Statistical Analysis Plan (SAP)

HISTORI – Home-Based Intervention with Semaglutide Treatment of neuroleptics-related prediabetes

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PRIMARY PUBLICATION

Change in HbA1c, metabolic measures, PANSS and SF-36.

SECONDARY PUBLICATIONS

- **Changes in PANSS – Mediators - Impact of Weight on Quality of Life-Lite (IWQOL-lite), Medication Adherence Rating Scale (MARS) and weight.**
- **Changes in cardiovascular risk markers and CAN assessment**
- **Changes in NAF PET CT scans and body composition.**
- **Changes in SF-36 – Mediators – Weight and Simple Physical Activity Questionnaire (SOMPAQ).**
- **Cost-utility analysis and Cost-effectiveness analysis.**
- **Insulin resistance a driving factor for prediabetes, dyslipidemia and elevated cardiovascular risk markers?**

INTRODUCTION

This document specifies the planned statistical analysis for the RCT-study "**HISTORI – Home-Based Intervention with Semaglutide Treatment of neuroleptics-Related prediabetes**"

" as carried out following the protocol published in BMJ Open: https://doi.org/10.1136/bmjopen-2023-077173, and in **ClinicalTrials.gov ID** NCT05193578

TRIAL DESIGN

A two-armed, multi-center, superiority, double-blinded, randomized trial investigating the effect of weekly injections of Semaglutide vs placebo. Each patient will be included in 30 weeks of treatment with weekly injection of Semaglutide 1.34 mg/ml (1 mg) or placebo. The treatment group will start at 0.25 mg Semaglutide per week for 4 weeks, then 0.5 mg per week for 4 weeks. The patients will remain at the highest tolerated dose. There is a 6 week lasting wash-out period for registration of side-effects after each last patient last visit. Setting: Mental health facilities in Region of Southern Denmark and Region of Zealand, Denmark.

RANDOMIZATION

Randomization 1:1 to Semaglutide or placebo. Block randomization including blocks of either 4 or 6 randomization numbers will be used, and the randomization list will be provided by the manufacturer of Semaglutide and placebo.

SAMPLE SIZE

PRIMARY OUTCOME (HBA1C CHANGE)

The study requires 154 participants, including an expected 15% dropout. If we conservatively assume that 30 weeks of Semaglutide reduces HbA1c by 0.2%, and that SD equals 0.35%, 65 subjects in each arm are required to obtain a power of 90%, using a 2-sided significance level of 5%.

FRAMEWORK

The trial has been designed to investigate whether the new treatment being tested is superior to placebo in terms of efficacy and other relevant outcomes.

STATISTICAL INTERIM ANALYSES

Early Identification of Safety Concerns: Monitoring safety outcomes during interim analyses allows for early detection of potential safety issues associated with the treatment. The analysis will be performed blinded through serious adverse events (SAE) and suspected unexpected serious adverse reaction (SUSAR) registrations.

TIMING OF FINAL ANALYSIS

All outcomes will be analyzed collectively when all participants have completed the study and when the researchers are still blinded.

SECTION 4: STATISTICAL PRINCIPLES

CONFIDENCE INTERVALS AND P VALUES

Statistical tests will be conducted as two-sided tests with a 5% significance level and a confidence interval of 95%.

1. **Holm's Method:**
    - A step-down procedure that adjusts p-values in a sequential manner.
    - It maintains strong control over the family-wise error rate (probability of making one or more Type I errors).

ADHERENCE AND PROTOCOL DEVIATIONS

Adherence to the intervention will be measured once weekly. Every pen of semaglutid/placebo has a specific number. Every week, the participants will have to inform the research staff of that number. The number will be inputted in the eCRF. Researchers can then track if the correct numbers are being reported. Furthermore, pens will be collected and checked to see if there are any medicine/placebo left.

Adherence to the intervention will be presented as number of weeks of injections completed. Not completing an injection or a questionnaire or measurement will be defined as a protocol deviation. Major protocol deviations will be summarized. Missing injections or missing data at baseline, week 15 and week 30.

ANALYSIS POPULATIONS

Analyses will be performed as intention-to-treat.

PLANNED ANALYSES FOR PRIMARY PUBLICATION: CHANGE IN HBA1C, METABOLIC MEASURES, PANSS AND SF-36

Primary outcome: Main analysis

HbA1c mmol/mol measured at baseline, week 15 and week 30.

SECONDARY OUTCOMES TO BE INCLUDED IN THE PRIMARY PUBLICATION

Secondary outcomes

- Positive and Negative syndrome scale (PANSS-6), the 36-Item Short Form Health Survey questionnaire (SF-36v2)
- Weight, BMI, Waist Circumference
- Biochemical screening at baseline and after 30 weeks: FBG, FBI, TG, LDL, HDL, C-peptide (proinsulin), liver and renal function test, hemoglobin, amylase and calcium, SHBG, testosterone, FSH, LH, uric acid, hs-CRP and choriogonadotropin (hCG) in all female participants.

SAFETY OUTCOMES

- Death - all causes
- SAE
- GI symptoms
- Worsening of psychiatric symptoms
- Changes in liver function and kidney function

PLANNED TABLES AND FIGURES AND CORRESPONDING ANALYSES

Characteristics (as listed in Table 1 below, numerical characteristics marked with [numerical]) of patients at baseline will be reported separately for the two treatment groups.

- Clinical results that align with the normal distribution will be presented as mean with SE, SD or two-sided 95% CI.

- Clinical results that do not align with a normal distribution will be presented as median, and 25 and 75 percentile.

Beside the descriptive tables spaghetti and margins plot will be constructed to graphically assess how the parametrization of our model has to be for the primary analysis.

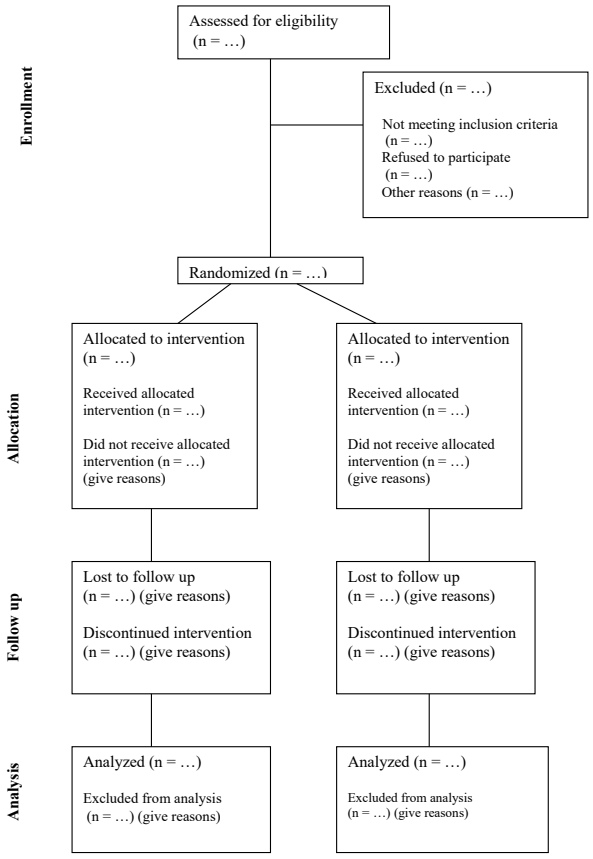
Mixed-effects linear models will be employed to assess the association between the treatment groups and the primary outcome (HbA1c) over time (baseline, week 15 and 30 weeks). These models efficiently handle missing values using the maximum likelihood estimator, assuming the dropout mechanism is missing at random (MAR). The models will include a random intercept per participant. The necessity of a random slope will be evaluated for each model using F-test, and it will be included if it significantly improves the model. In order to test whether the intervention has a statistically significant effect we will employ a likelihood ratio test and use G-estimation where the Q-model will be the mixed effect model to effectively estimate the overall effect size across all timepoints.

In relation to the secondary analysis we will again use the G-estimation framework. For the secondary analysis of PANSS-6 and SF-36 we will use the beta binomial regression as the Q-model and again employ likelihood ratio test to assess whether our intervention has an effect on our secondary outcomes. In relation to weight we will either employ mixed effect model or mixed effect gamma model and again use G-estimation to get interpretable effect sizes.

For the G-estimation approaches we will estimate the confidence intervals by way of bootstrap.

No adjustments for multiple testing will be carried out in Table 1 and no effect sizes for differences between groups will be estimated. Missing observations will be excluded from tests in Table 1, and number for missing observations in each group will be specified.

Sample template for the CONSORT diagram showing the flow of participants through each stage of a randomized trial. The text boxes can be modified by clicking on them.



Baseline Table 1	Active	Placebo	P-value
Age, mean (SD), Years			
Female, No. (%)			

Smoking no. (%)

Alcohol consumptions > 14 units weekly.

Diagnosis, No. (%)

Schizophrenia

Schizotypal disorder

Schizoaffective disorder

Duration of diagnosis, months, No(%) .

6-12 months

12-24 months

>24 months

Treatment, No. (%)

Quetiapine

Olanzapine

Risperidone

Ziprasidone

Paliperidone

Aripiprazole

Clozapine

Other

1 Drug

2 drugs

3+ drugs

Psychiatric evaluations

PANSS-6 Score

Positive Score

Negative Score

SF 36

Physical Component Summary

Mental Component Summary

Clinical characteristics, mean (SD)

Body weight, kg

Waist circumference, cm

BMI

Systolic Blood pressure, mm HG

Diastolic Blood pressure, mm HG

Glucose Metabolism

HbA1C, mmol/mol, mean (SD)

Fasting Blood Glucose

Fasting C-peptide

Fasting insulin level

Organ function

Creatinine Levels

ALAT

Amylase

BASP

Bilirubin

Cholesterol Level

Total

LDL

HDL

Triglycerides

Other biochemical variables

Testosterone

SHBG

Urate

CRP

Hemoglobin

PRIMARY OUTCOME AND SECONDARY OUTCOMES

Table 2: Changes in endpoints from baseline to week 30.

Variables	Active	Placebo	Estimated difference	P-value
Glucose Metabolism				
HbA1C, mmol/mol, mean (SD)				
Fasting Blood Glucose				
Fasting C-peptide				
Fasting insulin level				
PANSS Score				
Positive				

Negative

SF - 36

Physical Component Summary

Mental Component Summary

Clinical characteristics, mean (SD)

Body weight, kg

Waist circumference, cm

BMI

Systolic Blood pressure, mmHg

Diastolic Blood pressure, mmHg

Organ function

Creatinine Levels

ALAT

Amylase

BASP

Bilirubin

Cholesterol Level

Total

LDL

HDL

Triglycerides

Other biochemical variables

Testosterone

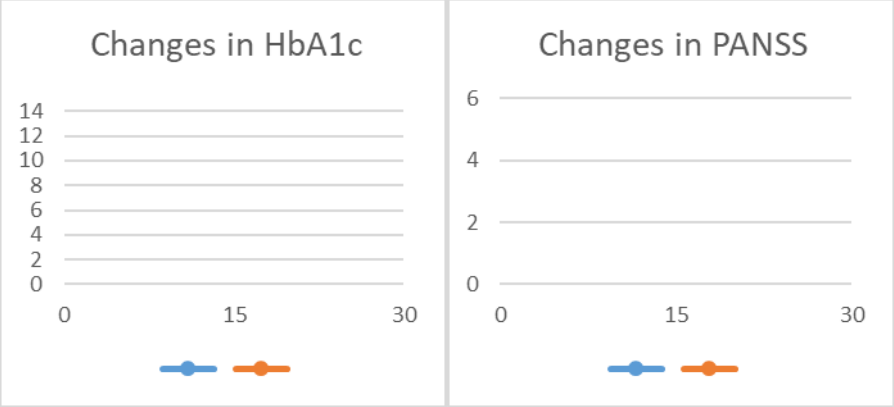
SHBG

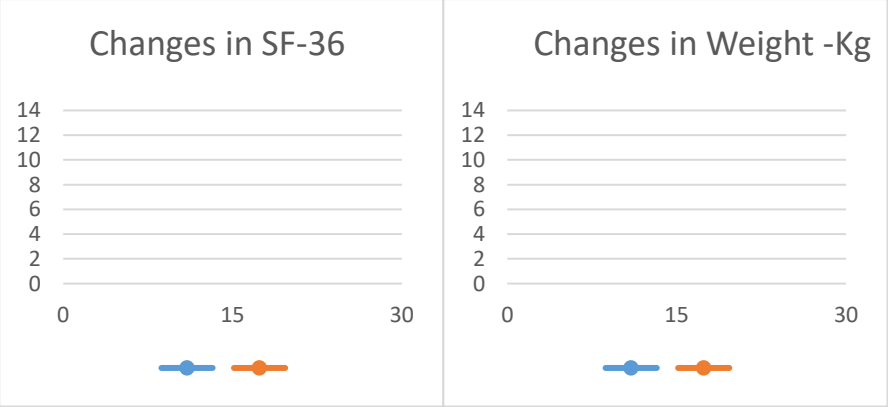
Urate

CRP

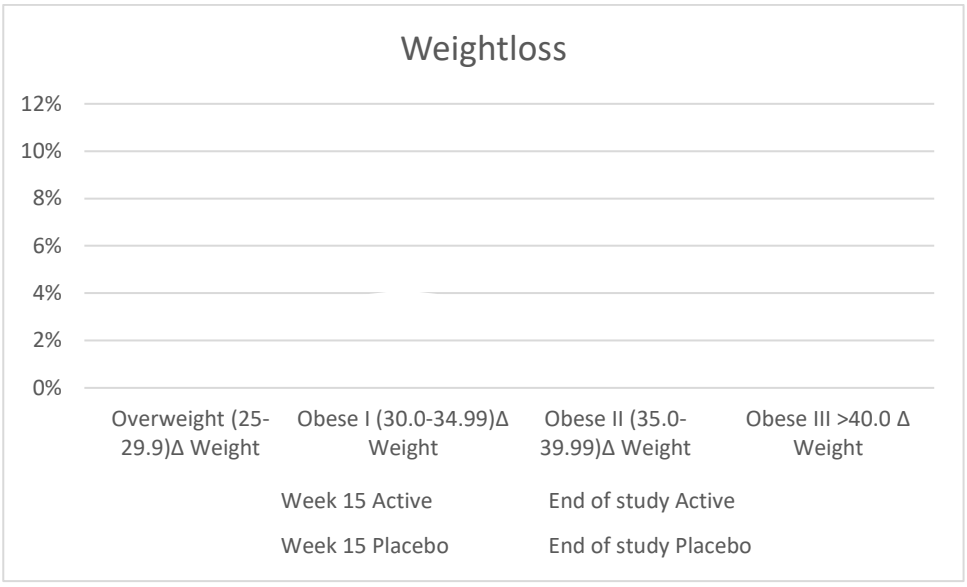
Hemoglobin

Table 3. Changes after 15 and 30 weeks treatment with Semaglutide or placebo				
	Active - Week 15	Placebo- Week 15	Mean difference	P-Value
HbA1c				
PANSS				
SF-36				
Weight				
	Active - end of study	Placebo - end of study	Mean difference	P-Value
HbA1c				
PANSS				
SF-36				
Weight				
HbA1c mol/mmol, PANSS and SF-36 are numerical values and weight Kg. Values are represented as number (%).				
p Values were obtained from chi square test.				





Blue: Placebo. Orange: Semaglutide.



SAFETY OUTCOMES

Severe safety outcomes will be reported in table 3.

Variables	Active	Placebo	Estimated difference	P-value
SERIOUS ADVERSE EVENTS				
Death				
Hopitalisation				
Somatic illness				
Hypoglycemia				
Psychiatric illness				
Admission – worsening of schizophrenia				
Voluntarily institutional care				

Compulsory institutional care

Expected adverse events and side effects will be reported table 4

Variables	Active	Placebo	Estimated difference	P-value
Side effects				
Nausea				
Diarrhea				
Abdominal pain				
Dyspepsia				
Flatulence				
Constipation				
Vomiting				
Other Adverse events				
Fatigue				
Headache				
Dizziness				
Infections				
Flue like symptoms				
Pneumonia				
Abdominal infection				
Urinal Tract infections				
Biochemical safety				
Change in liver function				
Change in Kidney function				
Elevated Amylase levels				

PLANNED ANALYSES FOR SECONDARY PUBLICATION: **CHANGES IN PANSS – MEDIATORS - IMPACT OF WEIGHT ON QUALITY OF LIFE-LITE (IWQOL-LITE), MEDICATION ADHERENCE RATING SCALE (MARS) AND WEIGHT.**

Characteristics (as listed in Table 1 below, numerical characteristics marked with [numerical]) of patients at baseline will be reported separately for the two treatment groups.

- Clinical results that align with the normal distribution will be presented as mean with SE, SD or two-sided 95% CI.

- Clinical results that do not align with a normal distribution will be presented as median, and 25 and 75 percentile.

Beside the descriptive tables spaghetti and margins plot will be constructed to graphically assess how the parametrization of our model must be for the primary analysis.

The primary analysis will utilize the G-estimation framework. The Q-model in this regard will be a structural equation model where the longitudinal aspect and effect modification will be incorporated into the framework. A directed acyclic graphs will be constructed to visualize which assumption we have made concerning the underlying causal structure. For the G-estimation approaches we will estimate the confidence intervals by way of bootstrap.

No adjustments for multiple testing will be carried out in Table 1 and no effect sizes for differences between groups will be estimated. Missing observations will be excluded from tests in Table 1, and number for missing observations in each group will be specified.

PLANNED ANALYSES SECONDARY PUBLICATION:. CHANGES IN CAN ASSESSMENT

Baseline Tabel 1	Active	Placebo	P-value
Age, mean (SD), Years			
Female, No. (%)			
Smoking no. (%)			

Alcohol consumptions > 14 units weekly.

Diagnosis, No. (%)

Schizophrenia

Schizotypal disorder

Schizoaffective disorder

Duration of diagnosis, months, No (%) .

6-12 months

12-24 months

>24 months

Treatment, No. (%)

Quetiapine

Olanzapine

Risperidone

Ziprasidone

Paliperidone

Aripiprazole

Clozapine

Other

Psychiatric evaluations

PANSS-6 Score

P1

P2

P3

N1

N4

N6

Clinical characteristics, mean (SD)

Body weight, kg

Waist circumference, cm

BMI

Systolic Blood pressure, mmHg

Diastolic Blood pressure, mmHg

Glucose Metabolism

HbA1C, mmol/mol, mean (SD)

Fasting Blood Glucose

Fasting C-peptide

Fasting insulin level

Organ function

Creatinine Levels

ALAT

Amylase

BASP

Bilirubin

Cholesterol Level

Total

LDL

HDL

Triglycerides

Other biochemical variables

Testosterone

SHBG

Urate

CRP

Hemoglobin

Table 2. Prevalence of CAN among patients with schizophrenia and obese psychiatric healthy controls

	Schizophrenia	Healthy control	P-value
Degree of CAN			
No diagnosis of CAN, n/N (%)			
Early CAN, n/N (%)			
Manifest CAN, n/N (%)			
	Schizophrenia	Diabetes mellitus type 2	
Degree of CAN			
No diagnosis of CAN, n/N (%)			
Early CAN, n/N (%)			
Manifest CAN, n/N (%)			
CAN: cardiovascular autonomic neuropathy. Values are represented as number (%).			
p Values were obtained from chi square test.			

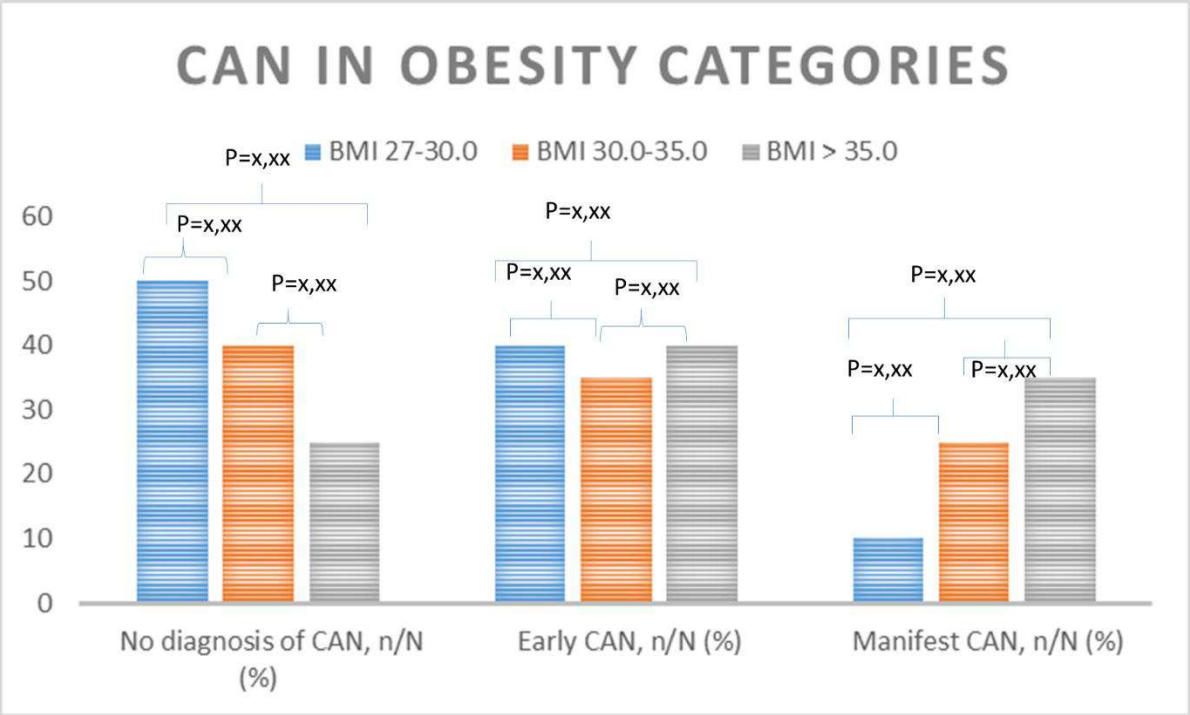


Table 3. Prevalence of CAN among patients with schizophrenia in various Antipsychotic treatment			
	No diagnosis of CAN, n/N (%)	Early CAN, n/N (%)	Manifest CAN, n/N (%)
Olanzapine			
Quetiapine			
Risperidone			
Ziprasiode			
Palliperidone			
Aripiprazole			
Clozapine			
Other			
CAN: cardiovascular autonomic neuropathy. Values are represented as number (%).			
p Values were obtained from chi square test.			

Table 4. Changes in CAN after weeks treatment with Semaglutide or placebo			
	Active - baseline	Placebo- baseline	P-value
Degree of CAN			
No diagnosis of CAN, n/N (%)			
Early CAN, n/N (%)			
Manifest CAN, n/N (%)			
	Active - end of study	Placebo - end of study	
Degree of CAN			
No diagnosis of CAN, n/N (%)			
Early CAN, n/N (%)			
Manifest CAN, n/N (%)			
CAN: cardiovascular autonomic neuropathy. Values are represented as number (%).			
p Values were obtained from chi square test.			

PLANNED ANALYSES SECONDARY PUBLICATION: CHANGES IN NAF PET CT SCANS AND BODY COMPOSITION – A PILOT STUDY IN PROJECT HISTORI

Baseline Tabel 1	Active	Placebo	P-value
Age, mean (SD), Years			
Female, No. (%)			
Smoking no. (%)			
Alcohol consumptions > 14 units weekly.			
Diagnosis, No. (%)			
Schizophrenia			
Schizotypal disorder			
Schizoaffective disorder			
Duration of diagnosis, months, No(%)			.
6-12 months			
12-24 months			
>24 months			
Treatment, No. (%)			
Quetiapine			
Olanzapine			
Risperidone			
Ziprasidone			
Paliperidone			
Aripiprazole			
Clozapine			
Other			

Psychiatric evaluations

PANSS-6 Score

P1

P2

P3

N1

N4

N6

Clinical characteristics, mean (SD)

Body weight, kg

Waist circumference, cm

BMI

Systolic Blood pressure, mmHg

Diastolic Blood pressure, mmHg

Glucose Metabolism

HbA1C, mmol/mol, mean (SD)

Fasting Blood Glucose

Fasting C-peptide

Fasting insulin level

Organ function

Creatinine Levels

ALAT

Amylase

BASP

Bilirubin

Cholesterol Level

Total

LDL

HDL

Triglycerides

Other biochemical variables

Testosterone

SHBG

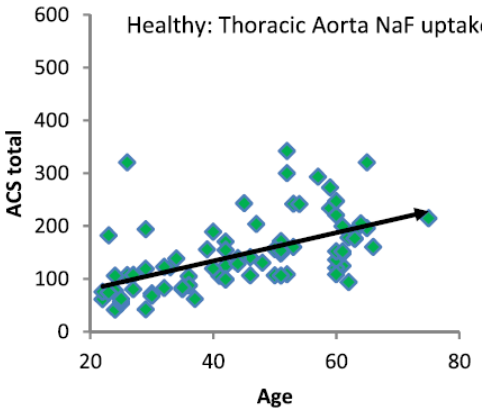
Urate

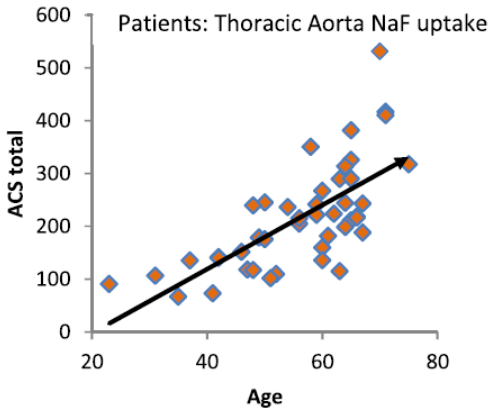
CRP

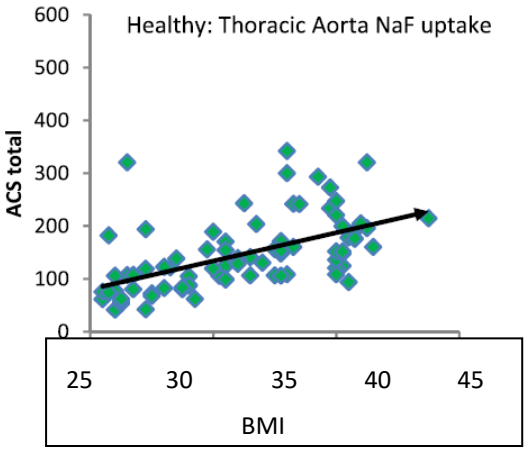
Hemoglobin

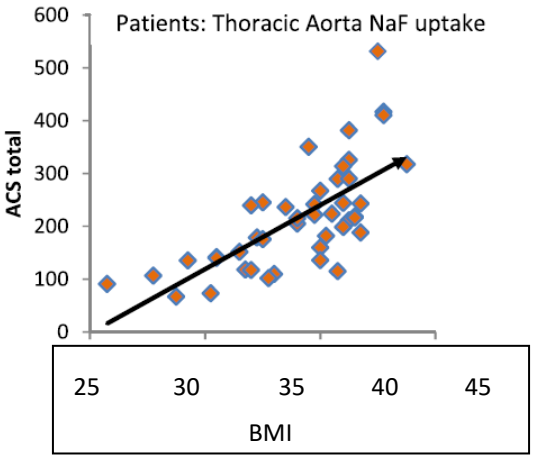
Table 2. NAF uptake in healthy population and study population			
	Non schizophrenic population	Study population	P-value
NAF uptake Total			
NAF uptake BMI 27 - 30			
NAF uptake BMI 30 - 40			
NAF uptake BMI 40+			
NAF: Sodiumflouride uptake. Values are represented as number (%).			
p Values were obtained from chi square test.			

Table 3. NAF uptake before and after 30 weeks treatment with Semaglutide or placebo			
	Active - baseline	Placebo- baseline	P-value
NAF uptake Total			
NAF uptake BMI 27 - 30			
NAF uptake BMI 30 - 40			
NAF uptake BMI 40+			
	Active - end of study	Placebo - end of study	
NAF uptake Total			
NAF uptake BMI 27 - 30			
NAF uptake BMI 30 - 40			
NAF uptake BMI 40+			
NAF: Sodiumflouride uptake. Values are represented as number (%).			
p Values were obtained from chi square test.			









Baseline Tabel 5	Active	Placebo	P-value
Adiponectin			
TNF-alfa			
IL-6			
Leptin			
IGF-1			
GDF-15			
NT ProBNP			
Homocysteine			
Fibrinogen			
Lp(a)			
ApoB			

PLANNED ANALYSES SECONDARY PUBLICATION: **CHANGES IN SF-36 – MEDIATORS – WEIGHT AND SIMPLE PHYSICAL ACTIVITY QUESTIONNAIRE (SOMPAQ).**

See analysis plan for PANSS mediators. The same statistical approach will be applied.

IS INSULIN RESISTANCE A KEY DRIVING FACTOR FOR PREDIABETES, DYSLIPIDEMIA AND ELEVATED CARDIOVASCULAR RISK MARKERS?

Baseline Tabel 1	Active	Placebo	P-value
Age, mean (SD), Years			
Female, No. (%)			
Smoking no. (%)			
Alcohol consumptions > 14 units weekly.			
Diagnosis, No. (%)			
Schizophrenia			
Schizotypal disorder			
Schizoaffective disorder			
Duration of diagnosis, months, No(%)			.
6-12 months			
12-24 months			
>24 months			
Treatment, No. (%)			
Quetiapine			
Olanzapine			
Risperidone			
Ziprasidone			
Paliperidone			
Aripiprazole			
Clozapine			
Other			
Psychiatric evaluations			
PANSS-6 Score			
P1			
P2			
P3			
N1			
N4			
N6			
Clinical characteristics, mean (SD)			
Body weight, kg			

Waist circumference, cm

BMI

Systolic Blood pressure, mmHg

Diastolic Blood pressure, mmHg

Glucose Metabolism

HbA1C, mmol/mol, mean (SD)

Fasting Blood Glucose

Fasting C-peptide

Fasting insulin level

Organ function

Creatinine Levels

ALAT

Amylase

BASP

Bilirubin

Cholesterol Level

Total

LDL

HDL

Triglycerides

Other biochemical variables

Testosterone

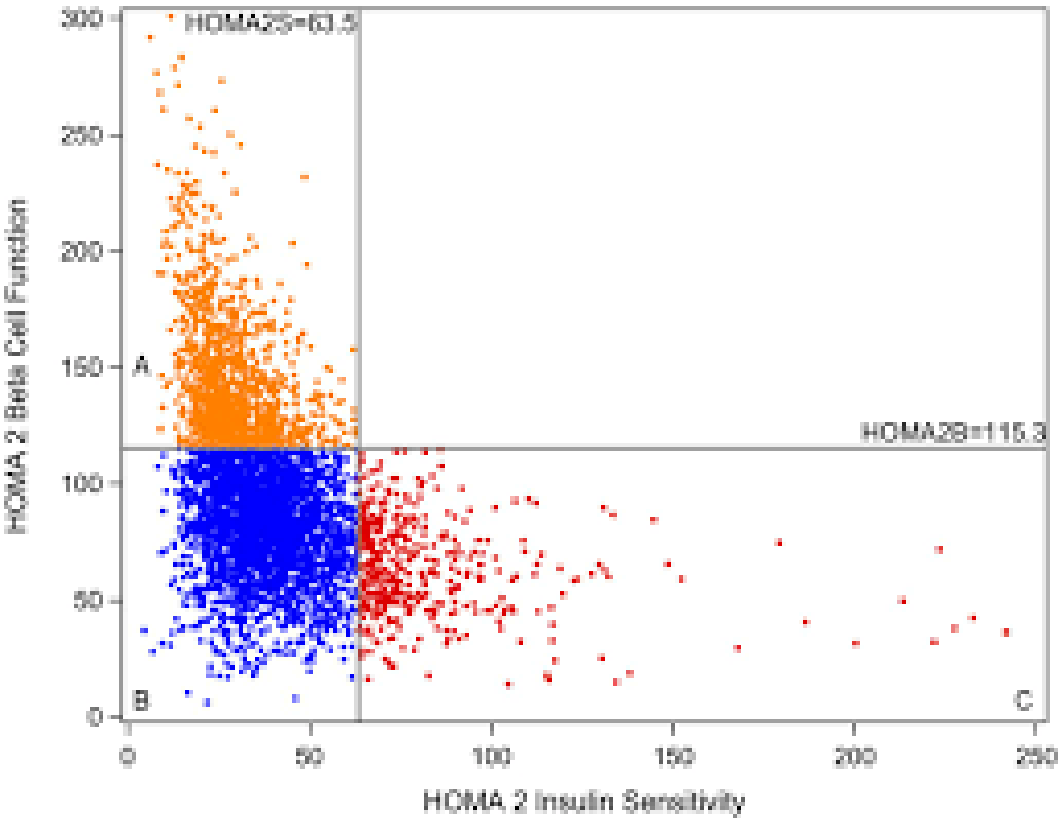
SHBG

Urate

CRP

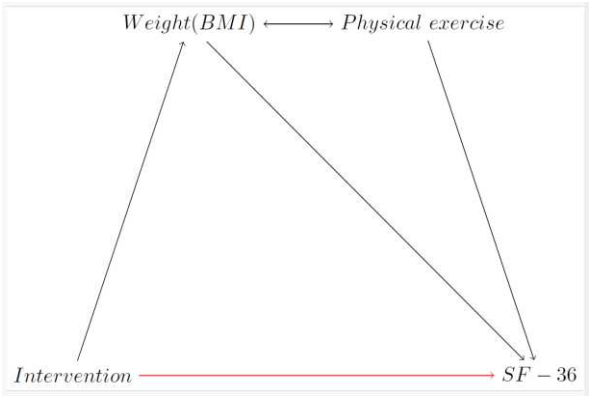
Hemoglobin

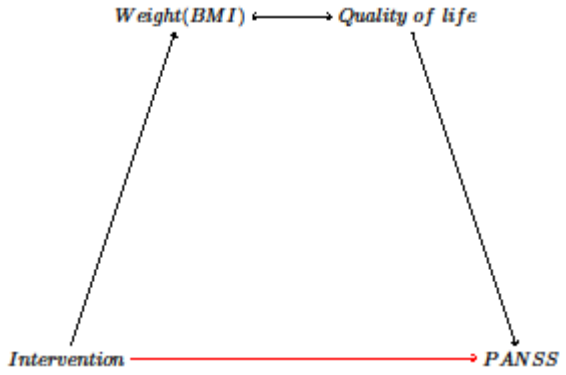
Baseline Tabel 2	Active	Placebo	P-value
HOMA-2B			
HOMA2S			



Baseline Tabel 4	Active	Placebo	P-value
Adiponectin			
TNF-alfa			
IL-6			
Leptin			
IGF-1			
GDF-15			
NT ProBNP			
Homocysteine			
Fibrinogen			
Lp(a)			
ApoB			

Mediator analysis


Weight(BMI)
Physical exercise
Intervention
SF − 36


Weight(BMI)
Quality of life
Intervention
PANSS

Alternative for table 3 for PRIMARY OUTCOME (HBA1C CHANGE)

	Active - Week 15	Placebo - Week 15	Mean difference	P-value
HbA1c PANSS-6 SF-36 Weight				
	Active - end of study	Placebo - end of study	Mean difference	P-value
HbA1c PANSS-6 SF-36 Weight				
	Active - marginal	Placebo - marginal	Mean difference	P-value
HbA1c PANSS-6 SF-36 Weight				
HbA1c mol/mmol, PANSS-6 and SF-36 are numerical values and weight is measured in Kg. The p-values are either based on the z or likelihood ratio test.				

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Version 1.0

Two different conclusions before unblinding. Treatment A vs. B.

Home-based Intervention With Semaglutide Treatment Of Neuroleptica-Related Prediabetes

Treatment A is the investigated drug.

Patients receiving investigated drug Semaglutide (group A) demonstrate significant reductions in HbA1c at week 15 and week 30, when compared to patients receiving placebo (group B). Cases of pre-diabetes were reduced in group A compared to group B. Both findings are clinically meaningful and provide evidence that Semaglutide can normalize glucose levels in patients with schizophrenia and prediabetes. Furthermore, patients receiving Semaglutide reported superior physical health status compared to patients receiving placebo at both week 15 and week 30. Thus, Semaglutide also improves patients' experience of their physical health. In contrast, there were no differences between the two interventions in self-reported mental health status or positive and negative symptoms of schizophrenia. In conclusion, we recommend treatment with Semaglutide for patients with schizophrenia and prediabetes.

Treatment B is the investigated drug.

Patients receiving investigated drug Semaglutide (group B) demonstrate significantly higher levels of HbA1c at week 15 or week 30, when compared to patients receiving treatment with placebo (group A). Reduction in cases with prediabetes were also less in patients receiving Semaglutide (group B). Differences are clinically meaningful and this provides evidence that Semaglutide is inferior to placebo in normalizing glucose levels in patents with schizophrenia and prediabetes. Furthermore, patients receiving Semaglutide reported inferior physical health status compared to the placebo group. In contrast, there were no differences between the two interventions in self-reported mental health status or positive and negative symptoms of schizophrenia. In conclusion, we cannot recommend treatment with Semaglutide for patients with schizophrenia and prediabetes.