Statistical Analysis Plan (SAP)

HYDRATION TO OPTIMIZE METABOLISM

Principal Investigator	Olle Melander, MD, PhD Professor of Internal Medicine Lund University and Skåne University Hospital Malmö, Sweden
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Author	Tommy Schyman, BSc Statistician Clinical Studies Sweden – Forum South Skåne University Hospital Lund, Sweden
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Principal Investigator

Olle Melander (2017-12-15)

Author

Tommy Schyman (2017-12-15)

Abbreviations

ACTH	Adrenocorticotrophic hormone
CI	Confidence Interval
DBP	Diastolic Blood Pressure
eGFR	Estimated glomerular filtration rate
HbA1c	Glycated hemoglobin
HDL	High Density Lipoprotein
IQR	Interquartile Range
ITT	Intention-to-Treat
LDL	Low Density Lipoprotein
OGTT	Oral glucose tolerance test
OR	Odds Ratio
PP	Per Protocol
RCT	Randomized Clinical Trial
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
VP	Vasopressin

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1. Introduction

The aim of this project is to test in a single-centre randomized clinical trial (RCT), if water supplementation in subjects with high plasma levels of vasopressin (VP) (measured by a stable VP marker of its precursor hormone called "copeptin") can reduce fasting levels of glucose (primary outcome measure), risk of new-onset diabetes and other cardiometabolic risk factors (secondary outcome measures).

This statistical analysis plan (SAP) will give more detailed descriptions of the endpoints in the study and the corresponding analyses.

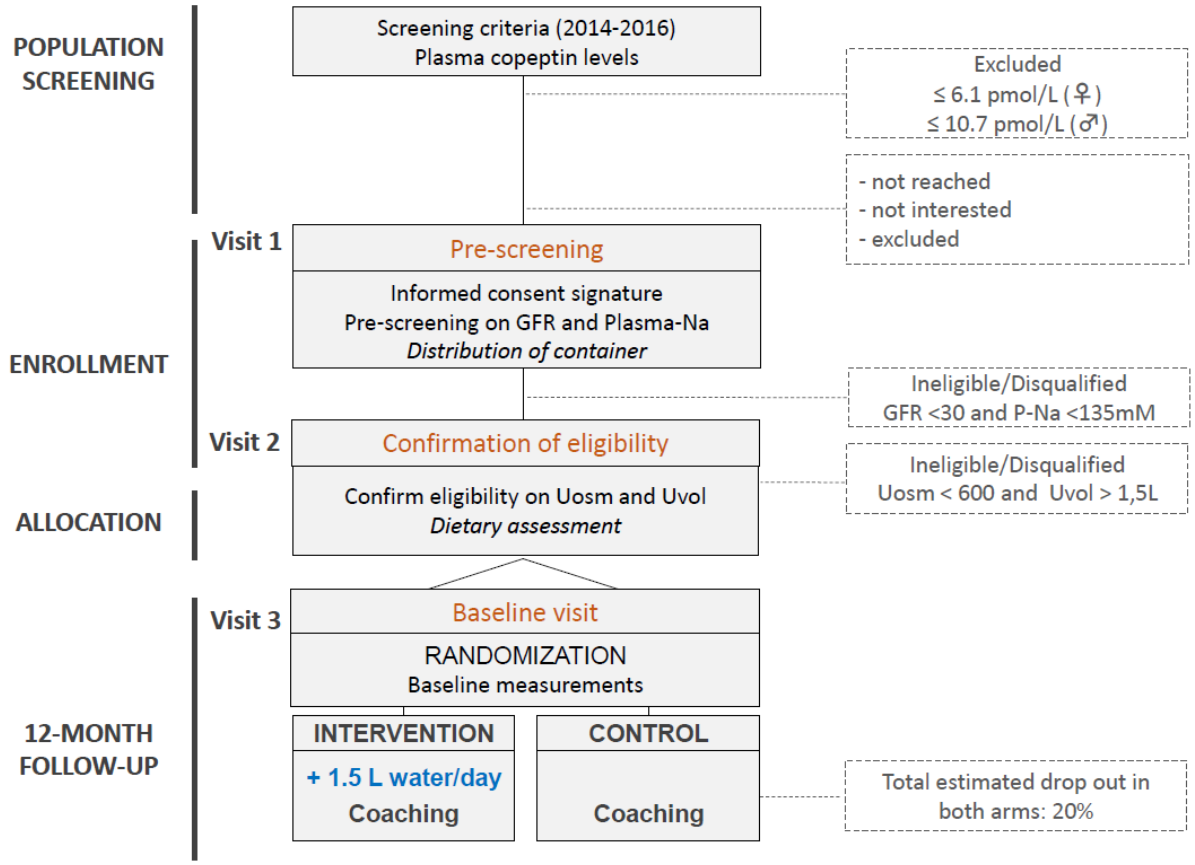
2. Study design

Study subjects will be recruited from 4 ongoing population studies in the Scania region encompassing altogether approximately 20 000 individuals within the current age span. Copeptin will be measured in -80 degree frozen plasma samples from these 4 studies. Individuals having a copeptin concentration of >6.1 pmol/L (women) or >10.7 pmol/L (men) will be invited to participate in the screening and inclusion process of this study. If fewer than expected will be recruited from these 4 studies, employees of the City of Malmö and Skåne University Hospital who are 20-75 years will be invited to undergo a fasting plasma determination of copeptin. The same cut-off values will be used for invitation to the study. If more study subjects are needed, a third source of recruitment will be advertisements in local press.

The study is a parallel-group RCT with two arms during 12 months. Subjects will be randomized to the water-intervention (1.5 L total in three (3) 0.5 L increments daily on the top of habitual intake) and control group (1:1). The randomization will be stratified by gender to pursue equal distribution of intervention and control group for both male and female subjects. Both groups will receive general life style advice (general oral and written advice on diet and physical activity). Smart bottles, which are volume sensitive and can be linked to an Android or iPhone application for individual monitoring purposes will be provided to subjects in the active treatment arm.

Clinic visits are performed at 8 time points: visit 1, visit 2, baseline, 3 weeks and 3, 6, 9 and 12 months at which cardiometabolic risk factors and hydration parameters are measured. The study design is visualised in Figure 1 below.

Figure 1 Flowchart of screening and inclusion process



2.1 Sample size calculation

The primary outcome measure for the power calculation is the difference between active and control treatment in the change of fasting plasma glucose between baseline and 12 months. We use prior effect estimates from the largest RCT for diabetes prevention study in Europe, i.e. the Finnish Diabetes Prevention Study (FDPS) (New Engl J Med 2001;344:1343-50), which compared individual life style counselling (active treatment) with general oral and written life-style advice (control treatment) in relation to risk of new onset type 2 diabetes and change of plasma glucose concentration and found a 58% decreased relative risk of diabetes. After 12 months, the fasting glucose in the active treatment group was reduced by 4±12 mg/dL vs a 1±12 mg/dL increase in the control group with the mean difference of 5 mg/dL of the 12-month change of fasting glucose being highly significant (P<0.001).

To obtain sufficient statistical power to detect a clinically significant effect size, we base our power calculation on an effect of water vs control that is at least 50% of what is considered an epoch changing effect of life style, i.e. the difference observed in the FDPS, while assuming the standard deviation for the change (s = 0.67 mmol/L) as observed in FDPS. In order to be able to detect ≥50% of that effect (a difference of 2.5 mg/dL = 0.14 mmol/L between treatments in 12-month change) we need 319 subjects in both the active and control treatment groups at a power of 80% and a 2-tailed significance level of <0.05. Experiences from the WIT (see above) indicate 8-10% lost to follow-up during 12 months. As our study subjects are "healthy subjects at risk" rather than patients, we anticipate a higher drop-out rate (up to 20%). Based on the power calculation (n=319+319) we will enrol 400 individuals in each treatment group (n=400 in active and n=400 in control arm), i.e. a total number of 800 individuals, to have a final sample size robust to lower compliance than anticipated.

3. Aims and objectives

To study if water supplementation in subjects with high plasma levels of VP (measured by a stable VP marker of its precursor hormone called copeptin) can reduce fasting levels of glucose, risk of new-onset diabetes and other cardiometabolic risk factors.

4. Outcomes

This section will present the outcomes investigated to answer the study aims and objectives. The analyses are described in section 6 Analyses.

4.1 Primary outcome

Fasting plasma glucose. It will be measured at baseline, 6 months and 12 months.

4.2 Secondary outcomes

Diabetes incidence

New-onset diabetes is defined as 2 consecutive fasting plasma glucose values ≥7.0 mmol/L or 2-hour post glucose challenge value of ≥11.0 mmol/L at an oral glucose tolerance test (OGTT). In addition, new onset diabetes is considered present if diagnosed by a physician outside of the current study, as assessed in the questionnaire (answering yes on having had a physician diagnosis of diabetes or having been prescribed medication for diabetes).

Cardiometabolic risk factors

- 2-hour glucose during OGTT
- HbA1c
- Waist circumference
- Body mass index
- Systolic and diastolic blood pressure
- Serum triglycerides
- HDL- and LDL-cholesterol
- Apo-B
- Apo-A1
- Cortisol
- Adrenocorticotrophic hormone (ACTH)
- Insulin (fasting and 2h post OGTT)
- Glucagon (fasting and 2h post OGTT)
- C-reactive protein
- Estimated glomerular filtration rate (eGFR)
- Creatinine clearance

All these risk factors will be measured at baseline, 6 months and 12 months.

Other blood laboratory parameters

Copeptin, Sodium, Potassium, Urea and Erytrocyte Volume Fraction (%).

Urine laboratory parameters

Volume, Osmolality, Creatinine, Sodium, Potassium, Urea, Albumin/creatinine ratio and Cortisol.

Surveys

Health-related quality of life, fluid/water and dietary intake (using Riksmaten 2010), stool form (Bristol Stool Scale).

4.3 Safety outcomes

Adverse events

Adverse events are reported at each clinic visit.

Concomitant medications
Usage of medications during study period will be recorded.

5. Populations and subgroups to be analysed

5.1 Populations

Intention-to-treat (ITT)
All randomised study subjects. This will be seen as the primary population for the analysis.

Per Protocol (PP)
All randomised study subjects completing the whole study period (complete cases). For a specific analysis, study subjects with missing data on any of the variables in the model will be excluded from the analysis. Analyses of this population is seen as a sensitivity analysis to investigate whether conclusions are sensitive to assumptions regarding the pattern of missing data.

5.2 Subgroups

Six subgroups will be analysed. All subgroups will be analysed using both ITT and PP populations.

High-high
All randomised study subjects having copeptin concentration above the previously specified cut-off values, 6.1 pmol/L (women) or 10.7 pmol/L (men), at both population screening and baseline visit.

Top tertile
All randomised study subjects having copeption concentration in the top tertile (gender specific) at baseline visit.

Diabetes mellitus
All randomised study subjects will be divided into two subgroups according to having diabetes mellitus or not at baseline visit.

Gender
All randomised study subjects will be divided into two subgroups according to gender.

6. Analyses

All outcomes will be presented using descriptive statistics; normally distributed data by the mean and standard deviation (SD) and skewed distributions by the median and interquartile range (IQR). Binary and categorical variables will be presented using counts and percentages. SAS 9.4 will be used for all statistical analysis.

The subsections below will describe analyses in addition to the descriptive statistics.

6.1 Primary outcome

The primary analysis will compare intervention groups (water supplementation vs control treatment) on their mean change in fasting plasma glucose between baseline and 12 months using a linear mixed model. Difference in fasting plasma glucose from baseline to time points where it is measured during the study (6 and 12 months) will be the dependent variable. Study subjects will be considered as random effects, treatment group and visit number as fixed effects. Baseline value of fasting plasma glucose will be included as a covariate. The estimated difference in mean change from baseline to 12 months and the corresponding 95 % confidence interval (CI) will be presented.

6.2 Secondary outcomes

Cardiometabolic risk factors will be analysed using the same method as for the primary outcome, including usage of the baseline value for the actual factor as a covariate. Diabetes incidence will be analysed using logistic regression, the odds ratio (OR) including 95 % CI will be presented.

In addition, correlation(s) between change in copeptin and change in cardiometabolic risk factors, other blood laboratory parameters and urine laboratory parameters may be calculated.

7. Missing data

When analysing using the ITT population, model based multiple imputation (MI) will be used for both primary and secondary outcomes. The number of imputations will be the largest value of:

- 10
- the proportion of missing values for the actual variable * 100

200 burn in iterations will be used for all analyses. Trace plots and distribution plots will be created to check the accuracy of the imputations.