May 2020

Research protocol.

Rec protocol number: 39113[1]

Application to carry out human intervention studies in the NFR project:
Short name: Lipidinflammagenes.
Full title**: "Effects** of lipids' composition and structure in meat and dairy foods on digestibility and low-grade inflammation in cells, animals and humans.
Funding: The Norwegian Agriculture Agency Grant no 281297
Project No 281297
Overall project leader: Professor Bjørg Egelandsdal (KBM Faculty, Norwegian University of Life Sciences, NMBU)

Work package 4 of the project describes the human intervention:

Responsible researcher: Professor Catia Martins (NTNU, Department of Clinical and Molecular Medicine, Clinical Nutrition)
Daily executive researcher: Professor Anna Haug (NMBU, nutrition), Milena-Monfort-Pires (NMBU, nutrition)

Background

Meat and milk production is a key part of value creation in Norwegian agriculture, i.e., 75% of primary production and more than 50% of industrial production. However, these two product groups contribute significantly to the total intake of saturated fats (SFA) in the diet; 42% and 20%, respectively. Norwegian health authorities recommend that the intake of saturated fats should be reduced from the current intake of about 14 energy percent to <10% of energy intake due to an assumed link between animal saturated fats and cardiovascular diseases. The mechanism of how saturated fat leads to cardiovascular diseases is poorly understood[2]. A recent study found a link between the intake of saturated fats and cardiovascular disease, and an inverse link to stroke. A clear signal of international disagreement is that the Australian[3] Heart Foundation recently (2019; https://www.heartfoundation.org.au/news/new-advice-from-the-heart-foundation-on-meat-dairy-and-eggs) removed its recommendations to avoid whole milk, whole-fat cheese and yogurt, claiming that no negative health relationship had been shown. The argument for changing the recommendation was, among other things, related to the calcium content of these products.

The ratio of saturated fat to calcium (SFA: Ca) in yellow cheese (27% fat) is 18.2; in butter 2688:1; in beef dough (14% fat) 743:1 and in pork dough (10% fat) 925:1. There is no definition of an optimal relationship between SFA and calcium from a health perspective. Mechanistic

[1] REK ID number is 139404
[2]Leroy, F: & Cofnas, N. (2019) Should dietary guidelines recommend low red meat intake? Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2019.1657063
[3]Dehghan M, Meant A Zhang X et al. (2017) Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet*; 390: 2050-2062

explanations are linked to the fact that calcium is anti-inflammatory and acts as an antioxidant in[4] complex systems (not in pure oils).[5]

Intake of saturated fats comes mainly from dairy, pork, and cattle products. However, the triglycerides in these 3 fat types cannot only be understood based on their saturated fat content because the stereo-specific position of the saturated fatty acids is not equal. This applies in particular to palmitic acid (C16:0) which has a preference for the SN2 position in Norwegian pork fat. In addition, pork triglycerides with a high n6:n3 ratio are produced for polyunsaturated fatty acids. The three sources of triglyceride must therefore to some extent be perceived as unique.

Oddly enough, there are no /few compared human intervention attempts of the most dominant animal fat sources in our diet. A new Australian study compares 'meat' and dairy fat, but the meat group was so heterogeneous that it is not suitable for mechanistic interpretation.[6]

Experiments are underway with 100 mice at NMBU fed different types of animal fats, and the results of this experiment may affect the final dietary choice for this human intervention study. The mouse trial will be completed in May 2020.

Satiety is another important parameter associated with fat intake because obesity is both an inflammatory condition and is linked to non-infectious diseases such as cancer, cardiovascular diseases, insulin resistance, dyslipidemia, and hypertension.

The intended human intervention study contains a crossed setup with **four different diets** combined in one study. The number of subjects is 32 (women and men).
The experiment is carried out as a 14-day intervention study x 4.

On day 1 in the morning, each individual will come fasting for sampling.
The intervention attempt is ongoing for 14 days (see Tables 1 and 2). Fourteen days are chosen as a balance between two factors; 1) short studies impose significant requirements to homogeneity among participants in order to gain the necessary **strength;** 2) longer studies require a locked diet over many days. The strength of the intervention falls proportionally to dietary abnormalities, cf. a statement from the director of the Journal of the American Medical Association in October 2019: 'but long-term studies are notoriously difficult in large part because of poor long-term adherence to prescribed diets' (https://jamanetwork.com/journals/jama/fullarticle/2752906).

A 14-day crossed randomized intervention study with four different diets
After 14 days of intervention, inflammation markers and upregulation of early biomarkers for CVD and changes in phospholipid turnover may be detected with sensitive analyses (-omics and gene analyses) (https://www.nature.com/articles/s41598-017-12254-1.pdf). Strong early indicators of atherosclerosis include changes in phospholipid metabolism, increased concentration of monoglycerides as well as reduced concentration of dehydroepiandrosterone

[4]JDeSousa J, Tong M, Wei J, et al. (2016) . The anti-inflammatory effect of calcium for preventing endothelial cell activation in preeclampsia. J. Hypertens. 30(5):303-8. doi: 10.1038/jhh.2015.73.
[5]Yi, G. et al, (2015). Compositional Factors that Influence Lipid Peroxidation in Beef Juice and Standard Sausages. Journal of Food Science, 80(12):C2692-700DOI: 10.1111/1750-3841.13123 PMID: 26579877
[6]Liu, Y: et al, (2019). Fat from dairy and 'meat' consumed within recommended levels is associated with favourable serum cholesterol level in institutionalized older adults. J. of Nutritional Science, e10., 1-8

sulfate (a neuron protector; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2894999/) in serum.

During 4 days of week 1 (Monday, Tuesday, Wednesday, Thursday), 8 people will come to the experimental laboratory daily and randomize to consume diet 1-4 (see Tables 1 and 2), so that all 32 subjects start the intervention in the same week. They arrive fasting and bring morning urine, and they are weighed, blood pressure and heart rate are taken, and blood is sampled. They receive diet rations for 14 days and start eating their test meals after the initial samples are taken. Help will be offered for the transportation of the food, if necessary.

Participants are given five meals per day, where the energy content is adapted to their individual energy needs (total energy expenditure, TEE) (test product 1, 2, 3 or 4). As large single meals increase inflammation, we recommend that food should be distributed evenly throughout the day, with the same amount of energy in each meal. Participants who find this difficult are asked to estimate deviations. In the test meals, necessary amounts, according to recommendations, of vitamin-mineral supplements are added so that participants should not take any kind of supplement during the experiment. Meals are obtained from commercial manufacturers. They should limit their intake of coffee and tea to a maximum of 5 cups per day and avoid alcohol and soft drinks with natural sugar. The test meals will get 40 E% from fat to make the meals as similar as possible.

After 14 days, they will enter fasting in the morning for sampling.

Pilot setup illustrated with 14 days

Table 1- The principle of intervention with respect to the distribution of 32*(4x 8) subjects.

	32 people: 14 days crossed intervention						
Diet	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1**	-	-	2	2	2	2	-
2**	-	-	2	2	2	2	-
3**	-	-	2	2	2	2	-
4**	-	-	2	2	2	2	-
Number	-	-	8	8	8	8	-

*) It is assumed that one must start up with more than 32 people in order to have 32 people in the end; **) controlled start-up diet, but in randomized order, i.e. 1/4 get diet 4, etc;

After a 14-day longitudinal study, a two-week wash out follows before it all repeats, but where the subjects are given a different diet. During the wash-out period, participants eat their normal diet.

This is done a total of four times so that each subject has eaten the four experimental diets for 14 days, i.e. for a total of 14 days x 4 diets.

In total, the intervention study (including wash out periods) will last for about 14 weeks (late winter - 2020 - spring 2021).

Table 2- Timeline of the intervention (14 weeks)

Week	1,2	3	4	5,6	7	8	9,10	11	12	13,14
Test	TEST			TEST			TEST			TEST

Description of the study

Recruitment of participants: inclusion and exclusion criteria

We want to recruit healthy men and women 18-40 years of normal weight, without very high physical activity level per week (< 10 hours of physical activity per week) during the trial period. Due to the nature of the intervention, the activity level is assumed to be stable through the intervention. We do not want to include people who take medications beyond oral contraceptives.

Subjects who do not like/tolerate milk, pork or cattle are excluded before starting.

50 people will be recruited to secure 32 in the end, as significant drop-out must be expected. Nor can we recruit participants who want to avoid eating to satiety considering they don't want to gain weight. As an exclusion criterion, a score > 3.5 in the DeBQ (Dutch Eating Behavior Questionnaire) will result in exclusion from the intervention. https://link.springer.com/referenceworkentry/10.1007%2F978-981-287-087-2_127-1. For women, the measurements will be carried out over different phases of the menstrual cycle since it is not appropriate to recruit only people in the same phase of the cycle. The mappings of participants to the groups will be randomized so that the effect of the cycle can be zeroed out. This means that each diet group has the same average value and spread regarding the phase of the cycle. We will nevertheless record phase to menstrual cycle so that the data can potentially be adjusted for this statistically. Two weeks have been chosen for the crossed intervention as this is enough to stabilize the level of blood lipids (Mensink, 2020; personal notice). Longer interventions impair the ability to comply with the "rules".

Upon inclusion, the blood will be analyzed for: LDL, HDL, ApoB, TG, and GLP-1, ALT, AST, GGT, albumin, IL1beta, IL6, IL8, TNFalpha, microCRP, - gas chromatography/liquid chromatography or core magnetic resonance will be selected for detailed blood serum analyses with respect to lipids (phospholipids, monoglycerides). Gene analyses will be limited to isolation of RNA from leucocytes (LeukoLOCK™ Total RNA Isolation System). Epigenetics can be performed on RNA and RNA sequences are analyzed in the project. Blood pressure, heart rate, height and weight will also be measured. Morning urine will be collected.

Intervention study (crossed design)

On the first day of testing (for example, Monday), 8 subjects will show up fasting for blood sampling and anthropometric measurements. They are randomized to diet 1-4, two people on each diet, and they are given their daily rations for each day for 14 days that are individually adapted to their energy needs. Resting metabolic activity will be based on height, weight and age and then adjusted with a physical activity factor. Usually, activity factor of 1.4 is used for

sedentary and a higher factor with moderate activity. The factor must be set after recruiting the participants.[7]

Compliance will be measured throughout the experiment and all food delivered will be registered. Participants will be asked to write down nonconformities. This applies to the fact that they have not eaten all the distributed food, any food they have eaten in addition and deviations in physical activity from what is used in the calculation. In addition, the daily executive or her closest associate will contact the participants midway through the 2-week intervention to motivate implementation. Participants are not allowed to eat food other than the one they are assigned to and, if they do not feel full, they are allowed to drink water. Water has no satisfactory effect, but it is nevertheless right to recommend that plenty of water can be drunk. If the participants deviate from the provided diet, this is requested to be noted down. It is not expected to be a major problem since the diet is planned to be normocaloric.

They should start eating the start-up day and continue with the diet for 14 days. The diets will contain all recommended nutrients by age group and the correct energy level.

Strength calculation

The effect size of what will be investigated is almost never known. It is therefore necessary to guess this based on related data in literature and own data.

Meta-analysis has shown that 2 weeks long interventions on healthy people are enough to get changes in lipid plasma concentrations. This is in accordance with other studies on the condition that one has a controlled diet. This was followed by Müller et al (1998) who used 27 young women and switched fatty acids at 30 E% fat in the diet. Reference is made to Table 3. The number of participants who will eventually join the intervention is approximately based on the need for stable blood lipid values, and in addition the number of participant must be a multiple of 4. Based on own data for collected standard [8][9][10]deviations (sigma estimate), the standard deviation in the population is so high (0.8mmol LDL cholesterol /L serum) that one – if possible should remove participants with extreme LDL cholesterol values to ensure an 80% chance of ending up with a p-value below 0.05 (estimated change of 0.3 mmol LDL cholesterol /L serum and 32 participants). Crossed interventions effectively reduce the negative effect of large standard deviations in the population.

Table 3 Crossed interventions relevant to strength calculation

Variable	Exclusion and design	Diet variety	Number of persons	Significance (p-value)

[7]Shirley Gerrior, S, RD, Wen,RD., Juan, Y. et al (2006) **An Easy Approach to Calculating Estimated Energy** Requirements
Gerrior S, Juan W, Basiotis P. An easy approach to calculating estimated energy requirements. Prev Chronic Dis.; 3(4): A129.
[8] Mensink RP &Katan MB (1992) Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arteriosclerosis and Thrombosis 12, 911–919. https://www.ahajournals.org/doi/pdf/10.1161/01.atv.12.8.911
[9]Müller, H., Jordal, O, Kierulf, P. et al. (1998) *Replacement of Partially Hydrogenated Soybean Oil by Palm Oil in Margarine Without Unfavorable Effects on Serum Lipoproteins Lipids, Vol. 33, no. 9 (1998)
[10]Pile, A. et al. (2020) Nutrient optimized beef enhance blood levels of vitamin D and selenium among young women. Manuscript, NMBU

Fasting **Blood glucose (mmol/L)**	BMI, young (19-25) 5 sugar interventions x **3 weeks**, 4 weeks cross over	Addition of 40-80 grams of **simple sugars** such as in drinks, otherwise free choice of food	29	Intended for **3 weeks** dietary intervention Blood sugar increased P < 0.01[11]
Lipid markers: LDL cholesterol (mmol/L serum) HDL cholesterol and TG, TC:HDL As well as triglycerides (TG)	Disease (-), Alcohol (-), young women, Crossed intervention, **2 weeks** (17d) with 1 week washout	Test with 3 diets in which fatty acids are replaced. 30 E% in the diet, Full control of the diet	27	LCl, HDL og TG changed **Min < 0.005[11]**
	Elevated cholesterol (+), BMI, both gender, age 25-64 år **4 weeks** x 2, crossed, 2 weeks washout	3 diets tested, changed fat % (max 34E%) and **fatty acids** **Total control of diet**	31	**LDL was reduced** (< 0.05) HDL unchanged, TC:HDL and TG were unchanged.[12]
Inflammation Markers Interleukins	Disease (-) BMI Gender and age (only young) **6 day** x 2 crossed, 2 weeks washout	Only on **one test product in 2 variants**, increased protein and fat (42E%) against reduced sugar. Free intake of other foods	34	IL-6 IL-8 **IL1 β /IL6** (< 0.038) IL1 β as well as TNFα, hsCRP were not significant[12]
	Disease (-), CRP, BMI, blood pressure urine, age (young, both sexes only),**6 days** x 2, ticked, 2 weeks washout	Test on 2 **diets** where fat levels vary (max 43 E%) as well as **protein and sugar levels** Total control of diet, **normocaloric**	32	**IL-6** and **TNF-alfa** (< 0.007) **hsCRP (**P=0.043)**[16]**
	3 groups with signs of illness and one without (25 persons), up to 1 year, men 60-67 years	No control of diet, only on CRP variety	25x4	Large increase in individual variation in hsCRP in the following order 4 (months):2 (4 weeks):1 (1 week): 0.3 (days variation) [13]

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As mentioned above, sticking 100% to the diet in the interventions is very important and is also becomes more difficult with time. Another thing is to select homogeneous test groups through frameworks for inclusion and exclusion. Fasting blood sugar indicates that it is wise to do selection of participants based on a larger sample. If possible, through the invitation to participate, potential participants with extreme blood lipid values are sorted away.

Many interventions do not get selected inflammatory markers significantly and there is reason to believe that short time (few weeks), total control of energy adaptation (normocaloric), choice of crossed intervention, selection on BMI, glucose tolerance and highly standardized time of withdrawal of last blood tests relative to last meal will reduce population variation relative to the effect and, thus, make it easier to have significant effects (see Table 3). Our intervention will be set up according to this principle. Based on separate estimates of population standard

[11]Aeberli. I. (2011). Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. Am J Clin Nutr. 94:479–85.
[12]. Binkoski A.E. et al. (2005) Balance of Unsaturated Fatty Acids Is Important to a Cholesterol-Lowering Diet: Comparison of Mid-Oleic Sunflower Oil and Olive Oil on Cardiovascular Disease Risk Factors. J Am Diet Assosc. 105(7):1080-6.
[13] Peter Bogaty et al (2013) Variability of C-Reactive Protein: Implications for Clinical Risk Stratification Plos One, 8, 4, e60759Time.

deviations[1412,] we find that 32 people are just enough to get p> 0.05 with 80% probability of detection (= strength).

ANALYSIS

Weight and waist circumference, blood pressure and blood glucose are recorded before and after each 14-day intervention sequence. Height is measured once. The blood samples are taken by qualified personnel. A medical doctor is always available on short notice.

Day 1 (see Table 1) in the morning at 07-10:32 (every day there are 8) people meet fasting and we measure height, waist circumference and weight, blood pressure, blood glucose, blood test and collect urine and feces sample (belongs to the crossed intervention).

Table 4- Analyses intended for cross-intervention

Which	What is measured?	Why are these needed.?	Who's going to analyze these?
Blood glucose		Central basal analysis	Fürst medical laboratory
Blood lipids	Triglycerides, LDL-HD cholesterol, HDL cholesterol, VLDL, ApoB, Lipidomics	The project aims to find out how the most consumed animal triglycerides in the diet affect	Fürst medical laboratory OWL Laboratory, Spain
Inflammation-markers in plasma:	hsCRP, adiponectin, PAI-1, C-peptide, TNFalfa, IL6: ILbeta, IL4, IL10), NFkappaB, STAT3-5, CTNNbeta, FOXO3A.	The project aims to compare these inflammation The markers, because together with blood lipids, will give a better assessment of the importance of the different sources of fat.	Fürst medical laboratory Norwegian University of Life Sciences (NMBU)
Leucocytes	RNA-SeeKvenation (RNA-Seq) analyses: RNA-Seq is performed on healthy leukocytes DNA sequencing for epigenetics on leukocytes	Can provide information on pro- and anti-inflammatory as well as regulation of metabolism in which the immune system plays a role,	The analyses are performed at NMBU and at the sequence centre at UiO.
Feces	Fats Calcium (calcium soaps) Microbiota	To be taken to identify and assess the amount of fatty acids in faeces depending on the position in the trigflycerides	NMBU

Purpose and research question

A better understanding of the relationship between this fat and cardiovascular diseases is the overriding goal. Our data will be linked to our mouse experiments where real endpoints are

[14]Emerson, S.R. (2017). Magnitude and Timing of the Postprandial Inflammatory Response to a High-Fat Meal in Healthy Adults: A Systematic Review. American Society for Nutrition. Adv Nutr.,8:213-25; doi:10.3945/an.116.014431.

possible. The human intervention focuses on the relationships between intake of the same lipids, but relies on biomarkers (metabolites, inflammation) and gene expression.

Hypotheses:
Ingestion of various types of fat affects the level of blood lipids, inflammatory cytokines in blood, and alters the gene expression of inflammation markers.

Selection
Subjects participating in the intervention trials will be persons who do not take medication (except for oral contraceptives/IUD), do not take supplements of cod liver oil and/or vitamin/mineral supplementation during the intervention period and who have a BMI below 30.) The participants will be recruited at NMBU (via postings or via social media). Other selection criteria (e.g., LDL cholesterol and blood glucose) mentioned above can be used if there are many who want to join the intervention. The aforementioned variables have significant population variation and reduce strength in the intervention.
Recruitment is done by persons other than the project manager. It will be a person where participants that are faculty students cannot be directly become dependent of the recruiting person (e.g., teacher-student).

Variables
The variables collected via questionnaires, blood tests and anthropometric examinations are exposure variables, health variables and other variables. Exposure variables are the independent variables in the problems. The health variables will be used to define or describe a health condition. The health variables will often be the dependent variables in a problem. Other variables that are collected will be included to correct for a known association, so that one can check for known association in relation to specific issues (*e.g.*, smoking and inflammation markers).
The health variables measured in blood serum/plasma are LDL cholesterol, HDL cholesterol, total cholesterol, inflammation parameters, triglycerides, and urine and faces metabolites.

Data collection
Questionnaires (Appendix 1) shall be used for the collection of data on age, health, diet, physical activity, tobacco habits, education and work, and the use of medicines. This questionnaire is based on the questionnaires from the Health Survey in Oslo (HUBRO).

Clinical examinations: Done for the collection of anthropometric data (height, weight, waist measurements).

1. Height: Measured in cm with one decimal place. Participants must stand straight with their heads in the Frankfurt plane. The altimeter to be used is the Charder HM200P Portstad Portable Stadiometer.
2. Weight: Measured in kg with one decimal place. The scale to be used is a Tanita TBF-300A Body Composition Analyzer.
3. Waist circumference: Measured in cm with one decimal place. The measurement is made at the midpoint between the lowest rib and hip bone comb. The measuring tape to be used is a Seca 203 Ergonomic Circumference Measuring Tape.
4. Physical activity level incl. type of activity, questions selected from GPAQ[15]

[15] Global Physical Activity Questionnaire (GPAQ) Analysis Guide. Global Physical Activity Questionnaire (GPAQ) Analysis Guide

5. Participants are measured and weighed with light, thin clothing and without shoes. For measuring waist measurements, the clothes on the upper body of the participants are lifted up.

Measurements in biological material: Blood tests, urine and faeces samples.
Blood samples are drawn in 8.5 ml gel tubes with yellow stopper (2 tubes per participant).
The blood samples are processed on the recommendation of the Fürst medical laboratory or (OWL laboratory): the tubes are put in a stand and immediately turned 8-10 times. The tubes are centrifuged in swing-out centrifuge after 0.5 – 2 hours at 1300 – 1500 g for 12 minutes. The stopper remains on. Inspect the tube (if the blood clot or gel is tilted), the serum is removed to a new tube and centrifuged for 5 min at 1300 g and then re-pipetted.
Measurement of waist circumference varies more if different people make the measurements than if one person makes all the measurements (WHO, 2008). Therefore, it is decided that all anthropometric measurements are made by the same person. Another person makes all the blood pressure measurements. A bioengineer will be responsible for all blood sampling. A bioengineer will be responsible for centrifuging, pipetting, cooling, and storage of the samples.
All data collected on site is recorded on the questionnaire and later entered on your own PC.
Name and telephone number, social security number is required at Fust for the implementation of analysis. Only codes are given to OWL Laboratory.
The coupling key should only be located on a secure computer and stored in a safe at KBM. Coupling key will be deleted by the end of 2025.

Personally identifiable information
The study cannot really be blinded because it must be assumed that participants recognize what they eat by identifying characteristic fat tastes that could see it. It is known that almost all fats can be identified on their own taste.
Information about participants will be indirect and directly personally identifiable. Direct personally identifiable information is initials and telephone number or email address. This information will only be stored if the participant wants feedback if the test results fall outside normal values.
Direct personally identifiable information is deleted and shredded when those concerned have received abnormal test results. The other participants receive information about their metrics on request.
Indirect personally identifiable information is information about age, gender, place of work and education, etc. This information will be anonymized before the data analysis has been carried out. In order for these to be unknown to the researchers, a routine has been prepared for any test results (See passive consent, Appendix 3, separate appendix). Procedure for processing direct personally identifiable data)

Statistical Analysis
The statistical analysis to be carried out is uploaded in a separate file**.**

Project organization

Responsible researcher: Professor Catia Martins (NTNU, clinical nutrition)
Daily executive researcher: Professor Anna Haug (nutrition)
Project staff: Researcher Milena Monfort -Pires (nutrition), Professor Bjørg Egelandsdal (Food science), Professor Siv Skeie (Food science), several recruited bio-engineers and technicians.

Research Director: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU).

The project has a steering group of people with a nutritional background; one with a food background. The chairman of the steering group is a nutrient physiologist with extensive management experience.

Chair of the steering group: Knut Hove, Professor IHA, NMBU

Personnel, equipment, and resources

1. Blood tests, urine and feces: doctor and bioengineers, Ås

Economy

Funding is covered by the the **Lipidinflammagenes** project.

Schedule

Planning phase:

1. Write application to NSD and REK: 2020
2. Planning the practicalities, obtaining equipment, planning with Fürst, Eurofins, AS
3. Recruiting and sending questionnaires: autumn 2020

Data collection phase:

4. Anthropometric measurements, urine, faeces and blood tests: 2021
5. Sending samples to Fürst / OWL: 2021

Analysis and reporting phase:

6. Entering and coding of data: 2021/22
7. Data analysis: 2021/22
8. Reporting of data/ publication of article: 2021/2022

Publishing

Results will primarily be published as scientific articles in international journals.
Popular presentation of the results will be given in laymen journals and elsewhere at conferences, seminars and in various newspapers and magazines

Ethics

Application for ethical approval submitted to the Regional Committees for Medical and Health Research Ethics (REK) South-East
Participants must give written consent by signing the declaration of consent accompanying the invitation letter. (Appendix 2, separate to clin trials reg). In the invitation letter, participants are informed of how the study will be carried out, which tests will be taken, that participation is voluntary, that participants can withdraw from the study at any time for no reason, and that their information will then be de-identified. The study will be carried out in accordance with the Declaration of Helsinki.

There is no known risk of physical damage when participating in this study. Some samples can be physically unpleasant and cause bruising to the arm (blood tests), while others can be mentally unpleasant (measurement of height, weight, waist, urine and feces samples).

Attachments*:*

These attachments are uploaded separately

1.Invitation letter (info form)

Purpose: Inform potential participants about the intervention and receive their consent signature.

2.Statistical method description

Purpose: describe the statistical methods chosen for the designed intervention.