Official title of the study: SalT Reduction InterVEntion: Examination of the metabolic, behavioral and health consequences of reducing salt intake. A randomized controlled trial in a real life setting.

NCT number: Not yet assigned

Date of the document: November 30, 2018

Statistical analysis plan STRIVE

Statistical Analysis Plan STRIVE

We are currently writing up a more in-depth plan of the statistical analysis plan, which will be uploaded when ready.

Aim: The SalT Reduction InterVENtion (STRIVE) aims to investigate the metabolic, behavioral and health effects of 14+ weeks of gradually salt reduced bread alone or in combination with dietary counselling compared to families receiving standard bread.

Design: Randomised, controlled trial with three parallel arms

Methods: Eligible families will be randomly allocated into three groups to investigate the effect of different salt reduction strategies: **A:** salt reduced bread; **A+B:** Salt reduced bread and dietary counseling; **C:** Bread with standard salt content. Participants will be instructed to replace their usual consumption of bread by bread products provided in the study. The primary outcome is changes in sodium intake measured by 24 hr urine excretion. Secondary outcomes are change in potassium and Sodium/ Potassium ratio, blood pressure, blood lipids, salt-taste sensitivity and renin, aldosterone and sympathetic nervous response. Participants will be examined before and by the end of the intervention. The flow of the trial is shown in figure. 1.

Sample size

The average daily intake of bread in Denmark is 139 grams (123 gram for children. One of the inclusion criteria is a daily intake of bread of 175 gram among adults. The average salt content of bread in Denmark is 1.3 gram/100-gram bread. Thus, the average daily intake of salt from bread among the participants is estimated to be approximately 2.3 gram. The expected reduction in salt intake due to the salt-reduced-bread intervention (average reduction of 55%) is 1.25 gram per day. The expected salt reduction in the intervention group with the combined intervention is on average 3 gram (based on the current average daily salt intake and the target for the intervention). The statistical power calculations were based on data from a population-based study at Center for Clinical Research and Prevention, DK (CCRP) showing an average daily intake of salt of 8.3 gram (SD 2.2). The analyses were done to fit the cluster-randomized design and showed that if the true difference in the intervention and control means after intervention is 1.2 gram per day we will need to include 50 subjects in each of the three groups to be able to reject the null hypothesis at a 5% level and with probability (power) 0.8. The intra-class correlation within families was assumed to be 0.33 and the average family was assumed to consist of four members. With an expected difference in salt intake of 1.2 gram/day we will expect to see clinical relevant differences in blood pressure, blood lipids, renin, aldosterone and albuminuria.

Randomization, allocation and concealment

Once included, baseline assessments will be conducted before the families will be randomized (1:1:1) to three groups (salt reduced bread, salt reduced bread and dietary counseling, standard bread no dietary counseling), using simple randomization.

A *data manager*, who has no other involvement in the trial than producing the data application will produce a computer-generated sequence of random group assignments (allocation sequence). *Participants* will be blinded to the treatment they receive. Bread will be coded by a red or blue label, and families collect

bread according to the color of their group. The color code will be known by two *bakers* responsible for baking the bread but blinded to the rest of the *bakery staff*, including those handing out the bread to the

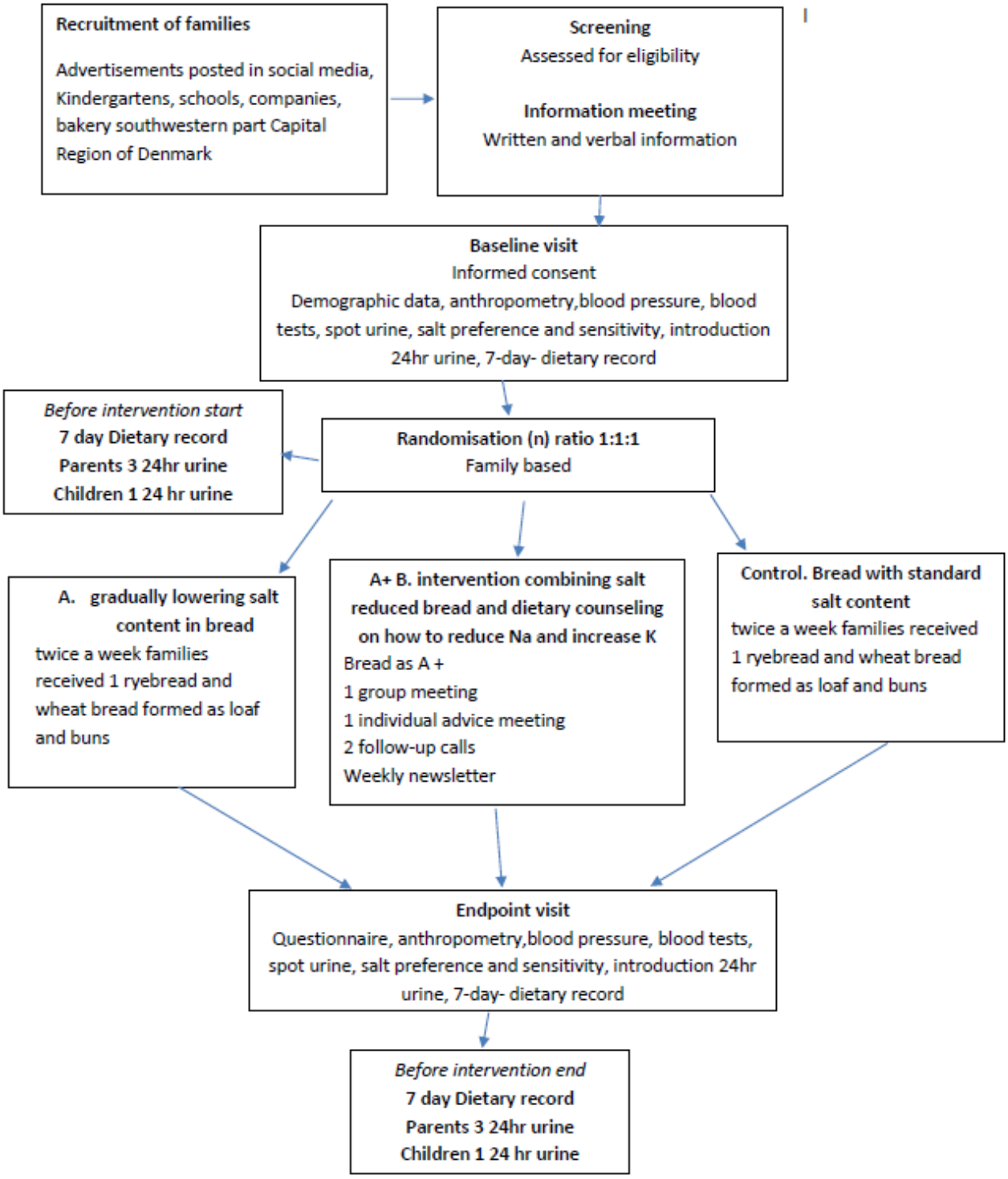

Figure 1. Trial flow diagram. *Participants are recruited as families, screened for eligibility, informed about the trial. After informed content, baseline visit completed ending with allocation to trial group. Then dietary*

record and urine collection, while eating own bread before intervention start. Four months later endpoint visit followed by 7-day-dietary record and 3-day urine collection while consuming intervention bread.

participants. Color-code for the bread will be revealed after the final participant has completed the second health examination.
The *secretary* screening and enrolling the participants will be blinded with respect to group allocation. The three *research nurses* conducting the clinical measurements, at baseline (before randomization) and at endpoint measurement will remain blinded to the group allocation. Given the nature of the intervention it is not possible to blind the *nutritionists* delivering the dietary counselling. Biochemical analyses will be carried out by *laboratories* blinded to the allocation of the samples. Thus, the primary outcome measures will be blinded to the outcome assessors.

Data management
The data will be handled in accordance with the rules from the Danish Data Protection Agency. All data will be entered blinded, with error control in the application or using double entry. Data entry will be validated by checks for valid values and range checks. For the analyses data will be exported to SAS Enterprice Guide 9.4, SAS institute, Inc., Cary, NC, USA: The electronic database and analyses will be stored on a secure computer server with personal login access authorized by the primary investigator.

Recruitment and withdrawals
Recruitment rates and numbers of withdrawals and dropouts will be reported along with reasons for exclusions, dropouts and withdrawals.

Compliance
To follow participants compliance with the intervention, bakers will inform the research team if bread is not being picked up. Families will fill in forms on bread consumption on a weekly basis.
Families receiving dietary counselling will be interviewed twice on their adherence to their own salt reducing goals.
Urine collections will be checked for completeness by the creatine and PABA method.

Table 1. Outcome variables and assessment tools

Assessment	Outcome	Method
Inclusion / Exclusion	Family structure, Bread consumption, Gluten intolerance Pregnancy, Diabetes, Hypertension	Screening questionnaire, Telephone interview, Interview by nurse
Informed consent		Written, Intro meeting Informed by nurse
Demographics	Gender, age, family, marital status, education, work	Questionnaire
Lifestyle	Physical activity, smoking, alcohol, salt and bread consumption, food habits	Questionnaire

Medical history	Hypertension, heart disease, cancer, diabetes, cholesterol, coeliac disease	Questionnaire, Interview
Anthropometry	Height, weight, BMI, arm and waist circumferences, fat percentage	Standardized measurements by nurse, impedance
Blood pressure	Pulse, diastolic BP Systolic BP	Average of three resting BP's, 5 min rest
Biochemistry	P-glucose, HbA1C, Triglyceride, Cholesterol, HDL, LDL, renin, aldosterone, adrenalin, noradrenalin, U-albumin, U-sodium, U-potassium, U Creatinine, U-albumin	Semi fasting (min 2 hr) blood samples after 30 min rest, spot urine, micral test
Salt preference/ sensitivity	Preference bread 0.4/0.8/1.2 g salt/ 100 g Recognition sweet, salt	Taste of bread Taste of sugar and salt solutions
24 hr urine collection	U-sodium, U-potassium, U Creatinine, PABA	<u>Adults:</u> 3 days, 24 hr urine + PABA <u>Children:</u> 1 day 24 hr urine
7 Day dietary history	Energy, macro- and micronutrients, foods, meal pattern, eating at home/ out	Web-based dietary record, estimated portion sizes from photos
Bread intervention	Bread collection twice a week and weekly response to questionnaire on consumption Weekly test of salt content in bread Participants evaluation of bread consumed	14+ weeks of bread intervention. Six weeks gradual reduction in salt. *Intro week* 1-2 after completion first dietary record and urine collection *End* after completion final dietary record and urine collection.
Randomization	Systematically into three groups	By allocation sequence
Dietary counseling	Salt intake, potassium Motivation, barriers, focus points Actions applied to reduce salt intake	Salt screening questionnaire, interview, follow up telephone interviews Evaluation questionnaire

Statistical analysis

Table 1 lists the outcome variables and assessment tools. The *primary study outcome* is the change in 24-h sodium (mean of 3 collections in adults, 1 collection in children). *Secondary outcomes* are change in potassium and Sodium/ Potassium ratio, blood pressure, blood lipids, salt-taste sensitivity and renin, aldosterone and sympathetic nervous response.

Baseline data will be presented as means (std) or medians (with range) for continuous data depending on the distribution of the variable. Categorical variables will be presented as frequencies and percentages.

Estimation of treatment effect for the primary outcome will be calculated using an "intention to treat" analysis, including all randomized participants regardless of the adherence to the intervention and dropouts. To create a full analysis data set, missing data for the primary outcome will be imputed using multiple imputation with 100 samples. A linear mixed model with change in sodium excretion as outcome variable, treatment group and baseline sodium excretion as fixed effects, and family ID as random effect.

The estimates or the size of treatment effects will be presented along with 95% confidence intervals and actual p-values. Levels of significance will be set at 0.05. The primary analysis will be unadjusted.
To account for differences in the adherence to the bread intervention, a per-protocol linear mixed model analysis will be undertaken following the principles outlined above.

Linear mixed models will also be applied to the analyses of the secondary outcomes.

Further analyses will be described in the statistical analysis plan, which we are currently writing up.