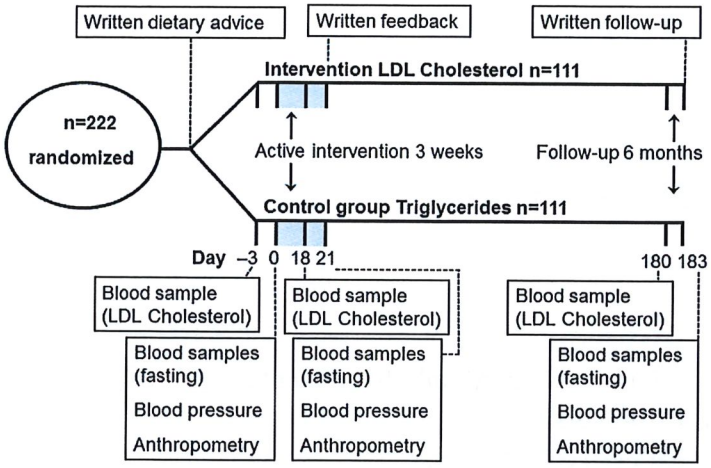

Statistical analysis plan (SAP)

Section 1. Administrative information

1a. Title, registration, versions and revisions

Full study title: Effectiveness of Written Dietary Advice on Lipoproteins - a Pragmatic Randomized Controlled Trial

Acronym: MYDICLIN

1b. Clinicaltrials.gov identifier: NCT03528252

2. SAP version: 1.4, 2019-04-18

3. Protocol version: Research plan version 18-09-20

4. SAP revisions: No major revisions.

5. Roles and responsibility

Author: David Iggman[1,2]

Principle Investigator: David Iggman[1,2]

Affiliations:

1. Unit for Clinical Nutrition and Metabolism, Deptartment of Public Health and Caring Sciences, Uppsala university.
2. Center for Clinical research Dalarna

6. Signatures:

I the undersigned, certify that I have read this SAP and approve it as adequate in scope of the main analyses of MYDICLIN.

6. Author and principle investigator

Name: David Iggman

.....

Date: 2019-04-18

Section 2. Introduction

7. Background and rationale

Dietary factors are of major importance for public health, but effective means are lacking, to improve dietary habits of patients in primary care. This pragmatic trial aims to investigate whether detailed written dietary advice may be an effective way of improving blood lipoproteins in a Swedish primary care setting.

8. Objectives

The main purpose is to investigate whether written dietary advice with individual written feedback can affect blood lipoproteins in the short-term (3 weeks) and medium-term (6 months).

Specific aims

- What effect does written dietary advice have on LDL cholesterol (primary outcome) and other lipoproteins and risk factors (secondary outcomes) after 3 weeks and 6 months, compared with a control group?
- What proportion of individuals achieve a ≥10% reduction in LDL cholesterol from baseline, after and 6 months?
- In the group of responders (≥10% reduction in LDL cholesterol from baseline), what is the effect after 3 weeks and 6 months?
- Is double sampling of LDL cholesterol required in clinical practice, i.e., how many would be misclassified as responders/non-responders if only one sample was taken at 3 weeks and 6 months?
- How does the intervention's cost compare with estimated future health care savings, as estimated per mean LDL cholesterol reduction, and per individual reaching ≥10% reduction in LDL cholesterol at 6 months?
- Which food choices are associated with reductions in LDL cholesterol in practice?
- What are the reasons given for wanting to reduce LDL cholesterol? Is there any association between reasons given and effect on LDL cholesterol?

Section 3. Study Methods

9. Trial design

The study design is a pragmatic randomized controlled dietary trial, with two parallel arms. The allocation ratio is 1:1. Both groups will receive dietary advice in sealed numbered envelopes. The intervention group will receive advice for reducing LDL cholesterol, whereas the control group will receive similar advice, but instead proven effective for reducing fasting triglycerides. The participants will not be aware of which group is considered as active intervention. Participants were recruited and randomized May to October 2018 and data collection is finished April 18th 2019.

10. Randomization

The allocation sequence is created by an external agent and performed in blocks (size unknown to investigators). The allocation sequence will be concealed until data collection has been completed.

11. Sample size

A primary power calculation was performed based on previous randomized intervention studies of dietary fat quality and effects on lipoproteins. At least n=222 participants were required (for alpha = 0.05 and beta = 0.10) to detect an effect in LDL cholesterol of 0.175 mmol/L (~5%), which would slightly exceed that in most previous studies, parallel within-individual day-to-day variation (at double sampling), and have some clinical significance. This calculation took into account a 20% loss to follow-up in the intervention group and a contamination effect of 5% in the control group.

In September 2018, during the end of recruitment process, another power calculation was performed in order to examine the power given the current recruitment status (n=104). For alpha = 0.05 and beta = 0.20, the minimum number of participants required was n=106 (n=94 without 5% contamination). Thus, the recruitment was considered as finished at Oct 16th, when n=113 participants were included (as the number of volunteer participants had faded, and power was considered sufficient, if not optimal).

12. Framework

The study is a dietary intervention with clinical and experimental outcomes.

13. Stopping interim analyses

N/A.

14. Timing of final analysis

All analyses will be performed once data collection is finished, April 18th 2019.

15. Timing of outcomes assessment

N/A.

Section 4. Statistical principles

Confidence intervals and P values

16. Level of statistical significance

P<0.05 will be considered as statistically significant for primary outcomes. For secondary outcomes, significance testing is not as essential as they are mainly hypothesis generating.

17. Adjustment for multiple analyses

The primary outcome is change in LDL cholesterol at 3 weeks and 6 months. The power calculations have been performed for between-group differences at 6 months and this analysis is considered the primary outcome of the study. Thus, no adjustment for multiple analyses will be performed.

18. Confidence intervals

N/A.

Adherence and protocol deviations

19a. Definition of adherence to the intervention

Being randomized and completing the trial (day 183) is considered as sufficient for adherence. For the visit at 3 weeks, no more than 2 days' deviation from protocol will be allowed. For the visit at 183 days, no more than 2 weeks' deviation will be allowed.

19b. Description how adherence will be presented

No specific information on adherence will be presented, besides no. of participants randomized and completing the trial at 3 weeks and 6 months, and no. excluded before and after randomization, with reasons.

19c. Definition of protocol deviations

Deviations from time schedule. Failing to be overnight fasting at time points 2, 4 or 6.

19d. Description of which deviations will be summarized

In cases in which blood samples are taken on the wrong day, the no. of deviations and number of days' deviation from protocol will be reported for each time point. Also, the number indivduals who failed to be completely fasting at time points 2, 4 and 6 will be given.

20. Analysis populations

Study populations will be analyzed according to intention-to-treat principles.

Section 5. Trial Population

21. Screening data

N/A (not collected).

22. Eligibility criteria

Inclusion criteria: patients listed at the health care centre, wish to improve blood lipoproteins, ages 18-99 years.

Exclusion criteria: drugs affecting lipid metabolism (statins, ezetemibe, fibrates, PCSK9 inhibitors, neuroleptics, cortisone, amiodarone, estrogen, progesterone, testosterone, cyclosporin, tacrolimus, loop diuretics, protease inhibitors and anti-convulsants, whereas beta-blockers, tiazid-diuretics and SGLT2 inhibitors are accepted at stable use), malignant disease, extreme diet (vegan diet, strict low-carbohydrate diet, other weight-loss diet), disturbed metabolism e.g. untreated hypothyroidism or hyperthyroidism, dementia or inability to understand written Swedish instructions, other participant from same household, current employment at the health care centre.

23. Recruitment

Information on no. enrolled (assessed for eligibility, excluded – with reasons and randomized), allocated, lost to follow-up or discontinued (with reasons) and analyzed or excluded from analysis (with reasons) will be presented in a CONSORT flow diagram.

Withdrawal/follow-up

24a. Level of withdrawal

No. of participants withdrawn or lost to follow-up will be presented for each group.

24b. Timing of withdrawal/lost to follow-up data

Timing of withdrawal or loss to follow-up will be presented for each group.

24c. Reasons and details of withdrawal/lost to follow-up data

Reasons and details will be presented in a CONSORT flow diagram and in text format (Results).

Baseline characteristics

25a. List of baselines characteristics

Age, sex (male/female), diabetes (yes/no), impaired fasting glucose (yes/no), body weight, BMI, waist circumference, systolic and diastolic blood pressure, LDL cholesterol, total cholesterol, HDL cholesterol, fasting triglycerides, apo B, apo AI, apo B:AI, fasting glucose and HbA1c will be presented for each group in table format (without significance testing of variable distributions).

25b. Details of baseline characteristics presentation

Non-normal variables will be presented as median,IQR, otherwise as mean±SD or n for categorical variables.

Section 6. Analysis

Outcome definitions

26a. Primary outcome: Change in LDL cholesterol at 6 months (primary analysis) and 3 weeks.

Secondary outcomes: Change in HDL cholesterol, total cholesterol, fasting triglycerides, apo B, apo AI, apo B:AI, fasting glucose, HbA1c, systolic and diastolic blood pressure, weight, BMI and waist circumference at 6 months and 3 weeks. No. and proportion of individuals that achieve a ≤0%, >0 to <10% or ≥10% reduction in LDL cholesterol from baseline, after 6 months and 3 weeks. Change in LDL cholesterol at 6 months and 3 weeks, in responders at 3 weeks (≥10% reduction in LDL cholesterol from baseline). No. and proportion of individuals misclassified at 6 months and 3 weeks with only sample 2, 4 and 6 included in analyses. Estimated cost of intervention. Associations of food choices (total no. and no. and proportion of total X:s for each dietary advice in food record table) with change in LDL cholesterol, and with HDL cholesterol, total cholesterol, fasting triglycerides, apo B, apo AI, apo B:AI, fasting glucose, HbA1c, systolic and diastolic blood pressure, weight, BMI and waist circumference at 6 months and 3 weeks. Reasons stated for wanting to improve blood lipoproteins, and associations with total no. and percentage of total of X:s in food record table and with changes in LDL cholesterol, HDL cholesterol, total cholesterol, fasting triglycerides, apo B, apo AI, apo B:AI, fasting glucose, HbA1c, systolic and diastolic blood pressure, weight, BMI and waist circumference at 6 months and 3 weeks. No. of individuals that develop impaired fasting

glucose, and elevated ALT at 6 months and 3 weeks. Change and average variation (CV) in LDL Cholesterol at 21 vs. 18 days and at 183 vs. 180 days.

Possible future analyses: Change in plasma Lipoprotein(a), serum PSCK9 and genes involved in lipid metabolism at 6 months and 3 weeks.

26b. Specific measurements and units

LDL cholesterol (direct method), HDL cholesterol, total cholesterol, fasting triglycerides and glucose in mmol/L.

Apo B and apo AI in g/L.

HbA1c in mmol/mol.

ALT in μkat/L.

TSH in mU/L.

Weight (in light clothing) in kg.

Height and waist circumference in cm.

BMI as kg/m^2.

Systolic and diastolic blood pressure in mm hg.

Age at randomization in X.X years

Type 2 diabetes is defined according to current diagnostic criteria.

Impaired fasting glucose is defined as fasting P-glucose 6.1 - 6.9 mmol/L.

26c. Specific calculations

For LDL Cholesterol baseline (BL) values will be calculated as the arithmetic mean of values at day -3 and day 0 [(d-3 + d0)/2], similarly, values at 3 weeks will be calculated as the arithmetic mean of values at days 18 and 21 [(d18 + d21)/2] and values at 6 months will be calculated as the arithmetic mean of values at days 180 and 183 [(d180 + d183)/2].

For all continuous variables, absolute change from baseline (Δ) will be calculated at 3 weeks and 6 months as [Δ = 3w - BL and 6m - BL]. Relative change from baseline (%Δ) will be calculated as [%Δ = (3w - BL)/BL and (6m - BL)/BL].

As a sensitivity analysis, variables in the GLM with non-normal distribution will be log transformed (otherwise, no transformations will be performed).

Analysis methods

27a. Analysis methods and presentation of results

Between-group differences for change from baseline (Δ) in continuous variables will be significance tested using a general linear model (GLM). For LDL cholesterol, the results will be presented as a figure with boxplots displaying values at BL, 3 weeks and 6 months for each group. Also, a boxplot of Δ-values at 6 months will be presented for each group. The results will be given in a table format, displayed as mean±SD change from baseline, and P-values for difference between groups.

Difference in mean change from baseline in LDL cholesterol (and triglycerides for control group) between 21 and 18 days and 183 vs. 180 days will be analyzed with t-tests.

Secondary outcome no. 4 (success rate) i.e. the no. and proportion of individuals that achieve a ≤0%, >0 to <10% or ≥10% reduction in LDL cholesterol from baseline will be analyzed by logistic regression, adjusted for age, sex and baseline BMI. No. of individuals that develop impaired fasting glucose will be compared between groups with Fisher's exact test (unadjusted).

Secondary outcome no. 8 (effective food choices) will be analyzed and presented as linear regressions between number of X:s for food choices 1-13 (and the total number of X:s) and change in LDL cholesterol (or Triglycerides within Control group) at 3 weeks and 6 months.

Secondary outcome no. 9 (stated reasons) will be categorized into main groups of reasons according to given responses (one, two or three compared to none stated), no. in each group and mean changes in LDL cholesterol (or triglycerides) as well as total number of X:s (and proportions of total number of possible X:s for both groups combined) will be given as means+SD in table format. For within-group effects on mean LDL cholesterol at 3 weeks and 6 months, paired t-tests will be performed in intervention group (the same for triglycerides in control group). For proportion of total X:s, differences compared with reference group (none stated) will be analyzed by Mann Whitney U-test.

27b. Adjustment for covariates

Comparisons of means in the GLM will be adjusted for the baseline value of the variable, baseline BMI, age and sex.

For success rate, the logistic regression will be adjusted for the covariates baseline LDL cholesterol, baseline BMI, age and sex.

Linear regressions for food choices and lipoproteins will be unadjusted.

27c. Tests for assumptions

Normality of data will be determined by visual inspection of histograms and QQ-plots. No other tests for assumptions will performed. The underlying assumptions of the GLM will be tested by examining residuals.

27d. Alternative methods if distributional assumptions do not hold

As a sensitivity analysis, variables in the GLM with non-normal distribution will be log transformed (otherwise, no transformations will be performed).

27e. Planned sensitivity analyses

For all comparisons of means of continuous outcomes, unadjusted Mann Whitney U-tests will be performed as sensitivity analyses.

27f. Planned subgroup analyses

In order to investigate whether different baseline characteristics are associated with success rate (outcome no. 4), responders (≥10% decrease in LDL cholesterol or Triglycerides) and non-responders at 3 weeks will be analyzed with descriptive statistics regarding age, sex, baseline cholesterol, no. with diabetes and impaired fasting glucose, systolic and diastolic blood pressure, no. of X:s (total and for each food choice), and reasons stated (according to categories), separately for both study arms. Comparisons between responders and non-responders will be analyzed by t-tests and Fisher's exact test. Similar analyses will be performed for successful individuals at 6 months (≥10% decrease in LDL cholesterol or Triglycerides).

Further, subgroup analyses will be performed in young (<65 years) vs elderly (65+) individuals, in order to establish whether results are homogenous in these subgroups, for guidance of future studies.

28. Missing data

When data are missing due to loss to follow-up, baseline values will be imputed. If only one time point 3 or 5 is missing (LDL cholesterol at d18 or d180), the mean LDL

cholesterol value will instead be assumed to be the exact value at time point 4 or 6 (d21 or d183).

29. Additional analyses

For possible future analyses (see 26a. secondary outcomes), analyses will be performed with similar methods as other continuous/categorical variables.

30. Harms

Reported adverse effects (if any) will be described in text or table format, depending on the outcome. No serious adverse effects are expected and severity will not be summarized separately.

31. Statistical software

IBM SPSS Statistics 25 will be used for all analyses.

32. References

Data management plan, standard operating procedures, dietary advice, food records and study letters will be stored separately and not provided at trial registry during the study. No statistical master file has yet been created and public access will not be granted.