Development of Hyponatremia in Highly Trained Rowers during a 32-Day Training Camp

Dissertation to obtain the doctoral degree of Medicine at the faculty of Medicine at Ulm University

Constantin Mayer

Born in Stuttgart

2014
Present Dean: Prof. Dr. Thomas Wirth

1. Reviewer: Prof. Dr.med.Dr.h.c. J. Steinacker

2. Reviewer: Prof. Dr. Radermacher

Date of Graduation: 29.10.2015
Table of contents:

**Abbreviations** ........................................................................................................................................ IV

I. **Introduction** ................................................................................................................................. 1

II. **Material and Methods** ............................................................................................................ 6
    1. Study design .............................................................................................................................. 6
    2. Subjects .................................................................................................................................. 6
    3. Study curriculum ...................................................................................................................... 6
    4. Laboratory assessment ............................................................................................................ 7
    5. Calculations ............................................................................................................................. 8
    6. Statistics .................................................................................................................................. 9

III. **Results** ....................................................................................................................................... 10
    1. Participants ............................................................................................................................. 10
    2. Weather conditions ............................................................................................................... 11
    3. Sodium .................................................................................................................................. 12
    4. Serum osmolality .................................................................................................................... 15
    5. Copeptin .................................................................................................................................. 15
    6. Fluid intake ............................................................................................................................ 16
    7. Hematocrit .............................................................................................................................. 17
    8. Secretion Index ....................................................................................................................... 18
    9. Urine volume and urine sodium content ............................................................................... 18
    10. Sodium intake ......................................................................................................................... 19
    11. Fluid balance .......................................................................................................................... 19
    12. Case study athlete ................................................................................................................... 20

IV. **Discussion** ............................................................................................................................. 21
    1. High volume rowing training in a training camp is associated with an incidence of hyponatremia ................................................................................................. 21
    2. Morning copeptin decreases during the course of the training camp ............................... 26
    4. Central down regulation ........................................................................................................ 29
    5. Case study athlete .................................................................................................................. 29
    6. Interaction with other hormones ............................................................................................ 30
    7. Limitations ................................................................................................................................ 31
    8. Conclusion ............................................................................................................................... 31

V. **Abstract** ..................................................................................................................................... 33

VI. **References** .................................................................................................................................. 34
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFI</td>
<td>ml/24h</td>
</tr>
<tr>
<td>ANP</td>
<td></td>
</tr>
<tr>
<td>AVP</td>
<td>m²</td>
</tr>
<tr>
<td>BSA</td>
<td>kg</td>
</tr>
<tr>
<td>BW</td>
<td></td>
</tr>
<tr>
<td>CFTR</td>
<td></td>
</tr>
<tr>
<td>COP</td>
<td>pmol/l</td>
</tr>
<tr>
<td>EAH</td>
<td></td>
</tr>
<tr>
<td>EAHE</td>
<td></td>
</tr>
<tr>
<td>ENaC</td>
<td></td>
</tr>
<tr>
<td>FB</td>
<td>ml</td>
</tr>
<tr>
<td>Hct</td>
<td>% volume</td>
</tr>
<tr>
<td>ILMA</td>
<td></td>
</tr>
<tr>
<td>Na⁺ fluid</td>
<td>mg</td>
</tr>
<tr>
<td>NaLo</td>
<td></td>
</tr>
<tr>
<td>NaNo</td>
<td></td>
</tr>
<tr>
<td>NaHi</td>
<td></td>
</tr>
<tr>
<td>NaSU</td>
<td>mg</td>
</tr>
<tr>
<td>P33</td>
<td></td>
</tr>
<tr>
<td>P66</td>
<td></td>
</tr>
<tr>
<td>P100</td>
<td></td>
</tr>
<tr>
<td>Pmax</td>
<td>W</td>
</tr>
<tr>
<td>RAAS</td>
<td></td>
</tr>
<tr>
<td>RFI</td>
<td>l/m²</td>
</tr>
<tr>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>pmol/mmol</td>
</tr>
<tr>
<td>UV</td>
<td>ml</td>
</tr>
<tr>
<td>VO₂max</td>
<td>ml/min/kg</td>
</tr>
<tr>
<td>[La] max</td>
<td>mmol/l</td>
</tr>
<tr>
<td>[Na⁺]</td>
<td>mmol/l</td>
</tr>
<tr>
<td>[Osm]</td>
<td>mosmol/kg</td>
</tr>
</tbody>
</table>
I. Introduction

During athletic training and competition, body water is lost mainly through respiration and via sweat loss [43]. This results in reduction of total body water and increase of plasma sodium concentration, as sweat is hypotonic compared to plasma. Consequently, water moves from intravascular to extravascular space. Water loss usually exceeds electrolyte loss resulting in hyperosmotic hypovolemia, increased plasma osmolality and decreased blood volume. Following, different mechanisms to reestablish deviations in fluid and electrolytes take place, especially during recovery periods.

Thirst is the major stimulus that controls fluid intake and therefore counteracts hypovolemia. Its development is multifactorial, but increase of plasma osmolality of only 2-3% is the most potent stimulus [99, 118, 119], followed by reduction of intravascular fluid of about 10% [67, 120]. Osmotic stimuli are sensed by osmo-Na\(^+\) receptors, which respond to the tonicity of interstitial fluid. Theses receptors belong to sensory neurons located within the central nervous system, however, outside the blood-brain-barrier. Volume changes are sensed by volume pressure receptors, located in the superior vena cava and the atria of the heart. Both triggers lead to a general response including renin-angiotensin-aldosterone-system (RAAS) activation, atrial natriuretic peptide (ANP) secretion as well as Vasopressin/AVP release from the posterior pituitary gland, later on sodium appetite and thirst[4, 52, 67, 99, 129]. Angiotensin induces aldosterone secretion from the zona glomerulosa of the cortex of the suprarenal gland and gives negative feedback to intercept its activation.

Short-term effects and interactions of fluid regulating hormones are complex, in the following particularly Aldosterone, RAAS, ANP and AVP are to be discussed.

Aldosterone itself induces the implantation of sodium channels (ENaC) into the epithelial membrane of kidney cells, which provides the reabsorption of sodium into the interstitial space and body. Nose et al. proofed that isolated plasma volume restoration was able to depress the renin-angiotensin-aldosterone system (RAAS), thus plasma volume changes seem to be a primary trigger for the RAAS [90]. Aldosterone is already up to 5-times elevated during exercise [106], to counteract the decrease of plasma volume that takes place during exercise and to re-establish and subsequently expanded plasma volume during recovery.
I. Introduction

ANP is released by myocyte cells located in the atria of the heart during stretch of the atria itself (volume overload) [51]. It inhibits renin secretion and therefore counteracts the RAAS, phosphorylates the ENaC channels and dilates afferent kidney vessels.

AVP, a nonapeptide produced in the hypothalamus, counts as the most sensitive hormone to blood osmolality due to its linear sensitivity to osmolality changes [120]. But current concepts suspect a more complex situation, where i.e. a non osmotic triggered AVP release comes into place [52]. AVP is in charge of free water clearance: via fusion of Aquaporine 2 channel containing vesicles to the collection duct cells of the kidney, a temporary permeability for water is implemented and water is reabsorbed.

Regardless of these immediate hormonal counter mechanisms, excessive fluid loss via sweating may not only lead to dehydration and impaired performance [14], which is commonly prevented by fluid intake. Dehydration accompanying electrolyte changes due to sweating are not as quickly restored as plasma volume changes and are known to potentially cause more severe complications. Hyponatremia, the depletion of sodium in blood plasma (hypoosmotic hypo- or hypervolemia), is the most frequent electrolyte disorder and is associated with increased morbidity and mortality in hospitalized patients [12, 40]. Recently this disorder has been more frequently observed in healthy athletes who may develop exercise-associated hyponatremia (EAH) with plasma sodium concentration ([Na⁺]) below 135 mmol/l during or within 24 hours after strenuous exercise [49]. EAH usually remains asymptomatic, but occasionally causes fatigue, nausea, dizziness, vomiting, non cardiac pulmonary edema [7] and headache [40]. Several studies describe an incidence of EAH in participants of long term endurance competitions of 2% up to 7% [102] or even 13 % [2]. Nowadays it is discussed as the most dangerous complication of long term endurance exercise [48]. In presence of major cerebral symptoms (like seizures, brain edema, and encephalopathy to the rare point of death), it is termed EAH encephalopathy (EAHE) [40, 134].

Former research, often driven by case reports of marathon runners, suggested water intoxication (hypoosmotic hypervolemia) to be the main singular cause of symptomatic hyponatremia [5, 40, 83, 87]. When subjects drink enormous amounts of fluid (up to 57 cups) during prolonged exercise duration (marathon running), such overhydration is a contributing factor towards hypoosmotic hypervolemia [47]. Accordingly, guidelines for fluid ingestions differ from a “biological driven approach” [27], meaning “ad libitum” to more constrict amounts [82]. When first cases of symptomatic hyponatremia regardless a
low fluid intake were studied, new contributors to EAH were identified [59, 103]. Data suggested a connection to the Schwartz-Bartter-Syndrome, a disorder of inadequate renal water excretion due to an elevated AVP production [49, 108, 111]. In more recent research, three contributors to the pathophysiology of EAH are identified: (i) inadequately high fluid intake (reactive overdrinking), (ii) inadequate suppression of antidiuretic hormone (AVP) secretion and (iii) the inability to mobilize sodium from internal stores [86].

Additionally, 6 risk factors have been identified for the pathogenesis of EAH: female sex [2, 7], low body weight [101], hot or cold environmental conditions [121], longer duration of exercise [15], weight gain during exercise [2] and cystic fibrosis transmembrane conductance regulator (CFTR) variant [74].

![Diagram of selected physiological responses to exercise induced dehydration](image)

**Figure 1**: diagram of selected physiological responses (bottom line) to exercise induced dehydration. Contributors (square boxes) and selected risk factors (circles) of EAH. Solid lines represent effects, dotted arrows represent negative feedback. AVP = Vasopressin ;CFTR = cystic fibrosis transmembrane conductance regulator; RAAS = Renin-angiotensin-aldosterone-system; EAH = exercise associated hyponatremia. Created by author.
Clinical studies regarding the involvement of AVP towards EAH are limited by severe issues of AVP measurements. Not only are relatively large amounts of blood (0.5-1.0 ml) needed for testing, but AVP additionally is highly instable at room temperature and rapidly cleared from plasma. Secondary, the analytical process is time consuming and platelet attachment in plasma of the peptide itself make routine measurements nearly impracticable [25, 49, 77, 96, 111].

Recently the stable 39 amino acid precursor COP has been shown to be a valid surrogate marker for AVP/vasopressin in field test settings [54]. COP is known to be secreted equimolar [76], is easier to be measured through an immunoluminometric assay [77, 111, 128] than AVP via an radioimmunoassay and is characterized by a long half-life (< 20% of loss for at least 7 days at room temperature and 14 days at 4°C ) [76]. Copeptin values correlate with AVP in healthy and ill individuals, but show no typical intraday secretion pattern [76]. Median values of 359 healthy individuals were 4.2pmol/l (range 1-13.8pmol/l, 97.5th percentile was 11.25 pmol/l, 2.5th percentile 1.7 pmol/l), median values differed for men and women [77]. Therefore, COP is expected to provide valuable information on hormonal, especially AVP induced antidiuresis.

Regarding the field test studies of EAH, not only variation within 24h prior to measurement, but long term changes due to physical training taking place earlier have to be taken into account to complete the picture of pathogenesis and risk factors. This may include altered physiological status compared to non-athletes, adoptions in hormonal responses as well as modified reactions to environmental conditions. Endurance athletes show higher hemoglobin mass and blood volume as long term adaption to training, usually resulting in unchanged Hct but raised total body hemoglobin mass [13]. Additionally, hormonal changes with training, detraining or overtraining, such as hypothalamic dysfunctions [11] have an influence on AVP [110]. At last, a higher rate, earlier onset, decreased sweat sodium content and augmented sweat gland responsiveness to aldosterone are known effects of acclimation on sweat production and will alter adaptive regulation to surroundings [61, 62].

Whereas endurance training leads to increased hemoglobin mass and further increased plasma volume and therefore results in a low Hct, contrarily relative higher Hct levels are found in rowers during periods of training and detraining, regardless a high fluid intake, indicating a potentially impaired or altered body water homeostasis (hyperosmotic hypovolemia) [63]. If elevated Hct levels are caused by dehydration or plasma volume reduction, performance may be impaired [14], whereas lowered blood viscosity and
elevated Hct levels caused by red cell mass increase, as a result of adaption to rowing training, are correlated with increased performance in elite rowing [28, 29, 126].

Overall, the appearance of hyponatremia due to prolonged exercise during a competition may not be explained by one single factor and is dependent on physiological adaptions during training prior to the event. This is more complicated when longer periods of training are considered; however, the incidence of EAH in athletes participating in a long lasting high volume training over several weeks has never been investigated.

**Hypotheses**

Healthy, highly trained rowers were studied while undergoing prolonged high volume training to examine the following scientific questions:

1. Evaluation of the incidence of hyponatremia during a training camp in endurance sports.

2. Characterization of the course and changes of resting morning copeptin (COP) as a marker for AVP over a period of 32 days.

3. Identification of possible relations between copeptin (COP), and its triggers (changes of $[Na^+]$, fluid intake (AFI, RFI), serum osmolality ($[Osm]$), and changes in fluid homeostasis (hematocrit (Hct), urine volume (UV)) during high volume training in a warm summer environment.
II. Material and Methods

1. Study design

Data were collected during a training camp in Berlin (Germany), preceding the Rowing World Junior Championships, its total duration was 32 days. After qualification for the national team, 54 athletes were included and 30 (21 males, 9 females) gave written informed consent. The study was an observational cohort study without control group and internal grouping according to drinking behavior. Training was recorded daily (distance, duration and intensity) (table 2). Weather information was retrieved from the DWD (Deutscher Wetterdienst), stationed at Berlin Tempelhof airport, daily for 6.a.m and noon (table 2).

2. Subjects

All athletes (table 1) were highly trained rowers in the sculling and sweep rowing disciplines. Coxswains were excluded from the study. The study was approved by the ethics committee of the University of Ulm (Germany). These athletes won 8 gold, 2 silver and 2 bronze medals at the World Junior Championships in Linz, Austria in 2008 immediately after this training camp.

3. Study curriculum

Pre-study training status was evaluated for maximal power output ($P_{\text{max}}$) and maximal oxygen uptake ($VO_{2\text{max}}$) during specific rowing ergometer incremental test. Starting at 200 W (male) / 150 W (female), 3 minute steps with increases of 50 W and breaks of 30 seconds were conducted to physical exhaustion (table 1).

BW, body height, COP, [Na$^+$], [Osm], Hct were measured prior to the training program (day 0) during day time, and again at 6.a.m. of day 7, day 13, day 18, day 24, and day 28, respectively in fasting state.

For COP, [Na$^+$] and [Osm], EDTA blood was drawn from an antecubital vein, for Hct, capillary blood was taken from the hyperaemized ear lobe.

In addition, BW, absolute fluid intake (AFI) and urine output (UV) measurements began 24 h prior to blood sampling at 6 a.m., at day 6, day 12, day 17, day 23 and day 27 and
ended after blood withdrawal and repeated body weight measurements at day 7, day 13, day 18, day 24 and day 28. AFI was monitored via a questionnaire, sodium intake via fluid (Na⁺ fluid) was calculated, UV in ml over 24h measured and sodium content (NaSU) determined in the lab.

4. Laboratory assessment

Capillary blood samples in 90 µl capillaries were centrifuged for 10 min at 10.000 rpm (Laborfuge A, Heraeus, Buckinghamshire, UK), Hct values were then measured with a measuring gauge.

EDTA blood was centrifuged at 5000 rpm for 10 min (Centrifuge, Braun GmbH, Germany), serum was aliquoted and immediately frozen at -20° C and then transferred to -80° C for long-term storage and for further analysis.

COP was measured using a commercially available immunoluminometric assay (ILMA) with 50 µl of collected plasma (CT-proAVP LIA, B.R.A.H.M.S. AG, Germany) [76].

[Na⁺] was measured from aliquoted plasma using automated chemical analysis via ion selective electrodes of the Central Laboratory of the Medical University of Würzburg, Germany, with a normal range for [Na⁺] between 135 - 145 mmol/l.

[Osm] was measured at the laboratory of the Medical University of Ulm, Germany, via freezing point reduction using an OSMOMAT 030 Osmometer, with a normal range of 280-300 mosm/kg.

BW was measured with a digital scale (Seca 862, Seca, Hamburg, Germany). Athletes wore underwear.

AFI questionnaires were collected after each 24h collection period. Drinking water was handed out only in 1 l bottles, and therefore athletes could easily assess their fluid intake. All athletes drank only the same kind of mineral water. Therefore Na⁺ fluid could be calculated (equation 3).

Urine was collected in 2 l containers. Each athlete had multiple containers, which were easily accessible and clearly labeled with his/her name. Exact amount of fluid was measured with a jug accurately to 10 ml. Before pouring away, 7 ml samples of each urine container were taken and frozen at -20° C for further analysis of NaSU.
NaSU was detected similar to \([\text{Na}^+]\) using ion selective electrodes in the Central Laboratory of the Medical University of Würzburg, Germany. Therefore, each sample was measured; final results were calculated according to volume of each container sample and total urine output.

## 5. Calculations

24 h relative fluid intake, fluid intake per body surface area (RFI), was calculated as follows: relative fluid intake is absolute fluid intake in liter divided by body surface area.

\[
\text{RFI (l/m}^2\text{)} = \frac{\text{AFI (ml)}}{1000} / \text{BSA (m}^2\text{)}
\]

with BSA (body surface area) calculated according to Mosteller [79] with the individual pre-study data and AFI describing absolute fluid intake.

A secretion index (SI) was defined as follows: Secretion index is plasma Copeptin per plasma sodium of the same blood withdrawal multiplied with 100.

\[
\text{SI (pmol/mmol)} = \frac{\text{Copeptin (pmol/l)}}{\text{[Na}^+\text{](mmol/l)}} \times 100
\]

\(\text{Na}^+\text{fluid}\) was calculated according to the beverage sodium content [41] (118 mg/l) multiplied with AFI (ml): Sodium intake is beverage sodium intake per liter multiplied with absolute fluid intake in liter.

\[
\text{Na}^+\text{fluid (mg)} = 118 \text{ (mg/l)} \times \frac{\text{AFI (ml)}}{1000}
\]

Fluid balance was calculated via fluid intake and urine output: fluid balance is absolute fluid intake minus urine volume.

\[
\text{FB (ml)} = \text{AFI (ml)} - \text{UV (ml)}
\]

Measurements of body mass 24h prior and right before blood withdrawal were taken to calculate weight changes within 24h:

Weight at blood withdrawal minus weight 24h before blood withdrawal.

\[
\Delta \text{BW} = \text{BW blood withdrawal (kg)} - \text{BW 24h before (kg)}
\]
6. Statistics

Regression analysis and ANOVA were calculated using SPSS 19 (IBM Germany GmbH, Ehningen, Germany). Data are presented as mean ± standard deviation, p-values ≤ 0.05 were considered significant, p-values ≤ 0.01 were considered highly significant. Correlations were calculated using Pearson’s coefficient of correlation (r) and considered trivial for r < 0.1, small for 0.1 - 0.3, moderate for 0.3 - 0.5, large for 0.5 – 0.7, very large for 0.7 – 0.9, nearly perfect for 0.9 < 1.0, and perfect for 1.0 [57]. Subgroup analysis was performed using Mann-Whitney-U-test and Kruskal-Wallis tests for unpaired samples.

Subgroups for AFI and RFI measurements between day 7 and day 28 were defined as follows: Group low was 0 - 33 percentile (P0 - P33), corresponding to RFI ≤ 2.36 l/m² (n = 7), ≤ 2.88 l/m² (n = 13), ≤ 2.79 l/m² (n = 18), ≤2.59 l/m² (n = 24), ≤3.56 l/m² (n = 28); Group high was 66 -100 percentile (P66-100), corresponding to RFI ≤ 3.21 l/m² (n = 7), ≤ 3.17 l/m² (n = 13), ≤ 3.78 l/m² (n = 18), ≤ 3.15 l/m² (n = 24), ≤ 3.86 l/m² (n = 28).

For [Na⁺] subgroups were defined according to the physiological range of sodium: Hyponatremia (NaLo) = [Na⁺] <135mmol/l; Normonatremia (NaNo): [Na⁺] =135 -145 mmol/l; Hypernatremia (NaHi): [Na⁺] >145mmol/l.

Hematocrit (Hct) subgroups were defined as follows: 0 - 33 percentile (P33), 33 - 66 percentile (P66) and 66 -100 percentile (P100).
III. Results

Due to the field experiment character of a training camp, single rowers did not show up at certain measurement points due to training, unawareness, physiotherapeutic intervention or practical reasons. In consequence, a lower percentage of scheduled samples were obtained and data therefore differs in absolute numbers. No injuries and no clinical symptoms of hyponatremia as described above were recorded; none of the included individuals had history of EAH.

1. Participants

All athletes completed the study. Daily exercise time amounted between 156 to 245 min/day, in average 176 min/day. Training included at least one low-intensity training session per day (athletics or stretching); rowing sessions lasted up to 100 min, in average 23.2 km of distance. The training schedule was organized in 3 - 4 sessions per day, allowing the athletes to recover (rest or sleep) between workouts. Athletes had time to consume sufficient food and beverages, which were unrestricted.

Due to sports-specific selection and age limitations for youth national teams, height and age showed very little inter-individual deviation. Body mass was conducted immediately before entering the ergometer incremental test, but there are no existing weight limitations in youth rowing. National team qualification took place 3 weeks before the start of the training camp; therefore, physical performance was already on elite level. Table 1 shows anthropometric data and athletic performance.

Medical baseline investigation showed physiological values for all laboratory assessment for the vast majority, while 3 (2 male, 1 female) athletes had insignificantly low [Na⁺] between 132 and 134 mmol/l, 9 athletes (7 male, 2 female) showed elevated [Na⁺] between 146 and 170 mmol/l. COP was within the physiological range (1.8 –13.8 pmol/l according to [76]) for males and females, amounting to 5.60 ± 3.49 pmol/l (males) and 5.36 ± 2.89 pmol/l (female). Body surface area averaged at 2.06 ± 0.17 m² overall, for males 2.14 ± 0.11m² (n=21), for female athletes (n=9) at 1.87 ± 0.11m².
Table 1: Subject characteristics. Subject characteristics of 30 adolescent rowers (21 male; 9 female) of the German junior national team at the beginning of a 32 day lasting training camp. Data is mean ± standard deviation. \( VO_{2\text{max}} \) = maximal oxygen uptake on rowing ergometer; \( P_{\text{max}} \) = maximal power output in rowing ergometer test. \([La]_{\text{max}}\): highest blood lactate concentration measured in ergometer test. See text for details.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>total</th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>17.9 ± 0.5</td>
<td>17.9 ± 0.4</td>
<td>17.8 ± 0.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>187.2 ± 7.99</td>
<td>190.8 ± 5.55</td>
<td>178.8 ± 6.47</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>82.0 ± 10.4</td>
<td>86.9 ± 7.3</td>
<td>70.6 ± 6.9</td>
</tr>
<tr>
<td>( VO_{2\text{max}} ) (ml/min/kg)</td>
<td>62.84 ± 3.85</td>
<td>52.67 ± 3.77</td>
<td></td>
</tr>
<tr>
<td>( P_{\text{max}} )/ body mass (W/kg)</td>
<td>5.50 ± 0.34</td>
<td>4.67 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>([La]_{\text{max}}) (mmol/l)</td>
<td>15.91 ± 2.93</td>
<td>14.52 ± 2.21</td>
<td></td>
</tr>
</tbody>
</table>

2. Weather conditions

Regarding outdoor surroundings and setting measurements of air humidity (%) and air temperature (°C) were collected each day at 6 a.m. (time of blood withdrawal) and at noon to conduct the conditions athletes were put in. There was no rainfall or thunderstorm on measurement days. Conditions were classified as warm but moderate temperatures throughout the experiment (table 2).

Table 2: Schedule for points of measurement and the training volume of the related training period during 32 day rowing training camp. Training is the total volume per week in minutes includes all exercises rowing, running, athletics, and resistance training. Rowing volume is displayed as distance in km/wk. Climate is represented as ambient temperature and air humidity; measurements being conducted at 6 a.m. and 12 p.m..

<table>
<thead>
<tr>
<th>Week of training camp</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training: Duration/wk (min/wk)</td>
<td>780</td>
<td>1715</td>
<td>1435</td>
<td>1240</td>
<td>840</td>
</tr>
<tr>
<td>Rowing: Distance/wk (km/wk)</td>
<td>104</td>
<td>180</td>
<td>202</td>
<td>170</td>
<td>133</td>
</tr>
<tr>
<td>Time point of measurement</td>
<td>day 7</td>
<td>day 13</td>
<td>day 18</td>
<td>day 24</td>
<td>day 28</td>
</tr>
<tr>
<td>Temperature at 6 a.m./12 a.m. (°C)</td>
<td>13.3/19.8</td>
<td>18.8/24.8</td>
<td>15.0/22.0</td>
<td>18.6/25.3</td>
<td>16.8/22.3</td>
</tr>
<tr>
<td>Humidity at 6 a.m./12 a.m. (%)</td>
<td>65/39</td>
<td>65/31</td>
<td>91/51</td>
<td>86/65</td>
<td>74/52</td>
</tr>
</tbody>
</table>
### 3. Sodium

[Sodium (Na⁺)] showed a highly significant nadir \((p<0.01)\) with the mean below the laboratory range for physiological values on day 18, compared to baseline, day 7 and day 28 measurements (Table 3). Overall values ranged from a minimum of 121 mmol/l (day 18, \(n=1\)), to a maximum of 157 mmol/l (day 28, \(n=1\)) and even 170 mmol/l (baseline, \(n=1\)), therefore hypo- as well as hypernatremia was observed. The time course of [Na⁺] was similar for both male and female athletes (figure 2).

**Figure 2a**: Time Course of sodium [Na⁺] and copeptin in 21 male rowers during a 32 day training camp. Measurements were taken before, after 7, after 13, after 18, after 24, and after 28 days. Data are represented as median, 25% and 75% -quartile (Boxplot); dots represent outliers. Dotted lines represent upper and lower physiological range for Sodium (135-145 mmol/l). * = sign. \((p<0.05)\) change to baseline measurement. # = sign. \((p<0.05)\) change to day 7 measurement.

Hyponatremia ([Na⁺] <135mmol/l) was observed at least once in 62 % of male (\(n=13\)) and 89 % of female (\(n=8\)) athletes with the highest prevalence at day 18 (43 %). Hyponatremia was found throughout the study period in several athletes; 4 male and 2 female athletes had hyponatremia on 2 consecutive measurements, 2 male athletes had recurrent hyponatremia, 2 hyponatremic values with intermittent normonatremic measurements. When data were combined, a small negative correlation was found between the changes in body weight within 24 h (\(Δ BW\)) to Sodium concentration (figure 3).
III. Results

Figure 2b: Time Course of sodium [Na⁺] and copeptin in 9 female rowers during a 32 day training camp. Measurements were taken before, after 7, after 13, after 18, after 24, and after 28 days. Data are represented as median, 25% and 75% -quartile (Boxplot); dots represent outliers. Dotted lines represent upper and lower physiological range for Sodium (135-145 mmol/l). * = sign. (p<0.05) change to baseline measurement. # = sign. (p<0.05) change to day 7 measurement.

Figure 3: Combined data of plasma sodium concentration (mmol/l) and weight change (kg) compared to weight 24h before sodium measurements. Data of 21 male and 9 female rowers was collected at 5 timepoints (day 7, 13, 18, 24, 28) during a 32 day lasting training camp. Grey highlighted area marks hyponatremia (plasma sodium < 135 mmol/l) and weight gain. Pearson correlation significant (r = -0.260, p<0.01).
III. Results

Table 3: Sodium, hematocrit, osmolality in fasting state, absolute and relative fluid intake and urine volume collected over 24h in 30 rowers during a training camp. [Na⁺] = Sodium in plasma, Hct = Hematocrit, [Osm] = Osmolality in plasma, RFI = relative fluid intake = absolute fluid intake per body surface area; AFI= absolute fluid intake; UV = urine volume. AFI and UV were collected over 24 hrs followed by blood withdrawal for [Na⁺], Hct, [Osm]. Blood withdrawal with baseline and at days 7, 13, 18, 24 and 28. Data are mean ± standard deviation. n = sample size.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sample</th>
<th>baseline</th>
<th>day 7 mean</th>
<th>SD</th>
<th>n</th>
<th>day 13 mean</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na⁺] [mmol/l]</td>
<td>total</td>
<td>144.0 ± 8.7</td>
<td>138.9 ± 1.5</td>
<td>30</td>
<td>137.4 ± 2.4</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>144.9 ± 9.7</td>
<td>138.7 ± 1.8</td>
<td>21</td>
<td>137.5 ± 2.6</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>141.8 ± 5.5</td>
<td>139.3 ± 0.7</td>
<td>9</td>
<td>137.4 ± 1.8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct [%]</td>
<td>male</td>
<td>48.6 ± 3.0</td>
<td>49.9 ± 2.0</td>
<td>21</td>
<td>49.1 ± 2.6</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>45.3 ± 3.7</td>
<td>44.0 ± 4.7</td>
<td>8</td>
<td>45.6 ± 2.9</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Osm] [mosm/kg]</td>
<td>total</td>
<td>277.90 ± 8.57</td>
<td>283.29 ± 6.89</td>
<td>29</td>
<td>283.86 ± 6.43</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>2.79 ± 0.87</td>
<td>3.09 ± 0.46</td>
<td>17</td>
<td>6798 ± 996</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>2.20 ± 0.70</td>
<td>2.76 ± 0.66</td>
<td>8</td>
<td>5181 ± 1320</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFI [l/m²]</td>
<td>male</td>
<td>5979 ± 1867</td>
<td>2458 ± 1140</td>
<td>17</td>
<td>2458 ± 1140</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>4194 ± 1491</td>
<td>2192 ± 1094</td>
<td>8</td>
<td>2192 ± 1094</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFI [ml]</td>
<td>male</td>
<td>2351 ± 1148</td>
<td>8</td>
<td>1724 ± 948</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>277.90 ± 8.57</td>
<td>283.29 ± 6.89</td>
<td>29</td>
<td>283.86 ± 6.43</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV [ml]</td>
<td>male</td>
<td>278.83 ± 8.58</td>
<td>282.63 ± 11.77</td>
<td>29</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>2.97 ± 0.55</td>
<td>3.88 ± 0.69</td>
<td>17</td>
<td>8162 ± 1612</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.26 ± 0.56</td>
<td>2.65 ± 0.93</td>
<td>7</td>
<td>4979 ± 1670</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6403 ± 1215</td>
<td>8162 ± 1612</td>
<td>17</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4293 ± 1115</td>
<td>4979 ± 1670</td>
<td>7</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3051 ± 1569</td>
<td>3726 ± 1711</td>
<td>21</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2813 ± 1139</td>
<td>2149 ± 900</td>
<td>9</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
III. Results

4. Serum osmolality

A significant decline (p<0.05) was found for [Osm] at day 18, but also at day 28 for the entire group (table 3). No significant differences were found between sexes. The correlation between [Osm] and COP was never higher than moderate (day 18: r = 0.368, p<0.05, n=29).

5. Copeptin

COP significantly decreased during the course of the training camp from baseline to day 18 (p<0.05), day 24 (p<0.001) and day 28 (p<0.001). Sex differences (fig.2 a-b) were significant at day 13 (p = 0.006; male: 7.48 ± 3.58 pmol/l n = 20; female: 3.56 ± 2.48 pmol/l, n = 9) and day 18 (p = 0.002; male: 6.41 ± 3.10 pmol/l n = 21; female 2.74 ± 1.21 pmol/l n = 9). Different fluid intake (Low/Mid/High) was not accompanied by significant changes in in COP in male rowers (Table 4).

Table 4: Copeptin(pmol/l) in male rowers grouped according to individual relative fluid intake (RFI). Athletes were grouped according to percentiles of their relative fluid intake (RFI), which was calculated as absolute fluid intake/ body surface area. Group low was percentile 0 - 33, Group mid was percentile 33 - 66, Group high was percentile 66 - 100. COP = Copeptin in plasma (pmol/l); n = sample size; measurements at day 7 of training camp, day 13, day 18, day 24 and day 28. Data is mean ± standard deviation.

<table>
<thead>
<tr>
<th>Group (Fluid Intake)</th>
<th>baseline COP</th>
<th>day 7 COP</th>
<th>day 13 COP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 7</td>
<td>day 13</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 18 COP</td>
<td>day 24</td>
<td>day 28</td>
</tr>
<tr>
<td>Low</td>
<td>6.5 ± 2.7</td>
<td>5.9 ± 2.9</td>
<td>3.1 ± 1.3</td>
</tr>
<tr>
<td>Mid</td>
<td>5.4 ± 3.0</td>
<td>2.7 ± 1.1</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>High</td>
<td>6.8 ± 4.0</td>
<td>2.6 ± 0.7</td>
<td>3.4 ± 1.3</td>
</tr>
</tbody>
</table>
III. Results

6. Fluid intake

RFI showed a tendency to increase (table 3). However, the increase in RFI was not paralleled by a decline in [Na\(^+\)]. Instead, the correlation between RFI and [Na\(^+\)] remained small over the duration of the training camp (overall correlation r = 0.116, p= 0.114). Increasing correlation was found between AFI and NaSU measurements, starting at day 7 (r = 0.217; n.s.), day 13 (r = 0.232; n.s.), day 18 (r = 0.367; n.s.), to day 24 (r = 0.567; p<0.01) and day 28 (r = 0.690; p<0.001). Correlation analysis between AFI and BSA (see equation 1) was calculated to support the assumption that large athletes drank more than small athletes. As correlation analysis revealed moderate to large correlations at every time point (correlation varied from r = 0.49 to r = 0.62), RFI was used throughout the study to analyze fluid intake.

Inter-group differences of fluid intake, body mass, hematocrit, osmolality and copeptin

When sodium measurements were grouped according to the [Na\(^+\)]-level ((NaLo): n = 30 (NaNo): n = 94; (NaHi): n = 9), no significant inter-group differences were found regarding Hct (p = 0.451), COP (p = 0.216), [Osm] (p = 0.096) and RFI (p = 0.632) for both sexes. Therefore, male athletes with low [Na\(^+\)] consistently had neither lower nor higher COP or [Osm] than the normonatremic athletes (table 5). Nonsignificant changes were found regarding fluid intake, when hyponatremic athletes (NaLo) at day 24 consumed less fluid than normo- and hypernatremic grouped athletes, whereas hyponatremic male and female athletes at day 18 (n = 13; [Na\(^+\)] 129.38 ± 2.76 mmol/l; COP 4.37 ± 2.53 pmol/l; [Osm] 275.75 ± 8.42 mosm/kg) showed higher RFI compared to normonatremic athletes. Δ BW (equation 5) was analysed for sodium groups: a significant difference between hyponatremic male and female athletes (who gained 1.03 ± 1.16 kg (n = 11)) and normonatremic male and female athletes (who lost 0.49 ± 0.64 kg (n = 14)) was observed at day 18, but not for day 7, day 13, day 24 and day 28.
### III. Results

Table 5: Copeptin in 21 male rowers grouped according to individual sodium level. 21 athletes were grouped according to their plasma sodium concentration (hyponatremia < 135 mmol/l, normonatremia 135 - 145 mmol/l, hypernatremia > 145 mmol/l). COP = copeptin in plasma (pmol/l); n = sample size; Measurements taken at baseline, at day 7 of training camp, day 13, day 18, day 24, and day 28. Data is mean ± standard deviation.

<table>
<thead>
<tr>
<th>Group (Sodium status)</th>
<th>baseline COP</th>
<th>n</th>
<th>day 7 COP</th>
<th>n</th>
<th>day 13 COP</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyponatremia (&lt; 135 mmol/l)</td>
<td>7.00 ± 3.81</td>
<td>2</td>
<td>0</td>
<td></td>
<td>7.53 ± 2.30</td>
<td>2</td>
</tr>
<tr>
<td>Normonatremia</td>
<td>5.75 ± 3.90</td>
<td>12</td>
<td>6.75 ± 2.78</td>
<td>18</td>
<td>7.47 ± 3.75</td>
<td>18</td>
</tr>
<tr>
<td>Hypernatremia (&gt;145 mmol/l)</td>
<td>4.29 ± 2.28</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group (Sodium status)</th>
<th>day 18 COP</th>
<th>n</th>
<th>day 24 COP</th>
<th>n</th>
<th>day 28 COP</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyponatremia (&lt; 135 mmol/l)</td>
<td>4.96 ± 3.05</td>
<td>8</td>
<td>3.53 ± 1.83</td>
<td>7</td>
<td>5.38 ± 3.68</td>
<td>2</td>
</tr>
<tr>
<td>Normonatremia</td>
<td>7.31 ± 2.88</td>
<td>13</td>
<td>3.37 ± 1.78</td>
<td>9</td>
<td>3.38 ± 1.45</td>
<td>17</td>
</tr>
<tr>
<td>Hypernatremia (&gt;145 mmol/l)</td>
<td>6.04 ± 4.24</td>
<td>4</td>
<td></td>
<td></td>
<td>2.97</td>
<td>1</td>
</tr>
</tbody>
</table>

7. Hematocrit

Up to day 7, Hct slightly increased after baseline measurements for males (48.6 ± 3.0 n = 21) and decreased for female athletes (45.3 ± 3.7 n = 8). However, Hct levels did not alter significantly between day 7 and day 28 for both genders, but continued to differ between male and female athletes (Table 3). Highest levels were found on day 24 for male athletes with mean over 50 % Hct, for females athletes on day 13 with 45.6 % mean Hct. Athletes with highest values (> Percentile 66) did not show significant different [Na⁺]-values, compared to athletes with lower Hct values.
8. Secretion Index

Calculated SI decreased from day 7 to 28 (fig. 4); values of day 24 (2.7 ± 0.32 pmol/mmol) and 28 (2.4 ± 0.20 pmol/mmol) were highly significant (p < 0.003) lower than values of day 7 (4.5 ± 0.40 pmol/mmol) and day 13 (4.3 ± 0.54 pmol/mmol).

![Figure 4: Course and distribution of secretion index.](image)

**Figure 4:** Course and distribution of secretion index. Secretion index (SI) in 30 rowers during a 32-day training camp. SI = (COP/[Na+]) x100 (pmol/mmol); at baseline, day 7, day 13, day 18, day 24 and day 28. Boxplot, dots represent outliers, arrows represent significant decrease of day 24 and day 28 values (p < 0.003).

9. Urine volume and urine sodium content

Urine volume significantly increased in male athletes values from day 7 (2351 ± 263 ml) to day 28 (3726 ± 383 ml) (p < 0.05), and significantly from day 7 (1723 ± 948 ml) to day 24 (2813 ± 1138 ml) in female rowers, but did not differ significantly between sexes. Sodium urine content NaSU differed likewise between sexes, but did not show a clear tendency with values ranging from 217.61 ± 98.26 mmol (approx. 12730 mg NaCl) at day 13 to 288.94 ± 115.73 mmol (approx. 16903mg NaCl) at day 24. NaSU was strongly correlated with AFI values at day 24 and 28.
III. Results

10. Sodium intake

Sodium intake with fluid was calculated for all athletes and differed between 654.24 ± 225.67 mg at day 7 up to 853.53 ± 256.32 mg at day 28, a congruent increase with the AFI/RFI values (table 6).

Table 6: Sodium intake and excretion at 5 time points during a 32-day training camp. \( \text{Na}_\text{fluid}^+ \) = Sodium intake with fluid in mg within 24h NaSU = Sodium excretion via urine in mg within 24h over the course of a 32 day training camp, measurements at day 7, day 13, day 18, day 24, day 28. Data are mean ± standard deviation. n = sample size.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sample</th>
<th>baseline</th>
<th>7 days</th>
<th>13 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>( \text{Na}_\text{fluid}^+ \text{ (mg)} )</td>
<td>male</td>
<td>721.4</td>
<td>213.3</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>494.9</td>
<td>176.0</td>
<td>8</td>
</tr>
<tr>
<td>NaSU (mg)</td>
<td>male</td>
<td>6291.2</td>
<td>1982.5</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>3836.3</td>
<td>1743.2</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sample</th>
<th>18 days</th>
<th>24 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>( \text{Na}_\text{fluid}^+ \text{ (mg)} )</td>
<td>male</td>
<td>855.8</td>
<td>202.8</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>498.6</td>
<td>191.4</td>
<td>8</td>
</tr>
<tr>
<td>NaSU (mg)</td>
<td>male</td>
<td>6450.7</td>
<td>2509.2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>3635.7</td>
<td>1337.5</td>
<td>9</td>
</tr>
</tbody>
</table>

11. Fluid balance

Calculated FB (equation 4), similar to AFI and UV, was gender specific, coming to a significant nadir at day 24 for male (3050.59 ± 1320.39 ml, n = 17) and female (1101.43 ± 1706.79 ml, n = 7) athletes. Values at day 28 (male 4271.18 ± 1642.76 ml n = 17; female 2647.14 ± 1388.58 ml n = 7) did not vary to day 7 (male 3762.11 ± 1533.60 ml n = 19, female 2470.0 ± 1147.17 ml n = 8), day 13 (male 4339.5 ± 1269.96 ml n = 20, female 2832.5 ± 1174.96 ml n = 8) or day 18 (male 4960.59 ± 2166.96ml, n = 17, female 1980.0 ± 1472.15 ml n = 8) measurements.
12. Case study athlete

To illustrate the common changes of the parameters in context to each other, data of a single male athlete was derived and compiled in a graph. This individual gained BW from day 17 to day 18 (1.1 kg). Hematocrit values showed increasing values, while FB decreased. See figure 5 for details.

![Graph](image)

**Figure 5: Case study athlete.** Course of changes in body weight (Δ BW) (kg), fluid intake (ml/d) and urine volume (ml/d) within 24h prior to measurements of capillary hematocrit [%], copeptin [pmol/l] and sodium [mmol/l] and SI (SI = (copeptin/sodium) x100 (pmol/mmol)) in a highly trained rower during a 32 day training camp. Baseline, at day 7, day 13, day 18, day 24 and day 28.
IV. Discussion

This study is the first to investigate the incidence of hyponatremia ([Na⁺]<135mmol/l) and samples of COP in a large sample of highly trained endurance athletes during a training camp with repeated bouts of exercise. Our study has the following key results:

- High volume rowing training in a training camp is associated with an incidence of hyponatremia.

- Morning copeptin continuously decreases during the course of a training camp in the presence of persisting high-normal Hct and increasing fluid intake.

- Plasma copeptin – plasma sodium ratio alters during a 32 day training camp.

1. High volume rowing training in a training camp is associated with an incidence of hyponatremia

The incidence of hyponatremia in our study is surprisingly high as 43 % of the athletes in this study experienced a clinically asymptomatic hyponatremia at day 18, exactly when weekly average training volume was at its highest. Overall, 70 % of the rowers developed hyponatremia at least once during four weeks of high volume rowing training. Due to the coincidence of exercise volume and hyponatremia it is questionable, whether the observed hyponatremia during this training camp meets the classic criteria of EAH, which has been defined as hyponatremia in individuals while or within 24 hours after “prolonged physical exercise”[49]. Hyponatremia is quantified by the normal range for plasma sodium according to the laboratory range [86]. Besides, a multi factor pathogenesis has to be taken into account to distinguish between hyponatremia and current EAH concepts. These concepts include the presence of exercise (1), excessive fluid intake (2), inadequate AVP levels (3) and the failure to mobilize sodium from internal stores [86], as well as several risk factors[49], which will be discussed below.

1a. Exercise
According to Noakes [86] “Prolonged physical exercise” is one factor contributing to EAH. However, “Prolonged physical exercise” has not been defined exactly, whereas EAH is clearly associated with marathon and ultra-marathon races [74, 75, 103, 111, 115, 122], while hyponatremia in athletes exercising at high intensities of less than 4h (240 min) in a single bout has been described rarely [75].

In this study, daily exercise time was shorter than 240 min, exercise varied in intensity and was additionally divided into shorter sessions 3-4 times daily up to 100 min. Still, the measurements confirm the appearance of asymptomatic hyponatremia in the presence of repetitive rowing training with a pathogenesis over days rather than hours. These conditions are not in line with the typical view on EAH.

1b. Inadequate ly high fluid intake

“Excessive overdrinking” is possibly the most important contributor to EAH [2, 24, 38, 47, 83, 86] and has even been suggested to be a monocausal trigger of EAH [87]. AFI of all athletes in this study (table 3) with enormous values over 8 liter per day may lead to drawing similar conclusions. But in literature, “ad libitum” fluid intake triggered through thirst has been postulated to be indifferent towards the development of EAH [88].

However, data is ambiguous regarding overdrinking: At day 18, RFI increased (not significantly) in 7 out of 8 male rowers who experienced hyponatremia. Male and female hyponatremic athletes on day 18 (n = 13) showed significant weight gain within 24 h before blood withdrawal, whereas normonatremic male and female athletes lost weight within this period. At day 24 and 28, in contrast, higher fluid intake was associated with normal and even elevated sodium levels, no significant difference was found in weight changes for day 24 and 28. Athletes with low sodium levels at day 24 consumed approximately 0.19 l/m² RFI (corresponding to about 300 ml AFI) less fluid, compared with athletes who maintained normal sodium values and not vice versa. Finally, RFI itself did not show any significant (negative) correlation with $[\text{Na}^+]$ levels.

This clearly indicates that drinking high volumes did not automatically lead to hyponatremia and drinking less did not consequently prevent the appearance of EAH. Weight gain within 24 h before blood withdrawal on the other hand were clearly correlated with lower $[\text{Na}^+]$ levels when data of all measurements was combined (figure 3), similar to previous data, i.e. from Noakes for acute EAH [2, 86, 94, 113-115, 131]. Consequently, excessive fluid intake and weight gain 24h before blood measurements contributed to the occurrence of hyponatremia in single athletes at particular time points, but does not explain
the onset of hyponatremia in most of the cases. This makes a clear difference to the classic view on EAH.

1c. Inadequate suppression of AVP

AVP counteracts an increase in plasma osmolality [99] and triggers water reabsorption. In consequence, inadequate high AVP levels will lead to a dilution of plasma sodium and hyponatremia, especially in an exercise setting [111, 114, 115, 117], similar to the syndrome of inappropriate antidiuretic hormone secretion (SIADH)[111]. In this study, AVP was measured via copeptin, its equimolar secreted precursor molecule[76]. In patients suspected to suffer from SIADH it has been demonstrated, that copeptin levels lower than 3 pmol/l plasma and low urine osmolality determined primary polydipsia[31]. Therefore, copeptin levels below normal would be the appropriate reaction to hypervolemia and hyponatremia.

Data of the present study are ambiguous regarding AVP suppression: [Na$^+$] in male athletes increases to normal values, when COP decreases below 4.0 pmol/l (figure 2a, day 24), supporting the theory of an inadequate AVP-suppression around day 18. Additionally, analysis on the individuals showed that COP levels in 5 of 8 hyponatremic males of day 18 were higher (6.55 ± 2.76 pmol/l) than levels conducted at day 28. Re-establishment of normal [Na$^+$] values in those individuals was therefore paralleled by a further reduction of COP towards day 24 (3.64 ± 2.49 pmol/l) and later day 28 (3.41 ± 1.26 pmol/l). Consequently, an intermittent and insufficient suppression of AVP might contribute to the hyponatremic situation in particular rowers.

On the other hand, it was obviously possible for most of the rowers to maintain normonatremia in spite of an “inappropriate” (above 3 pmol/l [31]) COP secretion (day 24 and 28) (table 5). Interestingly, in the female rowers the strongest suppression of AVP and the lowest COP values were found at day 18 and 24, when [Na$^+$] and FB were lowest as well. Moreover, normal values of [Na$^+$] were reestablished after 28 days in spite of a small increase in COP in females above 3pmol/l (figure 2b) and without hyponatremic measurements.

Taken altogether, a possible inadequate suppression of AVP resp. COP that contributes to hyponatremia cannot be ruled out in some of the observed male rowers, but data does neither underline this notion in the female rowers nor in the whole group.
1d. Inability to mobilize sodium from internal stores

The calculation of complete sodium uptake and sodium loss in context of blood sodium level can reveal changes in internal sodium stores.

Sodium fluid intake $\text{Na}^+_{\text{fluid}}$ was calculated (equation 3) and urine sodium loss was measured (NaSU). Sodium balance is indicated by the difference of $\text{Na}^+_{\text{fluid}}$ and NaSU, with negative values indicating sodium loss. The data support the incidence of hyponatremia.

$\text{Na}^+_{\text{fluid}}$ rises with increasing AFI (equation 3) and may contribute to maintaining $[\text{Na}^+]$ levels, especially when retrieving sodium from internal stores is impossible, either due to outspent stores or inability to mobilize those stores[86]. NaSU and AFI correlated at day 24 and 28, therefore sodium loss was in line with fluid (and sodium) intake when sodium levels were restored at day 28. As a limitation, sweat sodium loss and sodium food intake within a training camp setting with multiple exercise bouts and meals per day could not be retrieved, but are inter- and intra subject variables and directly responsible for water and electrolyte loss especially during exercise above 40%$\text{VO}_{2\text{max}}$ [21, 89, 95].

Rare reports of internal nonosmotic storage of sodium are found in microgravity studies [26], reproduced later under normal gravity conditions with storage in cartilage and skin, but only when intake rose from average (200mEq/d NaCl) to very high levels (550mEq/d NaCl) [46]. Daily food salt intake of elite athletes has not been evaluated, but salt intake has been found to be as high as 4.8 g/d sodiumchloride in an average Italian population[17], and was suggested not to exceed 5.8 g/d sodiumchloride ($\approx 2.3$ g sodium) for an individual [35]. Sweat Sodium loss, on the other hand, was stated to be up to 5g sodium during a single high-intensity workout [105], and has shown broad interindividual variability with a range from 20 to 80 mmol/l [109]. Meyer et al. suggested $0.47\pm0.23$ mEq for young adults males and $0.32\pm0.10$ mEq for adults females per hour activity per kg body mass [72]. The mechanisms leading to an activation or inactivation of osmotically inactive or active sodium storage are still unknown; therefore these data cannot support or neglect this theory.

1e. Presence of risk factors

Other potential contributors to the onset of EAH are female sex [2, 7-9, 24, 47, 114, 121], lower body weight [2, 15, 101, 111, 122], hot or cold environmental conditions [8, 33, 58,
IV. Discussion

97, 121], longer duration of exercise [2, 5, 15, 16, 27, 33, 38, 39, 47, 55, 60, 83, 92], weight gain during exercise [2, 5, 16, 27, 39, 45, 83-86, 94, 113-116, 131] and CFTR variant [10, 74, 93, 112].

In the present study the female rowers showed a higher prevalence for EAH, in line with findings by Hew-Butler et al., who studied 55 individuals treated with i.v. fluids in the medical area after the 2000 Houston Marathon, 34 without and 21 with hyponatremia [47]. A lower body mass was in female rowers also present, but overall there was a wide range of body weight in the observed group (65.0 to 97.5 kg) because there are no existing weight limits in youth rowing, and no “lightweights” were members of the team [101] (table 1).

The weather conditions in Berlin in July were moderately warm (table 2). While acclimation does not play a role during studies of a single exercise set, such as marathon running, a 32-day lasting study has to consider accumulated acclimation effects.

Heat acclimation is known to decrease the sweating threshold temperature and increase the rate of sweating in athletes [6, 61] as well as reduces the sodium content [1]. These mechanisms may support the regain of physiological sodium values at day 24 and 28. This study does not provide data towards the sweat sodium loss and cannot reflect athletes’ heat acclimation, even though this may play a role towards the incidence of EAH during a training camp. Exercise duration itself was not varied for different athletes, but declined over the course of the training camp, while exercise intensity increased (table 2). Weight changes during exercises or between single exercise sets respectively were not conducted, but changes within days were documented for single athletes, who suffered hyponatremia simultaneously (i.e. case study athlete, figure 5). Especially at day 18, hyponatremic athletes gained weight the day before, whereas normonatremic athletes had lost weight 24h prior to blood sampling. CFTR genotype was not conducted, but none of the participating athletes had history of symptomatic EAH.

1f. Conclusion regarding EAH

Taken all together, the hyponatremia being observed in the rowers during the training camp never completely met the criteria of “classic” EAH, but in some cases known pathomechanisms such as excessive fluid intake, osmotic stimulation and inadequate AVP-suppression can be identified. Furthermore, risk factors, such as weight gain during exercise, female sex and lower body weight as well as environmental conditions contribute
IV. Discussion

to the development of hyponatremia during a training camp. Future research is necessary to understand the underlying mechanism.

2. Morning copeptin decreases during the course of the training camp

A decrease in COP (or AVP, respectively) is induced by volemic or osmotic triggers [50]. According to Hew-Butler et al. COP reflects especially chronic volemic stimuli, whereas AVP is more reflective of acute osmotic stimuli [54]. Still, both triggers could cause a decrease in plasma level of COP (or AVP, respectively) and will be discussed below.

2a. Volemic triggers

An increase in plasma volume is a common adaption to endurance training [23], which normally takes place within the first days of a training program [18, 104]. Therefore we expected a decrease in Hct between baseline and the following measurements (day 7 to 28) as it has been documented for other endurance athletes during season [98], since an increase of red cell volume can be ruled out in normoxia [107]. This “hypervolemic” situation would potentially contribute to a decrease in COP.

In female rowers Hct, was reduced and [Osm] was significantly lower at day 18 (table 3). This indicates a hypovolemic situation compared to day 7 and day 13, which might contribute to the decrease in COP or AVP, respectively. This notion is supported by an increase in COP at day 24 (figure 2b), when Hct was reestablished, indicating a decrease in blood volume. Nevertheless, it has to be noted that COP did not return to baseline levels, even when Hct and [Osm] did, and the lowered morning COP levels at day 24 and 28 in the female rowers were not accompanied by plasma volume expansion (table 3, figure 2b).

However, Hct in male rowers did not decrease between baseline and day 28, as it was observed earlier by Lormes et al.[63], thus no sign for a plasma volume expansion in the male rowers during the whole period (table 3). It seems very likely that plasma volume did not increase because it was already high because the athletes were already highly trained, especially the male athletes [104, 130]. Assuming that the decrease in COP is mainly volume triggered[54], only two further mechanisms seem plausible: Negative feedback due to over proportional increase in fluid intake (in single cases: overdrinking [87]) or effect of repetitive plasma volume changes due to frequent exercise.
RFI (and AFI) had a tendency to increase in the male rowers, while urine excretion increased simultaneously (table 3) allowing body mass and Hct to remain unchanged in the whole group. As fluid intake increased and volemic triggers decreased, it seems plausible that COP (and therefore AVP) is lowered. Consequently, in athletes who drank more than others we expected a more pronounced decrease in COP. However, such a mechanism is not supported by the data: COP levels of male athletes with high fluid intake were not significantly lower or different compared with the other male athletes, even not at day 7 or day 24 (table 4). In other words, drinking huge amounts of fluid is not a prerequisite for reduced COP, although there seems to be a connection in individual cases and at single time points.

Plasma volume decreases during exercise due to fluid shifts from the vascular to interstitial space [19] and due to sweating. Hormonal mechanisms increase water retention and tubular sodium reabsorption [81], leading to a re-establishment and subsequent plasma volume expansion during recovery [22]. Possibly, the repeated increase of plasma volume as an immediate reaction to each training session is a hypervolemic trigger itself, independent of the absolute plasma volume. This is in line with an exercise triggered AVP release [44, 50, 124].

There is a lack of knowledge regarding the hormonal response on repeated bouts of exercise in highly trained subjects. Especially when COP is measured in the morning fasting state, reflection of the water regulation during a post exercise situation of the day before might be impaired, even though COP is said to reflect more chronic plasma volume changes in an exercise setting [54]. According to data of Morgenthaler et al. 2006, COP returns to pre-exercise levels within 1 hour after the end of exercise [76]. Moreover, COP is a surrogate of AVP and consequently there remains some uncertainty about the validity of this marker in a training camp setting.

2b. Potential osmotic triggers

A decrease in plasma osmolality is expected to induce a decrease in COP or AVP followed by an increased urine excretion leading to higher plasma osmolality at a given amount of electrolytes, glucose and urea [52]. As [Osm] was significantly decreased at day 18, the decrease in COP simultaneously was potentially osmotically triggered.

However, data again are ambiguous: [Osm] increased after day 18 (whole group, males, females), while COP decreased further (males) or remained unchanged (females), respectively (figure 2 a-b). Consequently, the decrease in COP after day 18 was not
solitarily osmotically triggered because in that case, COP was not expected to decrease further with increasing [Osm].

3. Plasma copeptin - plasma sodium ratio alters during a 32 day training camp

SI, the ratio of COP and [Na\(^+\)] had a clear tendency to decrease during the training camp (figure 4). In other words, at the end of the training camp, a given sodium concentration is associated with a lower COP concentration, even when sodium regained physiological values. With AVP, alterations in plasma levels associated with training status have been found[42]. During exercise, elevated AVP levels have been demonstrated due to short term training stimulus [18]. A 6 day cycling training test found reduced levels of AVP at the highest work load, which persisted after another 6 days of detraining [110]. Studies by Merry et al. found a decreased response of AVP in relationship to [Osm] in trained subjects compared to untrained subjects, although thirst, drinking response and baseline AVP values were comparable [71].

In this study, Hct remained constant and UV did not increase in relation to fluid intake. When applying calculated SI ratio to values collected before and after ultra endurance races, such as the Javelina Jundred 100 miles run (JJ100) [54], the pre-race values (n=6; 5.0 ± 2.9 pmol/l COP, 141.4 ± 2.2 mmol/l [Na\(^+\)]; [SI] ~3.5 ) are on a level with the pre-training camp values in our study, (n=30; 5.45 ± 3.28 pmol/l COP 143.97 ± 8.70 mmol/l [Na\(^+\)]; [SI] ~3.8).

Acute dehydration and immediate post exercise measurements, as observed in the JJ100 [54], seem to lead to an elevation of this index (post race: n=7; 19.4 ± 17.3 pmol/l COP , 138.9 ± 2.0 mmol/l [Na\(^-\)]; [SI] ~13.9 ), while long-lasting repetitive exercise, as seen in this study, reduces it (day 28: [SI] 2.5 ± 1.2 (n=29)). This change in COP to a lower blood level with similar outcome of antidiuresis could be conducive to the explanation may be understood as an adaption to training, as found for AVP [71]. Additionally, as exercise intensity increased during the course of training camp, an elevated COP response was expected [65]. But it may as well be the result from a lack of osmotic stimulus for COP secretion, when increasing fluid intake was observed (table 3) [53].
4. **Central down regulation**

Several studies describe the course of pituitary hormones and function in overtrained athletes [11] as well as in well trained subjects after repeated bouts of exercise [20, 36, 42, 66, 68, 71, 80]. Increasing training loads over a longer period, such as a training camp, always bare the risk of overtraining. Hypothalamic dysfunction is a well-known problem in overtrained athletes and correlated with decreased responsiveness of hypothalamic hormones (as AVP is) to hypoglycemia, which will be regaining physiological values after a resting period [11]. Consequently, a possible, exercise induced down regulation of AVP has to be taken into account to explain the related decrease in COP [132].

5. **Case study athlete**

In order to simplify the complex results of this study, data of one male athlete were depicted and illustrated separately (figure 5). Data does not exclusively meet trends of the whole group, but displays various typical characteristics:

The effect of overdrinking is known as major contributor to EAH [2, 24, 38, 47, 83, 86, 87]. This athlete increased fluid intake between day 13 and day 18 and, in difference to day 13, gained 1.1 kg body mass within 24 hours from day 17 to 18. Concurrently [Na⁺] clearly decreased to hyponatremic values.

Regarding a possible inadequate AVP suppression [5, 16, 49, 86, 136], this athlete showed increasing COP levels up to day 13, while simultaneously AFI rose, UV was low and [Na⁺] decreased to 134mmol/l. At day 24 and 28, similar AFI lead to lower COP and higher UV. The developed hyponatremia at day 18 was subsequently compensated at day 24, probably due to elevated UV, decreased FB (equation 4) and therefore decreased plasma volume, as indicated by elevated Hct. When the case study athlete persisted on a high drinking volume, consequently, COP (AVP) was inhibited and urine volume increased as volemic triggers for COP release decreased [54]. Regardless the regaining [Na⁺] levels, SI parallels Copeptin at day 18, 24 and 28, possibly as training induced adaption.
6. Interaction with other hormones

Besides AVP and its precursor COP, aldosterone and ANP are known to take part in the fluid balance regulation [44]. Aldosterone plasma levels are well studied and show a clear increase after high intensity and endurance exercise of 2 to 27 hours [3, 30, 37, 100]. Secretion stimuli to aldosterone are plasma volume contraction [51], ACTH stimulation via physical stress [56, 135] and stimulation of the RAAS system [32]; factors, which are clearly seen during a rowing training camp. Additionally, aldosterone plays a key role during recovery, as its inhibition prevents exercise induced plasma volume expansion [64, 123]. According to data of Milledge et al. [73], aldosterone is elevated 24 hours after long lasting exercise and it is even longer effective.

ANP secretion, stimulated by an increase in central blood volume, is elevated after shorter bouts of exercise [34, 127], but has proofed to decrease in long lasting exercise [70] and acclimatization of 30 days field training [80]. Its exercise induced increase was found to correlate with AVP and aldosterone increase [3, 69, 70] and was correlated to the extend of atrial distension [91]. As several tissues contain significant amounts of ANP, unexplored factors might influence the ANP plasma level [78, 125, 133].

We therefore assume that aldosterone is “chronically” elevated in the observed rowers (due to repeated exercise) leading to increased sodium re-absorption, whereas ANP, due to acclimatization and repeated exercise is suppressed. In this sense, the decrease in COP appears as a short time reaction to decrease ongoing antidiuresis. This notion is supported especially by data of the male rowers in this study: When COP (AVP) decreases at day 24, [Na⁺] increases again, since the re-absorption of [Na⁺] is one of the main functions of aldosterone. Simultaneously, COP (and AVP) decreases, avoiding dilution effects and leading to a well-balanced plasma volume, indicated by constant Hct.
7. Limitations

Data of this study is limited due to the field test setting that caused unavoidable biases. This includes the schedule of the testing days, which could not be realized strictly every 5th day, but had to be adjusted to the studied subjects, who were in preparation for world championships. Exact measurements of body mass changes during training session could not be provided due to spray water on sports dress and fear of respiratory tract infections/common cold for underwear measurements. Due to the field experiment character of a training camp, single rowers did not show up at certain time points due to training, unawareness, physiotherapeutic intervention or practical reasons. In consequence, a smaller percentage of scheduled samples was obtained and data therefore differs in absolute numbers. The sample size was not sufficient to draw safe conclusions, but it is the largest sample of highly trained rowers ever measured to evaluate the course of COP in a training camp.

8. Conclusion

Fluid loss during exercise can lead to dehydration, but also to severe electrolyte loss. The most frequent electrolyte disorder is hyponatremia, the decrease of plasma sodium level below physiological values. Among several risk factors, AVP secretion and fluid intake contribute to the pathophysiology of so-called exercise associated hyponatremia (EAH). Its incidence has been connected to endurance exercise, but has never been studied in training camp environment. To investigate changes in sodium, copeptin as surrogate marker for AVP, and identify possible confounders, 30 elite rowers were studied during a 32-day training camp. Repeated measurements of serum sodium ([Na⁺]), plasma copeptin (COP), serum osmolality ([Osm]) and hematocrit (Hct) were taken at baseline before entering the training camp (day 0) and at day 7, day 13, day 18, day 24, and day 28, respectively. In addition, daily fluid intake per body surface area (RFI) as well as urine output (UV) and training volume were assessed. [Na⁺], and [Osm] decreased with nadir at day 18, but returned to physiological values at day 28. COP values decreased, even when [Na⁺] regained physiological values, whereas RFI increased during training camp, so did urine volume (UV). Although, no symptomatic hyponatremia was observed.

This study is the first to investigate the incidence of hyponatremia ([Na⁺]<135mmol/l) in a large sample of highly trained endurance athletes during a training camp with repeated
IV. Discussion

bouts of exercise. The incidence of simultaneous hyponatremia up to 43% of the athletes was unexpectedly high. Although there is evidence for the participation of mechanisms that are also discussed in the onset of EAH, the contradictorily data indicates different mechanisms in the majority of the athletes.

An inadequate suppression of AVP leading to hyponatremia was studied through COP, which was used as a surrogate marker. Even though the discussed mechanism for the onset of EAH (overdrinking, inadequate AVP release, internal sodium store activation) could not be ruled out in particular athletes, the study showed a clear decrease in COP during the course of the training camp. Central down regulation seems very likely to explain the decrease, as well as an interaction with other volume regulating hormones, especially aldosterone. Moreover, the decreasing ratio of COP/[Na⁺] during the training camp might indicate an adaption to training as well. Future research is necessary to evaluate the exact mechanisms that can lead to hyponatremia and decreasing COP in a training camp setting.
V. Abstract

Purpose: The aim of this study was to determine the incidence of hyponatremia during a training camp consisting of high volume training and its relationship to fluid intake, urine volume and arginine vasopressin (AVP) secretion as assessed by measurements of plasma copeptin (COP).

Methods: 30 members (21 males, 9 females) of the German junior rowing team were studied during the training camp preceding the world championships. Samples for serum sodium ([Na\(^+\)], COP, serum osmolality [Osm] and hematocrit (Hct) were taken at baseline before entering the training camp (day 0) and at day 7, day 13, day 18, day 24, and day 28, respectively. In addition, daily absolute fluid intake (AFI) and fluid intake per body surface area (RFI) as well as urine output (UV) and training volume was assessed.

Results: A high incidence of hyponatremia was observed (with 43% of the rowers being hyponatremic at day 18, when training volume was highest). Mean [Na\(^+\)] decreased from 144 ± 8.7 mmol/l (day 0) to 134.5 ± 5.4 mmol (day 18). [Na\(^+\)] returned to normal values at day 28 (139.8 ± 3.9 mmol). [Osm] also decreased (day 18: 278.8 ± 8.6 mosm/kg). COP decreased from baseline (males: 6.74 ± 2.78 pmol/l; females: 4.78 ± 1.05 pmol/l) to day 28 (3.56 ± 1.70 pmol/l; 3.21 ± 1.50 pmol/l) (p<0.05; p<0.05), whereas RFI increased from day 7 (males: 2.79 ± 0.78 l/m\(^2\); females: 2.20 ± 0.70 l/m\(^2\)) to day 28 (3.88 ± 0.69 l/m\(^2\) and 2.65 ± 0.93 l/m\(^2\), respectively (p<0.05). Urine volume (UV) increased simultaneously in male and female athletes, while the difference between AFI and UV (FB) came to a significant low at day 24. None of the athletes developed symptomatic hyponatremia.

Conclusion: High volume rowing training frequently leads to hyponatremia despite decreasing copeptin levels. Overdrinking combined with inadequate suppression of AVP may in part explain these findings. However, additional mechanisms probably contribute to the development of hyponatremia under these conditions.
VI. References


Danksagung

Den Athleten und Betreuern der U19 Nationalmannschaft des deutschen Ruderverbandes, voran Bundestrainerin Brigitte Bielig, ohne deren Einsatz diese Studie niemals hätte durchgeführt werden können.

Mein besonderer Dank gilt meinen wissenschaftlichen Kollegen und Betreuer, insbesondere Dr. Gunnar Treff und Prof. Dr. Steinacker für Ihr stetes Interesse und konstruktive Kritik, welche die Anfertigung dieser Arbeit stets voran trieb.

Meiner Familie, ganz besonders meiner Frau, danke ich für die nötige Geduld und den grenzenlosen Rückhalt, diese Arbeit neben dem beruflichen Alltag fertig zu bringen.
Constantin Ulrich Mayer

Curriculum Vitae

Lebenslauf aus Gründen des Datenschutzes entfernt