indication[87]. In addition, the sodium levels or the correction magnitude that have to be strived for, are still debated.

7.4 DIAGNOSTIC AND ETIOLOGIES OF HYPONATREMIA – FOCUS ON SIAD

Hyponatremia is indicative of a disrupted water homeostasis and can occur secondary to a broad spectrum of diseases (e.g., heart failure, liver insufficiency, central nervous system disorders)[1], medications (e.g., diuretics, anticonvulsants)[88], or in stress situation such as in the postoperative period[89] or after extreme sport performances[90]. Combined aetiologies are more the rule than the exception making difficult both the diagnosis and clinical management of hyponatremia.

The diagnostic pathway starts with measuring plasma osmolality[3]. Hypertonic or isotonic hyponatremia can be caused by an excess in effective osmoles such as glucose or mannitol (i.e., translocation hyponatremia), or by an excess in ineffective osmoles (e.g., urea) that conceals hypotonic hyponatremia[3]. An isotonic hyponatremia can also result from an analytic pitfall caused by an excess in plasma proteins (e.g., monoclonal gammopathies, intravenous immunoglobulins) or in plasma lipids. After having been measured in plasma, osmolality should be measured in urine (U_{Osm}) as it mirrors AVP activity. $U_{Osm} \leq 100$ mOsm/kg is indicative of suppressed AVP levels and primary polydispia, low solute intake or beer potomania[3]. $U_{Osm} > 100$ mOsm/kg signalizes AVP activity and needs to be further differentiated by measuring urine sodium (U_{Na}), which mirrors EABV-dependent RAAS and natriuretic peptides activity[3]. $U_{Na} \leq 30$ mmol/l indicate either true hypovolemia (e.g., diarrhea, third spacing) or low EABV (e.g., heart failure, liver cirrhosis)[3]. $U_{Na} > 30$ mmol/l suggests SIAD or secondary adrenal insufficiency in euvolemic patients and primary adrenal insufficiency, renal/cerebral salt wasting or vomiting in hypovolemic patients[3]. In patients taking diuretics, urine sodium is of little utility, therefore the fractional uric acid excretion should be prefered[91]. As the last step, hyponatremia etiologies are classified according to volemic status, while keeping in mind the dubious accuracy of the clinical assessment of volemia[92], especially when differentiating euvolemia from modest hypovolemia.

7.4.1 The Syndrome of Inappropriate Antidiuresis (SIAD)

The most common etiology of euvolemic hyponatremia is the syndrome of inappropriate antidiuresis (SIAD) which is also the main etiology of hyponatremia overall[71,93-95]. It has first been described in 1957 by Schwartz and Bartter in two patients with bronchogenic carcinoma[96]. SIAD is characterized by an imbalanced AVP activity in regard to both osmolality and hemodynamics[97]. The impaired AVP regulation leads to free water retention, thereupon to extracellular volume expansion and a subsequent renal sodium loss resulting in hypotonic hyponatremia[98]. Patients with SIAD are euvolemic despite water retention, because ECF volume is determined by both water and sodium absolute content, the latter being restored because of the secondary natriuresis[35,95].

Different patterns of AVP secretion have been defined first by Robertson et al.[98] (types A, B, C and D) through measurement of AVP levels, and later by Fenske et al.[99] through measurement of the surrogate stochiometric marker of AVP copeptin, which lead to the discovery of a fifth pattern (type E). In type A, AVP secretion is totally erratic but constantly over the normal range [98,99]. In type B, the osmotic threshold for AVP to increase is lowered (“reset osmostat”) despite normal effective arterial volume[98,99]. In type C, there is a resting secretion of AVP at any range of hypoosmolality, although it increases normally when osmolality increases[98,99]. In type D, AVP secretion in response to hypertonic saline is completely normal, so that antidiuresis might rely on an increased renal sensitivity or on the ectopic production of a paraneoplastic AVP-like peptide[98,99]. Accordingly, a x-linked hemizygous gain-of-function mutation and constitutive activation of AVP2R leads to a so-called nephrogenic SIAD[100,101]. In type E, there is an inverse linear relationship between copeptin and serum osmolality[99].

SIAD may occur secondary to malignancies, pulmonary diseases, central nervous system processes (e.g. tumors, hemorrhages, infections), stress, pain, nausea or the intake or certain drugs[94,97]. Culprit drugs can be AVP analogs (dDAVP, oxytocin) that cross-react with AVP2R, drugs that increase central AVP release (e.g., vincristine, ifosfamide) and drugs that stimulate AVP2R (e.g., SSRI or carbamazepine)[102].

SIAD is a diagnostic of exclusion that can only be made after excluding hypothyroidism and adrenal insufficiency[103]. A lower plasma uric acid concentration[104] and a higher fractional uric acid excretion[91] are suggestive for SIAD. Cerebral salt wasting (CSW) is a contentious differential diagnosis in patients with intracranial pathology[105,106], in whom only clinical signs of hypovolemia are helpful, although some authors suggest a persistent high fractional uric acid excretion and a reduction of urine osmolality after saline infusion to be rather suggestive of CSW[107].

7.5 TREATMENT PRINCIPLES OF HYPONATREMIA – FOCUS ON SIAD

Hyponatremia treatment should evidently increase sodium levels effectively, however at a reasonable pace if hyponatremia is chronic. Brain cells need 48h to adapt to a hypotonic environment (*Section 7.2*) and also need several days to adapt to the restoration of a normal surrounding tonicity. The European clinical practice guidelines on diagnosis and treatment of hyponatremia (further referred to as "European guidelines") recommend a correction limit of 10 mmol/l in the first 24h and of 18 mmol/l in the first 48h[3]. The American expert panel for the diagnosis, evaluation, and treatment of hyponatremia (further referred to as "American guidelines") recommend a correction limit of 10-12 mmol/l over 24h and of 18 mmol/l over 48h[108]. A faster rise in sodium concentration can cause an osmotic demyelination syndrome (ODS), that is characterized by central myelin disruption and oligodendrocytes loss and can manifest with pseudobulbar palsy, tetraparesis and even locked-in syndrome[109].

Acute symptomatic hyponatremia requires intravenous boli of hypertonic saline regardless of hyponatremia etiology[110,111]. In contrast, treatments of chronic hyponatremia are tailored to the underlying etiologies. Whenever possible, the latter should be directly addressed and resolved, for instance hypovolemic hyponatremia can be treated successfully with volume repletion, adrenal insufficiency with glucocorticoids and hypothyroidism with thyroxine. Similarly, culprit drugs such as in thiazide-induced hyponatremia or in drug-induced SIAD should be discontinued. In case underlying diseases are chronic or accountable treatments cannot be abated, therapeutic measures should rather target the relative water excess than the relative lack of sodium. This can either be achieved by restricting fluid intake (*Section 7.5.1*) and/or by increasing renal electrolyte free water clearance (EFWC).

EFWC (*Figure 1C, Equation 5*), is more appropriate than free water clearance (FWC) (*Figure 1B, Equation 4*), in quantifying water loss contributing to plasma sodium rises, because potassium and sodium (and not urea), are determinants of plasma sodium concentration together with total body water[40,112].

Equation 4: Conventional solute free water clearance (FWC) calculation[113] (*Figure 1B*):

$$FWC = U_{Volume}(1 - \frac{U_{osm}}{P_{osm}}) = U_{Volume}(1 - \frac{2\ x\ U_{Na} + 2\ x\ U_K + U_{Glucose} + U_{Urea}}{2\ x\ P_{Na} + P_{Urea} + P_{Glucose}})$$

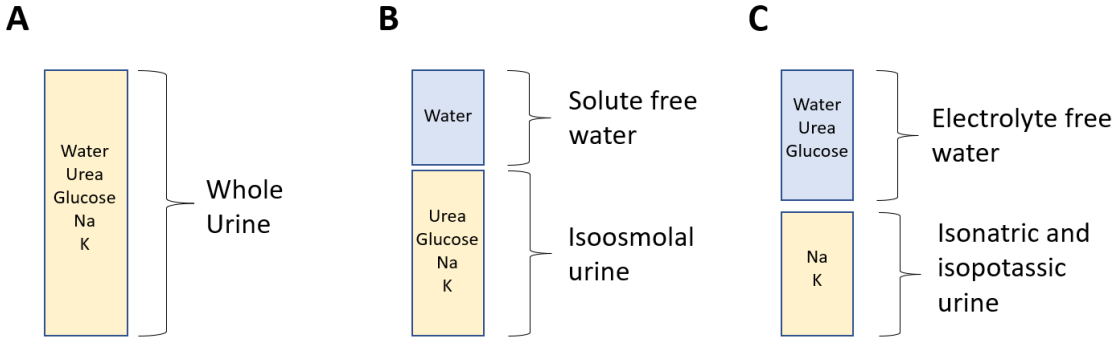
Equation 5: Conventional electrolyte free water clearance EFWC calculation[114] (*Figure 1C*):

$$EFWC = U_{Volume}\left(1 - \frac{U_{Na} + U_K}{P_{Na}}\right)$$

EFWC represents the urine volume free of sodium and potassium that should be virtually added/removed to make urine isonatric and isopotassic in regards to plasma. A positive EFWC indicates a renal loss of electrolyte free water resulting in an increase in plasma sodium, whereas

a negative EFWC indicates a renal retention of electrolyte free water resulting in a decrease in plasma sodium.

Figure 1: Gamblegrams of water and electrolytes contents in urine for visual theoretical representation of solute free water clearance (B) and electrolyte free water clearance (C) as compared to a simplified urine composition (A).



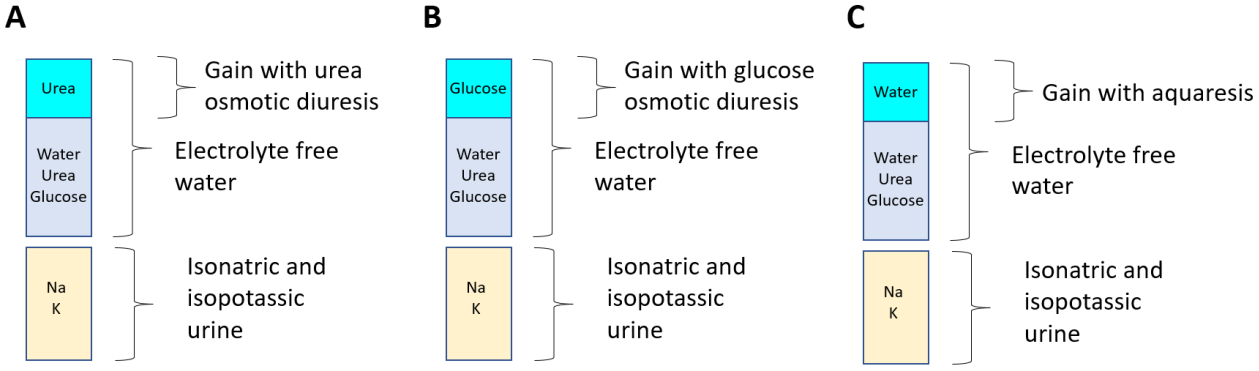
Twenty-four hours urine collection is required to compute exact EFWC which is not easily practicable in clinical practice. Nonetheless, EFWC can be approximated by calculating the urine/plasma electrolyte ratio drawn from a spot urine sample (*Equation 6*). A urine/plasma electrolyte ratio < 1 reflects positive EFWC and indicates a renal loss of electrolyte free water resulting in an increase in plasma sodium[115]. A urine/plasma electrolyte ratio > 1 reflects negative EFWC and indicates a renal gain of electrolyte free water resulting in a decrease in plasma sodium[115].

Equation 6: Urine/plasma electrolyte ratio[115]:

$$\left(\frac{U}{P}\right) Ratio = \frac{U_{Na} + U_K}{P_{Na}}$$

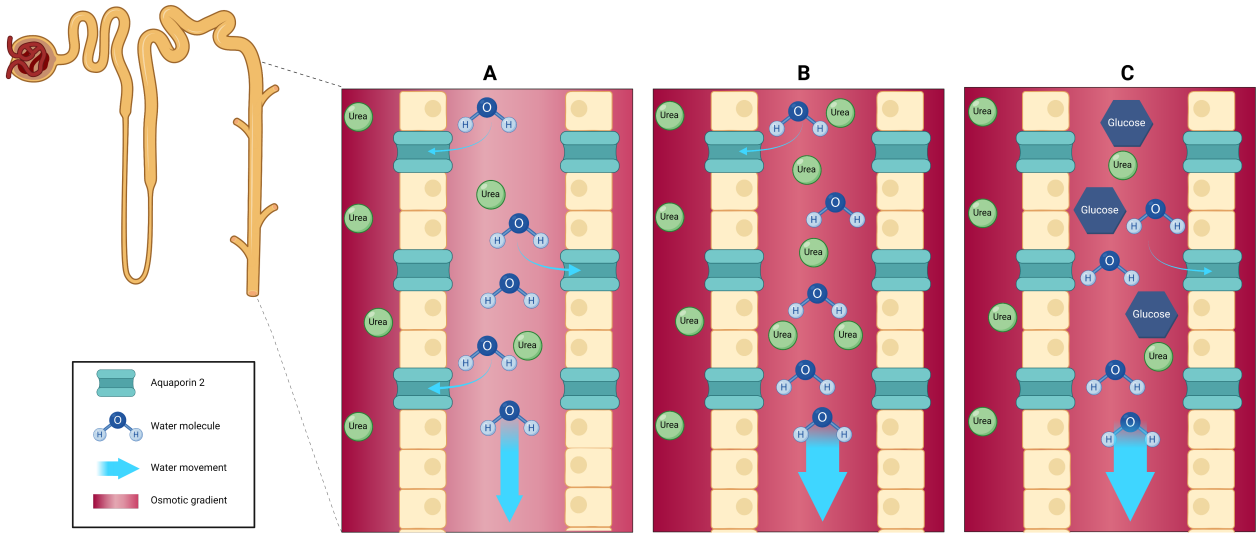
EFWC can be increased by either inducing aquaresis (*Figure 2C, Section 7.5.3* for tolvaptan) or osmotic diuresis with solute other than sodium and potassium (*Figure 2B, Section 7.5.4* for SGLT2 inhibitors and *Figure 2A*, *Section 7.5.5* for urea).

Figure 2: Gamblegrams of water and electrolytes contents in urine for visual theoretical representation of solute free water clearance (B) and electrolyte free water clearance (C) as compared to a simplified urine composition (A).



When AVP activity is higher, urine osmolality is fixed at a higher level so that the urine volume of patients is more dependent of the osmolar excretion rate than healthy patients. Administering solute reduces the osmotic gradient between the tubular fluid and the medullary interstitium, thus reducing water reabsorption[116] (*Figure 3*).

Figure 3: Simplified representation of solute osmotic diuresis. Osmotic gradient and water reabsorption can be reduced as compared to physiological state (A) by increasing tubular urea (B) or glucose (C) concentration. Original Figure created with BioRender.



7.5.1 Fluid restriction

Fluid restriction is the first line treatment of chronic non severe SIAD[30,103]. A recent randomized controlled trial (RCT) in 46 outpatient with chronic SIAD showed a plasma sodium increase by 3 mmol/l after 3 days and 4 mmol/l after 30 days (versus 1 mmol without treatment)[117]. However, its efficacy might be lower in real-life setting[30] and insufficient in patients with urine osmolality >500mOsm/kg[118], urine sodium >130 mmol/l[118] or a urine/plasma electrolyte ratio >1[115]. Furthermore, restricting fluid administration might not be possible in patients with malignant SIAD during chemotherapy cycles as large intravenous fluid volumes are required in certain treatment protocols. Fluid restriction has a rather favorable safety profile[117] although acute kidney injury and hypotension was observed in 10% of the patients treated with fluid restriction in one trial[119].

7.5.2 Loop Diuretics and Sodium Chloride Tablets

The European Guidelines recommend low-dose loop diuretics combined with sodium chloride tablets as a second-line treatment for patients with moderate to profound SIAD[3], whereas the American Guidelines reserve loop diuretics for hypervolemic hyponatremia and mention salt tablets as an option in CSW[108]. A retrospective study in patients with euvolemic hyponatremia suggest that a 48-h treatment with sodium chloride tablets leads to a higher increase in sodium as compared to a control group (5.2 mmol/l versus 3.1 mmol/l, $P < 0.001$)[120] and a case series of

2 patients suggest its efficacy in fluid restriction refractory SIAD[121]. However, an open-label RCT showed that a 28-day treatment with fluid restriction alone was as effective in increasing sodium levels as its combination with loop diuretics or with loop diuretics and salt tablets[119]. Furthermore, hypokalemia and acute kidney injury were more common in patients receiving furosemide.

7.5.3 Vaptans

Vaptans are AVP2R antagonists that promote aquaresis (*Figure 2C*) by reducing AQ2 expression. Tolvaptan is an oral agent available in Europe and the United States of America (USA)[103]. The randomized double-blind placebo-controlled SALT1 and SALT2 trials demonstrated the efficacy of tolvaptan in 448 patients with euvolemic hyponatremia due to SIAD or hypervolemic hyponatremia due to heart failure and liver cirrhosis[19]. An open-label extension of this trial in 111 patients over about 2 years suggested long-term efficacy and safety[122], although recent reports described patients with secondary tolvaptan resistance[123,124]. These pivotal trials led to the authorization of tolvaptan for SIAD treatment in Europe in 2009, and for both euvolemic and hypervolemic hyponatremia in the USA in 2008[125]. However, the authorization for liver cirrhosis was withdrawn by the Food and Drug Administration (FDA) in 2013 after severe liver toxicity has been linked to higher tolvaptan dosage in patients with autosomal dominant polycystic kidney disease[126]. The American Guidelines place tolvaptan as a second line treatment for profound or fluid restriction refractory symptomatic hyponatremia due to SIAD or heart failure[108], but European guidelines discourage the use of tolvaptan in SIAD[3]. The main concern is that tolvaptan needs to be titrated in inpatient setting due to the risk of overly rapid sodium correction[127],especially in patients with profound hyponatremia[128]. Caution is also warranted in patients receiving CYP3A4 inhibitors or inductors due to interaction potential. Furthermore, tolvaptan costs about 100 Swiss francs per day (which currently roughly equates to 100 euros and 100 American dollars) which often refrains physicians from its use. Economic analyses suggest cost-effectiveness of tolvaptan compared with fluid restriction in hospitalized patients[129,130], whether it is applicable to outpatients who should be hospitalized for titration is questionable.

7.5.4 SGLT2 inhibitors

Glucose is freely filtered by the glomerulus and to 99% actively reabsorbed in the proximal tubules, mainly by the luminal sodium/glucose cotransporter 2 (SGLT2) and to a lesser extent by SGLT1[131]. The renal threshold for glucose reabsorption is a plasma glucose concentration of > 10mmol/l, meaning that hyperglycemia over this level outreaches the SGLT2/1 reabsorption capacity and leads to glucosuria[131]. SGLT2 inhibitors have been developed as oral antidiabetic drugs. In Switzerland, 4 different SGLT2 inhibitors are authorized for the treatment of type 2 diabetes (T2DM), empagliflozin, canagliflozin, dapagliflozin and ertugliflozin, both as single substances and combined preparations. SGLT2 inhibition with empagliflozin results in renal

excretion of glucose, namely up to 90 g a day depending on dosage and underlying glucose tolerance[132,133]. SGLT2 inhibitors do not only optimize glycemic control but also slow chronic kidney disease progression and offer cardiovascular protection in patients with[134,135] and without[134,136,137] T2DM. Due to their glucosuric effect, SGLT2 inhibitors increase the risk for genital infections by 3 to 5 fold, whereas only dapagliflozin seems to also increase risk for urinary tract infections (UTIs)[138]. Risks for genitourinary infections is especially high in patients with a history of such complaints, while new onsets are more seldom[139]. Rare but life threatening side effects include euglycemic diabetic ketoacidosis in T2DM[140] and possibly Fournier's gangrene[141].

The increased tubular glucose concentration reduces the osmotic gradient with the renal medulla and promotes electrolyte free water excretion (*Figure 3C*). T2DM-independent benefits observed in heart failure are thought to be at least in part due the effect of SGLT2 inhibitors on salt water balance and to the elicited osmotic decongestion[142]. Some authors speculate that SGLT2 inhibitors better mobilize interstitial fluid and to a lesser extent intravasal fluid as compared to standard diuretics[116], and thus mitigate the compensatory RAAS activation. SGLT2 inhibitors increase urinary volume in healthy volunteers and in patients with acute heart failure[143] and more importantly increase EFWC in diabetic rats[144], healthy volunteers[116] and in patients with T2DM and heart failure[145]. A potential glucosuria-independent aquaretic effect has been hypothesized in rats studies in which empagliflozin increased the expression of AVP2R[144] and urine vasopressin excretion[146], but downregulated AQP2 expression[144].

We hypothesized that AVP-mediated free water reabsorption in SIAD could be balanced with the increase in EFWC from glucose-induced osmotic diuresis. To investigate this, we first performed a proof-of-concept study in which empagliflozin led to a significant increase in short-term urinary volume in 14 healthy volunteers with desmopressin-induced SIAD[147]. We then conducted a double-blind placebo-controlled randomized trial in 87 hospitalized SIAD patients showing that a 4-day treatment with empagliflozin in addition to standard fluid restriction <1000 ml/24h, increased plasma sodium levels in SIAD more efficiently than fluid restriction combined with placebo (10 versus 7 mmol/l, $P = 0.04$)[148]. Finally, we recently investigated the effect of a 4- week treatment with empagliflozin in chronic outpatients with SIAD in a randomized double-blind placebo-controlled cross-over study[149] in which empagliflozin was associated with a sodium increase of 4.1 mmol/l (95%-CI: 1.7 to 6.5; $P = 0.004$). Whether SLGT2 inhibitors might not only improve overt hyponatremia but also prevent its development was not known and has been investigated in Manuscript 2.

7.5.5 Oral Urea

Urea osmotic diuresis and electrolyte free water loss can be induced by administering urea powder. Urea is a non-protein-bonded molecule that is freely filtered by the glomerulus[131]. Half of the filtered urea is reabsorbed passively in the proximal tubules[131]. Urea is one of the main components of the corticopapillary osmotic gradient[131] and is therefore secreted into the thin descending limb and thin ascending limbs of the loop of Henle from the interstitium whose urea concentration is higher than the one of the tubular fluid[155]. The filtrate entering the thick ascending limb of the loop of Henle contains more urea than the primary filtrate and the maximal osmotic effect of urea takes place on the urea-impermeable connecting tubules, cortical collecting tubules and outer medullary collecting tubules[155] (*Figure 3B*). Some urea is reabsorbed in the inner medullary collecting duct by the vasopressin-dependent urea transporter (UT)-A1[155] and the vasopressin-independent UT-A3 and is in part secreted back to the loop of Henle by UT-A2 (urea recycling)[150], so that about half of filtered urea is secreted with the urine[131].

In Europe, oral urea is prepared by pharmacies at the behest of physicians. In the USA, ready-to-use flavored powders and tablets are available to treat euvolemic and hypervolemic hyponatremia, and authorized as medical food by the FDA[151-154]. Its efficacy for treating SIAD has been shown for the first time in 1980[157], and later confirmed in specific populations such as critically ill patients[158,159] and in pediatric patients[101,160]. A prospective study in 12 patients suggests that oral urea is safe, well tolerated and as effective as tolvaptan in treating SIAD over one year[161]. European guidelines recommend 0.25–0.50 g/kg per day of urea in moderate or profound SIAD[3]. A daily dosage of 15-60 g is usually required[103]. Thirty grams of urea provides 500 excretable miliosmoles which corresponds to about 15 one-gram sodium chloride tablets[152]. About 90% of orally ingested urea is absorbed in the intestine and its plasmatic half-life is 2h[155]. Ingested urea is completely excreted after 12h in patients with normal kidney function[156]. However, patient's acceptance is low due to the poor palatability of urea, the absence of ready-to-use preparations in Europe and the uncertain reimbursement by the health insurance[103].

Endogenous proteins and dietary proteins are metabolized into nitrogen which is metabolized to soluble excretable urea by the liver. Ten grams of protein intake approximates 50 mmol of urea[36]. Both a 20% protein diet and a low protein diet combined to oral urea increased sodium concentration, reduced natriuresis and increased inner medullary urea concentration in rats with induced SIAD, compared to the ones fed with a low protein diet[162]. Furthermore, a low-protein diet abolishes the diuretic effect of inhibiting renal urea transporter in rats[163]. Urea osmotic diuresis has been reported to cause hypernatremia catabolic patients[164,165], showing that urea from increased protein turnover can influence sodium levels in humans. This suggests that protein intake could represent a therapeutically relevant source of urea. Manuscript 3 consists of the first controlled trial on the effect of protein supplementation in patients with SIAD.

8 OBJECTIVES OF THE MD-PHD

Manuscript 1: An Increase in Plasma Sodium Levels is Associated With an Increase in Osteoblast Function in Outpatients with Chronic SIAD

- Objective: Characterize the clinical benefits of hyponatremia improvement in investigating the effect of an increase in plasma sodium on bone metabolism in outpatients with chronic SIAD.
- Hypothesis: An increase in plasma sodium is associated with an increase in the osteoblasts markers P1NP and osteocalcin and a decrease in the osteoclasts marker CTX, independently of empagliflozin treatment.

Manuscript 2: Prevalence of Admission Hyponatremia in Diabetic Patients Treated With and Without an SGLT2-Inhibitor - a Cross-Sectional Study

- Objective: Investigate the potential of chronic glucose-induced osmotic diuresis in preventing hyponatremia on admission using SGLT2 inhibitors.
- Hypothesis: Hyponatremia prevalence on hospital admission is lower in T2DM patients treated with an SGLT2 inhibitor as compared to T2DM patients who are not treated with an SGLT2 inhibitor.

Manuscript 3: Effect of Protein Supplementation on Plasma Sodium Levels and Urinary Urea Excretion in Patients with Chronic SIAD – a Monocentric Open-Label Proof-of-Concept Study – the TREASURE Study

- Objective: Investigate the efficacy of urea-induced osmotic diuresis using high-protein supplementation in outpatients with chronic SIAD.
- Hypothesis: Increasing protein intake promotes ureagenesis, “urearesis” and subsequent electrolyte free water loss and increase in plasma sodium levels.

9 MANUSCRIPT 1: AN INCREASE IN PLASMA SODIUM LEVELS IS ASSOCIATED WITH AN INCREASE IN OSTEOBLAST FUNCTION IN CHRONIC SIAD

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9.1 ABSTRACT

INTRODUCTION Hyponatremia is associated with an increased risk for osteoporosis and fragility fractures. Preclinical studies in untreated hyponatremia suggest osteoclast upregulation whereas a clinical study showed improved osteoblast function after hyponatremia normalization in hospitalized patients with a syndrome of inappropriate antidiuresis (SIAD).

METHODS This is a secondary analysis of a randomized double-blind, crossover, placebo-controlled trial investigating the effect of a 4-week treatment with empagliflozin 25mg/day as compared to placebo in outpatients with chronic SIAD (SANDx Trial, NCT03202667). The primary objective was to investigate the relationship between the change in bone formation index (BFI), defined as P1NP/CTX, and the change in plasma sodium levels. Secondary objectives included the relationship between the change in the osteoblasts markers procollagen type 1 N-terminal propeptide (P1NP) and osteocalcin and the osteoclasts marker C-terminal cross-linking telopeptide (CTX), and the change in plasma sodium levels over the treatment periods.

RESULTS Six out of the 11 outpatients with chronic SIAD were female (median [IQR] age 73 years [66, 78]). At baseline, median CTX concentration was 0.47 μg/l [0.29, 0.65], median osteocalcin was 18.00 μg/l [13.95, 25.87] and median P1NP was 64.64 μg/l [45.62, 68.45]. The calculated median BFI was 131.86 [96.21, 168.73]. Changes in sodium were positively correlated with changes in BFI and P1NP (BFI: $\rho = 0.55$, $P < 0.001$; P1NP: $\rho = 0.45$, $P = 0.004$) but not with CTX ($P = 0.184$) and osteocalcin ($P = 0.149$). A sodium increase of 1 mmol/l was associated with an increase of 5.21 in BFI (95%-CI: 1.41, 9.00, $P = 0.013$) and with an increase of 1.48 μg/l in P1NP (95%-CI: 0.26, 2.62, $P = 0.03$). The effect of sodium change on bone markers was independent of the study medication empagliflozin.

CONCLUSION An increase in plasma sodium levels in outpatients with chronic hyponatremia due to SIAD, even when mild, was associated with an increase in bone formation index (P1NP/CTX) mirrored by an increase in P1NP, a surrogate marker of osteoblast function.

9.2 INTRODUCTION

Hyponatremia is the most common electrolyte disorder encountered in clinical practice[1]. Chronic hyponatremia is associated with increased mortality and morbidity[166], including an increased risk for osteoporosis and fractures[9]. Both the risk for osteoporosis and the risk for fragility fractures increase with hyponatremia severity and duration[167]. Bones represent the biggest pool of mobilizable osmotically inactive sodium, thus hyponatremia-mediated bone destruction is evolutionary plausible[42]. This hypothesis is supported by the in-vitro activation of osteoclasts in a hyponatremic environment[48], and by histological bone loss[7,84] and reduced bone sodium content[84] in hyponatremic rats.

In humans, the use of biochemical markers of bone turnover allows non-invasive assessment of bone metabolism. Procollagen type 1 N-terminal propeptide (P1NP), a byproduct of type 1 collagen synthesis, and osteocalcin, a marker of bone mineralization, are indicators of bone formation and osteoblast function, while serum C-terminal cross-linking telopeptide (CTX) is a widely used marker of osteoclast-dependent bone resorption[168].

In two randomized double-blind placebo-controlled trials we have demonstrated that the SGLT2 inhibitor empagliflozin is a promising treatment option in patients with a syndrome of inappropriate antidiuresis (SIAD), first over 4 days in hospitalized patients[148] and more recently over 4 weeks in outpatients with chronic SIAD[29]. In a post-hoc analysis of our 4-day trial in hospitalized patients, P1NP/CTX remained stable and P1NP increased in patients in whom hyponatremia was corrected, whereas P1NP/CTX decreased and P1NP remained stable if hyponatremia persisted[86]. CTX increased in all patients but all of them were hospitalized, so that this might have been attributable to immobilization[169]. To circumvent this limitation, we now performed a predefined secondary analysis of our trial in 14 outpatients with chronic SIAD. We hypothesized that an increase in plasma sodium levels would be associated with an increase in osteoblast markers and the bone formation index (BFI), and with a decrease in osteoclasts markers, independently of empagliflozin treatment.

9.3 METHODS

9.3.1 Study Design and Participants

This is a predefined secondary analysis of a prospective randomized, crossover, double-blind, placebo-controlled trial performed at the University Hospital Basel, Switzerland from 12/2017 to 08/2021. The trial was registered at ClinicalTrials.gov (NCT03202667) and conducted according to the principles of the Declaration of Helsinki. The local ethics committee (EKNZ 2017-00701)

and the national agency for the authorization and supervision of therapeutic products (Swissmedic, 2017DR2127) approved the study protocol and study medication.

Eligibility criteria included being 18 years of age or older and having chronic SIAD-induced hyponatremia defined as: plasma sodium concentration < 135 mmol/l, clinical euvolemia, plasma osmolality < 275 mOsm/kg, urine osmolality > 100 mOsm/kg, urine sodium > 30 mmol/l, absence of uncontrolled hypothyroidism and hypocortisolism[97]. Exclusion criteria included acute or transient hyponatremia, severe symptomatic hyponatremia in need of hospitalization, pregnancy or breastfeeding, renal insufficiency (glomerular filtration rate (GFR) < 45 ml/min), heart failure, diabetes mellitus type 1, liver cirrhosis or acute hepatic impairment (ALAT / ASAT > 3x ULN), or patients under treatment with SGLT2 inhibitors, lithium chloride, urea or glitazone.

Participants were randomized 1:1 to undergo first the empagliflozin phase followed by the placebo phase, or first the placebo phase followed by the empagliflozin phase. Treatment consisted in 1 capsule per day (empagliflozin 25 mg or placebo respectively) for 28 days. Further treatment included limitation of daily fluid intake to ≤ 1.5 l/day. Further details can be found in the initial report of the study[29].

For this present analysis, patients treated with an antiresorptive or anabolic osteoporosis treatment or with active bone processes (i.e., surgery, fracture, infection) were excluded.

9.3.2 Bone Markers

Fasting blood samples were drawn between 08:00 and 11:00 a.m. and fresh serum aliquots were stored at -80 °C until analysis. The parameters CTX and P1NP were measured in serum with Elecsys® assays on the automated analyzer cobas e 411 (Roche Diagnostics International, Rotkreuz, Switzerland). The intra- and interassay variations were 2.0 and 8.4% for CTX and 1.2 and 3.3% for P1NP, respectively. P1NP and CTX were measured at baseline and after 1 week and 4 weeks of each treatment regimen (empagliflozin or placebo). Osteocalcin was measured with the automated IDS N-MID® Osteocalcin-Assay (Immunodiagnostic Systems Ltd., UK). Osteocalcin was measured at baseline and after 4 weeks of each treatment regimen (empagliflozin or placebo). Baseline values correspond to the first visit of the first treatment phase. Bone formation index was calculated by dividing the P1NP concentration by the CTX concentration, so that a higher value suggests bone anabolism.

9.3.3 Study Outcomes

The primary outcome was association between the change in BFI (serum P1NP (μg/l) divided by serum CTX (μg/l)) over each treatment regimen (empagliflozin 25mg or placebo) with the change in plasma sodium levels (mmol/l).

Secondary outcomes were the association between change in P1NP (μg/l), osteocalcin (μg/l) and CTX (μg/l) respectively and plasma sodium levels (mmol/l) as well as the association between BFI or each bone marker and hyponatremia persistence/resolution using a cut-off of 135 mmol/l, over each treatment regimen (empagliflozin 25mg or placebo).

9.3.4 Statistical Analysis

Baseline characteristics are summarized using descriptive statistics. Discrete variables are expressed as frequencies (percentage (%) and number of patients (n)). Continuous variables are expressed as median and interquartile range [IQR]. Changes in bone markers are represented graphically with boxplots.

For the primary outcome, the association between changes in BFI and plasma sodium levels was investigated by fitting a linear mixed-effects model[170,171], with BFI as outcome variable, patients as random effect and the following fixed effects: change in plasma sodium levels, baseline BFI, baseline serum cortisol, baseline serum 25-OH vitamin D, age, gender, smoking status as well as the week of treatment. Because there was no evidence for either carryover or sequence effect, treatment sequence and study phase were not included in the models.

For secondary outcomes, identical linear mixed-effect models were used with P1NP, osteocalcin and CTX as outcome variables and the baseline bone marker levels as fixed predictor. In a second step, the continuous fixed effect plasma sodium levels was replaced by the dichotomous predictor hyponatremia persistence/resolution. The impact of sodium levels and urine osmolality at baseline on changes in bone metabolism was investigated by sequentially adding these variables as fixed effects to the linear mixed-effect models described above.

A correlation analysis between changes in each bone marker and changes in serum sodium levels was computed.

All analyses were performed using the statistical program R (version 4.2.1)[172]. A two-sided significance level of 0.05 was used for every analysis.

9.4 RESULTS

9.4.1 Baseline Characteristics

Fourteen patients completed both treatment cycles. Two patients treated with antiresorptive drugs and one patient who underwent hip arthroplasty 2 months before inclusion were excluded from the analysis, leading to a total of 11 evaluable patients. Median [IQR] age was 73 years [66, 78], with 6 (54.5%) participants being female. Median [IQR] serum sodium level at baseline was 131 mmol/l [130, 132] (*Table M1.1*).

The etiology of chronic SIAD ranged from drug-induced (n = 3), to pulmonary diseases (n = 2), central nervous system disorders (n = 2) and chronic pain (n = 1). In 3 patients, the etiology remained idiopathic. Hyponatremia duration ranged from a minimum of 11 months to a maximum

of 90 months. Three patients took calcium supplementation and 6 patients took vitamin D supplementation. Two patients had a distant history (> 1 year) of traumatic fractures. Of note, no patient received glucocorticoids. The majority (75%) of patients had an history of falls (n = 8) and a third reported gait instability (n = 3) and fear of falling (n = 4). Detailed baseline characteristics are shown in *Table M1.1*.

Table M1.1 Baseline Characteristics

Baseline Characteristics	
Characteristics	**Participants n = 11**
Age, years	73 [66, 78]
Sex: female, n (%)	6 (54.5)
BMI, kg/m^2	23.2 [21.1, 27.5]
Ethnicity: Caucasian, n (%)	11 (100.0)
Alcohol, glasses per week	4.00 [1.00, 10.50]
Active smoker	2 (18.2)
Comorbidities, n (%)	
Arterial hypertension	8 (72.7)
Depression	1 (9.1)
Diabetes mellitus type 2	1 (9.1)
Polyneuropathy	7 (63.6)
Malignancy	1 (9.1)
Obstructive lung disease	2 (18.2)
Subarachnoid hemorrhage	1 (9.1)
Tuberculosis	1 (9.1)
Medication, n(%)	
Antidepressant	4 (36.4)
Corticosteroid	0 (0)
Diuretic	1 (9.1)
Hormone replacement therapy	0 (0)
Proton pump inhibitor	1 (9.1)
Narcotic	1 (9.1)
Bone health	
History of fractures (traumatic)	2 (18.2)
Osteoporosis prophylaxis	
Calcium supplementation	3 (27.3)
Vitamin D supplementation	6 (54.5)
Gait	
Gait impairment	3 (27.3)
Fear of falling	4 (36.4)
History of falls	8 (72.7)
SIAD	
Duration, months	50 [16, 69]
Etiology	
Central nervous system	2 (18.2)
Chronic pain	1 (9.1)
Drug-induced	3 (27.3)
Idiopathic	3 (27.3)
Pulmonary disease	2 (18.2)

(continued)

Table M1.1 Baseline Characteristics (continued)

Characteristics	Participants n = 11
Laboratory values	
P-sodium, mmol/l	131 [130, 132]
P-osmolality, mOsm/kg	271 [265, 277]
P-creatinine, mmol/l	60 [55, 63]
GFR, ml/min	90 [86, 103]
P-glucose, mmol/l	4.90 [4.6, 5.6]
P-calcium, mmol/l	2.3 [2.2, 2.7]
P-albumine, g/l	38 [37, 41]
25-hydroxy vitamin D, nmol/l	64 [51, 97]
S-cortisol, nmol/l	416 [317, 456]
TSH, mlU/l	1.49 [1.06, 2.84]
U-osmolality, mOsm/kg	521 [392, 580]
U-sodium, mmol/l	105 [77, 133]

Categorical variables are shown as frequencies (%), numerical variables as median [interquartile range]. BMI: body mass index; GFR: glomerular filtration rate; N = number; P = plasma; S = serum; U = urine.

9.4.2 Bone Formation Index (BFI)

Median [IQR] BFI was 131.9 [96.2, 168.7] at baseline (n = 11), 115.1 [110.4, 138.8] at the end of the placebo phase (n = 11) and 152.6 [108.9, 165.4] at the end of the empagliflozin phase (n = 11) (*Table M1.2*).

Changes in BFI were correlated with changes in sodium levels ($\rho = 0.55$, $p < 0.001$). An increase of 1 mmol/l in plasma sodium concentration was associated with a change of 5.2 in BFI (95%-CI: 1.4, 9.0; *P* = 0.013) (*Supplementary Material M1.2*). Treatment with empagliflozin was not a predictor for changes in BFI (β = 5.8; 95%-CI: -15.1, 26.7; *P* = 0.591). The effect of plasma sodium concentration on BFI was independent of empagliflozin (interaction: *P* = 0.779). The effect was independent of baseline plasma sodium levels and urine osmolality (data not shown).

After adjustments, hyponatremia persistence clearly tended to be associated with a reduction in BFI (β = -26.3, 95%-CI: -51.5, -1.0; *P* = 0.053) (*Figure M1.1A, Supplementary Material M1.3)*. Treatment with empagliflozin was not a predictor for changes in BFI (β = 16.5; 95%-CI: -1.3, 34.3; *P* = 0.082). The effect of hyponatremia persistence/resolution on BFI was independent of empagliflozin (interaction: *P* = 0.439).

9.4.3 Osteoblast Markers

9.4.3.1 Procollagen Type 1 N-Terminal Propeptide (P1NP)

Median [IQR] P1NP was 64.6 µg/l [45.6, 68.5] at baseline (n = 11), 59.2 [47.9, 65.2] at the end of the placebo phase (n = 11) and 70.4 µg/l [45.7, 75.0] at the end of the empagliflozin phase (n = 11) (*Table M1.2*).

Changes in P1NP were correlated with changes in sodium levels ($\rho = 0.45$, *P* = 0.004). An increase of 1 mmol/l sodium was associated with a change in 1.4 µg/l in P1NP (95%-CI: 0.3, 2.6;

P = 0.025) (*Supplementary Material M1.2*). Treatment with empagliflozin was not a predictor for changes in P1NP (β = 1.4; 95%-CI: -4.9, 7.6; *P* = 0.591). The effect of sodium change on P1NP was independent of empagliflozin (interaction: *P* = 0.398). The effect was independent of baseline plasma sodium levels and urine osmolality (data not shown).

After adjustments, hyponatremia persistence was not associated with a change in P1NP (β = -6.2, 95%-CI: -14.2, 1.9; *P* = 0.145) (*Figure M1.1B*). Treatment with empagliflozin was not a predictor for changes in P1NP (β = 4.7; 95%-CI: -0.8, 10.1, *P* = 0.107).

9.4.3.2 Osteocalcin

Median [IQR] osteocalcin was 18.0 µg/l [14.0, 25.9] at baseline (n = 10), 17.6 µg/l [15.6, 24.8] at the end of the placebo phase (n = 11) and 19.2 µg/l [16.1, 25.1] at the end of the empagliflozin phase (n = 9) (*Table M1.2*).

Changes in osteocalcin were not correlated with changes in sodium levels (ρ = 0.34, *P* = 0.149). Plasma sodium concentration was not associated with a change in osteocalcin (β = 0.3; 95%-CI: -0.1, 0.7; *P* = 0.117) (*Supplementary Material M1.2*). Treatment with empagliflozin was not a predictor for changes in osteocalcin (β = 2.0; 95%-CI: -0.9, 4.9; *P* = 0.233). The effect was independent of baseline plasma sodium levels and urine osmolality (data not shown).

After adjustments, hyponatremia persistence was not associated with a change in osteocalcin (β = -1.7, 95%-CI: (-4.9, 1., *P* = 0.349) (*Figure M1.1C, Supplementary Material M1.3*). Treatment with empagliflozin was not a predictor for changes in osteocalcin (β = 2.8; 95%-CI: 0.2, 5.5; *P* = 0.084).

9.4.4 Osteoclasts Marker

9.4.4.1 C-Terminal Cross-Linking Telopeptide (CTX)

Median [IQR] CTX was 0.47 µg/l [0.29, 0.65] at baseline (n = 11), 0.57 µg/l [0.34, 0.59] at the end of the placebo phase (n = 11) and 0.46 µg/l [0.27, 0.63] at the end of the empagliflozin phase (n = 11) (*Table M1.2*).

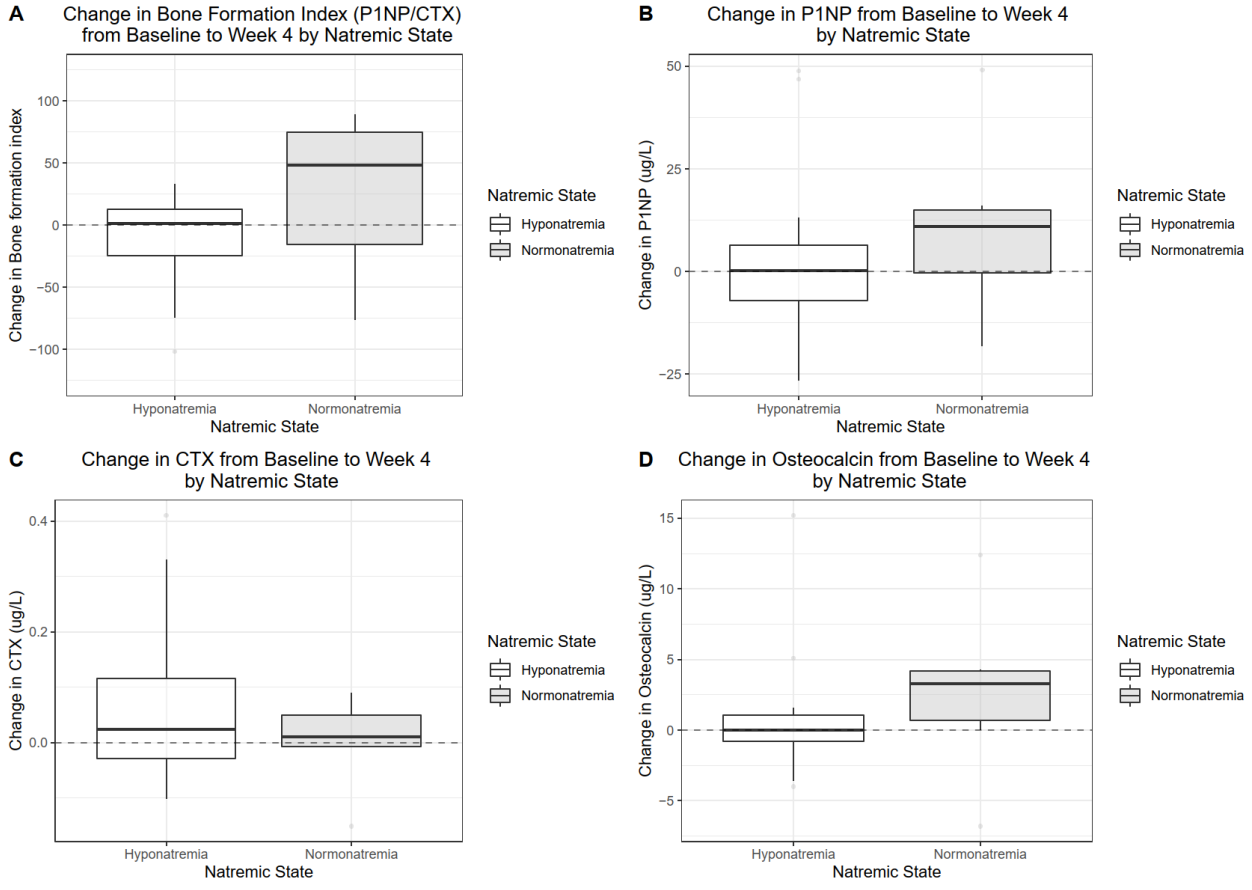
Changes in CTX were not correlated with changes in sodium levels (ρ = − 0.21, *P* = 0.184). Plasma sodium concentration was not associated with a change in CTX (β = 0.003; 95%-CI: -0.008, 0.014; *P* = 0.627) (*Supplementary Material M1.2)*. Treatment with empagliflozin was not a After adjustments, hyponatremia persistence was not associated with a change in CTX (β = 0.020, 95%-CI: -0.051, 0.090, *P* = 0.594) (*Figure M1.1D, Supplementary Material M1.3*). Treatment with empagliflozin was not a predictor for changes in CTX (β = -0.043; 95%-CI: -0.092, 0.006; *P* = 0.098).

Table M1.2 Bone Markers by Timepoint and Treatment: Median concentration of bone markers and sodium levels at baseline and over the two treatment phases. Bone formation index (BFI) was calculated by dividing P1NP concentration by CTX concentration for each patient.

Laboratory Parameter	Baseline	Placebo		Empagliflozin	
		Week 1	Week 4	Week 1	Week 4
BFI (P1NP/CTX)	131.9 [96.2, 168.7]	107.4 [95.3, 119.5][b]	115.1 [110.4, 138.8]	128.2 [117.2, 150.8][a]	152.6 [108.9, 165.4]
CTX (µg/L)	0.47 [0.29, 0.65]	0.55 [0.44, 0.78][b]	0.57 [0.34, 0.59]	0.41 [0.36, 0.60][a]	0.46 [0.27, 0.63]
P1NP (µg/L)	64.6 [45.6, 68.5]	63.3 [53.4, 73.0][b]	59.2 [47.9, 65.2]	56.2 [49.2, 70.9][a]	70.4 [45.7, 75.0]
Osteocalcin (µg/L)	18.0 [14.0, 25.9][a]	n.a.	17.6 [15.6, 24.8]	n.a.	19.2 [16.1, 25.1][c]
P-sodium (mmol/L)	131 [130, 132]	130 [128.8, 131][b]	130 [128, 132]	134 [132, 135][a]	134 [132.0, 136.0]

Summary statistics are shown as median [interquartile range]. CTX: C-Terminal Cross-Linking Telopeptide; BFI: Bone formation index; P: Plasma; P1NP: Procollagen Type 1 N-Terminal Propeptide; n.a.: not applicable. Data are from the whole cohort (n=11) unless otherwise specified: [a]10 patients, [b]8 patients, [c]9 patients

Figure M1.1 Changes in Bone Markers by Natremic State: Changes in bone markers were computed by subtracting the baseline value from week 4. Values above the dashed line represent an increase and values below the dashed line a decrease in bone marker levels. CTX: C-Terminal Cross-Linking Telopeptide; BFI: Bone formation index; P1NP: Procollagen Type 1 N-Terminal Propeptide.



9.4.5 Tolerability and Safety

A detailed description of safety outcomes is given in the primary report of the trial[29]. In brief, no case of overly rapid sodium correction, hypoglycemia, hypotension, or urinary tract or genital infection occurred. Five out of 7 adverse events during the empagliflozin phase were potentially related to the study intervention (mild headache: n = 1, gastrointestinal disorders: n = 2, fatigue: n = 1, xerostomia: n = 1). No serious adverse event was reported.

9.5 DISCUSSION

This preplanned secondary analysis of our randomized placebo-controlled cross-over study in chronic SIAD patients[29] has two main findings. First, an increase in sodium levels in patients with chronic SIAD was associated with an increase in bone formation index (P1NP/CTX). Second, this increase was mediated by an increase in the osteoblast marker P1NP. This relationship was independent of empagliflozin and was confirmed by a positive correlation between changes in plasma sodium levels and changes in bone formation index and P1NP respectively. This supports the importance of treating hyponatremia, particularly in older adults in whom chronic hyponatremia is also associated with falls[4], as illustrated by the high fall incidence in our cohort. Importantly, most patients had mild hyponatremia at baseline and the average sodium increase was 3 mmol/l, which underlines the clinical implication of increasing sodium levels, even slightly, and even in patients with a sodium concentration ≥130 mmol/l. To our knowledge, we provide the first cross-over data on the effect of an increase in sodium levels on bone metabolism in chronic SIAD patients.

We observed a clear association between an absolute increase in sodium and an increase in bone formation, as well as a strong trend towards an increase in bone formation index (P1NP/CTX) in patients in whom sodium levels normalized. Our original study was not powered for this analysis and therefore the sample size might not have sufficed to detect significance for a dichotomous predictor. Our findings align however, with our previous analysis in 88 hospitalized patients with SIAD[86], in which we showed that hyponatremia normalization led to an increase in P1NP and bone formation index after 4 days. Garrahy and colleagues investigated the effect of acute hyponatremia development on bone metabolism in patients with subarachnoid hemorrhage, and also showed an association between hyponatremia and reduced P1NP and P1NP/CTX ratio, while no change in osteocalcin levels were seen[85]. The reason why we, like others, did not see any association with osteocalcin could be that P1NP is a marker of collagen synthesis as the first step of new bone formation, whereas osteocalcin is a marker of the subsequent bone mineralization[173]. Consequently, our observation period might not have been long enough to detect a change in osteocalcin. Although this hypothesis is supported by the fact that time from baseline was a significant predictor for osteocalcin change both in this current analysis of hyponatremia treatment and in the study of Garrahy and colleagues on acute hyponatremia development[85], this is not supported by the INSIGHT trial, which showed an osteocalcin increase upon the AVP receptor antagonist tolvaptan over a similar observation period of 22 days[20]. The increase in sodium was greater in the INSIGHT trial (129 to 136 mmol/l) and the sample size larger (n = 107) which might account for the observed effect on osteocalcin. In addition, certain in-vitro data suggest a direct effect of AVP antagonism on this marker so that the use of tolvaptan might also be contributive[174]. Other preclinical studies showed that a

hyponatremic environment makes human bone marrow-derived mesenchymal stromal cells more prone to differentiate to adipocytes rather than to osteoblasts[49]. One could therefore hypothesize that correcting hyponatremia reverses this shift towards osteoblast differentiation. In rodents, there was a tendency toward reduced indexes of bone formation (i.e., mineral apposition rate and bone-formation rate per trabecular bone surface) in hyponatremic animals compared to controls[7].

A change in sodium levels in our study did not have an impact on CTX levels. This is in contrast to our previous analysis in hospitalized patients, where we observed an increase in the osteoclast marker CTX independently of sodium change, probably because of immobilization[169]. Moreover, the INSIGHT trial in hyponatremic outpatients showed a decrease in another osteoclast marker, i.e. urine N-telopeptide-creatinine ratio at the end of the 22-day treatment with tolvaptan while a slight increase was observed in the placebo group[20]. The use of this different osteoclast marker and again, the larger sample size, the greater sodium increase and the use of tolvaptan might explain this discrepancy. Both AVP receptor 1 and 2 are indeed expressed on osteoblasts and osteoclasts and their pharmacological inhibition in vitro stimulates bone synthesis and inhibits bone resorption[174]. Inducing hyponatremia for 3 months in rodents led to a significant histologic bone loss confirmed by a 30% bone mineral density loss in dual energy X-ray densitometry (DXA), and increased the number of osteoclasts in hyponatremic as compared to normonatremic rats[7]. Because osteoclasts are also activated in a hyponatremic environment in absence of AVP[48], an additive independent effects of low sodium and AVP could explain the discrepancy between human data with and without tolvaptan, and animal data. The separated impact of low sodium is shown in in-vitro studies in which an increase in osteoclasts differentiation[48,175], numbers[48] and activity[48,175] due to hyponatremia is observed independently of osmolality and AVP[48].

Importantly, our analysis confirms the neutral effect of empagliflozin on bone turnover. The CANVAS Trial raised concerns about a possible increased fracture risk under canagliflozin (HR = 1.26; 95%-CI: 1.04-1.52)[176]. However, this was not confirmed in the CREDENCE Trial (HR = 0.98; 95%-CI: 0.70-1,37) in which 4401 patients received canagliflozin 100mg for a median of more than 2 years[177], or in any other large randomized trial with other SGLT2 inhibitors[178-180]. A metanalysis suggests a neutral effect of SGLT2 inhibitors on bone mineral density (BMD)[181] but this should be interpreted cautiously due to the low number of included trials (n = 3). Since AVP antagonism might have an additional direct effect on bone[20,174], further studies should also investigate the effect of different hyponatremia treatments on bone metabolism.

The main strength of our study relies upon its cross-over and double-blind design. However, several limitations should be mentioned. First, our sample size (n = 11) and the average sodium change (131 to 134 mmol/l) are modest, which might negate meaningful findings for osteocalcin and CTX. Second, although the P1NP/CTX ratio used for the primary endpoint has been used in

our previous study[86] and by others[182], it is not broadly established and has never been shown to correlate with BMD. Further studies should include BMD measurement with dual energy X-ray densitometry (DXA) and comprise a longer observation period. This would allow to investigate the relationship between hyponatremia duration and BMD at baseline, taking into account that retrospective data suggest an increased risk for osteoporosis with hyponatremia duration[167]. Reliable diagnosis of osteoporosis in all study patients would be important, given that one case report describes osteoporosis recovery in a patient after the resolution of a paraneoplastic SIAD[23].

9.6 CONCLUSION

An increase in plasma sodium levels in outpatients with chronic hyponatremia due to SIAD, even when mild, was associated with an increase in bone formation index (P1NP/CTX) mirrored by an increase in P1NP, a surrogate marker of osteoblast function. The long-term impact of sodium correction on bone health in patients with chronic hyponatremia needs further research.

9.7 ACKNOWLEDGMENTS

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9.8 AUTHORS CONTRIBUTION

JR conceived and planned the original study as well as this preplanned secondary analysis. SM and JR selected the samples for the analysis. SM performed the statistical analysis and wrote the first draft of the manuscript. MCC supervised all steps of this work. All authors critically reviewed the manuscript.

9.9 FUNDING

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9.10 DISCLOSURE STATEMENT:

SM, JR, LP, CM and MCC have nothing to disclose.

9.11 SUPPLEMENTARY MATERIAL

<u>Supplementary Material M1.1 Bone Markers by Natremic State:</u> Median concentration of bone markers at baseline and over the two treatment phases by natremic state, i.e., persistent hyponatremia or normonatremia (corrected hyponatremia). Bone formation index (BFI) was calculated by dividing P1NP concentration by CTX concentration for each patient.

Variables	Baseline	Placebo						Empagliflozin					
		Week 1			Week 4			Week 1			Week 4		
	Hyponatremia	Hyponatremia	Normonatremia	*P value*	Hyponatremia	Normonatremia	*P value*	Hyponatremia	Normonatremia	*P value*	Hyponatremia	Normonatremia	*P value*
Patients, n (%)	11	8 (100)	0		9 (82)	2 (18)		6 (60)	4 (40)		7 (64)	4 (36)	
BFI (P1NP/CTX)	131.9 [96.2, 68.7]	107.4 [95.3, 119.5]		n.a.	114.1 [108.5, 128.4]	154.8 [140.2, 169.4]	0.220	128.2 [119.4, 132.0]	137.6 [117.8, 165.3]	0.914	114.2 [102.0, 152.7]	165.4 [157.7, 192.4]	0.073
CTX (µg/L)	0.47 [0.3, 0.7]	0.55 [0.44, 0.78]		n.a.	0.57 [0.39, 0.60]	0.32 [0.3, 0.4]	0.330	0.50 [0.39, 0.60]	0.36 [0.33, 0.53]	0.610	0.48 [0.45, 0.72]	0.27 [0.26, 0.37]	0.32
P1NP (µg/L)	64.6 [45.6, 68.5]	63.3 [53.4, 73.0]		n.a.	47.9 [47.1, 48.7]	61.5 [58.5, 65.4]	0.40	53.3 [47.7, 73.3]	63.0 [50.7, 70.9]	0.380	53.0 [44.4, 71.0]	71.7 [58.4, 75.0]	0.260
Osteocalcin (µg/L)	18.0 [14.0, 25.9][a]			n.a.	16.80 [16.4, 17.2]	21.0 [15.2, 26.6]	0.730			n.a.	17.9 [14.6, 22.9]	19.2 [16.2, 25.1][b]	0.730

Summary statistics are shown as median [interquartile range]. P values are computed with a non-paired two-sided Wilcoxon Rank Sum Test, a value <0.05 is considered to be statistically significant. CTX: C-Terminal Cross-Linking Telopeptide; BFI: Bone formation index; P1NP: Procollagen Type 1 N-Terminal Propeptide; n.a.: not applicable. Data are shown for the number of patients of each natremia category unless otherwise specified. [a]10 patients, [b]5 patients.

Supplementary Material M1.2 Adjusted Relationship between Bone Markers and Plasma Sodium Levels: Estimate of the linear mixed models are shown with 95% confidence interval and p value. Significant predictor are displayed bold.

Independent variable	Dependent variable: Change in Bone Markers			
	Bone Formation Index	P1NP	CTX	Osteocalcin
Bone Markers at Baseline	0.950 (-0.034, 1.934) *P* = 0.132	0.806 (0.052, 1.560) *P* = 0.105	**1.433**** **(1.077, 1.788)** ***P* = 0.002**	**1.294**** **(0.991, 1.598)** ***P* = 0.004**
Change in Sodium (mmol/l)	**5.207*** **(1.410, 9.004)** ***P* = 0.013**	**1.438*** **(0.255, 2.622)** ***P* = 0.025**	0.003 (-0.008, 0.014) *P* = 0.627	0.315 (-0.088, 0.719) *P* = 0.177
Empagliflozin	5.817 (-15.126, 26.761) *P* = 0.591	1.371 (-4.871, 7.613) *P* = 0.671	-0.058 (-0.116, 0.001) *P* = 0.065	1.961 (-0.931, 4.852) *P* = 0.233
Active Smoking	25.345 (-30.142, 80.832) *P* = 0.422	8.002 (-31.735, 47.740) *P* = 0.714	-0.206 (-0.403, -0.009) *P* = 0.110	6.090 (1.886, 10.293) *P* = 0.066
Male Sex	10.593 (-39.597, 60.782) *P* = 0.701	5.692 (-24.947, 36.330) *P* = 0.735	0.042 (-0.106, 0.191) *P* = 0.605	**6.149*** **(3.050, 9.249)** ***P* = 0.031**
Age	-0.392 (-1.826, 1.041) *P* = 0.621	-0.033 (-1.068, 1.002) *P* = 0.954	-0.002 (-0.007, 0.003) *P* = 0.501	0.189 (0.052, 0.326) *P* = 0.074
25-hydroxy vitamin D (nmol/l)	-0.711 (-1.872, 0.449) *P* = 0.296	-0.249 (-0.635, 0.138) *P* = 0.276	0.002 (-0.001, 0.004) *P* = 0.200	**-0.149*** **(-0.215, -0.084)** ***P* = 0.022**
Serum Cortisol (nmol/l)	0.120 (-0.026, 0.265) *P* = 0.183	0.001 (-0.116, 0.118) *P* = 0.983	0.0001 (-0.0004, 0.001) *P* = 0.648	-0.039 (-0.073, -0.006) *P* = 0.104
Week 4	3.134 (-13.813, 20.082) *P* = 0.720	1.107 (-3.828, 6.043) *P* = 0.664	-0.004 (-0.051, 0.043) *P* = 0.863	**16.497**** **(10.503, 22.491)** ***P* = 0.002**
Intercept	13.124 (-128.568, 154.815) *P* = 0.858	27.466 (-80.375, 135.307) *P* = 0.622	-0.172 (-0.641, 0.298) *P* = 0.480	-10.764 (-31.407, 9.879) *P* = 0.347
Significance levels: *p<0.05; **p<0.01	*CTX: C-Terminal Cross-Linking Telopeptide; BFI: Bone formation index; P1NP: Procollagen Type 1 N-Terminal Propeptide*			

Supplementary Material M1.3 Adjusted Relationship between Bone Markers and Hyponatremia: Estimates are shown with 95% confidence interval and p value. Significant predictor are displayed bold.

Independent variables	Dependent variable: Change in Bone Markers			
	Bone Formation Index	P1NP	CTX	Osteocalcin
Bone Markers at Baseline	0.621	0.743	**1.412****	**1.267****
	(-0.542, 1.784)	(-0.041, 1.526)	**(1.039, 1.784)**	**(0.946, 1.588)**
	P = 0.355	*P* = 0.137	***P* = 0.002**	***P* = 0.005**
Hyponatremia	-26.254	-6.167	0.020	-1.655
	(-51.525, -0.982)	(-14.207, 1.874)	(-0.051, 0.090)	(-4.847, 1.537)
	P = 0.053	*P* = 0.145	*P* = 0.594	*P* = 0.349
Empagliflozin	16.486	4.637	-0.043	2.829
	(-1.335, 34.308)	(-0.804, 10.079)	(-0.092, 0.006)	(0.155, 5.503)
	P = 0.082	*P* = 0.107	*P* = 0.098	*P* = 0.084
Active Smoking	25.382	11.888	-0.198	**7.291***
	(-42.433, 93.197)	(-29.396, 53.171)	(-0.402, 0.006)	**(3.273, 11.310)**
	P = 0.504	*P* = 0.603	*P* = 0.130	***P* = 0.038**
Male Sex	9.656	2.242	0.033	**5.522***
	(-51.391, 70.703)	(-29.559, 34.044)	(-0.119, 0.185)	**(2.348, 8.695)**
	P = 0.773	*P* = 0.897	*P* = 0.690	***P* = 0.043**
Age	-0.433	0.016	-0.001	0.206
	(-2.188, 1.322)	(-1.063, 1.094)	(-0.007, 0.004)	(0.064, 0.349)
	P = 0.655	*P* = 0.979	*P* = 0.601	*P* = 0.066
25-hydroxy vitamin D (nmol/l)	-0.539	-0.306	0.002	**-0.163***
	(-1.938, 0.861)	(-0.706, 0.094)	(-0.001, 0.004)	**(-0.228, -0.098)**
	P = 0.493	*P* = 0.209	*P* = 0.239	***P* = 0.017**
Serum Cortisol (nmol/l)	0.060	-0.017	0.0001	-0.044
	(-0.113, 0.233)	(-0.138, 0.104)	(-0.0004, 0.001)	(-0.078, -0.009)
	P = 0.534	*P* = 0.794	*P* = 0.707	*P* = 0.091
Week 4	5.812	1.994	-0.001	**15.646****
	(-11.039, 22.664)	(-3.120, 7.108)	(-0.047, 0.045)	**(9.213, 22.080)**
	P = 0.505	*P* = 0.452	*P* = 0.969	***P* = 0.004**
Intercept	92.205	44.340	-0.187	-6.558
	(-83.316, 267.727)	(-68.112, 156.793)	(-0.684, 0.310)	(-27.453, 14.338)
	P = 0.313	*P* = 0.447	*P* = 0.467	*P* = 0.562
Significance levels: *p<0.05; **p<0.01	*CTX: C-Terminal Cross-Linking Telopeptide; BFI: Bone formation index; P1NP: Procollagen Type 1 N-Terminal Propeptide*			

10 Manuscript 2: Prevalence of Admission Hyponatremia in Diabetic Patients Treated With and Without an SGLT2-Inhibitor - a Cross-Sectional Study

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10.1 ABSTRACT

BACKGROUND Hyponatremia often reflects a free water excess. Sodium/glucose co-transporter 2 (SGLT2) inhibitors increase free water excretion through glucose-induced osmotic diuresis. In two randomized double-blind placebo-controlled trials in patients with a syndrome of inappropriate antidiuresis (SIAD), we showed that empagliflozin increased plasma sodium concentration more effectively than placebo. We hypothesized that long-term therapy with SGLT2 inhibitors might reduce the prevalence of hyponatremia on hospital admission.

METHOD In this retrospective analysis, we extracted data of adult patients with type 2 diabetes (T2DM), hospitalized at the University Hospital Basel between 2015 and 2020. Patients with an SGLT2 inhibitor on admission were matched 1:1 according to age, gender, diagnosis of heart failure and principal diagnosis, to patients without an SGLT2 inhibitor on admission. The primary outcome was the prevalence of hyponatremia (plasma sodium concentration corrected for glycemia < 135 mmol/l) on admission.

RESULTS We analyzed 821 patients with T2DM treated with and 821 patients with T2DM without an SGLT2 inhibitor on admission. Hyponatremia prevalence on admission was 9.9% in the treated group, and 8.9% in the matched control group (P = 0.554), i.e., the risk for hyponatremia did not differ (multivariable adjusted OR = 1.08, 95%-CI: 0.72-1.44, P = 0.666). There was no difference in the median [IQR] plasma sodium concentration between both groups (treated: 140 mmol/l [138-142], controls: 140 mmol/l [138-142]; P = 0.1017).

CONCLUSION Based on these retrospective findings, treatment with SGLT2 inhibitors does not prevent hyponatremia. However, prospective randomized data suggest their efficacy at a higher dosage in overt SIAD.

10.2 INTRODUCTION

Hyponatremia, defined as plasma sodium concentration < 135 mmol/l, is the most common electrolyte disturbance in hospitalized patients[56]. Its prevalence on hospital admission ranges from 3% to 38%, depending on the severity of hyponatremia[53,54,56,65]. Hyponatremia on hospital admission is associated with increased in-hospital[53,56,65], 1-year[53,58] and 5-year mortality[53]. Furthermore, it is associated with an increased risk for intensive care unit (ICU) admission and mechanical ventilation[54], longer hospital stays[54,65], higher hospital costs[54] and discharge to care facilities[65].

In prevailing guidelines, treatments for hypovolemic hyponatremia and acute severely symptomatic hyponatremia are well established[3,108,183]. By contrast, patients with chronic euvolemic or hypervolemic hyponatremia are often discharged still hyponatremic because of the limited efficacy of the available therapeutic options[30].

Both euvolemic and hypervolemic hyponatremia result primarily from arginine vasopressin (AVP) mediated free water retention[36]. Accordingly, fluid restriction is usually the first-line therapy but has limited efficacy[117]. AVP antagonists (vaptans) lead to increased aquaresis and can be used as a second-line treatment[3,19,108,184]. However, they are costly and carry the risk for overly rapid plasma sodium correction, requiring close plasma sodium monitoring on treatment initiation[127,185,186].

The sodium/glucose co-transporter 2 (SGLT2) is expressed in the proximal tubules of the kidneys and reabsorbs approximately 90% of the filtered glucose[187]. Blockade of SGLT2 with the oral antidiabetic drugs SGLT2 inhibitors results in renal excretion of glucose[133] and subsequent osmotic diuresis[147]. The SGLT2 inhibitor empagliflozin reduces the risk of major adverse cardiovascular event[178] and heart failure[188] and slows progression of kidney disease in diabetic patients with high cardiovascular risk[189]. Empagliflozin has also cardiovascular and renal benefits regardless of diabetes mellitus in patients with heart failure and reduced (HFrEF)[190] or preserved (HFpEF) ejection fraction[191]. Furthermore, we showed in a randomized double-blind placebo-controlled trial in 87 hospitalized euvolemic hyponatremic patients with a syndrome of inappropriate antidiuresis (SIAD), that a 4-day treatment with empagliflozin combined with fluid restriction leads to a higher plasma sodium increase as compared to fluid restriction alone[148]. We recently confirmed these findings in a randomized double-blind placebo-controlled crossover trial in 14 outpatients with a chronic SIAD[29].

To our knowledge, the effect of long-term treatment with SGLT2 inhibitors on hyponatremia prevalence in hospitalized patients has never been investigated. We, therefore, aimed to compare hyponatremia prevalence on admission in patients with type 2 diabetes mellitus (T2DM) treated with an SGLT2 inhibitor with that in patients with T2DM but without SGLT2 inhibitors. We

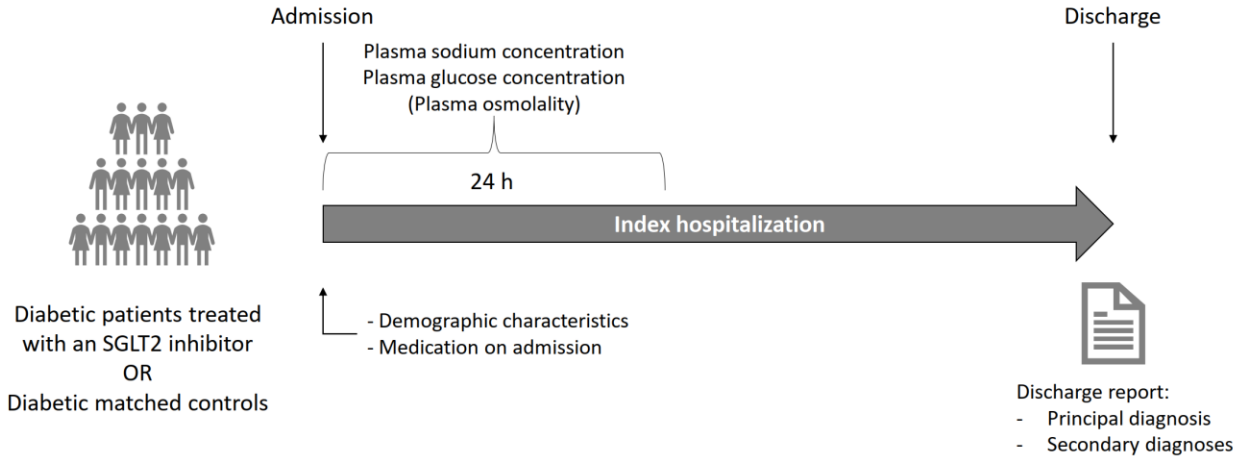
hypothesized that hyponatremia prevalence is lower in patients treated with an SGLT2 inhibitor. This would support their use as a prophylaxis for hyponatremia recurrence in patients with chronic hyponatremia or as a prophylaxis for hyponatremia in general in at-risk patients.

10.3 METHODS

10.3.1 Patients Selection and Extraction

This retrospective cross-sectional study selected all patients with T2DM hospitalized at the University Hospital of Basel, Switzerland, between 2015 and 2020 with available plasma sodium measurements within the first 24h following admission (*Figure M2.1*). Demographic characteristics, medication on admission, plasma glucose and osmolality (if available at the timepoint of sodium measurement), and comorbidities were extracted from the electronic health records at once by the Information and Communication Technologies (ICT) Department of the University Hospital of Basel and transmitted to the first author for statistical analysis. Diagnoses were coded with the International Statistical Classification of Diseases and Related Health Problems (ICD) 10-GM (version 2014, 2016 and 2018)[192-194] and taken out discharge reports. The extraction of the health-related data from the electronic health records of the University Hospital of Basel required for this study was approved by the local ethics committee (Ethikkommision Nordwest- und Zentralschweiz, EKNZ 2021-00649).

Figure M2.1 Study Diagram: Study diagram showing patients selection. All patients with T2DM *hospitalized between 2015 and 2020 and with a plasma sodium and a glucose measurement within the first 24h following admission were selected.*



10.3.2 Laboratory

Plasma concentrations of sodium, glucose, and osmolality were from the same timepoint and measured by the central laboratory of the University Hospital Basel. Plasma sodium concentration was measured by the indirect ion selective electrode (ISE) method (cobas® 8000 modular

analyzer, Roche Diagnostics) in centrifuged lithium-heparin plasma. Plasma osmolality levels were measured using the freezing point depression osmometric method.

At higher concentrations, glucose can cause translocational isotonic or hypertonic hyponatremia[195]. Because all selected patients were diabetic, we corrected plasma sodium values for glycemia according to the linear model of Hillier et al.[195], as recommended by European guidelines[3]. For each patient in whom glycemia was above 5.5 mmol/l, sodium levels were corrected by adding 2.4 mmol/l per 5.5 mmol/l glucose using the following equation:

$$Corrected\ sodium\ levels\ (mmol/l) = measured\ sodium\ levels\ (mmol/l) + 2.4\ x\ \frac{glucose\ levels\ (mmol/l) - 5.5\ mmol/l}{5.5\ mmol/l}$$

Hyponatremia was defined as a plasma sodium concentration < 135 mmol/l and further subclassified according to biochemical severity (mild: plasma sodium concentration: 130-134 mmol/l, moderate: plasma sodium concentration 125-129 mmol/l, profound: plasma sodium concentration <125 mmol/l)[3]. Hypernatremia was defined as a plasma sodium concentration > 145 mmol/l.

10.3.3 Study Outcomes

The primary outcome was the prevalence of hyponatremia on hospital admission in patients with T2DM treated with an SGLT2 inhibitor versus matched control patients with T2DM without an SGLT2 inhibitor.

The secondary outcomes included the difference in plasma sodium concentration, the prevalence of hyponatremia severities and hypernatremia in patients with T2DM treated with an SGLT2 inhibitor versus matched control patients with T2DM who were not treated with an SGLT2 inhibitor. We additionally computed the prevalence of hyponatremia, hyponatremia severities and hypernatremia in a subset excluding patients with an ICD10 code for hypovolemia or hypotension and in a subset containing only hypervolemic patients, i.e., with heart failure, chronic kidney disease or liver cirrhosis as comorbidities. Furthermore, we investigated the association between SGLT2 inhibitors and hyponatremia/plasma sodium levels on admission adjusted for medication and comorbidities.

10.3.4 Statistical Analysis

Baseline characteristics are summarized using descriptive statistics. Discrete variables are expressed as frequencies (percentage (%) and number of patients (n)). Continuous variables are expressed as median and interquartile range (IQR, 25th to 75th percentiles).

Eight hundred twenty-one patients treated with an SGLT2 inhibitor, and 15 999 control patients met the selection criteria. We performed a 1:1 propensity score matching using the package *MatchIt*[196]. The Average Treatment effect on the Treated (ATT) was estimated by fitting a

generalized linear model with the variable *SGLT2 inhibitor* as the dependent variable and the covariates *gender, age (+/-5 years), heart failure diagnosis* and *main diagnosis* (as ICD-10 chapter) as independent variables. Patients were matched 1:1 using the nearest neighbor matching (i.e., “greedy matching”) method without replacement; therefore, each treated patient was matched to one control patient and 15 178 controls were discarded. Further details on matching specification and covariate balance can be found in the appendix (*Supplementary Material M2.1*).

Prevalence in each group was compared using a chi-squared test. Plasma sodium concentration in each group was compared using a Wilcoxon-Mann-Whitney test. The independent effect of SGLT2 inhibitors on hyponatremia occurrence on admission was investigated by fitting a univariable and a multivariable logistic regression model. The model with the lowest Akaike information criterion (AIC) was selected in a stepwise way using the *step* function, with *SGLT2 inhibitors* as a fixed predictor[197]. The association between plasma sodium concentration and SGLT2 inhibitors was investigated in the same way, i.e., with a univariable and multivariable linear model, with additional verification of assumptions and multicollinearity. Detailed covariables fitting and outputs can be found in the appendix (*Supplementary Material M2.2, Supplementary Material M2.3*).

All analyses were performed using the statistical program R (version 4.0.5 or higher). A two-sided significance level of 0.05 was set for every analyses.

10.4 RESULTS

10.4.1 Baseline Characteristics

Eight hundred twenty-one patients treated with an SGLT2 inhibitor were matched to 821 control patients. Covariate balance was achieved as emphasized by the final matching specification including a standardized mean difference (SMD) of -0.0001 (SMD before matching = 0.3751), a variance ratio of 0.9994 (variance ratio before matching = 0.9869) and a mean empirical cumulative density function (eCDF) of 0.0001 (mean eCDF before matching = 0.0954). Twenty-nine percent of patients (n = 238) were female, and median [IQR] age was 70 years [61;78] in each group. Detailed baseline characteristics including comorbidities, medications, and laboratory parameters of each group were well balanced and are shown in *Table M2.1, Table M2.2* and *Table M2.3.*

Table M2.1: Demographic Characteristics: Demographic characteristics in diabetic patient treated with an SGLT2 inhibitor and in diabetic matched controls

Table 1. Demographic characteristics

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)
Female, n (%)	238 (29)	238 (29)
Admission year, n (%)		
2015	567 (69.1)	18 (2.2)
2016	129 (15.7)	56 (6.8)
2017	55 (6.7)	124 (15.1)
2018	30 (3.7)	144 (17.5)
2019	24 (2.9)	198 (24.1)
2020	16 (1.9)	281 (34.2)
Age, y, median (IQR)	70.00 (61.00, 78.00)	70.00 (61.00, 78.00)

Demographic characteristics in patients with T2DM treated with an SGLT2 inhibitor and in matched controls with T2DM.

Table M2.2 Admission Diagnoses and Comorbidities: Admission principal diagnosis as ICD10 chapter and comorbidities in diabetic patient treated with an SGLT2 inhibitor and in diabetic matched controls.

Table 2. Admission diagnoses and comorbidities

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)
ICD10 chapter of admission diagnosis, n (%)		
I: Certain infectious and parasitic diseases	52 (6.3)	53 (6.5)
II: Neoplasms	47 (5.7)	48 (5.8)
III: Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	3 (0.4)	2 (0.2)
IV: Endocrine, nutritional and metabolic diseases	50 (6.1)	47 (5.7)
V: Mental and behavioral disorders	6 (0.7)	7 (0.9)
VI: Diseases of the nervous system	29 (3.5)	32 (3.9)
VII: Diseases of the eye and adnexa	3 (0.4)	4 (0.5)
VIII: Diseases of the ear and mastoid process	3 (0.4)	5 (0.6)
IX: Diseases of the circulatory system	258 (31.4)	256 (31.2)

(*continued*)

Table 2. Continued

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)
X: Diseases of the respiratory system	95 (11.6)	86 (10.5)
XI: Diseases of the digestive system	57 (6.9)	55 (6.7)
XII: Diseases of the skin and subcutaneous tissue	12 (1.5)	15 (1.8)
XIII: Diseases of the musculoskeletal system and connective tissue	40 (4.9)	40 (4.9)
XIV: Diseases of the genitourinary system	42 (5.1)	44 (5.4)
X: Diseases of the respiratory system	95 (11.6)	86 (10.5)
XI: Diseases of the digestive system	57 (6.9)	55 (6.7)
XII: Diseases of the skin and subcutaneous tissue	12 (1.5)	15 (1.8)
XIII: Diseases of the musculoskeletal system and connective tissue	40 (4.9)	40 (4.9)
XIV: Diseases of the genitourinary system	42 (5.1)	44 (5.4)
XVII: Congenital malformations, deformations and chromosomal abnormalities	0 (0.0)	0 (0.0)
XVIII: Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	1 (0.1)	1 (0.1)
XIX: Injury, poisoning and certain other consequences of external causes	42 (5.1)	43 (5.2)
XXI: Factors influencing health status and contact with health services	81 (9.9)	82 (10.0)
All diagnoses, n (%)		
Acute kidney injury	45 (5.5)	107 (13.0)
Coronary heart disease	308 (37.5)	399 (48.6)
Acute coronary syndrome	69 (8.4)	91 (11.1)
Chronic kidney disease	262 (31.9)	252 (30.7)
Heart failure	149 (18.1)	149 (18.1)
Hypertension	429 (52.3)	470 (57.2)
Hyponatremia diagnosis	25 (3.0)	29 (3.5)
Hypotension	11 (1.3)	23 (2.8)

(*continued*)

Table 2. Continued

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)
Hypovolemia	19 (2.3)	32 (3.9)
Liver cirrhosis	13 (1.6)	16 (1.9)
Lung cancer	16 (1.9)	16 (1.9)
Pneumonia	86 (10.5)	86 (10.5)
Seizure	42 (5.1)	26 (3.2)
Stroke	47 (5.7)	56 (6.8)
Ischemic stroke	43 (5.2)	54 (6.6)
Subarachnoid hemorrhage	1 (0.1)	0 (0.0)
Syndrome of inappropriate antidiuresis (SIAD)	3 (0.4)	2 (0.2)
Tuberculosis	4 (0.5)	3 (0.4)

Admission principal diagnosis as ICD10 chapter and comorbidities in patients with T2DM treated with an SGLT2 inhibitor and in matched controls with T2DM.

Table M2.3 Medication and Laboratory Values on Admission: Medication and laboratory values on admission in diabetic patients treated with an SGLT2 inhibitor and in diabetic matched controls. Raw plasma sodium values and plasma sodium values corrected for glucose are displayed. Plasma sodium values are the first available in the 24 hours following admission. Plasma osmolality and glucose values are from the same samples as plasma sodium values. The two groups were compared with a Wilcoxon-Mann-Whitney test

Table 3. Medication and laboratory values on admission

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)	*P* value
Medication on admission, n (%)			
Antihypertensive agents and diuretics			
ACE inhibitor	191 (23.3)	268 (32.6)	
ARB	172 (21.0)	236 (28.7)	
ARNI	1 (0.1)	32 (3.9)	
Beta blocker	286 (34.8)	406 (49.5)	
Calcium channel antagonists	198 (24.1)	221 (26.9)	
Loop diuretic	222 (27.0)	244 (29.7)	
Mineralocorticoid receptor antagonist	74 (9.0)	110 (13.4)	
Renin inhibitor	6 (0.7)	0 (0.0)	
Thiazide or thiazide-like diuretic	145 (17.7)	183 (22.3)	
Antidiabetic drugs			
Acarbose	0 (0.0)	0 (0.0)	
Biguanide	329 (40.1)	514 (62.6)	
DDP4 inhibitor	189 (23.0)	217 (26.4)	
Glinide	5 (0.6)	4 (0.5)	
GLP-1 receptor agonist	31 (3.8)	100 (12.2)	
Insulin or insulin analog	226 (27.5)	325 (39.6)	
SGLT2 inhibitor	0 (0.0)	821 (100.0)	
Canagliflozin	0 (0.0)	39 (4.8)	
Dapagliflozin	0 (0.0)	184 (22.4)	
Empagliflozin	0 (0.0)	599 (73.0)	

(*continued*)

.

Table 3. Medication and laboratory values on admission (continued)

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)	*P* value
Ertugliflozin	0 (0.0)	0 (0.0)	
Sulfonylurea	77 (9.4)	82 (10.0)	
Thiazolidinedione	5 (0.6)	5 (0.6)	
Antidepressants			
Other antidepressant	5 (0.6)	8 (1.0)	
SNRI	11 (1.3)	13 (1.6)	
SSRI	62 (7.6)	97 (11.8)	
Tetracyclic antidepressant	28 (3.4)	17 (2.1)	
Tricyclic antidepressant	17 (2.1)	15 (1.8)	
Psycholeptics			
Atypical neuroleptics	61 (7.4)	51 (6.2)	
Lithium	8 (1.0)	0 (0.0)	
Typical neuroleptics	16 (1.9)	5 (0.6)	
Anticonvulsants	86 (10.5)	93 (11.3)	
Laboratory values on admission, median (IQR)			
Plasma sodium corrected for glucose (mmol/L)	140 (138, 142), (n = 821)	140 (138, 142), (n = 821)	.1017
Raw plasma sodium (mmol/L)	138 (136, 140), (n = 821)	138 (136, 141), (n = 821)	.2473
Plasma osmolality (mOsm/kg)	300 (290, 317), (n = 23)	296 (287, 314) (n = 26)	.4056
Plasma glucose (mmol/L)	8.8 (6.9, 11.9), (n = 821)	9.1 (7, 12.1), (n = 821)	.2555

Medication and laboratory values on admission in patients with T2DM treated with an SGLT2 inhibitor and in matched controls with T2DM. Raw plasma sodium values and plasma sodium values corrected for glucose are displayed. Plasma sodium values are the first available in the 24 hours following admission. Plasma osmolality and glucose values are from the same samples as plasma sodium values. The 2 groups were compared with a Wilcoxon–Mann–Whitney test. Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ARNI, angiotensin receptor/neprilysin inhibitor; GLP, glucagon-like peptide; SGLT2, sodium/glucose cotransporter 2; SNRI, serotonin–norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitors.

10.4.2 Prevalence of Hyponatremia on Admission and Association with SGLT2 Inhibitors

Patients treated with SGLT2 inhibitors showed no difference in hyponatremia prevalence on admission in comparison to the matched control group (9.9%, n = 81 vs 8.9%, n = 73, *P* = 0.554) (*Table M2.4*). There was no difference in the different hyponatremia severities, i.e., mild (7.9%, n = 65 vs 6.9%, n = 57, *P* = 0.510), moderate (1.2%, n = 10 vs 1.3%, n = 11, *P* = 1.0) and profound (0.7%, n = 6 vs 0.6%, n = 5, *P* = 1.0), and in hypernatremia prevalence (4.0%, n = 33 vs 5.6%, n = 46, *P* = 0.116) (*Table M2.4*).

SGLT2 inhibitors were not associated with hyponatremia (unadjusted OR = 1.12, 95%-CI: 0.79-1.45, *P* = 0.499; multivariable adjusted OR = 1.08, 95%-CI: 0.72-1.44, *P* = 0.666). Furthermore, there was no difference in the median [IQR] plasma sodium concentration between both groups (treated: 140 mmol/l [138-142], controls: 140 mmol/l [138-142]; p=0.1017) (*Figure M2.2, Table M2.3*). SGLT2 inhibitors were not associated with a significant change in plasma sodium levels (unadjusted β = -0.08, 95%-CI: -0.35-0.51, *P* = 0.712; multivariable adjusted β = -0.24, 95%-CI: -0.20-0.68, *P* = 0.280). Detailed statistical models can be found in the appendix (*Supplementary Material M2.2, Supplementary Material M2.3*).

After excluding patients with an ICD10 code for hypovolemia or hypotension, there was still no difference in hyponatremia prevalence on admission between patients treated with SGLT2 inhibitors and their matched control patients (9.2%, n = 71 vs 9.0%, n = 71, *P* = 0.936). There was no difference in the different hyponatremia severities, i.e., mild (7.4%, n = 57 vs 7.0%, n =

55, *P* = 0.806), moderate (1.0%, n = 8 vs 1.4%, n = 11, *P* = 0.687) and profound (0.8%, n = 6 vs 0.6%, n = 5, *P* = 0.964), and in hypernatremia prevalence (3.4%, n = 26 vs 5.1%, n = 40, *P* = 0.128) (*Supplementary Material M2.4)*.

Similarly, hyponatremia prevalence was similar in hypervolemic patients treated with an SGLT2 inhibitor and without an SGLT2 inhibitor (13.3%, n = 43 vs 10.7%, n = 37, *P* = 0.363). There was no difference in the different hyponatremia severities, i.e., mild (10.8%, n = 35 vs 7.8%, n = 27, *P* = 0.228), moderate (1.0%, n = 8 vs 1.4%, n = 11, *P* = 0.860) and profound (0.9%, n = 3 vs 0.9%, n = 3, *P* = 1.0), and in hypernatremia prevalence (5.2%, n = 17 vs 5.8%, n = 20, *P* = 0.894) (*Supplementary Material M2.5).*

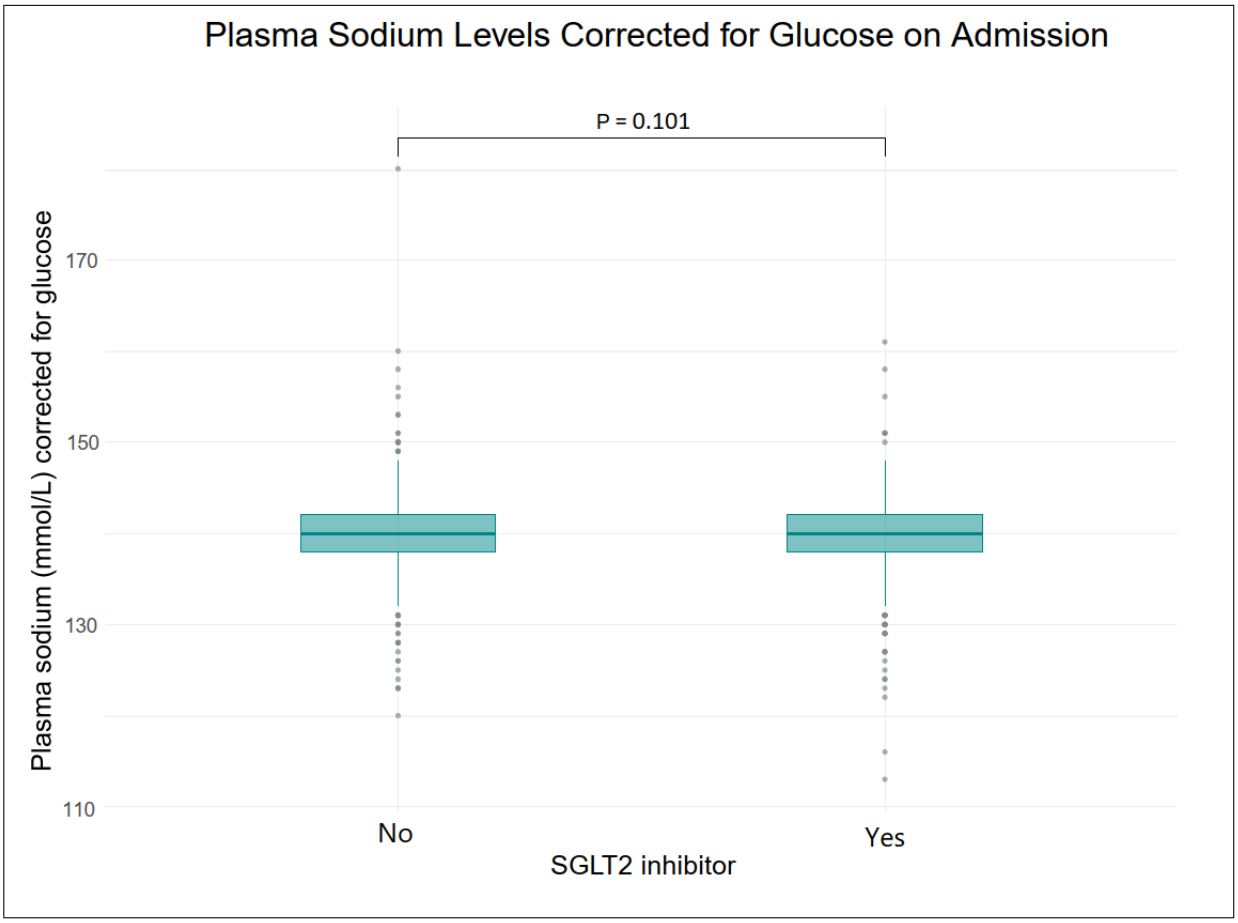
Table M2.4 Prevalence of Dysnatremia on Admission: Prevalence of dysnatremia on admission in diabetic patient treated with an SGLT2 inhibitor and in diabetic matched controls (plasma sodium corrected for glucose). The two groups were compared with a chi-quared test. SGLT2 = sodium glucose co-transporter 2, 95%-CI = 95% confidence interval.

Table 4. Prevalence of dysnatremia on admission

	No SGLT2 inhibitor on admission (n = 821)	SGLT2 inhibitor on admission (n = 821)	*P* value
Hypernatremia (plasma sodium >145 mmol/L), n (%)	46 (5.6)	33 (4.0)	.166
Normonatremia (plasma sodium 135–145 mmol/L), n (%)	702 (85.5)	707 (86.1)	.777
Hyponatremia (corrected plasma sodium <135 mmol/L), n (%)	73 (8.9)	81 (9.9)	.554
Mild hyponatremia (plasma sodium 130–134 mmol/L), n (%)	57 (6.9)	65 (7.9)	.510
Moderate hyponatremia (plasma sodium 125–129 mmol/L), n (%)	11 (1.3)	10 (1.2)	1
Profound hyponatremia (plasma sodium <125 mmol/L), n (%)	5 (0.6)	6 (0.7)	1

Prevalence of dysnatremia on admission in patients with T2DM treated with an SGLT2 inhibitor and in matched controls with T2DM (plasma sodium corrected for glucose). The 2 groups were compared with a chi-squared test.

<u>Figure M2.2 Plasma Sodium Levels on Admission:</u> Plasma sodium levels in mmol/l for each group, i.e., diabetic patients treated with a SGLT2 inhibitor (n = 821) and matched controls (n = 821). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR. All other values are considered to be outliers and plotted as individual points. A Wilcoxon-Mann-Whitney test was performed to compare hyponatremia levels in both groups.



10.5 DISCUSSION

The main finding of this cross-sectional study is that hyponatremia prevalence and plasma sodium concentration were the same in patients with T2DM treated with and without SGLT2 inhibitors, irrespective of comorbidities and comedications.

To our knowledge, this is the first study providing data on hyponatremia prevalence in patients with T2DM treated with an SGLT2 inhibitor. Falhammar et al. investigated the association between hyponatremia and glucose-lowering drugs, however, the number of patients treated with an SGLT2 inhibitor (n = 2) in their study was too low to investigate their effect on plasma sodium levels[198]. In the current analysis, we chose hyponatremia on admission to investigate the effect of SGLT2 inhibitors because these drugs are commonly paused during hospitalization. Contrary to our hypothesis, we found no difference in the hyponatremia prevalence and plasma sodium levels on admission between patients with T2DM treated with SGLT2 inhibitors and control patients with T2DM without SGLT2 inhibitors. The prevalence of the different hyponatremia severities and of hypernatremia did not differ either.

A first plausible explanation is that we, unfortunately, could not truly differentiate hyponatremia subtypes, especially not precisely identify hypovolemic hyponatremia, which is one of the most common causes for hyponatremia[71]. A recent meta-analysis by Rong et al. suggested that SGLT2 inhibitors are not associated with orthostatic hypotension[199]; however, they reduce blood pressure[200] and induce volume depletion[147]. In hypovolemic hyponatremia, SGLT2 inhibitors might therefore show no effects or even lower plasma sodium levels through hemodynamic AVP stimulation, and thus counterbalance the benefit of SGLT2 inhibitors on plasma sodium levels in euvolemic and hypervolemic patients in the full dataset. The subgroup analysis we performed was inconclusive, however we only extracted ICD10 codes for hypovolemia and hypotension but were not able to account for the other diverse etiologies for hypovolemic hyponatremia (e.g., bleeding, third spacing or gastrointestinal fluid loss) and therefore, a reliable subset analysis was not possible. In addition, there was no difference in the subgroup of patients with heart failure, liver cirrhosis or chronic kidney disease as comorbidities. Because we did not perform chart review, we were not able to recognize patients with decompensated aforementioned conditions that might have been causative for hyponatremia.

Second, the inhibition of the SGLT2 increases glucosuria and natriuresis[201]. One could argue that it would increase urinary sodium clearance and worsen hyponatremia. However, hyponatremia is not a side effect of SGLT2 inhibitors, mainly because the pathophysiology of hyponatremia relies more on a relative water excess than an absolute sodium deficit[202]. Interestingly, our data showed no difference in urine sodium concentration, and fractional excretion of sodium between patients with SIAD treated with empagliflozin or a placebo[29,148]. In patients with T2DM, natriuresis seems to be transient as well[203].

Of note, all patients in this study have T2DM. Even though benefits from SGLT2 inhibitors in heart failure[191,204] and CKD[136] are irrespective of T2DM, the current findings cannot be extended to patients without T2DM. Glucosuria is more prominent in T2DM[133,205], therefore osmotic diuresis might be greater and favor hypovolemic hyponatremia. Furthermore, we were not able to record treatment duration. Patients with T2DM treated with an SGLT2 inhibitor initially show a reduction of extracellular fluid and an activation of the renin angiotensin aldosterone system (RAAS), both of which do not persist after 6 months of treatment[206], whereas reduction of extracellular volume persists after 12 weeks in patients with heart failure independently of diabetes [142], which support the hypothesis that the effect of SGLT2 inhibitor might differ in patients with a relative water excess (e.g., heart failure, SIAD). In support of this, a recent post-hoc analysis of the DAPA-HF placebo-controlled trial investigating the effect of dapagliflozin 10mg in patients with HFrEF, showed a higher prevalence of hyponatremia after 14 days (11.3% vs 9.4%; *P* = 0.04) but a reduced prevalence of hyponatremia after 12 months (4.6% vs 6.7%; *P* = 0.003) in the dapagliflozin group[207].

Two of our randomized double-blind placebo controlled trials provided evidence that empagliflozin is an effective treatment first, in hospitalized patients with SIAD[148] and second, in outpatients with chronic SIAD[29]. Furthermore, a post hoc analysis in patients with HFrEF[207] and case reports in patients with liver cirrhosis[208] suggest that SGLT2 inhibitors might represent an effective option these hyponatremic subgroups. Long-term SGLT2-inhibitor treatment might only influence plasma sodium levels in patients with overt euvolemic or hypervolemic hyponatremia, i.e., with a relative body water excess. The effect of SGLT2 inhibitors in hypervolemic hyponatremic patients with heart failure or liver cirrhosis is currently investigated in a multicentric, randomized, double-blind, placebo controlled trial (NCT04447911).

Finally, cross-sectional studies provide helpful insight into associations but yield poor information about causal relationships. Therefore, findings should be cautiously interpreted[209]. The incongruence between our retrospective observational results and our prospective randomized data[29,148] underlines this limitation.

10.6 Conclusion

Based on this cross-sectional retrospective study, SGLT2 inhibitors do not prevent hyponatremia development. These findings do not support their use as hyponatremia prophylaxis in at-risk patients. Prospective randomized data suggest their efficacy at a higher dosage in overt SIAD[29,148], but their efficacy in other hyponatremia subtypes remains to be demonstrated. An ongoing randomized placebo-controlled studies will help better define the role of empagliflozin in overt euvolemic and hypervolemic hyponatremia (NCT04447911).

10.7 Acknowledgments

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10.8 Authors Contribution

SM conceived, designed and performed the analysis and wrote the first draft of the manuscript. CA and JR reviewed the manuscript. MCC revised the manuscript and supervised all steps of the work.

10.9 Funding

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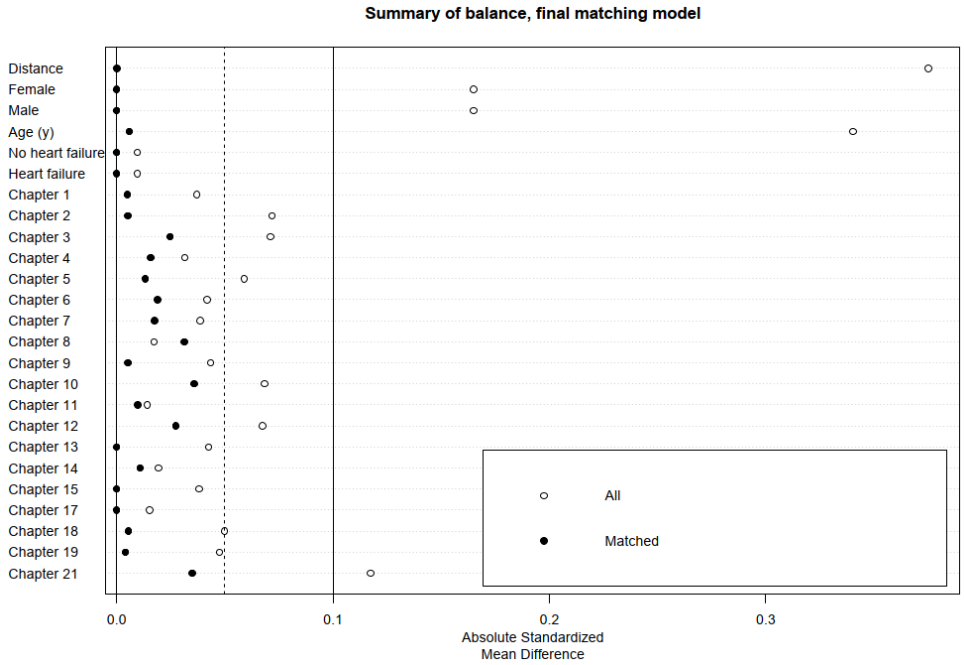
10.10 Disclosure Statement:

SM, CA, JR and MCC have nothing to disclose.

10.11 Supplementary Material

Supplementary Material M2.1 Covariate Balance Before and After Matching (Love Plot): Covariate balance before (all) and after (Matched) matching. All covariates are balanced within a threshold of 0.1 for the absolute standardized mean difference. Chapters refer to ICD10 chapters, details are displayed in Table M2.2.



<u>Supplementary Material M2.2 Logistic Regression:</u> Summary output of the univariable and best multivariable logistic regression model (selected by AIC). Significant predictors are displayed bold. Estimates represent odds ratio and are displayed with 95% confidence interval.

Logistic regression: output				
Dependent variable: hyponatremia (plasma sodium < 135mmol/l, corrected for glucose)				
	Univariable model		Best multivariable model	
Fixed predictor (OR, 95%-CI)				
SGLT2 inhibitor	1.12 (0.79-1.45)	*P* = 0.499	1.08 (0.72-1.44)	*P* = 0.666
Predictors after fitting (OR, 95%-CI)				
Insulin			**0.51 (0.10-0.92)**	***P* = 0.002**
Loop diuretic			**0.49 (0.016-0.96)**	***P* = 0.004**
Mineralocorticoid receptor antagonist			**3.56 (3.04-4.08)**	***P* < 0.001**
SSRI			1.55 (1.02-2.07)	*P* =0.104
Sulfonylureas			0.60 (-0.09-1.29)	*P* = 0.148
Tetracyclic antidepressant			**3.71 (2.95-4.47)**	***P* = 0.001**
Thiazide			1.41 (1-1.82)	*P* = 0.103
Acute kidney injury			**3.75 (3.28-4.22)**	***P* < 0.001**
Chronic kidney disease			**1.49 (1.09-1.88)**	***P* = 0.048**
Coronary heart disease			**0.56 (0.18-0.94)**	***P* = 0.003**
Pneumonia			**2.15 (1.68-2.63)**	***P* = 0.002**
Stroke			0.45 (-0.50-1.41)	*P* = 0.104
Tuberculosis			**6.19 (4.48-7.91)**	***P* = 0.038**
Intercept				
Constant	0.098 (-0.14-0.34)		0.092 (-0.24, 0.42)	
Statistics				
Observations	1642		1642	
Log Likelihood	-510.79		-473.75	
AIC	1025.57		946.37	

95%-CI = 95% confidence interval, AIC = Akaike information criterion, P = p-value, SGLT2 = sodium glucose co-transporter 2, SSRI = selective serotonin reuptake inhibitors.

Supplementary Material M2.3 Linear Regression: Summary output of the univariable and best multivariable linear model (selected by AIC). Significant predictors are displayed bold. Estimates represent sodium changes (in mmol/l) and are displayed with 95% confidence interval.

Linear regression: output Dependent variable: plasma sodium (mmol/l), corrected for glucose				
	Univariable model		Best multivariable model	
Fixed predictor (OR, 95%-CI)				
SGLT2 inhibitor	0.08 (-0.35-0.51)	*P* = 0.712	0.24 (-0.20-0.68)	*P* = 0.280
Predictors after fitting (OR, 95%-CI)				
Acute kidney injury			**-1.10 (-1.87-(-0.32))**	***P* = 0.006**
Hypovolemia			**2.14 (0.89-3.4)**	***P* = 0.001**
Chronic kidney disease			-0.39 (-0.87-0.09)	*P* = 0.113
Liver cirrhosis			-1.39 (-3.03-0.24)	*P* = 0.095
Seizure			0.96 (-0.13-2.05)	*P* = 0.085
Tuberculosis			**-3.53 (-6.73-(-0.34))**	***P* = 0.031**
Beta blocker			0.45 (-0.002, 0.89)	*P* = 0.052
Biguanide			**-0.47 (-0.91-(-0.03))**	***P* = 0.036**
Calcium channel antagonist			**0.58 (0.06-1.10)**	***P* = 0.03**
Insulin			0.46 (-0.01-0.92)	*P* = 0.053
Mineralocorticoid receptor antagonist			**-1.50 (-2.19-(-0.80))**	***P* < 0.001**
SSRI			-0.70 (-1.43-0.02)	*P* = 0.06
Tetracyclic antidepressant			-1.17 (-2.46-0.14)	*P* = 0.08
Thiazide			-0.429 (-0.99,0.13)	*P* = 0.132
ICD-10 chapter of admission diagnosis				
II - Neoplasms			0.69 (-0.52-1.89)	*P* = 0.267
III - Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism			2.33 (-1.51-6.18)	*P* = 0.235
IV - Endocrine, nutritional and metabolic diseases			**1.68 (0.48-2.87)**	***P* = 0.007**
V - Mental and behavioural disorders			1.30 (-1.18-3.78)	*P* = 0.306
VI - Diseases of the nervous system			1.26 (-0.14-2.65)	*P* = 0.078
VII - Diseases of the eye and adnexa			1.87 (-1.41-5.16)	*P* = 0.264
VIII - Diseases of the ear and mastoid process			-0.60 (-3.70-2.49)	*P* = 0.703
IX - Diseases of the circulatory system			**1.98 (1.06-2.90)**	***P* < 0.001**
X - Diseases of the respiratory system			0.73 (-0.31-1.78)	*P* = 0.169
XI - Diseases of the digestive system			**1.37 (0.21-2.53)**	***P* = 0.021**
				(continued)

Linear regression: output (continued) Dependent variable: plasma sodium (mmol/l), corrected for glucose			
		Best multivariable model	
Predictors after fitting (OR, 95%-CI)			
XII - Diseases of the skin and subcutaneous tissue		-0.54 (-2.36-1.29)	*P* = 0.564
XIII - Diseases of the musculoskeletal system and connective tissue		0.613 (-0.65-1.87)	*P* = 0.341
XIV - Diseases of the genitourinary system		-0.35 (-1.58-0.88)	*P* = 0.579
XVII - Congenital malformations, deformations and chromosomal abnormalities		1.053 (-4.94-7.05)	*P* = 0.731
XVIII - Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified		0.92 (-0.32-2.17)	*P* = 0.148
XIX - Injury, poisoning and certain other consequences of external causes		0.945 (-0.13-2.02)	*P* = 0.085
XXI - Factors influencing health status and contact with health services		0.13 (-8.36-8.61)	*P* = 0.977
Intercept			
Constant	139.77 (139.47, 140.07)	138.75 (137.83-139.68)	
Statistics			
R^2	0.0001	0.078	
Adjusted R^2	-0.001	0.059	
Residual Std. Error	4.41 (df = 1640)	4.27 (df = 1608)	
Observations	1642	1642	
AIC	9533.4	9464	

95%-CI = 95% confidence interval, AIC = Akaike information criterion, df = degree of freedom, ICD = International Classification of Diseases, P = p-value, SGLT2 = sodium glucose co-transporter 2, SSRI = selective serotonin reuptake inhibitors

Supplementary Material M2.4 Prevalence of Dysnatremia on Admission in Non-Hypovolemic Patients: Prevalence of dysnatremia on admission in non-hypovolemic diabetic patient treated with an SGLT2 inhibitor and in diabetic matched controls (plasma sodium corrected for glucose). The two groups were compared with a chi-quared test.

	No SGLT2 inhibitor on admission (n=791)	SGLT2 inhibitor on admission (n=770)	*P* Value
Hypernatremia (Plasma sodium > 145 mmol/l), n (%)	40 (5.1)	26 (3.4)	0.128
Normonatremia (Plasma sodium 135-145 mmol/l), n (%)	680 (86.0)	673 (87.4)	0.447
Hyponatremia (Plasma sodium < 135 mmol/l) , n (%)	71 (9.0)	71 (9.2)	0.936
Mild hyponatremia (Plasma sodium 130-134 mmol/l), n (%)	55 (7.0)	57 (7.4)	0.806
Moderate hyponatremia (Plasma sodium 125-129 mmol/l), n (%)	11 (1.4)	8 (1.0)	0.687
Profound hyponatremia (Plasma sodium < 125 mmol/l), n (%)	5 (0.6)	6 (0.8)	0.964
SGLT2 = sodium glucose co-transporter 2, 95%-CI = 95% confidence interval			

Supplementary Material M2.5: Prevalence of Dysnatremia on Admission in Hypervolemic Patients: Prevalence of dysnatremia on admission in hypervolemic diabetic patient treated with an SGLT2 inhibitor and in diabetic matched controls (plasma sodium corrected for glucose). The two groups were comparedwith a chi-quared test.

	No SGLT2 inhibitor on admission (n=346)	SGLT2 inhibitor on admission (n=324)	P Value
Hypernatremia (Plasma sodium > 145 mmol/l), n (%)	20 (5.8)	17 (5.2)	0.128
Normonatremia (Plasma sodium 135-145 mmol/l), n (%)	680 (86.0)	673 (87.4)	0.447
Hyponatremia (Plasma sodium < 135 mmol/l) , n (%)	71 (9.0)	71 (9.2)	0.936
Mild hyponatremia (Plasma sodium 130-134 mmol/l), n (%)	27 (7.8)	35 (10.8)	0.228
Moderate hyponatremia (Plasma sodium 125-129 mmol/l), n (%)	11 (1.4)	8 (1.0)	0.860
Profound hyponatremia (Plasma sodium < 125 mmol/l), n (%)	3 (0.9)	3 (0.9)	1
SGLT2 = sodium glucose co-transporter 2, 95%-CI = 95% confidence interval			

11 MANUSCRIPT 3: EFFECT OF PROTEIN SUPPLEMENTATION ON PLASMA SODIUM LEVELS AND URINARY UREA EXCRETION IN PATIENTS WITH CHRONIC SIAD – A MONOCENTRIC OPEN-LABEL PROOF-OF-CONCEPT STUDY - THE TREASURE STUDY

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[+] SM and CA have contributed equally and share first authorship.

Article status: submitted for publication

11.1 ABSTRACT

INTRODUCTION The syndrome of inappropriate antidiuresis (SIAD) can be treated with oral urea. Proteins are metabolized into urea by the liver. We hypothesized that dietary protein could increase free water clearance through urea-induced osmotic diuresis and aimed to investigate the effect of a high-protein supplementation on plasma sodium levels in chronic SIAD.

METHODS This is a monocentric open-label proof-of-concept trial conducted at the University Hospital of Basel, Switzerland, between 10/2021 and 02/2023. Adult outpatients with chronic SIAD of any etiology were eligible. Patients received 90 g protein daily for 7 days in the form of protein powder dissolved in a maximum of 1l of liquid of choice. After a wash-out period of at least a week, patients received 30 g of oral urea daily for 7 days. The primary endpoint was the increase in sodium levels from baseline to the end of the 7-day protein supplementation.

RESULTS Seventeen patients were included (14 females, median age 68 [61, 79]). After 7 days of 90 g daily protein supplementation (n = 17), plasma sodium levels increased by a median of 3 mmol/l [0, 5] (*P* = 0.01), plasma urea by a median of 3 mmol/l [1.7, 4.9] and urinary urea corrected for urine creatinine by a median of 21.2 mmol/mmol [6.2, 29.1]. After 7 days of oral urea (n = 10), sodium levels increased by a median of 2 mmol/l [1, 3], plasma urea by a median of 5.8 mmol/l [2.7, 9.2] and urinary urea corrected for urine creatinine by a median of 31.0 mmol/mmol [18.7, 45.1].

CONCLUSION Our findings suggest that a high-protein supplementation with protein powder increases plasma sodium levels in patients with chronic SIAD through protein-induced ureagenesis.

11.2 INTRODUCTION

Hyponatremia, defined as a plasma sodium concentration < 135 mmol/l, is the most frequent electrolyte disorder in both in- and outpatient setting[1]. Chronic hyponatremia is associated with increased mortality[67] and morbidity, such as neurocognitive and neuromuscular disorders[4,29], resulting in increased rates of falls and fractures[167]. There is increasing evidence that correcting hyponatremia could improve clinical outcomes[4,21,26,29,86,210].

The most common etiology of euvolemic hyponatremia is the syndrome of inappropriate antidiuresis (SIAD)[211]. SIAD is characterized by an imbalanced arginine vasopressin (AVP) secretion or an increased renal AVP sensitivity, leading to excessive free water retention[94,98]. This excess in water can be therapeutically addressed by either restricting fluid intake[117] and/or increasing renal free water excretion. The latter can be achieved by diluting urine with AVP receptor antagonists (vaptans)[19] or by inducing osmotic diuresis with SGLT2-inhibitors[29,148] or oral urea[155].

The efficacy of urea has been demonstrated in several cohorts either as an adjunct treatment to fluid restriction or in patients with SIAD in whom fluid restriction is not possible or is contraindicated[161,212]. A prospective study in 12 patients suggested that oral urea is safe, well tolerated, and as effective as tolvaptan in treating SIAD over one year[161]. A daily dosage of 15 to 60 g is usually required and is best dissolved in a strongly flavored beverage (e.g., orange juice) to cover its bitter taste. Unfortunately, this poor palatability is also the main reason for low long-term patient acceptance.

Endogenous and dietary proteins are metabolized into nitrogen which is metabolized to soluble excretable urea by the liver. Both a 20% protein diet and a low protein diet combined with oral urea increased sodium concentration, reduced natriuresis, and increased inner medullary urea concentration in rats with induced SIAD, compared to the ones fed with a low protein diet[162]. It has further been reported that osmotic urea diuresis can cause hypernatremia in catabolic critically ill patients in the intensive care unit[164,165], indicating that urea generated from endogenous proteins can affect sodium levels.

To our knowledge, the effect of a high-protein diet on sodium levels in patients with chronic SIAD has never been investigated in a controlled trial. Therefore, we aimed to investigate the effect of a 1-week daily administration of 90 g protein versus 30 g oral urea on plasma sodium and urea levels and urinary urea excretion. We hypothesized that a high-protein supplementation would increase plasma sodium levels in patients with SIAD through protein-induced ureagenesis and resulting osmotic diuresis.

11.3 METHODS

11.3.1 Study Design and Participants

This prospective monocentric open-label proof-of-concept study was performed at the University Hospital of Basel, Switzerland, from October 2021 to February 2023. The local ethic committee (EKNZ 2021-01116) approved the study protocol. The study was registered at ClinicalTrials.gov (NCT04987385) and conducted in accordance with the Declaration of Helsinki.

Eligible patients were 18 years of age or older and had a previously documented diagnosis of chronic SIAD that was confirmed at inclusion based on established diagnostic criteria[3]: plasma sodium concentration < 135 mmol/l, plasma osmolality < 300 mOsm/kg, urine osmolality > 100 mOsm/kg, urine sodium concentration > 30 mmol/l, clinical euvolemia (absence of orthostasis, tachycardia, decreased skin turgor, dry mucous membranes, edema, and ascites[97]) and the absence of uncontrolled hypothyroidism or uncontrolled adrenal insufficiency. Main exclusion criteria included known hypersensitivity or allergy to one of the components of the protein supplementation; inborn metabolic disorders; severe symptomatic hyponatremia in need of treatment with 3 % NaCl-solution or of intensive/intermediate care treatment; risk factors for osmotic demyelination syndrome: hypokalemia (K < 3,4 mmol/l), malnutrition, advanced liver disease, alcoholism; type 1 diabetes mellitus and uncontrolled type 2 diabetes mellitus (defined as HbA1c > 8.0 %); estimated glomerular filtration rate (eGFR) < 60 ml/min/1,73 m^2, severe hepatic impairment (ALAT/ASAT > 3x upper limit) or advanced symptomatic liver disease defined as past or current hepatic encephalopathy, liver cirrhosis Child C or decompensated (bleeding, jaundice, hepatorenal syndrome); leucocytes < 2G; treatment with a diuretic, an SGLT2 inhibitor or a corresponding combined preparation, lithium chloride, urea, vaptans or demeclocycline in the 7 days before screening; pregnancy and breastfeeding; end of life care.

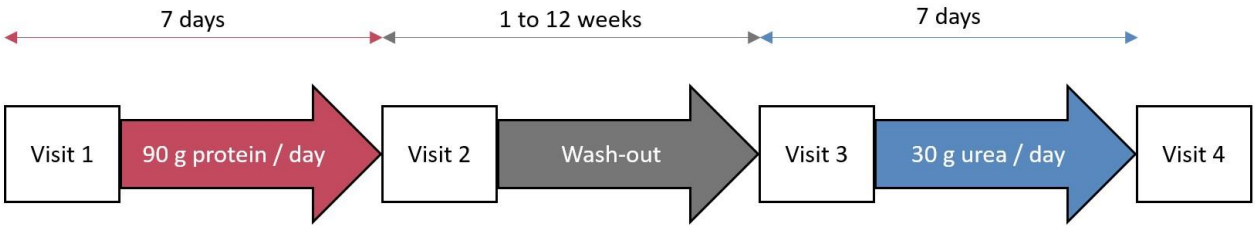
11.3.2 Study Intervention and Procedures

Outpatients with chronic hyponatremia due to SIAD from the University Hospital of Basel and patients referred from endocrinologists, neurologists, and family doctors in practice were asked to participate. Each patient provided written informed consent.

During the first intervention period, 90 g of protein in the form of protein powder were administered daily for seven days (*Supplementary Material M3.1*) which corresponds roughly to 30 g urea when using the Jones' factor of 6.25 to convert nitrogen to protein equivalent[213]. Daily doses were weighed in advance in seven sealable containers and labeled with the intake date. Patients dissolved the content of one container every day in a maximum of 1 l of liquid of choice that was integrated in the usual daily hydration volume documented at baseline. After a wash-out phase of at least one week and a maximum of twelve weeks, patients underwent a second analog treatment regimen but with oral urea instead of protein for seven days (*Figure M3.1*). Patients

were asked to document their daily fluid intake during each treatment phase and during the wash-out week preceding the start of the urea treatment phase, while keeping their usual fluid intake and meal composition unchanged throughout the whole study. In order to account for the volume of the protein/urea drinks the remaining beverages were reduced in order to keep the daily hydration volume constant. Three patients were on fluid restriction (1 000 to 1 300 ml per day) on inclusion which was maintained during the whole study.

Figure M.3.1 Study Diagram



Every visit included a routine physical examination, a control of body weight and vital signs, a questionnaire about dietary habits and hyponatremia symptoms, as well as fasting blood and urine samplings. Patients with profound hyponatremia (< 125 mmol/l) were asked to come for an additional blood sampling on the day following treatment start to timely recognize any overly rapid correction and initiate relowering counteractions if needed. An increase in plasma sodium levels > 10 mmol/l in the first 24 h of treatment was defined as overcorrection[3].

11.3.3 Laboratory Measurements

Plasma and urine parameters were measured by the central laboratory of the University Hospital Basel. Plasma sodium levels were analyzed by indirect ion selective electrode (ISE) method (cobas 8000 modular analyzer; Roche Diagnostics). Serum and urinary osmolality were measured using the freezing point depression osmometer method.

11.3.4 Sample Size Estimation

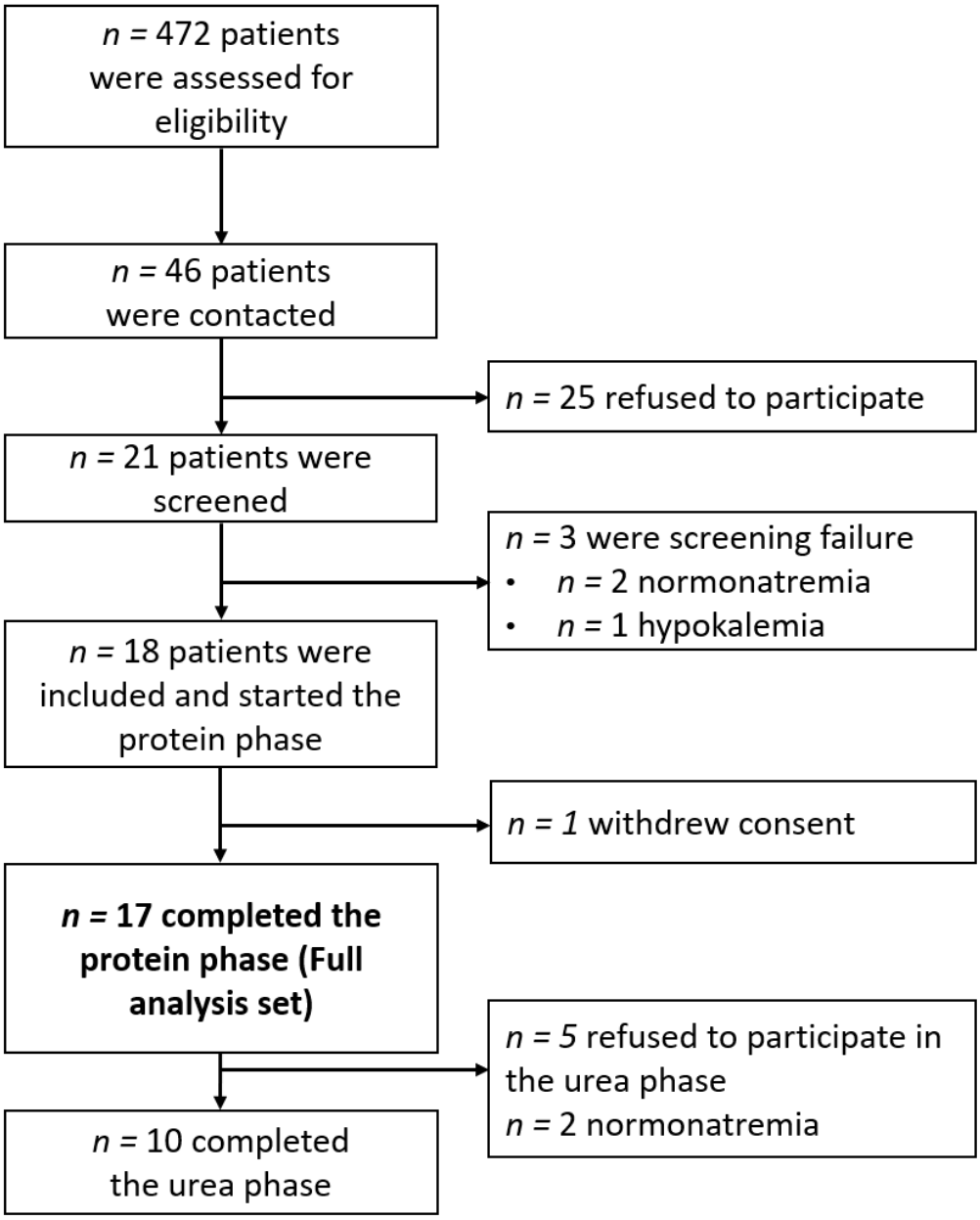
We used the A'Hern's single-stage phase 2 approach to determine sample size[31] and defined a sodium improvement goal of ≥ 3mmol/l from baseline to day 7 as endpoint for efficacy. We set the maximal unacceptable level of efficacy (p0) (i.e., a response rate inferior to which the intervention is considered ineffective) to 20%, the expected level of efficacy (p1) to ≥ 50%, the type I error probability α to 0.05 and the type II error probability β to 0.20, which led to a target sample size of n = 17 patients.

11.3.5 Analysis Sets

In total, 18 patients with chronic SIAD were included (*Figure M3.2*). Of those, n = 17 completed the protein phase and could be evaluated for the primary endpoint. Four patients wished to

terminate the study at the end of the protein phase. After the wash-out period, n = 13 patients came to visit 3, of which n = 2 (15%) were not eligible for the urea phase due to normonatremia, n=1 (8%) refused the second phase despite being hyponatremic, and n = 10 (77%, respectively 59% of the whole cohort) completed the urea phase. The two patients who remained normonatremic had and chronic mild recurrent hyponatremia for three years and 6 months respectively. We assessed the primary endpoint first for all patients (n = 17) and then for the subset of patients who completed both phases (n = 10).

Figure M3.2 Study Flow Chart



11.3.6 Objectives and Statistical Analysis

The primary objective was to determine the effect of a 7-day 90 g protein supplementation on plasma sodium levels (in mmol/l) from baseline to the end of treatment. Secondary objectives were the assessment of changes from baseline to end of treatment (after 7 days) with 7-day 90 g protein supplementation and 7-day 30 g urea intake in blood and urine -sodium, osmolality, -potassium, -creatinine, -urea, -uric acid, -glucose, eGFR, course of general well-being measured by visual analog scale (VAS, 0-10), symptoms of hyponatremia (vertigo, headache, nausea, attention deficit, mental slowness, forgetfulness, gait instability) assessed by a questionnaire (yes/no), change in oral daily fluid intake assessed by a self-completed drinking protocol and change in vital parameters.

Baseline characteristics are summarized using descriptive statistics. Discrete variables are expressed as frequencies (percentage (%) and number of patients (n)). Continuous variables are expressed as median and interquartile range (IQR, 25^{th} to 75^{th} percentiles). The primary objective was assessed using the Wilcoxon rank-sum test and visualized using boxplots. Hypothesis testing was two-sided, and p-values < 0.05 were considered statistically significant. The correlation between changes in plasma sodium levels and change in urine urea corrected by creatinine and change in plasma urea were assessed using the Pearson correlation coefficient and are presented visually with scatterplots. We further assessed the predictive value of baseline plasma sodium, eGFR, BMI, urine sodium, urine osmolality, and urine/plasma ratio on the increase in plasma sodium or treatment response based on the predefined sodium improvement goal (responder: ≥ 3 mmol/l, non-responder: sodium increase ≤ 2 mmol/l) in a multivariable linear or logistic regression model. Secondary objectives were assessed using descriptive statistics. In addition, we performed sensitivity analyses by excluding patients consuming less than 5 out of 7 doses of 90 g protein or 30 g urea and those with changes in concurrent medications or treatments. All analyses were performed in R version 4.2.2 (2022/10/31)[172].

11.4 RESULTS

11.4.1 Baseline Characteristics

From October 01, 2021, to February 01, 2023, 18 patients with chronic SIAD were included. The median [IQR] age was 68 years ([61, 79], 83% (n = 15) females). Aetiologies of SIAD were drug-induced (60%, n = 10), malignant disease (6%, n = 1), lung disease (12%, n = 2), and disease of the central nervous system (24%, n = 4). Median duration of SIAD was 6 years [1, 10], and the last plasma sodium level prior to inclusion was 129 mmol/l [127, 132]. Baseline characteristics are summarized in *Table M3.1.*

Table M3.1 Baseline Characteristics

Variables	Patients with chronic SIAD (n = 17)
Sex, female	14 (82)
Age, years	68 [61, 79]
Ethnicity, caucasian	17 (100)
Weight, kg	58 [56, 61]
Height, cm	165 [164, 168]
BMI	21 [20, 24]
Estimated daily fluid intake, ml	1750 [1300, 2000]
Hyponatremia treatment: mild (1 - 1.3 l) fluid restriction	3 (19)
Last sodium prior to inclusion, mmol/l	129 [127, 132]
Hyponatremia severity at inclusion	
Mild, plasma sodium 130 - 134 mmol/l	11 (65)
Moderate, plasma sodium 125 - 129 mmol/l	4 (24)
Profound, plasma sodium < 125 mmol/l	2 (12)
Aetiology of SIAD	
Drug induced	10 (60)
Malignant disease	1 (6)
Lung disease	2 (12)
CNS disease	4 (24)
Treatment hypothyroidism	3 (19)
Comorbidities	
Pulmonal disease	4 (24)
Cardiovascular disease	5 (29)
Epilepsy	3 (18)
Psychiatric disease	4 (24)
Osteoporosis	3 (18)
Neurological disease	3 (18)
Malignant disease	2 (12)
History of falls in the last 12 months	5 (29)
Diet	
Omnivorous	16 (94)
Vegetarian	1 (6)
Meat consumption	
Daily	1 (6)
≥ 3x/week	9 (53)
1-2x/week	5 (29)
Never	2 (12)
Egg/dairy products/soy consumption	
At every meal	5 (29)
Daily	7 (41)
≥ 3x/week	2 (12)
Never	3 (18)
Regular consumption of protein supplements	2 (12)

Data are presented in median [IQR] and n (%). BMI = Body mass index, CNS = Central nervous system

11.4.2 Effect of Protein Supplementation

In total, 17 patients underwent the protein phase; 16 patients consumed the minimum required total protein dose of 90 g for ≥ 5 days and one patient consumed 90 g for 2 days. At baseline, the median plasma sodium concentration was 131 mmol/l [124, 135]; 65% (n = 11) had mild hyponatremia, 24% (n = 3) had moderate hyponatremia, and 12% (n = 2) had profound hyponatremia. After 7 days of daily protein supplementation, plasma sodium levels significantly increased to 133 mmol/l [132, 137], resulting in a median increase of 3 mmol/l [0, 5] (*P* = 0.01) (*Figure M3.3, Table M3.2, Supplementary Material M3.2*). After excluding patients with insufficient protein intake (n = 1) and potential concurrent treatment effects (i.e., one patient with reduction in antiepileptic drug dose during the intervention phase, and one patient with intravenous fluid administration), our sensitivity analysis demonstrated an unchanged plasma sodium increase of 3 mmol/l [0, 5] in the remaining patients (n = 14) (*P* = 0.01). After 7 days of daily protein supplementation, plasma urea increased by 3.0 mmol/l [1.7, 4.9], and urine urea corrected for urine creatinine increased by 21.2 mmol/mmol [6.2, 29.1] (*Figure M3.3, Table M3.2, Supplementary Material M3.2*). In the subgroup analysis including only patients who completed both study phases (n = 10), courses of plasma sodium, plasma urea, and urine urea corrected for urine creatinine were comparable to the full analysis set (*Supplementary Material M3.3)*.

Changes in fractional excretion (FE) of sodium, urea, and uric acid are demonstrated in *Figure M3.4* and *Table M3.2*. Change in plasma sodium levels showed a significant moderate correlation with the change in urine urea corrected for urine creatinine (R = 0.50, *P* = 0.01), whereas no correlation was observed with the change in plasma urea (R = 0.05, *P* = 0.80) (*Figure M3.5*).

In a multivariable linear regression model, baseline plasma sodium, eGFR, BMI, urine sodium, urine osmolality, and urine/plasma ratio showed no predictive value for the increase in plasma sodium (*Supplementary Material M3.4*). In a multivariable logistic regression model, the same variables showed no predictive value for a successful response defined as a sodium increase of ≥ 3mmol/l (*Supplementary Material M3.5*).

11.4.3 Effect of Oral Urea Administration

In total, 10 patients underwent the urea phase, 9 consumed the minimum required total urea dose of 30 g for ≥ 5 days, and one patient consumed 30 g for 3 days. At the beginning of the urea phase, the median plasma sodium concentration was 132 mmol/l [130, 133]; 80% (n = 8) had mild hyponatremia, 10% (n = 1) had moderate hyponatremia, and 10% (n = 1) had profound hyponatremia. After 7 days of daily urea intake, plasma sodium levels increased to 134 mmol/l [131, 136], resulting in a median increase of 2 mmol/l [1, 3] (*Figure M3.3*). After excluding one patient due to insufficient urea intake (n = 1), our sensitivity analysis demonstrated an unchanged plasma sodium increase of 2 mmol/l [2, 3] in the remaining patients (n = 9). Upon 7 days of daily urea intake, plasma urea increased by 5.8 mmol/l [2.7, 9.2], and urine urea corrected for urine

creatinine increased by 31.0 mmol/mmol [18.7, 45.1] (*Figure M3.3, Table M3.2*). Changes in FE of sodium, urea, and uric acid are demonstrated in *Figure M3.4* and *Table M3.2*.

Figure M3.3. Plasma Sodium, Plasma Urea, and Urine Urea/Creatinine Ratio by Treatment Phase: (A) Course and (B) change in plasma sodium levels, (C) plasma urea, and (D) urine urea/creatinine ratio visualized by treatment phase (protein phase in red; urea phase in blue). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

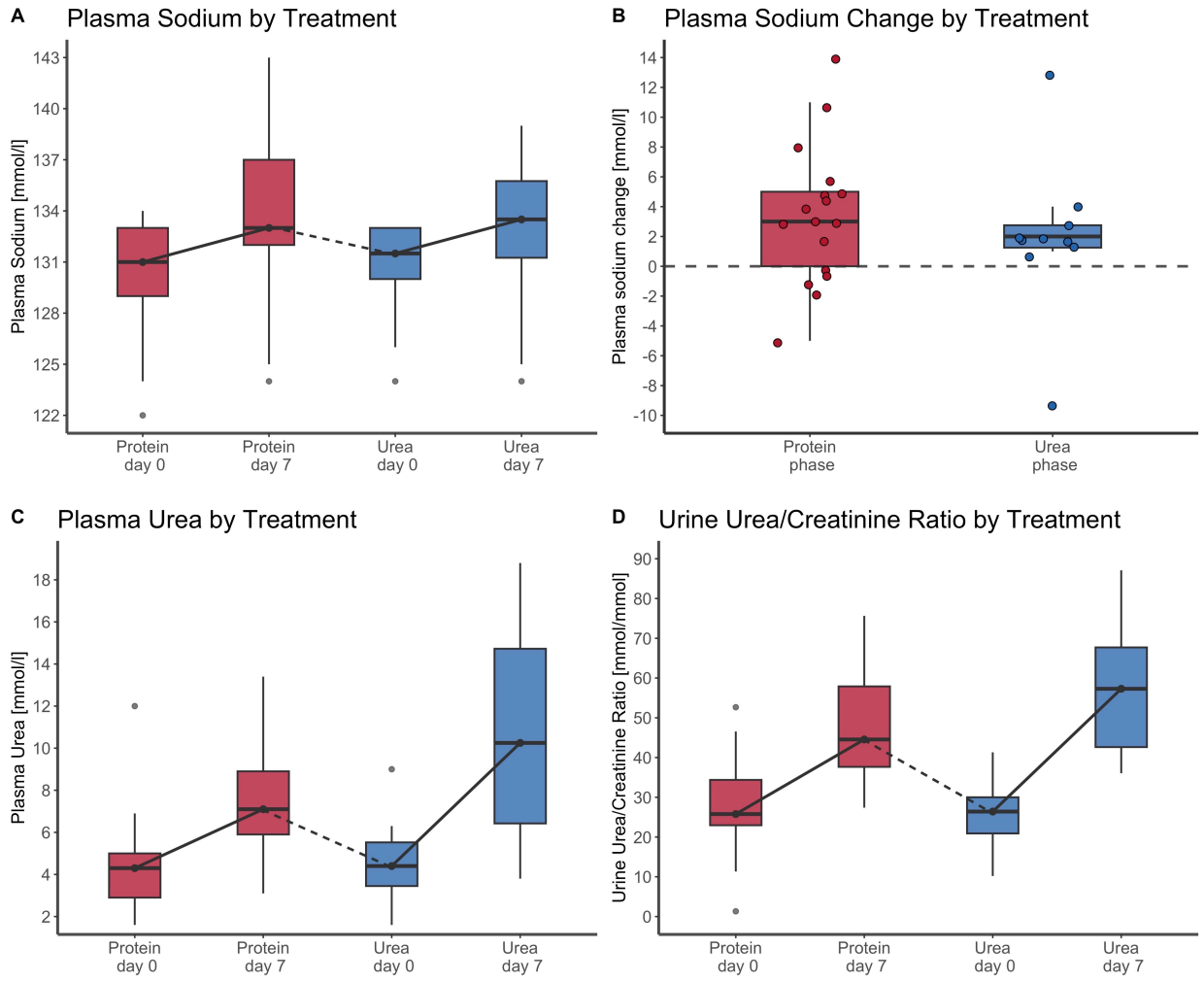


Table M3.2. Clinical Parameters and Laboratory Results

	Protein phase day 0 (n=17)	**Protein phase day 7 (n=17)**	**Urea phase day 0 (n=10)**	**Urea phase day 7 (n=10)**
Weight, kg	58 [56, 61]	57 [56, 61]	56 [55, 60]	58 [56, 61]
Blood pressure, systolic, mmHg	131 [124, 135]	127 [111, 131]	127 [122.8, 135.2]	131 [110, 138]
Blood pressure, diastolic, mmHg	73 [73, 87]	78 [71, 82]	84.5 [75, 86.8]	75 [71, 82]
Heart rate, BPM	68 [65, 75]	72 [68, 79]	68 [62, 75]	75 [70, 76]
Plasma sodium, mmol/l	131 [129, 133]	133 [132, 137]	132 [130, 133]	134 [131, 136]
Plasma urea, mmol/l	4.3 [2.9, 5.0]	7.1 [5.9, 8.9]	4.4 [3.4, 5.5]	10.2 [6.4, 14.7]
Plasma osmolality, mmol/kg	274 [265, 279]	287 [278, 289]	275 [272, 279]	290 [281, 292]
Plasma potassium, mmol/l	4.3 [4.2, 4.6]	4.3 [4.2, 4.5]	4.2 [4.1, 4.4]	4.2 [4.1, 4.4]
Plasma creatinine, µmol/l	62 [57, 72]	59 [53, 67]	63 [59, 69]	66 [60, 73]
Plasma uric acid, µmol/l	217 [167, 262]	210 [182, 232]	228 [191, 252]	194 [184, 235]
Plasma albumin, g/l	38 [36, 42]	37 [36, 40]	37 [34, 38]	37 [35, 39]
Plasma glucose, mmol/l	5.3 [4.9, 5.6]	5.2 [4.8, 5.9]	5.3 [4.6, 5.8]	5.2 [5.1, 5.8]
Estimated GFR, ml/min	88 [75, 96]	89 [83, 98]	84.5 [79, 98]	76 [74, 90]
Urine sodium, mmol/l	47 [40, 83]	60 [29, 68]	61 [53, 87]	52 [42, 60]
Urine urea, mmol/l	124 [86, 184]	300 [189, 433]	103 [102, 205]	349 [260, 467]
Urine osmolality, mmol/kg	354 [259, 514]	548 [443, 709]	324 [292, 537]	601 [413, 783]
Urine potassium, mmol/l	50 [36, 67]	54 [39, 84]	43 [35.2, 54.5]	49 [29, 72]
Urine creatinine, mmol/l	4.6 [3.2, 7.9]	6.8 [4.5, 8.5]	5.1 [3.6, 9.5]	6.1 [3.6, 10.5]
Urine uric acid, µmol/mmol	1 812 [1 000, 2 279]	1 825 [1 229, 2 493]	1 643 [1 448, 2 414]	1 579 [1 210, 2 634]
Urine glucose, mmol/l	0.2 [0.1, 0.3]	0.3 [0.2, 0.5]	0.2 [0.2, 0.4]	0.2 [0.1, 0.3]
Urine/Plasma Ratio	0.8 [0.6-1.0]	0.9 [0.7-1.1]	0.8 [0.7-1.3]	0.7 [0.6-1.0]
FENa, %	0.5 [0.4, 0.6]	0.3 [0.2, 0.5]	0.6 [0.4, 0.8]	0.4 [0.3, 0.6]
FEUrea, %	45 [35, 50]	38 [33, 43]	42 [29, 46]	44 [35, 54]
FEUricAcid, %	9 [8, 13]	9 [7, 10]	8 [8, 12]	9 [7, 13]
Urine urea corrected for urine creatinine	26 [23, 34]	45 [38, 58]	26 [21, 30]	57 [43, 68]

Data are presented in median [IQR]. BPM = Beat per minutes, GFR = glomerular filtration rate, FENa = Fractional excretion of sodium, FEUrea = Fractional excretion of urea, FEUricAcid = Fractional uric acid excretion

Figure M3.4 Course of Fractional Excretion of Sodium, Urea, and Uric Acid by Treatment Phase: Course in fractional excretion of (A) sodium (FENa), (B) urea (FEUrea), and (C) uric acid (FEUricAcid) visualized by treatment phase (protein phase in red; urea phase in blue). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

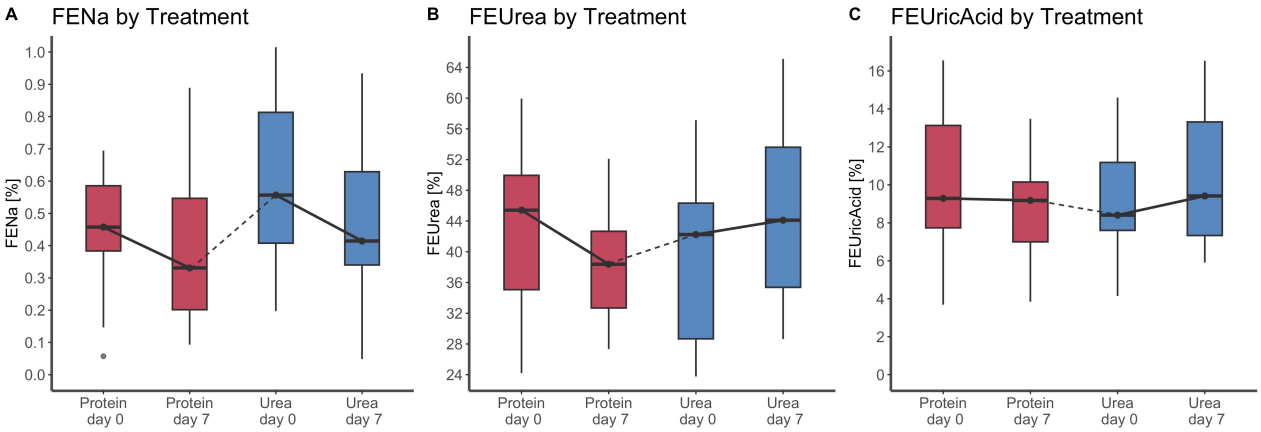
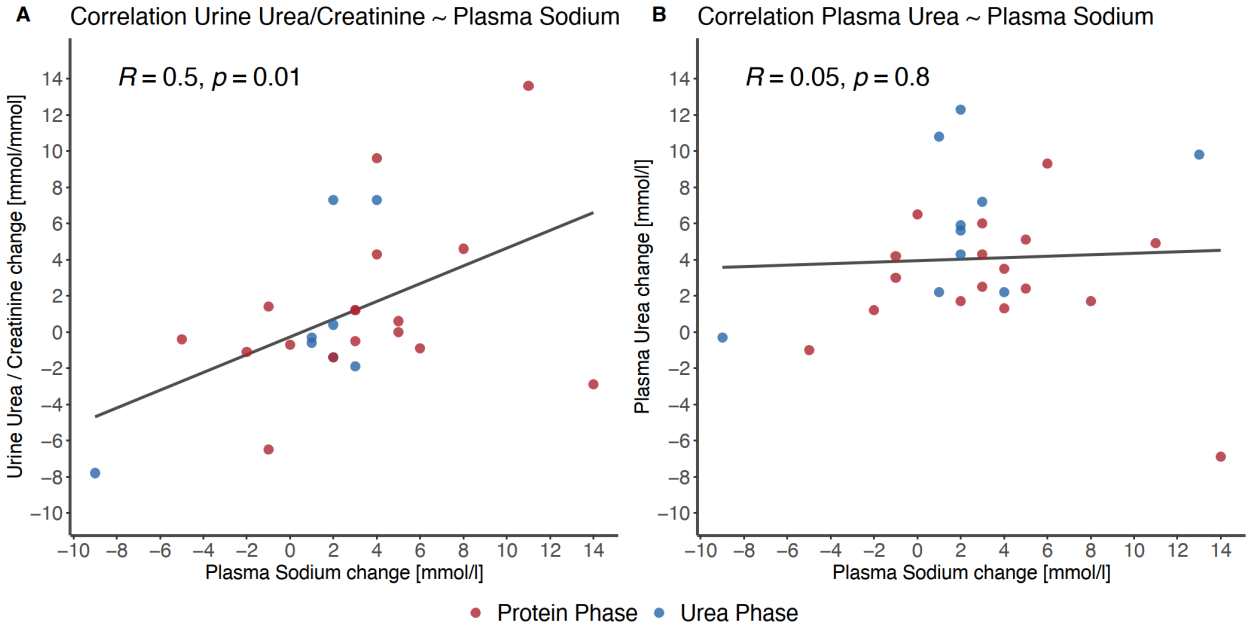


Figure M3.5 Correlation Between Changes in Urine Urea by Creatinine and Changes in Plasma Sodium by Treatment Phase: (A) The change in urine urea by creatinine versus the change in plasma sodium, and (B) the change in plasma urea versus the change in plasma sodium visualized by scatterplot and correlation line; the protein phase displayed in red, and the urea phase displayed in blue. Pearson's correlation coefficient R is given for the pooled data set.



11.4.4 Clinical Outcomes Tolerability and Safety

Vital parameters and weight remained stable throughout the study phases (*Table M3.2*). *The* estimated daily fluid intake at study inclusion was 1 750 ml [1 300, 2 000], and no relevant change was recorded throughout the study phases (*Supplementary Material M3.6*). Overall, protein supplementation and oral urea intake were safe and well-tolerated in all patients. The rating for overall well-being slightly improved from 7 VAS points [6, 8] to 8 VAS points [7, 8] upon protein

intake, whereas it slightly worsened from 7 VAS points [6, 7] to 6 VAS points [6, 7] upon urea intake.

At the beginning of the protein supplementation phase, the most commonly reported symptoms were fatigue (71%), nocturia (59%), orthostasis (53%), and gait instability (53%). After 7 days of protein supplementation, these self-reported symptoms improved or remained stable, i.e., fatigue (24%), nocturia (53%), orthostasis (12%), and gait instability (29%) (*Table M3.3*). After a wash-out period of at least one week, i.e., at the beginning of the urea phase, the most commonly reported symptoms were fatigue (40%), nocturia (50%), forgetfulness (40%), and gait instability (40%). After 7 days of urea supplementation, these self-reported symptoms mostly remained stable, i.e., fatigue (50%), nocturia (50%), forgetfulness (30%), and gait instability (30%) (*Table M3.3*).

Table M3.3 Self-Reported Clinical Symptoms

	Protein phase day 0 (n=17)	Protein phase day 7 (n=17)	Urea phase day 0 (n=10)	Urea phase day 7 (n=10)
General well-being	7 [6, 8]	8 [7, 8]	7 [6, 7]	6 [6, 7]
Pollakiuria	3 (18)	3 (18)	1 (10)	0 (0)
Nocturia	10 (59)	9 (53)	5 (50)	5 (50)
Orthostasis	9 (53)	2 (12)	2 (20)	1 (10)
Fatigue	12 (71)	4 (24)	4 (40)	5 (50)
Nausea	3 (18)	3 (18)	0 (0)	2 (20)
Vomiting	1 (6)	0 (0)	0 (0)	1 (10)
Diarrhea	1 (6)	1 (6)	0 (0)	2 (20)
Vertigo	6 (35)	3 (18)	0 (0)	0 (0)
Headache	5 (29)	2 (12)	3 (30)	1 (10)
Attention deficit	5 (29)	2 (12)	1 (10)	2 (20)
Mental slowness	5 (29)	2 (12)	3 (30)	1 (10)
Forgetfulness	7 (41)	3 (18)	4 (40)	3 (30)
Gait instability	9 (53)	5 (29)	4 (40)	3 (30)
Other	0 (0)	3 (18)	1 (10)	2 (20)
Constipation	0 (0)	1 (6)	0 (0)	0 (0)
Bloating	0 (0)	2 (12)	0 (0)	0 (0)
Depression	0 (0)	0 (0)	0 (0)	1 (10)
Acute pain finger joint	0 (0)	0 (0)	0 (0)	1 (10)
Data are presented in median [IQR] and n (%) according to visual analogue scale assessment				

In total, one adverse event of sodium overcorrection (11 mmol/l in 24 h) during the protein phase occurred and was adequately treated with oral water intake. Upon urea, no adverse events were documented. A hospitalization due to an underlying malignant disease was recorded as serious adverse event and followed-up until hospitalized patient's death six weeks after the study termination. In patients who completed both phases (n = 10), 60% (n = 6) preferred the protein intake, 30% (n = 3) preferred the urea intake, and 10% (n = 1) preferred none of both options.

11.5 DISCUSSION

This proof-of-concept study has two main findings. First, a 7-day high-protein supplementation increased plasma sodium levels in patients with chronic SIAD. Second, protein intake led to an increase in both plasma and urine urea concentration comparable to the increase upon oral urea intake, supporting the underlying mechanism of protein-induced ureagenesis.

Urine volume is not only determined by renal function, fluid intake, and AVP activity but also by solute intake and excretion[34]. Depending on the dilution capacity of the kidneys, 50-100 mOsm are required to produce 1l of urine[3]. Consequently, daily solute intake determines the volume of urine that can be excreted, especially in SIAD patients in whom urine osmolality is fixed at a higher concentration[214]. SIAD can therefore be treated by increasing electrolyte free water clearance with solute osmotic diuresis using oral urea[155,215]. Verbalis and colleagues demonstrated in a hyponatremic animal model of SIAD a comparable increase in sodium levels in rodents fed with high-protein chow compared to a low-protein chow combined with oral urea[162]. While the *American expert panel for the diagnosis, evaluation, and treatment of hyponatremia* mentioned that in humans, the osmotic effect of urea could theoretically also be achieved with dietary protein, the required amounts of protein was judged to be impractical[108]. To our knowledge, we here provide the first controlled human data on the effect of a standardized high-protein intake in patients with chronic SIAD.

We observed a median sodium increase of 3 mmol/l after 7 days of high-protein compared to 2 mmol/l after 7 days of oral urea. Our treatment effects hence stay in line with available therapeutic options such as fluid restriction of 1 000 ml/day showing a 3 mmol/l increase after 3 days[117], tolvaptan leading to an average daily sodium change of 5 mmol/l after 4 days[125] and the SGLT2 inhibitor empagliflozin demonstrating an increase of 4 mmol/l[29] after 7 days. A high-protein diet might not suffice to normalize plasma sodium levels in some patients but could be recommended in combination with other mentioned treatments, i.e., fluid restriction, vaptans, or SGLT2 inhibitors. For instance, a dual osmotic diuresis with oral urea and an SGLT2 inhibitor has been shown to be effective[216].

Mechanistically, in the animal study mentioned above[162], the authors observed an increase in urinary urea nitrogen, a stable plasma urea, and a reduction in natriuresis in both urea and protein groups. The latter was thought to be conferred by an increased inner medullary urea concentration upon protein and urea. In our study, we also showed a reduction in FENa in the protein and urea phases. One could assume that not only the increased medulla concentration but also the reduced extracellular fluid expansion reduces secondary natriuresis. In support of this, a more recent study investigating kidney urea transporter blockade in rats showed that “urearesis” increased 24-h urine volume more efficiently than furosemide and led to a positive

free water clearance, whereas furosemide did not[163]. Notably, this urea-induced osmotic diuresis prevented hyponatremia development in rats with artificial SIAD and disappeared in rats fed with a low-protein chow. In our study, we used the urine/plasma ratio as a surrogate marker for electrolyte free water clearance[115] but did not see any change.

Correcting hyponatremia with protein powder represents a tempting holistic approach in older adults and malnourished patients (e.g., oncologic patients). The hyponatremia associated gait instability and increased rate of falls[4], is often accompanied by sarcopenia in the elderly or chronically ill patients[217]. Interestingly, hyponatremia additionally increases loss of lean mass in aging rats[84]. A Japanese study showed that mild hyponatremia was independently associated with sarcopenia and weaker grip strength in older outpatients[218], although the effect of hyponatremia correction on muscle strength could so far not be demonstrated in humans[26,82,210]. Nevertheless, increasing dietary protein intake in hyponatremic patients could improve sodium levels and, at the same time, increase muscle strength[219] and mass[220], thus synergistically reducing the risk of falling. This is supported by our findings showing an improvement in self-reported fatigue and gait instability upon protein supplementation.

Concerns might be raised about the possible deleterious effect of a high-protein intake in patients with advanced chronic kidney disease and liver cirrhosis. However, these patients do not comply with the strict definition of SIAD[3] and the respective guidelines recommend against a low protein diet[221,222]. Accumulation of urea and uremic toxins is not expected in patients with a normal kidney functions since urea is completely excreted within 12h[156]. In addition, we showed that urinary urea excretion correlate with sodium increase so that patients in which urea is retained will not profit from its osmotic effect. Meat, fish and seafood are rich in purine, therefore patients with gout should prefer low fat dairy products[223]. Plasma uric acid did not increase in our study upon protein powder originating from milk.

Our study has some limitations. First, our cohort is rather small and not all patients performed the positive control phase with oral urea. In addition, patients sequentially underwent the protein phase followed by the urea phase, whereas a randomized cross-over design would have increased internal validity. We chose a 1-week observation period to minimize changes in external factors that could impact sodium levels but this prevents us from drawing conclusion on long-term efficacy and safety.

Further studies should investigate the effect of more modest daily protein amount over a longer time period. Dietary counseling is an attractive treatment approach, especially in patients with mild hyponatremia in whom physicians might be reluctant to administer second-line treatments. We chose to administer 90 g protein per day to roughly equate to the usual daily 30-g dose of urea powder. Although the 90 g is slightly beyond the recommended 0.8 per kg body weight for

adults[224], it is possible to incorporate this into the diet, as provided in the exemplary "SIAD daily diet menus" (omnivorous and vegetarian) in the appendix (*Supplementary Material M3.7*).

11.6 Conclusion

High-protein supplementation increased plasma sodium levels without additional fluid restriction in outpatients with chronic SIAD. The induced ureagenesis was similar to oral urea, suggesting a urea-induced osmotic diuresis with following electrolyte free water clearance.

11.7 Acknowledgments

The authors thank the patients and their families for their efforts in participating in the study, our Study Nurses Cemile Bathelt and Nina Hutter, as well as Rakithan Murugesu for his help in the logistical aspects of the study; and Larissa Rinkes for the support in the preparation of the dietary plans.

11.8 Authors Contribution

SM and CA elaborated the study design with MCC and then shared equally all of the following study tasks: writing of the study protocol, approval by the Ethic Committee, trial registration, on-site study planning and on-site study conduct (screening, recruitment, study visits, logistics, preparation of the powder containers, safety reporting). FB performed study visits and helped with logistics. SM performed the initial statistical analysis necessary for abstract writing for submission to conferences. CA performed the complete final statistical analysis for the manuscript. SM wrote the first draft of the whole introduction, parts of the method and the whole conclusion sections. CA wrote the first draft of parts of the methods and the whole results section. All authors reviewed the complete manuscript.

11.9 Funding

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11.10 Disclosure Statement:

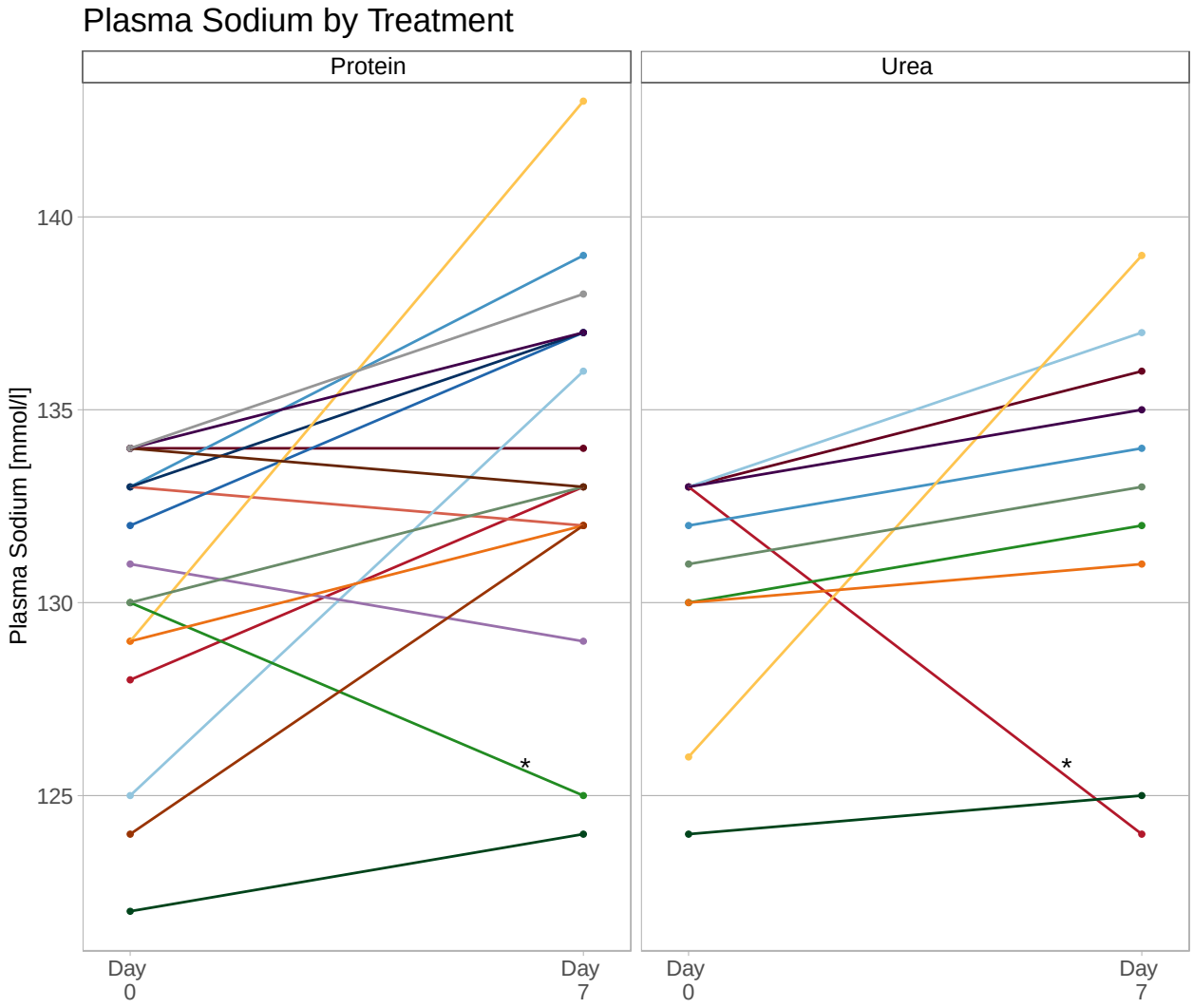
OMANDA AG provided the protein powder for free but was involved neither in the study planning, design, and conduct nor in the analysis and interpretation of the study results. SM, CA, FB, JR, MD, and MCC have nothing to disclose.

11.11 SUPPLEMENTARY MATERIAL

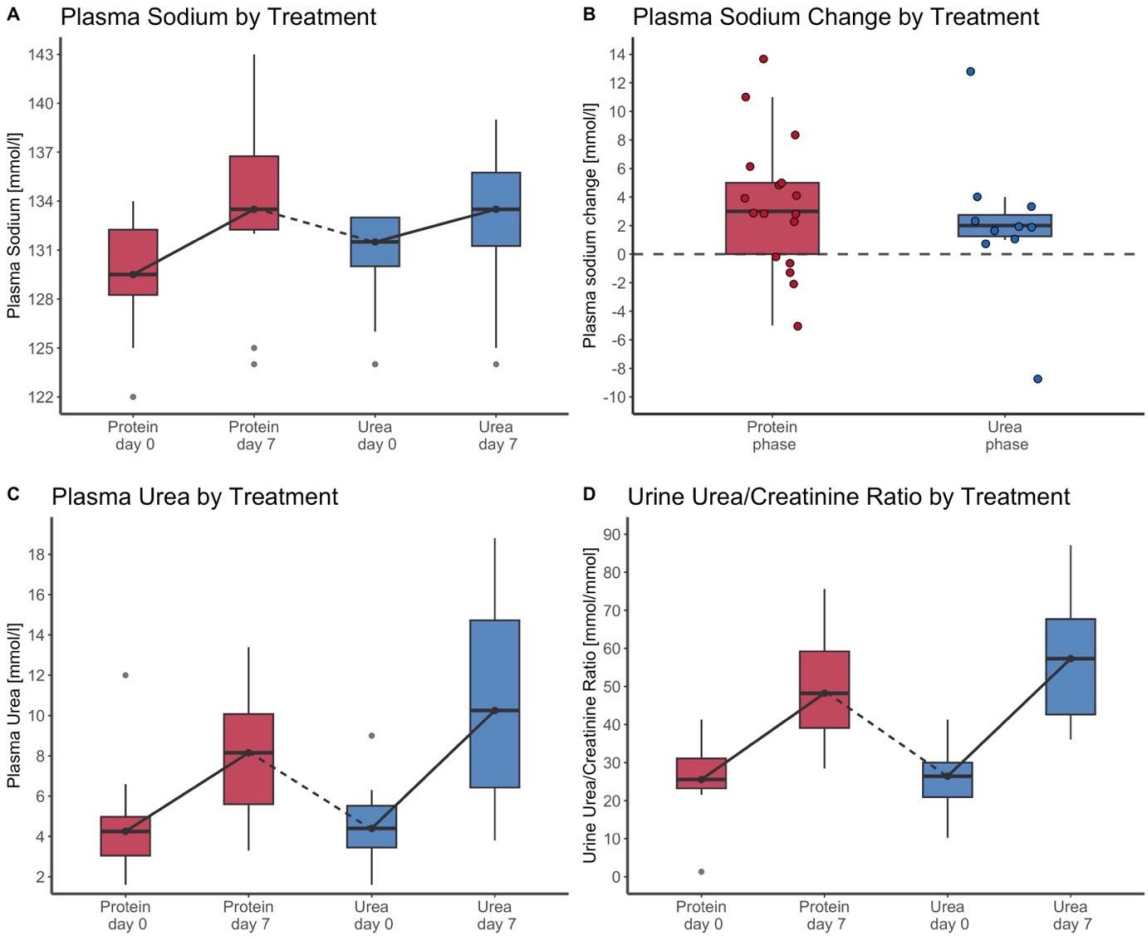
Supplementary Material M3.1 Type and Brand of Protein Powder per Patient: Three types of protein powder were used in the study to offer patients a variety of flavors. Patients always consumed one single brand during the whole study. Each type of powder was weighed in order to exactly correspond to 90 grams of protein per daily dose.

Protein Type	Moltein PURE®, OMANDA AG, Switzerland	Whey Protein®, foodspring GmbH, Germany	Clear Whey Isolate®, MyProtein THG Company, United Kingdom
Number of patients (%)	8 (47)	8 (47)	1 (6)

*Supplementary Material M3.2 Individual Change in Plasma Sodium by Treatment Phase: Plasma levels by treatment phase and individual patient (each represented with a different colour). * Insufficient protein or urea intake.*



Supplementary Material M3.3 Plasma Sodium, Plasma Urea, and Urine Urea/Creatinine Ratio by Treatment Phase in Patients Who Participated in Both Protein and Urea Phases: (A) Course and (B) change in plasma sodium levels, (C) plasma urea, and (D) urine urea/creatinine ratio visualized by treatment phase (protein phase in red; urea phase in blue). Patients participated in both phases were excluded (n = 10).



Supplementary Material M3.4 Predictive Linear Regression Model Output

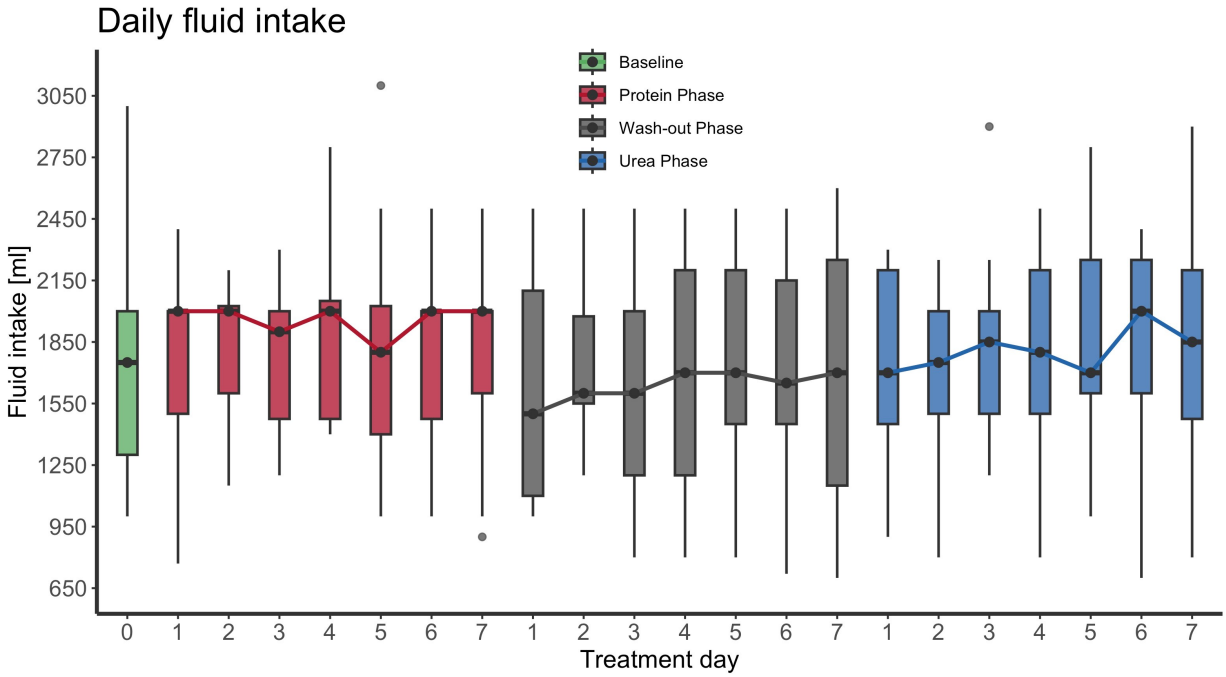
Dependent variable: change in plasma sodium (mmol/l)		
Independent variables	**Estimate**	***P***
P-Sodium at baseline	-0.6 (SE 0.4)	0.146
GFR at baseline	-0.1 (SE 0.1)	0.354
BMI	-0.1 (SE 0.2)	0.581
Urine sodium at baseline	0.02 (SE 0.1)	0.888
Urine osmolality at baseline	-0.004 (SE 0.01)	0.757
Urine/Plasma ratio	-0.2 (SE 12.5)	0.990
Intercept	96.1 (SE 54.2)	

BMI: body mass index; GFR: glomerular filtration rate; P: plasma; SE: standard error

Supplementary Material M3.5 Predictive Logistic Regression Model Output

Dependent variable: treatment response yes/no (defined as a sodium increase ≥3 mmol/l)			
	Odds Ratio	**95%-CI**	***P***
P-Sodium at baseline	0.8	0.5 – 1.2	0.381
GFR at baseline	1.0	0.9 – 1.0	0.194
BMI	0.9	0.6 – 1.2	0.585
Urine sodium at baseline	1.0	0.9 – 1.1	0.790
Urine osmolality at baseline	1.0	1.0 – 1.0	0.247
Urine/Plasma ratio at baseline	1.0	(0.0 - 2490419)	0.692
Intercept	9.5		
BMI: body mass index; GFR: glomerular filtration rate; P: plasma; SE: standard error			

Supplementary Material M3.6 Course of Daily Fluid Intake: Course in daily fluid intake in ml visualized by treatment phase (protein phase in red; wash-out phase in grey; urea phase in blue).



Supplementary Material M3. 7 Exemplary Daily Menus (Omnivorous and Vegetarian)

Omnivorous menu 1 (≈ 95.9 g protein)

Breakfast: Quark/Skyr with fruit (of choice) and rolled oats

Quantity	Food	Protein content (g)
150 g	Skyr/Quark	15 g
	Fruit of choice	0 g
2 tablespoons	Rolled oats	2.5 g
	Sweetener of choice	0 g
		Total: 17.5 g

Lunch: Cheese sandwich with vegetables and hart-boiled egg

Quantity	Food	Protein content (g)
2 100-g slices	Protein bread	22 g
30 g	Hard cheese of choice	8 g
1	Egg	7 g
	Vegetables of choice	0 g
		Total = 37 g

Dinner: Vegetables and chicken stir-fry with rice

Quantity	Food	Protein content (g)
150 g	Chicken	40 g
50 g (dry)	Rice	1.4 g
	Broccoli, zucchini, red pepper, onions	0 g
	Spices of choice	0 g
		Gesamt = 41.4 Gramm

Omnivorous menu 2 (≈ 92.6 g protein)

Breakfast: Porridge with berries and protein powder

Quantity	**Food**	**Protein content (g)**
40 g (≈ 4 tablespoons)	Rolled oats	5 g
200 ml	Milk	7 g
2 tablespoons	Protein powder	≈ 12 g
	Beries of choice	0 g
		Total = 24 g

Lunch: Whole-grain bread with dried meat, herb dip and vegetables sticks

Quantity	**Food**	**Protein content (g)**
50 g	Dried meat	16.3 g
65 g	Whole-grain bread	5.3 g
100 g	Cottage cheese	12 g
	Cucumber/red pepper sticks	0 g
		Total = 33.6 g

Dinner: Salmon with baked potatoes, vegetables and quark dip

Quantity	**Food**	**Protein content (g)**
150 g	Salmon	30 g
	Potatoes	0 g
	Broccoli, herbs	0 g
50 g	Quark	5 g
		Total = 35 g

Vegetarian menu 1 (≈ 90.7 g protein)

Breakfast: Quark/Skyr with fruit (of choice) and rolled oats

Quantity	Food	Protein content (g)
150 g	Skyr/Quark	15 g
	Fruit of choice	0 g
2 tablespoons	Rolled oats	2.5 g
	Sweetener of choice	0 g
		Total: 17.5 g

Lunch: Cheese sandwich with vegetables and hart-boiled egg

Quantity	Food	Protein content (g)
2 100-g slices	Protein bread	22 g
30 g	Hard cheese of choice	8 g
1	Egg	7 g
	Vegetables of choice	0 g
		Total = 37 g

Snack: 1 handful nuts (60 g ≈ 10 g)

Dinner: Vegetables and tofu curry with rice

Quantity	Food	Protein content (g)
150 g	Tofu	22.5 g
	Vegetables of choice	0 g
50 g	Rice	1.4 g
100 ml	Coconut milk	2.3 g
	Curry paste of choice	0 g
		Total = 26.2 g

Vegetarian menu 2 (≈ 91.5 g protein)

Breakfast: Porridge with berries and protein powder

Quantity	**Food**	**Protein content (g)**
40 g (≈ 4 tablespoons)	Rolled oats	5 g
200 ml	Milk	7 g
2 tablespoons	Protein powder	≈ 12 g
	Beries of choice	0 g
		Total = 24 g

Lunch : **Whole-grain bread with avocado, tomato, herb dip and vegetables sticks**

Quantity	**Food**	**Protein content (g)**
100 g	Avocado	2 g
65 g	Whole-grain bread	5.3 g
100 g	Cottage cheese	12 g
	Cucumber/red pepper sticks	0 g
	Tomatoes	0 g
		Total = 19.3 g

Snack: 1 handful nuts (60 g ≈ 10 g)

Dinner: Lentils-Bolognese with whole-grain pasta and salat

Quantity	**Food**	**Protein content (g)**
60 g	Lentils	14 g
	Carrots, celery	0 g
	Tomato sauce, spices	0 g
60 g	Whole-grain pasta	7.8 g
30 g	Parmesan	11.4 g
		Total = 33.2 g

12 DISCUSSION AND OUTLOOK

My MD-PhD focused on the therapeutic potential of osmotic diuresis in hypotonic hyponatremia, either by inducing glucosuria with SGLT2 inhibitors or by inducing “urearesis” with a high-protein diet. This thesis has three main findings:

1) Increasing sodium levels in chronic SIAD upregulates osteoblasts independently of empagliflozin treatment.
2) SGLT2 inhibitors do not prevent hyponatremia in T2DM patients on hospital admission.
3) A high-protein supplementation promotes ureagenesis and increases sodium levels in patients with chronic SIAD.

12.1 EFFECT OF HYPONATREMIA ON BONE HEALTH

Population aging makes osteoporosis an important and increasing public health issue. A systematic review and meta-analysis reported doubled odds for fractures as well as a 23% increase in odds of having osteoporosis in hyponatremic individuals as compared to people with normal sodium levels[225]. Importantly, Manuscript 1 suggests that even mild hyponatremia and even a modest sodium increase have an influence on bone metabolism.

Given the growing preclinical and clinical evidence, it is worth reflecting on whether hyponatremia should be considered as a secondary cause of osteoporosis and whether it should be included in fracture risk assessment tools such as the FRAX tool[226]. Although more prospective interventional studies on the influence of hyponatremia correction on BMD are required before asserting such assumptions, the available data should encourage physicians to screen for chronic hyponatremia in osteoporotic patients and patients with risk factors for osteoporosis. Similarly, patients with chronic hyponatremia, even if mild, should be screened for osteoporosis. Which osteoporosis treatment would be best in addition to hyponatremia correction in osteoporotic patients with hyponatremia is unclear. Manuscript 1 and our previous work[86] suggest that an increase in sodium promotes osteoblasts activation, independently of empagliflozin treatment. Adding an anti-resorptive treatment might complementarily target the hyponatremia-mediated osteoclast activation that has been described consistently in animal studies[9]. In case hyponatremia cannot be sufficiently addressed, the dual antiresorptive and anabolic effects of the anti-sclerostin monoclonal antibody romosozumab[227] might target the negative effect of hyponatremia on both osteoclasts and osteoblasts. Future studies need to investigate the effect of different extents of sodium increase in different hyponatremia severities and the use of different hyponatremia and osteoporosis treatments on bone metabolism, including on BMD. Assuming an additive deleterious effect of AVP on bones, tolvaptan might represent the best treatment option, however because the contribution of AVP to hyponatremia-mediated bone loss is contentious, a head-to-

head study would be essential before drawing any conclusion. Chronic hyponatremia increases the risk for fractures not only by increasing the risk for osteoporosis but also by increasing the risk for falling[4,76-81]. In this perspective, correcting hyponatremia with a high-protein diet might increase muscle strength[219] and mass[220] and thus globally address the threatening consequences that might be provoked by the combination of gait instability and hyponatremia-mediated bone fragility.

12.2 Glucose-Induced Osmotic Diuresis in Hyponatremia

Our group has now been investigating the therapeutic potential of empagliflozin to treat SIAD for more than half a decade. We first showed in healthy volunteers with artificially-induced SIAD that empagliflozin increased urine output[147]. We then demonstrated in a randomized, double-blind, placebo-controlled trial that plasma sodium could be increased more efficiently with a 4-day treatment with empagliflozin combined with fluid restriction, as compared to fluid restriction and placebo in 87 hospitalized patients with SIAD[148]. More recently, we confirmed these findings in a randomized, double-blind, placebo-controlled, crossover study in 14 outpatients with chronic SIAD[29]. Given this efficacy in overt SIAD, I aimed to investigate whether chronic glucose-induced osmotic diuresis reduced hyponatremia prevalence on admission in patients with T2DM treated with an SGLT2 inhibitor and found no difference in glucose-corrected plasma sodium concentration as compared to T2DM patients without SGLT2 inhibitors. These findings suggest against a broad prophylactic SGLT2 inhibitor administration in patients at risk for hyponatremia development. The postoperative period after pituitary surgery is the only clinical situation in which hyponatremia prophylaxis with fluid restriction revealed to be efficient[228]. Whether SGLT2 inhibitors or low-dose oral urea might also be beneficial in this specific patient group might be worth further investigation[229]. Besides, the biggest untapped potential of SGLT2 inhibitors in hyponatremic disorders lies in the treatment of patients with hypervolemic hyponatremia due to heart failure or liver cirrhosis, who could also profit from an increase in free water clearance.

Hyponatremia occurs in 15%[11]-20%[230] of patients with heart failure and is an independent predictor for hospitalization[11] , adverse in-hospital outcomes[230] and mortality[11,207,230]. Treatment options include fluid restriction, loop diuretics and vaptans[108]. However, the latter is not authorized in Europe and not recommended by the European guidelines[3]. The addition of acetazolamide was recently shown to improve decongestion with loop diuretics[231], but its effect on sodium levels seems to be neutral[232]. SGLT2 inhibitors have revolutionized heart failure management by preventing hospitalization for heart failure and cardiovascular death[233], remarkably also in heart failure with preserved ejection fraction[191,234]. SGLT2 inhibitors thus represent an attractive treatment approach for hypervolemic hyponatremia since they would simultaneously address the underlying disease. A post-hoc analysis of a placebo-controlled trial investigating the effect of

dapagliflozin 10mg in patients with heart failure with reduced ejection fraction (DAPA-HF)[204], showed a higher prevalence of hyponatremia after 14 days (11.3% vs 9.4%; *P* = 0.04) but a reduced prevalence of hyponatremia after 12 months (4.6% vs 6.7%; *P* = 0.003) in the dapagliflozin group[207]. There was a tendency towards higher normalization rate in the subgroup with hyponatremia at baseline and a lower incidence of new-onset hyponatremia in the dapagliflozin group as compared to the placebo group. Another RCT suggested that adding dapagliflozin to standard of care favors an increase in sodium levels during hospitalization in patients with acute heart failure, especially in patients with hyponatremia at randomization[235]. However another study of empagliflozin in acute heart failure did not see any change in sodium levels[143]. More data are needed to better characterize the effect of a targeted use of SGLT2 inhibitors in patients with hypervolemic hyponatremia secondary to heart failure.

Hyponatremia is a very common complication of advanced liver cirrhosis. A prospective multicentric study in 1000 patients reported a prevalence of 60% in hospitalized patients and of 40% in ambulatory patients[236]. Hyponatremia is associated with increased risks for hepatic encephalopathy, hepatorenal syndrome, spontaneous bacterial peritonitis and gastrointestinal bleeding[236]. Hyponatremia is also associated with increased mortality and has been identified as a predictor for neurological complications and poor survival after liver transplantation[18,236]. Therefore, sodium concentration has been added as an additional parameter to the Model for End-stage Liver Disease (MELD) score used for 3-month survival prediction[18]. Treating hyponatremia in these patients is extremely difficult. A report of the hyponatremia registry demonstrated how inconsistent and ineffective its management often is, with the consequence that two thirds of patients are discharged still hyponatremic[237]. Treatment options include diuretics discontinuation, fluid restriction, isotonic saline, tolvaptan and hypertonic saline[237]. Despite its efficacy[238], the use of tolvaptan in liver cirrhosis has been put to an end by a FDA warning in 2013 after severe cases of hepatotoxicity were reported in treated patients with polycystic kidney diseases[239]. Intravenous albumin has recently regained attention[240-242] and surely represents a welcome therapeutic alternative in inpatients settings. However, the practicability of its regular administration in outpatients is uncertain. In view of these elements, new effective, easy and safe treatments options are urgently required. Data on the SGLT2 inhibitors in liver cirrhosis are scarce. To my knowledge, 3 case reports with a total of 5 patients with T2DM and liver cirrhosis have been published to date. Among them, plasma sodium increased in 2 patients, of whom 1 had profound hyponatremia at baseline. A small open-label study (NCT05013502) will provide data on the effect of empagliflozin on resistant ascites in patients with liver cirrhosis but no T2DM. In view of these preliminary data and the lack of adequate therapeutic strategies or guidelines, SGLT2 inhibitors might offer a desperately needed treatment for hypervolemic hyponatremia due to liver cirrhosis.

In order to adequately investigate the use of SGLT2 inhibitors in hypervolemic hyponatremia. We designed a multicentric randomized double-blind placebo-controlled trial in outpatients or inpatients with either euvolemic hyponatremia due to SIAD or hypervolemic hyponatremia due to heart failure, liver cirrhosis or chronic kidney diseases (the EMPOWER Study, NCT04447911). In my MD-PhD, I wrote the study protocol, set up the study and expanded it to several centers in Switzerland. I have been recruiting patients, supervising local investigators and coordinating the different centers.

12.3 Urea-Induced Osmotic Diuresis in Hyponatremia

Some authors had hitherto suggested that SIAD patients should strive for a higher protein intake[108,243], however this had been derived from animal data[162] and physiological considerations, because no controlled data in human were available. A study published in 1988 in rodents with artificially-induced SIAD showed that both a 20% protein diet and a low protein diet combined with oral urea increased sodium concentration, reduced natriuresis and increased inner medullary urea concentration as compared to a low protein diet[162]. Thirty-five years later, we were able to fill this evidence gap by translating animal findings to human patients. We used the Jones' factor of 6.25 that is commonly used to convert nitrogen to protein equivalent[213] and assumed an average nitrogen content of 16% in protein (100 g protein / 6,5 = 16 g nitrogen). Urea (CH_4N_2O) contains 46.6% nitrogen (atomic weight of nitrogen = 14 g/mol, atomic weight of urea = 60,1 g/mol), therefore we needed 87,5 g (≈ 90 g) protein to achieve the 14 g nitrogen contained in 30 g urea. This high-protein supplementation of 90 g per day for 7 days increased sodium levels along with plasma and urine urea concentrations to a similar extent than the treatment with the equivalent 30 g of oral urea per day for 7 days. The increase in sodium levels correlated with urine urea concentration (corrected for creatinine) but not with plasma urea concentration, supporting the hypothesis that urea-induced osmotic diuresis was the underlying mechanism of action. Our findings in Manuscript 3 open prospects for a paradigm change in SIAD treatment, for which not only fluid restriction but also diet change could belong to first-line therapeutic measures. Increasing protein intake represents an elegant treatment approach in the often frail older adults who could also benefit from its effect on muscle strength[219] and mass[220]. Based on these promising results, future studies should consists of 1) a double-blind placebo-controlled crossover trial investigating the effect of protein powder against a non-protein powder, 2) a longer trial investigating the effect of a high-protein diet over a longer observation period including measurements of muscle mass and strength, 3) a head-to-head trial with other SIAD treatments (e.g., fluid restriction, SGLT2 inhibitors).

We currently use oral urea on a daily basis to treat patients with fluid-refractory SIAD. The results of Manuscript 3 do not invalidate the rationale of using oral urea, which remains an important

treatment option. Until the early 2010s, only small studies of mainly one same Belgian group were carried out[157-159,244-251], which limited the external validity of this approach. However, interest in urea has been growing in the last 10 years[159,161,212,251-258], in part probably because the hope put in the AVP receptor antagonists has been dimmed by the costs and safety concerns resulting from their use. As a result, European[3] and American[108] Guidelines adopted urea as a possible second-line treatment for SIAD. Nonetheless, the evidence for urea mainly comes from case reports, retrospective studies (cumulative n ≈ 350 patients) and prospective observational studies (cumulative n ≈ 20 patients). The only comparative studies are one prospective study comparing the effect of a one-year treatment with tolvaptan followed by a one-year treatment with urea[161] and one retrospective study that compared urea-treated patients with matched control patients[252]. In this respect, we report in Manuscript 3 one of the rare controlled use of oral urea in chronic SIAD, although due to the study design not in a randomized or double-blind manner. Two RCTs are ongoing (NCT04588207, NCT04552873) and will soon provide the first randomized data. In Europe, oral urea is a medical food prepared as a compounding agent by pharmacies at the behest of physicians. This lack of ready-to-use formulation and the poor palatability[255,256] tend to limit its broader use. In the USA, ready-to-use flavored powders (Ure-Na®[151,152], UreaAide®[153]) and tablets (UreaTabs®[154]) are available to treat euvolemic and hypervolemic hyponatremia, and authorized as medical food by the FDA. I have recently contacted a Swiss Company and initiated the first steps of the development of a similar alternative in Switzerland and hope it will improve patients care.

Case series in genetic nephrogenic SIAD provide reassuring data on the efficacy and safety of urea over a therapy duration of several years or decades[257]. A logical increase in urea concentration is observed upon urea and protein powder administration, so that concerns might be raised about potential uremic symptoms (i.e., fatigue, anorexia, nausea, cramps, itching) or toxic effects of uremia (e.g., insulin resistance, anemia, platelet dysfunction)[259]. However, urea concentrations observed upon urea therapies do not reach levels of patients with end-stage renal disease (e.g., 7.1 mmol/l [5.9, 8.9] upon protein and 10.2 mmol/l [6.4, 14.7] upon urea in Manuscript 3) and the deleterious effects of uremia are thought to be mediated not only by urea but by many unmeasured uremic solutes whose concentrations correlate with plasma urea concentration[259]. Nevertheless, the latter should be controlled regularly during treatment. An unusual rise (> 20 mmol/l) might suggest a tendency toward accumulation and usually goes together with a poor treatment efficacy.

An important safety aspect of all treatments for chronic hyponatremia is the risk of overly rapid correction. In Manuscript 3, one overly rapid correction (11 mmol/l in 24h) occurred during the protein phase (1/17 patients, 6%) while no case was documented in the urea phase. Two studies on urea in the ICU reported cases of overly rapid correction (> 12 mmol/l) in 15.3% (13/85

patients)[159] and 9.5% (4/42 patients)[251] while other studies did not report any case. We had no case of hypernatremia but three other studies reported hypernatremia cases. The two same studies on the ICU reported an hypernatremia rate of 7.1% (6/85 patients)[159] and 4.8% (2/42 patients)[251], as well as the one-year study in outpatients reporting one case of hypernatremia in a context of dehydration during an acute infection[161]. Importantly, not a single case of ODS has ever been reported secondary to urea treatment in humans. Interestingly, an animal study induced overly rapid sodium correction with vaptans, hypertonic saline or urea in rats and showed reduced mortality and neurological damage in the urea-treated rats[260]. The underlying mechanism remains elusive although a more gradual sodium increase with urea in the first hours might be conducive to this difference. Taken together, the risk for overly rapid correction and its clinical consequences appears to be low. However, to keep overcorrection risk at a minimum, urea should be started at its lower dose of 15 g in patients with risk factors for ODS (*Section 7.5*), with transient causes of SIAD or with profound hyponatremia. In every other patient, a starting dose of 30 g can be considered.

Urea has also been investigated in hypervolemic hyponatremia. Case series reported the successful use of urea in hyponatremic patients with heart failure and a GFR > 30 ml/min/1.73 m^2 (cumulative n = 36 patients)[248,261,262]. Plasma urea increased from an average of 14.3 mmol/l to an average of 23 mmol/l. Notably, one case of mild uraemic encephalopathy was reported in a patients with a baseline GFR of 32 ml/min/1.73 m^2 and plasma urea levels > 25 mmol/l which resolved after urea discontinuation. The use of urea in selected cases, especially in which hyponatremia limits diuretic titration seems to be a reasonable approach in inpatient settings with a close monitoring. Nevertheless, the efficacy in patients with high plasma urea concentration at baseline (> 20 mmol/l) is rather doubtful because it indicates a poor renal clearance of (endogenous) urea. A couple of case reports also described the successful intermittent use of urea in patients with liver cirrhosis (cumulative n = 5 patients)[246,247]. Again, due to the lack of evidence, this should only be considered on a case-by-case basis. The same precautions should be taken regarding the implementation of a high-protein diet.

12.4 Future Directions in Hyponatremia Prevention

Due to the clinical implications and the healthcare burden associated with hyponatremia, further research should aim at identifying patients at risk for hyponatremia to take preventive measures in a timely manner, especially in the at-risk population described below.

Although some risk factors have been identified for postoperative SIAD after pituitary surgery, we are still not able to recognize patients prior to hyponatremia development, which alas often develops after discharge. An increased urinary oxytocin excretion on the 4th postoperative day has been associated with postoperative transsphenoidal surgery-related SIAD but this needs

further investigation[263]. Copeptin helps predict postoperative AVP deficiency in this context, but is of no utility for predicting SIAD[264].

Another patients group with a high hyponatremia prevalence is the one of patients receiving thiazide and thiazide-like diuretics. Both are diuretics of choice for hypertension in patients without advanced CKD and without congestive disorders[265], and are thus very commonly prescribed. Hyponatremia and hypokalemia are the main electrolyte disturbances under thiazides[266,267], and are observed in about one fifth of patients taking thiazides in the emergency department[268]. Whereas hyponatremia usually develops within weeks[266], a delayed occurrence after months or years is also possible[267,269]. Interestingly, patients with a history of thiazide associated hyponatremia (TAH) are very likely to develop hyponatremia by rechallenge[270,271]. Recognized independent risk factors are older age and female gender[266,268,272]. There might be a hormone-dependent variation in thiazide-sensitive sodium-chloride cotransporters (NCC) density as well as a greater NCC sensitivity to thiazides in females[273,274]. In addition, a genome-wide association study found an association with a polymorphism of the gene SLCO2A1 (rs34550074, p.A396T) coding for the apical prostaglandin transporter (PGT) in the collecting duct[275]. This mutation was present in half of the patients with TAH and in only a quarter of normonatremic controls with thiazides. An increase in urinary prostaglandin E_2 (PGE2) was observed in these patients, especially when taking thiazides. Elevated luminal prostaglandins activate the prostaglandin E_2 receptor (EP) 4 that promotes AVP-independent aquaporin 2 externalization and do not reach the basolateral prostaglandin receptors EP1 and EP3 that mediate AVP receptor internalization[275]. Whether urinary prostaglandin could predict TAH is unknown and currently investigated by our group (the PROPHECY study, NCT05542056).

Beyond TAH, pharmacogenomics is a pivotal aspect of personalized medicine and might also allow for recognition of patients at-risk for developing drug-induced SIAD[276].

12.5 Future Direction in Diagnostics

As mentioned in the previous section, hyponatremia is a common side effect of thiazide and thiazide-like diuretics. These patients can be divided into hypovolemic patients requiring volume repletion and euvolemic SIAD-like patients requiring fluid restriction, in addition to thiazide withdrawal. Distinguishing one group from the other can be challenging since thiazides induce natriuresis and prevent the use of from using the standard diagnostic algorithm[3], and clinical assessment of volemia has poor accuracy[92]. We aimed to circumvent this limitation by investigating the diagnostic potential of the Stewart Approach[277-279], a bicarbonate-independent alternative model for the analysis of acid-base disturbances. In this model, the body always strives for electroneutrality, i.e., for an equal sum of anions and cations. Any variation in electroneutrality is corrected by a shift in water dissociation, so that a relative excess of anions will compensated

by protons (and thus acidosis) and a relative excess of cations will be compensated by hydroxide ions (and thus alkalosis). The difference in strong cations and anions (or Strong Ions Difference = SID) is calculated by summing plasma sodium and potassium and subtracting plasma chloride concentration (P_{Na} + P_K - P_{Cl}). SID has a physiological value of 40 mmol/l. Any variation of this value (i.e., in electroneutrality) is corrected by a shift in water dissociation, so that a value < 40 mmol/l will be compensated by protons (and thus acidosis) and a value > 40 mmol/l will be compensated by hydroxide ions (and thus alkalosis). An excess of free water (whose SID = 0) reduces the SID and shift water dissociation towards protons and acidosis. In contrast, a free water loss and/or a chloride loss increases the SID and shifts water dissociation toward hydroxide ions and alkalosis. We were recently able to retrospectively use these changes in SID to identify volume-depleted TAH patients from SIAD-like TAH patients with a water excess[280]. An SID > 42 mmol/l had a positive predictive value (PV) of 79.1% in identifying patients with volume-depleted TAH, whereas a value < 39 mmol/l excluded it with a negative PV of 76.5%. Whether this approach can be used in other hyponatremia subtypes should be investigated further.

12.6 PROSPECTS FOR TREATMENT

12.6.1 Choice of Treatment and Combination Therapies

Hyponatremia patients are often older and polymorbid. It is hence desirable that hyponatremia treatments not only increase plasma sodium levels but also bring collateral benefits, in the same way that treatments for T2DM not only focus on glycemic control. Both a high-protein diet and SGLT2 inhibitors confer a more holistic treatment approach. Whether a patient would benefit more from the improvement in muscle mass and strength or more from cardiorenal protection depends on the individual patient's comorbidities and preferences. For instance, oncologic patients with a lower BMI and susceptibility to infection would rather profit from protein, whereas patients with hypertension and/or T2DM would be ideal candidates for SGLT2 inhibitors. A combination of the two seems conceivable and has been shown to be effective in a recent case report[216]. Except for vaptans whose potency precludes from adding them to other treatments, there is a need for investigating treatments in combination, in the same way that fluid restriction alone was compared to its combination with loop diuretics and sodium chloride tablets[119].

Interestingly, tolvaptan has been shown to blunt proliferation and invasion of human cancer cells in vitro[281]. Because tolvaptan is effective in malignant SIAD[282], next studies should investigate the prognostic implication of correcting hyponatremia with vaptans as compared to other treatment options. As mentioned in *Section 12.1*, vaptans might also be the most suitable treatment for osteoporotic patients.

Overall, future trials should on the one hand be designed as head-to-head trials and on the other hand investigate the efficacy and safety of established treatments in combination.

12.6.2 Urea Transporter Inhibitors

Urea osmotic diuresis has so far been induced by increasing urea intake with urea powder or protein in humans. In rats, the salt-sparing diuretic action of urea has additionally been demonstrated by inhibiting the UT-A1 and B[163], which led to an increase in free water clearance and prevented sodium decrease in rodents with artificially induced SIAD[163]. This new class of diuretics would be of great therapeutic interest, not only for hyponatremia but also for diverse congestive disorders[283]. For instance, UT inhibition reduced ascites and reversed sodium lowering in cirrhotic rats[284]. Several candidate compounds have been investigated in rodents in the last 15 years but the development is still at the preclinical stage[283].

12.6.3 Interleukin-6 Antagonism

The immune and endocrine system must be able to interact in order to best face infections and inflammation. Interleukin (IL) 6 stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary [285,286] and AVP from the posterior pituitary[286,287]. IL-6 is therefore a potential cause of SIAD, notably in COVID-19 as supported by our own data[288]. Others showed an increase in sodium levels in eight patients with COVID-19, whose IL-6 levels decreased after treatment with the anti-IL-6 antibody tocilizumab[289]. Similarly, chronic SIAD of a child with juvenile idiopathic arthritis resolved upon tocilizumab[290]. IL-6 antagonism could represent an unexploited treatment strategy for SIAD secondary to inflammatory disorders. Further research should identify the IL-6-dependent hyponatremia subtypes and then investigate the targeted use of IL-6 blockers in these patients. In addition, sodium levels could help guide anti-IL 6 treatment.

12.6.4 Apelin System

The apelin receptor is a g-protein coupled receptor whose structure resembles the angiotensin 2 type 1 receptor[291]. It has two endogenous ligands apelin and elabela, whose different isoforms are present in different organs and are thought to work in a autotocrine and/or paracrine manner[292]. Apelin is, *inter alia*, expressed in the magnocellular neurons of hypothalamic supraoptic and paraventricular nuclei together with AVP and oxytocin[293,294]. Apelin has a broad spectrum of effects and thus represents an attractive new target for many medical specialties. For instance, it displays vasodilatory and inotropic effects and promotes glucose uptake and lipolysis[295]. In salt and water homeostasis, apelin counteracts the effects of AVP by inhibiting central AVP release and AVP renal effect. It also antagonizes the vasoconstricting effects of angiotensin II on renal afferent arterioles, and thus increases renal blood flow and glomerular filtration[295]. AVP and apelin have been shown to change in opposite directions upon hypo- and hyperosmotic challenges in healthy humans[296]. A cross-sectional study in hyponatremic patients

with SIAD or heart failure suggests that not only an increased copeptin level but also a relative insufficient apelin level contribute to renal water reabsorption[297]. Re-establishing a physiological copeptin apelin ratio by administering exogenous apelin appears therefore as a possible future treatment. This concept was validated in hyponatremic rats in whom an apelin-17 analog increased urine output, decreased urine osmolality and increased sodium levels[298].

Whether a similar effect could be reached in humans is not known. I am currently setting up a randomized double-blind placebo-controlled cross-over proof-of-concept study, in which I will investigate the effect of apelin in healthy volunteers with artificially-induced SIAD.

13 CONCLUSION

In my thesis, I was able to show that an increase in sodium in chronic SIAD stimulates bone formation, that a non-targeted use of SGLT2 inhibitors does not prevent hyponatremia and that plasma sodium correction with urea-induced osmotic diuresis could also be reached with a high protein diet. This MD-PhD hence contributed to better define the therapeutic potential and limits of osmotic diuresis in the treatment of hyponatremia with a focus on SIAD, and further provided an insight into the possible clinical implication of correcting chronic hyponatremia.

After my MD-PhD, my upcoming research will focus on the further coordination of my multicentric study investigating the effect of empagliflozin in eu- and hypervolemic hyponatremia and the investigation of the therapeutic potential of apelin in SIAD.

Overall, future research should focus on developing innovative treatment approaches, investigating available treatments in different combinations, and identifying which patients would most profit from which treatment option. In addition, the pathophysiological mechanisms behind the clinical symptoms and prognostic implications of hyponatremia should be further explored[299]. Clinical studies should always include broad spectrum clinical endpoints to precisely characterize how patients can profit from an increase in sodium and which extent of correction should be strived for.

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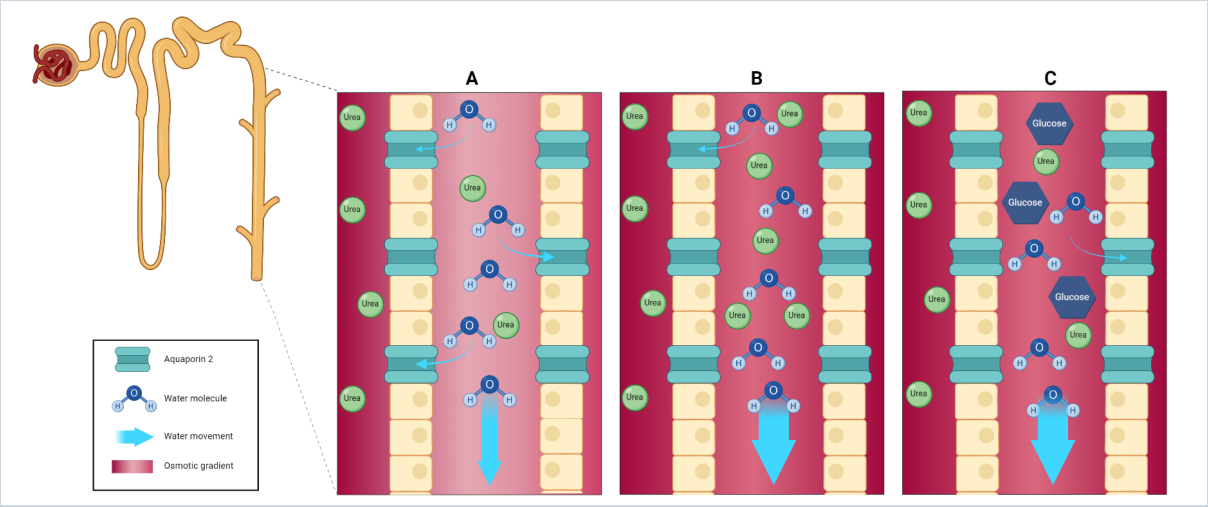

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