Research protocol.

Rec protocol number: 139404[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 researchers: Professor Anna Haug (NMBU, nutrition), Researcher Milena-Monfort-Pires (NMBU, nutrition)

This intervention is an add on to ISRCTN39863778 available at: https://doi.org/10.1186/ISRCTN39863778
The postprandial intervention explores dairy and pork fat in animal products based on the results obtained from the clinical trial described in ISRCTN39863778.

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. These two product groups contribute significantly to the total intake of saturated fats (SFA) in the diet; 42% and 20%, respectively. I Norway 50 % of the protein intake is from dairy and meat sources.[2]
Understanding the mechanisms behind appetite regulation/dysregulation is essential for reducing metabolic diseases like type 2 diabetes, cancer, and CVD. [3,4]

The consumption of animal products has been regarded as deleterious for metabolic outcomes, and a broad approach has hitherto been used to discriminate various animal products (e.g. dairy products, red meat etc.). Our knowledge regarding healthiness, especially for specific meat products needs to be improved. Our knowledge about satiety and satiation mechanisms is still limited regarding most food products, and when the food products are at the same macronutrient level. For animal foods this would commonly refer to the same fat and protein level. The general

[1] Regional ID number Regional Committees for Medical and Health Research Ethics (REK) South-East.
[2] Kilde til protein (Source of protein) Available at: https://www.helsedirektoratet.no/rapporter/utviklingen-i-norsk-kosthold-2023/kostens-innhold-av-energi-og-naeringsstoffer/fett-protein-og-karbohydrat [Accessed 10.17.2024].
[3] Valenzuela, P. L., Carrera-Bastos, P., Castillo-García, A., Lieberman, D. E., Santos-Lozano, A. & Lucia, A. 2023. Obesity and the risk of cardiometabolic diseases. *Nature Reviews Cardiology*, 20**,** 475-494.
[4] WORLD OBESITY FEDERATION. 2023. *World Obesity Atlas 2023* [Online]. https://data.worldobesity.org/publications/?cat=19. [Accessed 10 Oct 2024].

view is that lipids affect human satiety at a lower degree when compared to protein (Maher and Clegg, 2019, Marmonier, Chapelot and Louis-Sylvestre, 2000).
Aside from macronutrients, there is evidence that micronutrients, such as calcium content, could affect satiety and postprandial metabolic response but this needs further studies. Few studies analyze the effects of animal products with distinct amino acid and fatty acid composition in subjective satiety ratings and postprandial metabolism and results are controversial.

The intervention aims to investigate if differences in satiety hormones, lipids, amino acids and calcium and subjective measurements of hunger and fullness (VAS scale) could be identified when comparing two breakfast meals, one with gouda-type cheese and another with pork sausages as test products, keeping the macronutrient contents similar. Pork fat is quite different for dairy fat; all major fat categories like SFA, MUFA and PUFA are different. Pork muscle and dairy proteins are both so called complete proteins with all essential amino acids present in consistent but not identical amounts. How satiation is impacted by fat in a high fat product with significant amounts of protein is not clear.
Dairy fat is in addition speculated to be more satiating than pork fat due to a more significant part of SFA being short (< C12: 0) fatty acids. Triglycerides with fatty acids that have 6 to 10 carbon atoms are suggested as more satiating than longer fatty acids because these are taken straight from the liver via the portal vein which can provide a more immediate experience of satiety. Coconut fat, however, has been compared with cattle fat without finding differences according to these hypotheses.[5]

Description of the intervention

General

Participants (men and women) $\geq$ 24 are recruited. The postprandial will be crossed for the diets. In the morning, everyone will come fasting for fasting blood sampling followed by their first test-product. After 4 weeks, each participant will return, and they will be served the second test-product (Table 1). During the wash-out period, participants eat their normal diet. A difference of 4 week between participations was chosen to adapt sampling to the periods of women.
The set up was a Latin design with 2 treatments A and B (Table 2).

In total, the intervention study (including wash out periods) will lasts 6 weeks.

Table 1- Timeline of the intervention (6 weeks)

Week	1,2	3	4	5,6
Test	TEST			TEST

[5] Poppitt, S. D., Strik, C. M., Macgibbon, A. K., Mcardle, B. H., Budgett, S. C. & Mcgill, A. T. 2010. Fatty acid chain length, postprandial satiety and food intake in lean men. *Physiol Behav,* 101**,** 161-7.

Table 2- The principle of intervention design with respect to the distribution of 24 subjects

	24 people: crossed postprandial intervention; Diet A and B.						
	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday*	Saturday
Week 1	-	-	1A	2B	2A	1B	-
	-	-	2B	1A	1B	2A	-
Week 2	-	-	1B	2A	2B	1A	-
	-	-	2A	1B	1A	2B	-
SUM			**6**	**6**	**6**	**6**	
Week 3-4	-	-	-	-	-		-
Week 5	-	-	2B	1A	1B	2A	-
	-	-	1A	2B	2A	1B	-
Week 6	-	-	2A	1B	1A	2B	-
	-	-	1B	2A	2B	1A	-
SUM			**6**	**6**	**6**	**6**	-

*) controlled start-up diet, but in randomized order, i.e. Half of the participants get diet1 on a specific date

A crossed randomized postprandial intervention study with two different diets

During 4 days of week 1 (Tuesday, Wednesday, Thursday, Friday), 3 people will come each day to the experimental laboratory to consume either diet A or B (see Table 2), so that 12 subjects start the intervention during the same week; and the remaining 12, the following week. They arrive fasting and they are weighed, blood pressure and heart rate are taken, and blood is sampled. The first time their heights are measured, A cannula is then inserted by an experienced nurse (medical doctor or paramedic).
Four weeks later the participants will again enter fasting in the morning for new sampling and testing of their second diet.

Participants start eating their test meals after the initial blood samples are taken, in a nearby room that is equipped for preparing and serving food and drinks (Taverna type). The test-meal (breakfast) and buffet are served in booths.

Meals are obtained from commercial manufacturers or produced at authorized producers. The test meals/ ingredients will be described regarding macronutrients and detailed regarding amino acid and fatty acid composition.

The two test breakfasts will each provide 28 E% based on totally 2000 kcal/ day.

Table 3 – Energy % divided into micronutrients

	Fat	Protein	Carbohydrates incl sugar and fiber
Energy %	47	20	33

Postprandial crossed randomized study two different diets

It is important to carry out postprandial studies because these will be able to clarify relevant variations related to various protein and fatty raw materials/products in the diet and their absorption mechanisms. This type of intervention is a 4-hour test.
Satiety/hunger will be measured by two different methods; satiety and hunger hormones as well as a subjective Visual Analogue Scale (VAS) and an *Ad.libitum* buffet test.

The main objectives of the experiment are:

- map different measures **of satiety** as related to 2 differently composed diets described by their micronutrients and amino acids and fatty acid contents
- satiety assessment by blood markers
- subjective ratings of hunger, fullness, desire to eat and prospective food consumption using a scale with anchor statements (VAS scale)
- quantitative measurement of amount ate from an *Ad Libitum* buffet

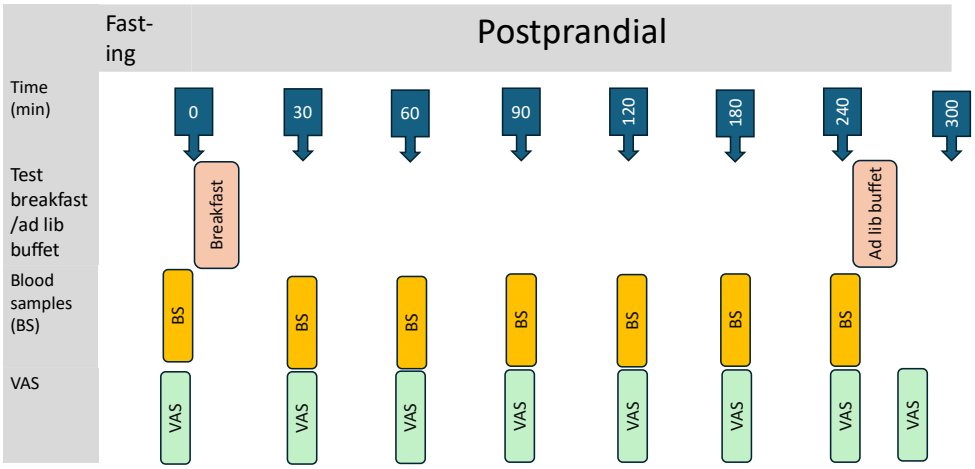

Figure 1- A graphical description of the analysis.

More detailed description of the intervention study

Test food description: Total fat and protein, amino acid and fatty acids. Commercial analytical laboratories will be used (Labtek, NMBU, Ås and VITAS, analytical services, Oslo). Micronutrients that are accepted as widely different between the test products will be acquired from the Food Composition database (https://www.matvaretabellen.no/en/)

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) generally or during the trial period. Due to the nature of the intervention (short time), 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 dairy or pork products are excluded.

We will recruit a small surplus (like 2) of people as drop out is not regarded as prevalent. We will not 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[6].

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.

Blood pressure, heart rate, height and weight will also be measured.

Upon inclusion, the blood will be analyzed for:

Appetite hormones: CCK, Ghrelin, GIP, GLP-1 and leptin

Blood lipid markers: LDL, HDL Total cholesterol, Triglycerides

Other blood markers: ferritin, glucose, insulin

Finally, the visual analogue scale (VAS) will be used to determine:

VAS-Desire to eat

VAS-Fulness

VAS-Hunger

VAS- Prospective food consumption

Ad Libitum buffet: gram and kcal consumed

Intervention study (crossover design)

On the first day of testing (for example, Tuesdays), 3 subjects showed up fasting for blood sampling and anthropometric measurements. They are randomized to diet 1-2, that are individually adapted to their energy needs (to the nearest 100kcal). Resting metabolic activity will be based on height, weight and age and then adjusted with a physical activity factor (self-reported). 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]

The breakfast meals provided 28% of the total daily energy requirements, calculated using the Norwegian Government online tool Kostholdsplanleggeren to the nearest 100 (e.g., 1500 kcal, 1600 kcal;). [8]

[6] Dutch Eating Behavior Questionnaire (DEBQ). Available at : https://link.springer.com/referenceworkentry/10.1007%2F978-981-287-087-2_127-1 Downloaded 20.05.2020

[7] Gerrior S, Juan W, Basiotis P. An easy approach to calculating estimated energy requirements. *Prev Chronic Dis.;* 3(4): A129.

[8] Kostholdsplanleggeren - Helsefremmende arbeid - NDLA Available at: https://ndla.no/en. Accessible : 20.10.2024

Table 3- Ingredients in the breakfast meals tested (28% of resting metabolic rate, 46.5% fat 32% carbohydrates, 21.5% protein, 60% Energy from test product)

Breakfast 1 (A)	Breakfast 2 (B)
Orange juice "Healthy" bread slices Pork based sausage product Tomatoes	Orange juice "Healthy" bread slices Semi-hard full fat gouda type cheese butter Tomatoes

The two **breakfast meals** will contain the two test products: either semi-hard full fat gouda type cheese and a pork lean meat or fat-based breakfast sausage.

Postprandial - First day example:

On the first day (for example, next Tuesday), the subjects enter fasting in the morning between 08-10. They are given an artery cannula for multiple sampling and they are allowed to eat a **test diet** (described below**)**. Blood samples are taken at time -10, (0=period for starting the test diet intake), 30, 60, 90, 120, 180 and 240 minutes (7 withdrawals/person*2 = 14 samples). During this time, participants record their subjective experience of satiety. The cannula needle is removed after 4 hours, and the participant get access to an *Ad-Libitum* **buffet** that provides a measure of satiety/hunger. The principle of the analysis will be a so-called 'preload- ad-libitum' test. Short-term saturation is recorded in the preload (test diet) section and long-term saturation is recorded in the "buffet" meal. [9]

Subjective Visual Analogue Scale

The participants fill in the Visual analogue scale (VAS) [10] upon arrival, 15 min, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min and after the *Ad-libitum* buffet meal.

«*Ad Libitum buffet»-test*

This test for satiation is taken 4 hours after the test-meal was served. The cannula needles are then removed. The lunch is developed like this: the participants get to rank 5 offers in advance according to how much they like these.

[9] Frode, C.G: (2017) Measuring Satiation and Satiety. In book: Methods in Consumer Research, Volume 2: Alternative Approaches and Special Applications. 1st Edition. Woodhead Publishing. Editors: Gaston Ares and Paula Varela

[10] Hill AJ, Rogers PJ, Blundell JE. (1995) Techniques for the experimental measurement of human eating behaviors and food intake: a practical guide. *Int J Obes Relat Metab Disord*. 19(6):361–75

This can be sandwiches with cooked ham, cheese 1, mackerel in tomato sauce, cheese 2, liver pate. The test is then done on the 2nd and 3rd choice. The slices of bread are cut into 2 equal pieces and

each person is offered half pieces.

After the participants have eaten their fill, the remaining food will be weighed.

The lunch is eaten in the taverna (see above). There are booths so that the participants do not see each other.

'Preload- ad-libitum' tests can easily be expanded to many more participants without major costs, but to include many tests of blood values are costly.

Strength calculation

The effect size of what will be investigated is almost never known. It is therefore necessary to estimate this based on related data in literature and own data. (See **Table 4**). Crossed interventions reduce the negative effect of large standard deviations in the population.
The number of participants who will eventually join the intervention is primarily based on the need for power regarding the blood measurements and VAS scale, as the *Ad Libitum* test is recognized as being much more demanding regarding power than the VAS (**Table 4).**

Table 4- Crossed postprandial intervention relevant to strength calculation.

Variable	Participants	Diet variation	Partici-pants	Diet difference Significance (p-value)
Ghrelin* (total)	Men only Age: 20.5±2.5 (sd) y BMI: 21.6±1.9 kg/m^2	Protein (19.3 -58.1 E%) & carb. (14.1-47.3 E%)	15	P<0.0001 [11]
	Men: (8) & women (6) Age: 22.6±0.6 y BMI: 22.6±0.6 kg/m^2	Protein (15-83 E%); fat (17-86 E%)	14	P<0.05 [12]
GLP-1	Men (5) & women (11), overweight Age: 45.6 ±6.2 y BMI: 29.8±2.9 kg/m^2	Fat (3.2- 50.3%) & carb. (38-83.6%)	16	P<0.05 [13]

[11] Blom, W. AM., Lluch, A., Stafleu, A., Vinoy, S., Holst, J.J.,Schaafsma, S.J. & Hendriks, H.FJ. (2006). Effect of a high-protein breakfast on the postprandial ghrelin response, *The American Journal of Clinical Nutrition*, 83(2), https://doi.org/10.1093/ajcn/83.2.211.

[12] Erdmann, J., Töpsch, R., Lippl, F., Gussmann, P., Schusdziarra, V. (2004) Postprandial Response of Plasma Ghrelin Levels to Various Test Meals in Relation to Food Intake, Plasma Insulin, and Glucose, *The Journal of Clinical Endocrinology & Metabolism.* 89 (6), 3048–3054,

[13] Gibbons, G., Caudwell, P., Finlayson, G., Webb, D.-L., Hellström, P.M., Näslund, E., Blundell, J.E. (2013) Comparison of Postprandial Profiles of Ghrelin, Active GLP-1, and Total PYY to Meals Varying in Fat and Carbohydrate and Their Association With Hunger and the Phases of Satiety, *The Journal of Clinical Endocrinology & Metabolism.* 98 (5), E847–E855. https://doi.org/10.1210/jc.2012-3835

GIP	Men (8) & women (6), diabetic T2 Age: 26-69 y BMI: 26.2±3.1 (sd) kg/m^2	Protein (29.8) & starch (50) types (in gram)	14	P = 0.072 [14]
	Male only Age: 40 ±9 y BMI: 25.2±2 kg/m^2 Obese subjects	Protein (14.2-20.5%) Fat (22.6-34.4%)	21	P< 0.05 [15]
TG	Men (11) & women (10) Age: 21-54 y Non obese subjects	Different fats/ oils	21	P<0.05 [16]
	Men only Age: mean about 33 BMI: mean between 22- 26 kg/m^2	Different TG types per BMI group	25	P <0.001 [17]
VAS scale	Men (12) & women (12) Age: 25±7 y BMI: 22.4±2.4 kg/m^2	Protein (20-10 E%) &fat (20-35 E%)	24	Hunger, desire to eat P <0.05 [18]
	Men only Age: 26±4 (sd) BMI: 23.9±2.1 (sd) kg/m^2	Carb./fat/protein (56-41/11-38/32-18 %) Lunch meal	13	Hunger, desire to eat P <0,05 [19]
			22 or 16	Adequate for VAS see [16-17]
Ad libitum buffet	Men only Age: 23±1.8 (sd) BMI: 22.2±1.5 kg/m^2 (sd)	Carb/protein/fat (36-53/12.6-0/1.8-0 in gram) Drinks	22	P= 0·42 [20]
	Women only Age: 18-40 y BMI: 30-39.9 kg/m^2	Fat categories at 70 % total fat	16	Not significant [21]

*sd means standard deviation

[14] Frid, A.H., Nilsson, M., Holst, J.J. and Björck, I.ME.(2005) Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects2, *The American Journal of Clinical Nutrition*, 82 (1), 69-75, https://doi.org/10.1093/ajcn/82.1.69.

[15] Sakane, N., Osaki, N., Takase, H. *et al*. (2019) The study of metabolic improvement by nutritional intervention controlling endogenous GIP (Mini Egg study): a randomized, cross-over study. *Nutr J*.,18 (52). https://doi.org/10.1186/s12937-019-0472-0

[16] Harris, W.S., William E. Connor, W.E., Alam,N. and Illingwoath R. (1988). Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids, *Journal of Lipid Research*. 29 (1451-1460). https://doi.org/10.1016/S0022-2275(20)38424-8

[17] Kasai, M., Maki,H., Nosaka,N., Aoyama, T., Ooyama, K., Uto, H., Okazaki, M., Igarashi, O., Kondo, K., (2003) Effect of Medium-chain Triglycerides on the Postprandial Triglyceride Concentration in Healthy Men, *Bioscience, Biotechnology, and Biochemistry*, 67(1), 46–53. https://doi.org/10.1271/bbb.67.46

[18] Hochstenbach-Waelen, A., Westerterp-Plantenga, M.S., Margriet Veldhorst, M.A.B., Nieuwenhuizen, A.G., Westerterp, K.R. (2009) Comparison of 2 diets with either 25 or 10 energy % gelatin on energy expenditure, substrate balances and appetite profile, *e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism*. 4 (6), e329-e336. https://doi.org/10.1016/j.eclnm.2009.10.001.

[19] Poortvliet PC, Bérubé-Parent S, Drapeau V, Lamarche B, Blundell JE, Tremblay A. (2007) Effects of a healthy meal course on spontaneous energy intake, satiety and palatability. *British Journal of Nutrition*. 97(3),584-590. doi:10.1017/S000711450738135X

[20] Harper A, James A, Flint A, Astrup A. (2007) Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent ad libitum lunch intake. *British Journal of Nutrition*.97(3), 579-583. doi:10.1017/S0007114507339846

[21] Stevenson, J.L., Clevenger, H.C. and Cooper, J.A. (2015), Hunger and satiety responses to high-fat meals of varying fatty acid composition in women with obesity. Obesity, 23, 1980-1986. https://doi.org/10.1002/oby.21202

It is important 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.

The participants will be asked to eat the same meal before both their diet interventions and no later than 8pm the day before the intervention, selection on BMI, glucose tolerance and highly standardized time of withdrawal of blood tests will reduce population variation relative to the effect and, thus, make it easier to have significant effects. Our intervention will be set up according to this principle. Based on existing knowledge we find that 24 people are just enough to get P> 0.05 with 80% probability of detection (= strength).

Analysis

Weight and waist circumference, blood pressure and fasting blood samples are recorded before each post-prandial intervention. Height is measured once. The blood samples are taken by qualified personnel. A medical doctor is always present.

The participants appear the morning around 8.30 (every day there are 3 participants). More details are present in Figure 1.

Table 4- Analyses intended for postprandial cross-intervention

Which	What is measured?	Why are these needed.?	Who's going to analyze these?
Blood glucose insulin		Central basal Analysis	Fürst medical laboratory
Blood lipids	Triglycerides, LDL-HD cholesterol, HDL cholesterol,	The project aims to find out how the most consumed animal triglycerides in the diet affect lipid blood markers	Fürst medical laboratory
Satiety markers	Ghrelin, GLP-1, GIP, CKK	These markers are highly relevant to understand how selected animal foods affect satiety	NTNU medical faculty, Trondheim Rigshospitalet, Copenhagen
VAS *Ad libitum* buffet	Hunger, fullness Amount of food intake (gram, kcal) chosen to eat	These variables are highly important to understand the blood markers	NMBU, Ås

Measurements

Biological material: Blood samples are drawn in 8.5 ml gel tubes with yellow stopper (2 tubes per participant). The blood samples are processed according to the recommendation of the Fürst medical laboratory, NTNU, Trondheim and Rigshospitalet, University of Copenhagen; the latter for analysis of satiety markers. The tubes are put in a stand and immediately turned 8-10 times. Tubes are centrifuged in swing-out centrifuge, time pending on plasma or serum collection after 0.5 – 4 hours for 12 minutes at recommended g and temperate and then transferred to labelled cryotubes and frozen at -80°C for later analysis. For analysis of satiety markers inhibitors are added before centrifugation (e.g. aprotinin).

All anthropometric measurements, and blood pressure measurements are made by the same person. 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 a PC.
Name and telephone number, social security number is required at Fust for the implementation of analysis. Only codes are given to other analytical laboratories
The coupling key should only be located on a secure computer and stored in a safe at KBM. Data handling including security) from the project is decribed in an extensive document that according to a required Data Management Plan that is approved by the administrative leader of KBM/NMBU and funding source. The Data Management Plan contains security, storage and ethics elements.

Data collection

Questionnaires 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 was assesses by using the International Physical activity Questionnaire (IPAQ).[22]
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.

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. 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, triglycerides, satiety hormones (ghrelin, GLP-1, GIP and CKK).

[22] Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F., & Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine and science in sports and exercise*, *35*(8), 1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FBe

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. Only candidates that are regular consumers of the products to be tested will be included,
Recruitment is done by persons other than the project manager. Flyers that describe the project will be used. It will be a person where participants that are faculty students cannot be directly become dependent of the recruiting person (e.g., teacher-student).

Purpose and research question

Based on the results of an intervention described in ISRCTN3986377; animal fats and foods that are different in terms healthiness (cardiovascular disease focus), are further studied in a postprandial study where the major goal is to differentiate animal products in terms of satiety.

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.
Animal fats/ products can impact different on satiety.

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. 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 (**Attachment 1**). Procedure for processing direct personally identifiable data)

Statistical Analysis

The statistical analysis to be carried out is uploaded in a separate file **(Attachment 2)**

Publishing

Results will primarily be published in one scientific article in an international journal. Popular presentation of the results will be laymen publications.

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 1**). 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. Cannula 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).

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), several recruited paramedics, nurses, bio-engineers, technicians and one medical doctor.

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: doctor and bioengineers, Ås

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

Schedule
Planning phase:
1. Planning the practicalities, obtaining equipment, planning with Fürst, NTNU and Rigshospitalet
2. Recruiting and sending questionnaires: spring 2021

Data collection phase:

3. Anthropometric measurements, and blood tests: fall 2021
4. Sending samples to Fürst /NTNU and Rigshospitalet: 2021/2022

Analysis and reporting phase:

5. Entering and coding of data: 2021/22
6. Data analysis: 2022-2024
7. Reporting of data/ publication of article: 2024/2025

Attachments*:*

These attachments are as follows

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.