Study Protocol

Official title of the study: HYDRATION TO OPTIMIZE METABOLISM

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STUDY PROTOCOL

Study Protocol, drafted by the Sponsor, LUND UNIVERSITY, through the Principal Investigator, Prof. Olle MELANDER, hereinafter referred to as "Sponsor" or "we".

PURPOSE AND AIMS

(numbered references refer to applicant´s list of 10 most project relevant publications listed below; other references are spelled out)

The number of patients with type 2 diabetes mellitus increases and represents a major public health threat as a potent risk factor for cardiovascular morbidity and mortality (CVD). Both diabetes and the pre-diabetic state also show clustering of other cardiovascular risk factors (e.g. hypertension and abdominal obesity) called the metabolic syndrome. Interestingly, many trials aimed at reducing risk of CVD in patients with established type 2 diabetes by treating hyperglycemia have failed to do so (N Engl J Med 2008;358:2545-59 & N Engl J Med 2013;369:1317-26). One likely reason for this is that the diabetes-related macrovascular disease is manifest already at diagnosis of diabetes and difficult to reverse after years of exposure to hyperglycemia and other diabetes-related CVD risk factors. *Thus, the most efficient way of reducing both the galloping epidemic of diabetes and its CVD complications is to prevent the disease.* As the diagnosis of diabetes is based on elevation of the fasting plasma glucose concentration, *any preventive actions, which result in lowering of plasma glucose levels in the population, will reduce the incidence of type 2 diabetes.* Although dietary changes and physical exercise are the cornerstones in the prevention of obesity and diabetes, these preventive interventions have not been able to satisfactorily reduce obesity and diabetes rates and new preventive easy-to-implement life style interventions need to be discovered. As described below, high plasma concentration of vasopressin (VP) (i.e. antidiuretic hormone) is a novel and independent risk factor for type 2 diabetes, the metabolic syndrome, CVD and premature death **(1-2, 6-8)**. *As VP can be suppressed by increasing water intake, we hypothesize that water supplementation in individuals with high VP can lower plasma glucose and prevent diabetes.*

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 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).

SURVEY OF THE FIELD

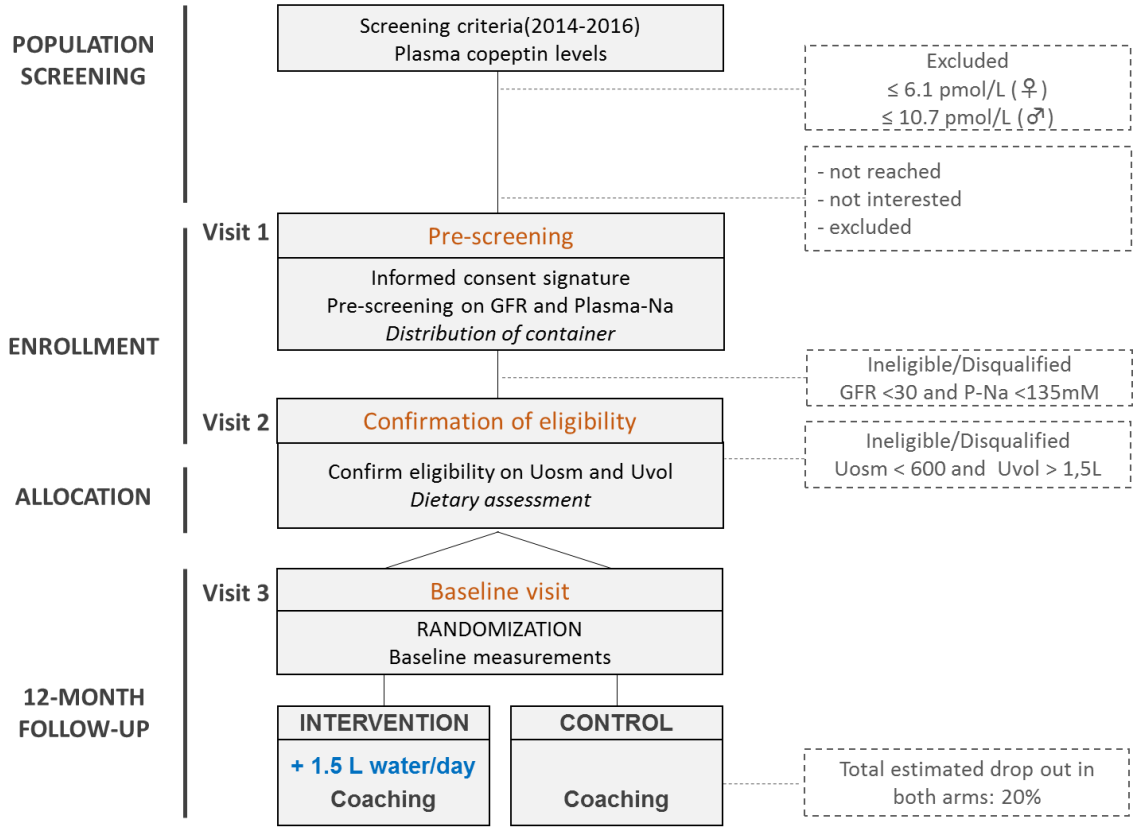
The main physiological role of VP is to maintain constant plasma osmolality. When water intake is low, increased pituitary VP secretion prevents hyper-osmolality by enhancing renal water reabsorption through VP stimulation of the V2-receptor in the renal collecting ducts, whereas at higher levels of water intake, VP secretion is supressed and circulating VP is low. Another effect of VP is that it increases release of adrenocorticotrophic hormone (ACTH) though binding to the VP-1B receptor in the pituitary gland and thereby leads to elevated adrenal cortisol secretion and a Cushing´s syndrome-like phenotype, i.e. diabetes, insulin resistance, hypertension, abdominal obesity and high risk of CVD morbidity and mortality (J Physiol 2007;584:235-44; JCI 2004;113:302-9; Clin Endocrinol 2004;60:192-200). We showed in 2010 that fasting plasma concentration of VP, (measured by copeptin, a stable fragment of the VP precursor hormone)

strongly predicts new onset type 2 diabetes independently of all other diabetes risk factors **(8)**, a finding that has later been replicated by other groups in large independent cohorts (Diabetologia 2012;55:1963-70; JCEM 2015;100:3332-9). Healthy subjects in the top quartile of copeptin (corresponding to plasma concentration of copeptin of >6.1 pmol/L in women and >10.7 pmol/L in men) had a 3-4 fold multivariate adjusted risk of developing diabetes as compared to subjects in the lowest quartile of copeptin **(8)**. Just like Cushing´s syndrome patients, subjects with high VP concentration are not only susceptible to diabetes. We showed that they are more likely to suffer from all components of the metabolic syndrome; i.e. abdominal obesity, insulin resistance, hypertension **(6-8)** and that high VP is an independent risk factor for CVD and premature mortality **(1-2)**. As a low water intake is the most likely cause of elevated VP levels, we tested in animals if high water intake leads to suppression of VP and amelioration of dysmetabolic phenotypes. Interestingly, whereas rats with high VP had deteriorated glucose tolerance, high water intake suppressed VP, ameliorated insulin resistance and markedly prevented hepatic steatosis, one of the hallmarks of the metabolic syndrome **(4)**. These data support a causal relationship between high VP, diabetes and metabolic syndrome, and suggest that water supplementation in humans with high VP may reduce the risk of diabetes and cardiometabolic risk.

PROJECT DESIGN (PICO)

Population: *We previously showed that subjects with high VP (copeptin of >6.1 pmol/L in women and >10.7 pmol/L in men), which corresponds to 25% of the population aged 20-75y, has 3-4 times elevated risk for diabetes development, i.e. a magnitude of risk that is similar to that of obesity, and a doubled risk of CVD mortality. In addition, this high VP target group has a urine osmolality of >600 mosm/kg water, i.e. concentrated urine concordant with a low water intake.*

FIGURE 1. Flowchart of screening and inclusion process



Inclusion criteria will be provision of informed consent, age 20-75 years with high plasma concentration of VP (plasma concentration of copeptin of >6.1 pmol/L in women and >10.7 pmol/L in men) and 24-hour urine osmolality >600 mosm/kg water.
Exclusion criteria will be 24-hour urine volume ≥1.5 L, pregnancy or breastfeeding, plasma sodium <135 mM, use of diuretics, lithium or SSRI drugs, chronic kidney disease (eGFR<30mL/min), heart failure, inflammatory bowel disease, type 1 diabetes or type 2 diabetes treated with insulin and vulnerable subjects (subjects with legal guardian, with loss of personal liberty).
Study subjects will be recruited from ongoing population studies in the Scania region encompassing altogether 20 000 individuals within the current age span: The Swedish CArdioPulmonary Imaging Study (SCAPIS) (n=6000) (https://www.hjart-lungfonden.se/scapis), BIG3 (n=4000) (http://www.skane.se/webbplatser/fou_centrum_skane/big3/), EpiHealth (n=9000) (https://www.epihealth.se/) and the Malmö Offspring Study (n=1000) (http://www.med.lu.se/mos). Copeptin will be measured in -80 degree frozen plasma samples from these four studies. Assuming that the prevalence of having plasma copeptin concentration of >6.1 pmol/L in women and >10.7 pmol/L in men will be approximately 25%, this will give us a pool of candidates for participation in the intervention trial of approximately 6500 individuals. If fewer than expected subjects fulfilling the criteria for inclusion will be generated from this source of inclusion, we will invite employees of the City of Malmö and employees of Skåne University Hospital who are 20-75 years of age to undergo a fasting plasma determination of copeptin. If the plasma concentration of copeptin is >6.1 pmol/L in women and >10.7 pmol/L in men, subjects will enter the same procedure to further test inclusion and exclusion criteria as participants of the population studies (detailed above). As a third source of recruitment, advertisements in local press will be used to invite subjects from the population aged 20-75 years to be tested for plasma copeptin followed by an identical test of inclusion and exclusion criteria as described above.

Intervention: Parallel-group RCT with two arms during 12 months. Subjects will be randomized to the water-intervention (1.5 L daily on the top of habitual intake) and control groups (1:1) by computer-generated block randomization. Both groups will receive general life style advice (general oral and written advice on diet and physical activity). Reusable Bluetooth water bottles will be provided by Sponsor to participants of the intervention group to track their daily water intake. We benefit from shared experience of how to successfully perform the water (and control) intervention by collaboration with Prof William Clark, London Health Science Centre, Canada (co-investigator in current project) who is PI of the Chronic Kidney Disease Water Intervention Trial (WIT) (same water vs control intervention as in current study but primary outcome = effect of water on decline of GFR) whose Data Safety Monitoring Report was completed June 2015 and showed excellent separation in water intake during follow-up (measured by 24-hour urine volume) and safety. In accordance with the protocol of the WIT, adherence to high water intake will be achieved by coaching at the clinic visits and by regular telephone contacts with the participants. Furthermore, we will provide urine color charts for intervention group participants to use at home to facilitate adherence to target urine concentration.

Control: Group that receive the general life style advice. The control group will be subject to coaching at clinic visits and regular telephone calls in order to maintain their water intake (i.e. keep water intake low).

Outcome Active water treatment group versus control group comparison of reduction (12-month value – baseline value for continuous outcomes & odds ratios for the dichotomous secondary outcome of diabetes incidence) of: **(A)** ***fasting plasma glucose (primary outcome measure)*** and

(B) diabetes incidence and level of 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, ACTH, insulin (fasting and 2h post OGTT), glucagon (fasting and 2h post OGTT), C-reactive protein, estimated GFR, Creatinine clearance) ***(secondary outcome measures)*** in 800 individuals (400 in each treatment arm, see power calculation below). Similar to previous studies on e.g. prevention of high cholesterol, we selected the *difference in continuous change of fasting glucose as the primary outcome*, rather than dichotomous outcome of new-onset diabetes. The rationale is that a difference of glucose reduction in active vs control treatment (for example 0.5 mM) would be equally important when being 6.3 vs 6.8 mM or 7.1 vs 7.6 mM, without "crossing" the diagnostic threshold for diabetes (7.0 mM), as when the same 0.5 mM difference is 6.7 vs 7.2. Thus, individuals with type 2 diabetes will not be excluded (unless on insulin therapy) except in the analyses of the secondary outcome of new-onset diabetes. We expect the proportion of participants with diabetes to correspond to the prevalence in the population (i.e. approximately 4-6%).

Assessment of outcome and baseline characteristics (TABLE 1): Clinic visits are performed at baseline, 3 weeks, 3, 6, 9 and 12 months at which cardiometabolic risk factors and hydration parameters are measured as detailed in **TABLE 1**. Please note that the main outcome variables are measured at baseline, 6 months and 12 months. The purpose of the other clinic visits is to optimize adherence to the allocated treatment arm. All laboratory analyses will be performed using certified methods. In addition, we will record medication, fluid/water and dietary intake and medical history. The gut microbiota composition has been suggested to mediate systemic metabolic changes. As stool consistency, in turn highly affected by water intake, has been shown to be one of the main factors determining the microbiota composition (Science 2016;352:560-4), we will measure the gut microbiota in order to test if such changes may mediate part of any beneficial effects of water supplementation on metabolism. Stool consistency will be assessed using a Bristol score chart. Furthermore, as recent work shows that genetic factors play a role in determining basal level of VP (measured by copeptin) (J Clin Endocrinol Metab 2016;101:2432-9), it can be hypothesized that the magnitude of the copeptin lowering effect by water – and the potential effects of metabolism – varies according to such genetic factors. Therefore, we will also isolate DNA from buffy coats in order to determine such genetic variation.

TABLE 1. Schedule of visits and measures

				Follow-up[b,c]				
	Visit 1 [a,c]	Visit (Inclusion) [a,c]	Baseline (Week 0)	3 weeks	3 months	6 months	9 months	12 months
				+/- 4 days	+/- 14 days	+/- 14 days	+/- 14 days	+/- 14 days
Informed Consent Process and Informed Consent Form Signature	+							
Exclusion criteria check-up including pregnancy	+							
Randomization			+					
SURVEY								
Demographics			+					

Diet (4-day report)			+			+		+
Health history			+			+		+
Health-related quality of life			+			+		+
Hydration coaching [d]			+	+	+	+	+	
CLINICAL								
Height (cm)			+			+		+
Weight (Kg)			+			+		+
Waist circumference (cm)			+			+		+
Blood pressure (mm Hg)			+			+		+
Medications			+			+		+
BLOOD								
Blood sample	+		+	+	+	+	+	+
Fasting glucose (mmol/L)			+			+		+
Oral glucose tolerance test (OGTT) (mmol/L)			+			+		+
Fasting and 2h insulin (during OGTT) (mIE/L)			+			+		+
Fasting and 2h glucagon during OGTT) (pmol/L)			+			+		+
Fasting cortisol (nmol/L)			+			+		+
Fasting ACTH (pmol/L)			+			+		+
Fasting copeptin (pmol/L)			+			+		+
Fasting Lipids/lipoproteins[e] (mmol/L)/(g/L)			+			+		+
C-reactive protein (mg/L)			+			+		+
Creatinine (µmol/L)	+		+	+	+	+	+	+
eGFR (mL/min/1,73 m^2)	+		+			+		+
Sodium (mmol/L)	+		+	+	+	+	+	+
Potassium (mmol/L)			+	+	+	+	+	+
Urea (mmol/L)			+	+	+	+	+	+
Osmolality (mOsm/kg)			+	+	+	+	+	+
Erytrocyte Volume Fraction (%)			+					+
HbA1c (mmol/mol)			+					+
Plasma samples for long term storage			+			+		+
Whole blood for DNA isolation			+					
URINE								
24-hour urine sample		+		+	+	+	+	+
Urine volume (ml)		+		+	+	+	+	+
Creatinine (mmol/24h)		+		+	+	+	+	+
Sodium (mmol/24h)		+		+	+	+	+	+
Potassium (mmol/24h)		+		+	+	+	+	+
Urea (mmol/24h)		+		+	+	+	+	+
Osmolality (mOsm/kg)		+		+	+	+	+	+
Albumin/creatinine ratio (g/mol)		+				+		+
Cortisol (nmol/24h)		+				+		+

Urine for long term storage		+				+		+
FAECES								
Sample for microbiome seq			+					+
Bristol stool score			+					+
Check for adverse events				+	+	+	+	+

[a]Pre-randomization.
[b]Time after randomization.
[c]Clinic visits 8 time points: visit 1, visit 2, baseline, 3 weeks & 3, 6, 9 and 12 months after baseline.
[d]In addition to coaching during telephone contacts and by a Bluetooth water bottle.
[e]triglycerides, LDL, HDL, ApoA1, ApoB.

Study procedures and timelines: All visits will take place at Skåne University Hospital. 1st patient's 1st visit is planned to be held in the first quarter of 2018. Recruitment and intervention will be ongoing until 2021 (rolling enrolment, 12-month follow up). Coaching and enquire about adherence and tolerance to water treatment will take place both during all clinical visits after baseline, and during telephone contacts (see below) to optimize compliance. The reusable Bluetooth water bottles will be available to subjects at Visit 3 after randomization to water treatment. During this visit, clinical site staff will help participants download the smartphone app, provide instructions on how to use the app and the Bluetooth water bottle, pair the bottle with participant's smartphone and set a daily target goal of 1.5L. In case participants do not have a smartphone compatible with the Bluetooth water bottle, they will be provided with a smartphone for the duration of the study. Throughout the term of the intervention, participants will refill their Bluetooth water bottle with tap water on a daily basis to achieve the target daily goal of 1.5L.
Colour charts will be used at all telephone contacts, and measures of urine volume and urine osmolality will be used at clinical visits, to motivate the participants of the active treatment group to increase their water supplementation/compliance if lack of compliance is indicated, suspected or documented based on these parameters.

For the sequence of events during visits please see TABLE 1.

Blood pressure and pulse is measured after a 5-minute rest.

Regular coaching contacts (in total 10 personal or telephone contacts) will be scheduled with all participants to check for adverse events (by asking everyone if it has occurred any new or worsened health problems or any medical treatment or hospitalization since last contact) and to coach the hydration intervention based on urine colour charts. Furthermore, contacts using electronic text messages will be used in between telephone contacts and study visits (in total 5 text messages during the study period) to remind participants about the hydration intervention.

Fluid and dietary intakes will be assessed using "*Riksmaten 2010*", a web-based 4-day record tool developed by the Swedish National Food Administration and used in the latest national diet survey in Swedish adults. In addition to Riksmaten recording, participants will fill in a food propensity questionnaire to assess foods that may not be consumed within the 4 days (like fish, berries or sugar-sweetened beverages). To help with the registration participants will receive a food/drink dairy and a portion guide with pictures of 24 different food categories. In addition, there is an instruction video available via YouTube https://www.youtube.com/watch?v=DB3bzD0FJMg. The method has been validated (Nybacka et al, J Nutr Sci 2016; 5:e39).

A check for adverse events will be done at every visit after baseline and during each telephone contact by asking all participants if it has occurred any new or worsened health problems or any medical treatment or hospitalization since last contact (see section "safety reporting" below).

Participation in the present study, starting from Visit 1 until 12-month follow-up Visit, will not last longer than 18 months.

Handling of blood samples: The amount of blood drawn is 3 ml at visit 1, 61 ml at baseline visit, 6 ml at 3-week, 3-month and 9-month visits, and finally 57 ml at the 6 and 12 months visits. This includes blood for direct analyses of routine chemistry (TABLE 1) as well as backup samples (biobank samples) corresponding to 16 x 250 µl vials of EDTA plasma, 8 x 250 µl vials of citrate plasma, and 8 x 250 µl vials of serum at baseline, 6 months and 12 months. Note that in contrast to routine chemistry, copeptin will not be measured continuously during the trial but in back-up samples (one batch) after all study subjects have completed the 12-month protocol.

The buffy-coat of one of the centrifuged plasma tubes will be collected and stored frozen for later DNA extraction. We are planning to genotype genes coding for AVP and its receptors, loci associated with urine and plasma osmolality and genes associated with altered copeptin concentration.

The routine (directly analyzed) blood/urine samples are transported in air temperature from the clinical site to the University Hospital's central clinical lab by site staff. The lab is located in the area of Skane University Hospital Malmö, only a 3 minute-walk from the clinical site, which is located at Inga Marie Nilssons gata 50, Skåne University Hospital, Malmö.

Blood samples for long-term storage (biobank) are centrifuged immediately after blood draw. Then they are stored in the fridge for approximately 1-3 hours before being aliquoted and transferred (transported on ice) to the -80 degree freezer in another close-by building, approximately a 5 minute-walk from the clinical site. These samples are also transported by site staff. Assays on the biobank samples will be performed after all study subjects have completed the protocol in the same batch of reagents (includes analysis of copeptin, see above).

Blood sample measures: All routine laboratory analyses will be performed using certified methods at the University Hospital's central clinical lab, including measurement of plasma glucose (COBAS, Roche Diagnostics, Rotkreuz, Switzerland), serum insulin, serum glucagon, plasma cortisol, plasma ACTH, lipids/lipoproteins, c-reactive protein, serum creatinine, serum sodium, serum urea, serum osmolality, serum haematocrit and glycated hemoglobin (HbA1c).

Plasma copeptin will be measured in our lab (located at Jan Waldenströms gata 35, 91:12, Skåne University Hospital, Malmö) in 50 µl from fasting plasma samples stored at -80°C using a commercially available chemiluminescence sandwich immunoassay copeptin ProAVP kit with coated tubes (B.R.A.H.M.S AG, Hennigsdorf, Germany).

Procedure for OGTT: After an over-night fast (no meals or drinks after 10PM the evening before), subjects will ingest 75 g of glucose over a maximum period of 3 minutes, starting sometime between 730 and 9 AM, followed by blood sampling for glucose measurement at 30, 60 and 120 min.

Handling of urine samples: 24-hour urine samples will be collected at baseline and again at 3-weeks, and 3, 6, 9, 12 months after randomization. 24-hour urine collections follow procedures developed at the Department of Endocrinology, Skåne University Hospital, and consists of a comprehensible written instruction aimed at ensuring accurate and complete collection of urine. The procedure has been used by us in many large research studies (see for example applicant references 5 and 10). The participants start the 24-hour urine collection one day before a planned visit, and bring their container on the day of visit. For example, if their visit is on a Wednesday they start collecting urine on a Tuesday. The first urine on Tuesday morning is not collected. The

subject notes the time of collection start on a form for urine collection. From this point and 24 hours forward they collect ALL urine. They are instructed to try and collect the first morning urine on Wednesday at approximately the same time as they started the collection on Tuesday morning. Prior to the urine collection they are provided with one big container and 3-4 small ones. They are asked to keep the big container in the fridge at all time, if possible. Subjects are instructed to bring the small containers with them when they leave home for work and such. These containers should also be kept in fridge if possible, but if this is not possible the participants are allowed to keep them in room temperature during the day and then pour the content into the large container when they get home.

Urine measures: All routine laboratory analyses except for urine osmolality will be performed using certified methods at the University Hospital's central clinical lab, including 24-hour urine urea, 24-hour sodium and potassium, 24-hour urine albumin to creatinine ratio and 24-hour urine cortisol.

24-urine osmolality will be measured on site at Jan Waldenströms Gata 15, Skåne University Hospital, Malmö, (clinical site) using an i-Osmometer basic (Löser, Germany).

Procedure for stool collection: Participants will be instructed via a video how to collect their faeces sample at home. The participants will be asked to store the samples in a freezer until delivery to the clinic. At the clinic, the 4 aliquots will be stored in a -80-degree freezer.

Data handling: The case report form (eCRF) of the present study consists of two separate databases, Redcap and Vattenstudien Content Management System (V-CMS). The questionnaire based data and 4-day web-based dietary and fluid intake data will be electronically stored in the Redcap database, using code linkage. The V-CMS is built to manage bookings, laboratory analyze results, follow-up documentation and study logging. The laboratory results from the hospital clinical chemistry are automatically imported into the V-CMS when analyses are available and validated using logical computerized checks on the data. Data that does not match valid patterns, reference values and/or expected criteria has to be validated manually by authorized nurse before assigned to patient data. All analyzed results, both external and locally generated, are subject for manual input/modification if needed. Anthropometric data, blood pressure and data on p-glucose and urine osmolality (which is analyzed on site and not sent to the lab) are entered manually into the V-CMS. All data are stored in a procedural database and the V-CMS also contains (backend-to-backend) export of data when required.

Archiving of data: Data is archived for at least 10 years.

Sample size: 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. The power calculations and statistics of our study are performed by the chief statistician Professor Jonas Björk at "Region Skåne Clinical Trials".

Statistical Analysis Plan: ***(Responsible statistician: Prof Jonas Björk)*****: Please see separate file**.

Ethical considerations: this study is conducted in compliance with the principles of the 'World Medical Association Declaration of Helsinki' (59th WMA General Assembly, Fortaleza, Brazil, October 2013), ICH guidelines for Good Clinical Practice as appropriate for nutritional products, and local legislation of the country in which the research is conducted, whichever affords the greater protection to the participants. Therefore, this study, as any study involving human subjects, will be registered in a publicly accessible database (ClinicalTrials.gov) before recruitment of the first subject.

This protocol and supportive documentation provided to the subjects, such as information and informed consent sheets and Forms, were submitted to the applicable Ethics Committee in Lund by Olle Melander according to local regulations. Approval from the Ethics Committee was obtained on 15 Dec 2016. Ethics Committee meetings are planned every 6 weeks. An amendment will be submitted to the Ethics Committee in Lund in December 2017, prior to the commencement of any study activities.

The signature of the study informed consent form marks the inclusion of the subject in the study. This signature will be obtained after having fully briefed the subject about the study and answered all of his/her questions about the study.

Safety reporting: The following variables will be followed at each visit in order to assess safety measurements: serum creatine, serum osmolality and serum sodium to control for water balance problem.

Definition and registration of adverse events: An adverse event (AE) is defined as any untoward medical occurrence in a participant who is administered an investigational product. The medical occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

AEs are defined as either a laboratory measurement outside the reference range (i.e. a value outside the reference values) or signs or symptoms reported by the participants during regular follow-up contacts (telephone calls and visits). During these contacts the participants will be asked if it has occurred any new or worsened health problems or any medical treatment or hospitalization since the last contact. AEs are documented in the eCRF. We will specifically ask about pregnancy, lactation and new use or change of medication.

AEs are categorized as serious (SAEs) if they result in death, illness requiring hospitalization, events deemed life-threatening, or if they result in persistent or significant disability/incapacity, or in a congenital anomaly/birth defect or medically important condition. SAEs will be reported to the sponsor within 24 hours, as well as recorded in the eCRF (V-CMS). The sponsor will report all SAEs to Danone Research within 3 days. The Adverse Event Form will be sent by email/fax to the following contact:

Position(*)	**Name**	**Cell Phone**	**Email**
Clinical Operations Leader	Jean-François Jeanne	+33 6 25 13 86 96	jean-francois.jeanne@danone.com
Hydration Physiology and Metabolism Junior Scientist	Tiphaine Vanhaecke	+33 6 01 65 59 16	tiphaine.vanhaecke@danone.com

Registration of AEs will start with the 1st telephone contact (which is the first follow up after start of treatment). The registration of AEs ends at the 12-month visit (end of treatment).

10 RELEVANT PUBLICATIONS OF APPLICANT (INCL REFERRED ONES IN RESEARCH PLAN)

1. Tasevska I, Enhörning S, Persson M, Nilsson PM, **MELANDER O; SENIOR AUTHOR**. Copeptin predicts coronary artery disease cardiovascular and total mortality. ***Heart***. 2016 Jan 15;102(2):127-32. ***(Relevance: Vasopressin epidemiology / pathophysiology)***
2. Enhörning S, Hedblad B, Nilsson PM, Engström G, **MELANDER O; SENIOR AUTHOR**. Copeptin is an independent predictor of diabetic heart disease and death. ***Am Heart J***. 2015 Apr;169(4):549-556. ***(Relevance: Vasopressin epidemiology / pathophysiology)***
3. Mega JL, Stitziel NO, Smith JG, Chasman DI, Caulfield MJ, Devlin JJ, Nordio F, Hyde CL, Cannon CP, Sacks FM, Poulter NR, Sever PS, Ridker PM, Braunwald E, **MELANDER O**, Kathiresan S, Sabatine MS. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. ***Lancet***. 2015 Jun 6;385(9984):2264-71. ***(Relevance: Clinical trial)***
4. Taveau C, Chollet C, Waeckel L, Desposito D, Bichet DG, Arthus MF, Magnan C, Philippe E, Paradis V, Foufelle F, Hainault I, Enhorning S, Velho G, Roussel R, Bankir L, **MELANDER O**, Bouby N. Vasopressin and hydration play a major role in the development of glucose intolerance and hepatic steatosis in obese rats. ***Diabetologia***. 2015 May;58(5):1081-90. ***(Relevance: Vasopressin epidemiology / pathophysiology)***
5. Tasevska I, Enhörning S, Burri P, **MELANDER O; SENIOR AUTHOR**. High salt intake increases copeptin but salt sensitivity is associated with fluid induced reduction of copeptin in women. ***Int J Hypertens***. 2014;2014:641587. doi: 10.1155/2014/641587 ***(Relevance: Vasopressin epidemiology / pathophysiology)***
6. Enhörning S, Bankir L, Bouby N, Struck J, Hedblad B, Persson M, Morgenthaler NG, Nilsson PM, **MELANDER O; SENIOR AUTHOR**. Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmö Diet and Cancer Study cardiovascular cohort. ***Int J Obes*** (Lond). 2013 Apr;37(4):598-603. ***(Relevance: Vasopressin epidemiology / pathophysiology)***
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   (Relevance: Vasopressin epidemiology / pathophysiology)
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