Changes in Intestinal Permeability and Systemic Inflammation Following Fast Food Overfeeding (ChIPS)

STUDY PROTOCOL

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Introduction

Obesity is a major global health challenge, driving chronic low-grade inflammation that contributes to conditions such as type 2 diabetes, cardiovascular disease, liver disease, and neurodegenerative disorders. Increasing evidence suggests that impaired gut barrier function allows bacteria and bacterial products to enter the circulation, triggering systemic inflammation. Diets high in fat and ultra-processed foods, combined with a sedentary lifestyle, appear to weaken gut barrier integrity, potentially worsening obesity-related complications.

Molecular analyses will explore how diet, microbes, and host immunity interact to influence gut barrier integrity and inflammatory responses. Preliminary findings from our group indicate that a short-term hypercaloric diet alters gut barrier markers in healthy individuals, and that these changes are reversible upon return to a normocaloric diet. This study will explore the impact of a hypercaloric fast food diet on gut barrier permeability, and our overall goal is to clarify how gut barrier dysfunction may drive obesity-associated systemic inflammation.

Aims

1) Characterize the acute effects of a hypercaloric fast food diet combined with sedentary behavior on gut barrier integrity and systemic and tissue-specific inflammation in healthy young adults.
2) Assess the reversibility of diet-induced changes in gut permeability and inflammatory markers following a recovery period, thereby identifying early-stage intervention opportunities.
3) Investigate whether a short-term hypercaloric fast food diet alters gut microbiota function and proinflammatory potential and whether these changes are associated with impaired gut barrier integrity and systemic inflammation.

Background

Obesity is a rapidly escalating global health concern and among the leading contributors to mortality worldwide *(1, 2)*. It drives a spectrum of cardiometabolic conditions, including heart disease, type 2 diabetes, high blood pressure, and metabolic dysfunction-associated steatotic liver disease (MASLD)*(3, 4)*. A hallmark of obesity is chronic systemic low-grade inflammation, which plays a pivotal role in the pathophysiology of these conditions*(5–7)*. Understanding the mechanisms linking obesity and systemic inflammation is therefore critical for developing new therapeutic strategies.

The gut microbiome has emerged as a key modulator of obesity*(8)*. Diets high in fat, low in fiber, and enriched with ultra-processed foods and additives alter microbial composition and function, thereby contributing to obesity-related pathophysiology*(9–11)*. The gut barrier provides a first line of defense against luminal microbes and harmful particles. Its mucus layer shields the epithelium, which is composed of enterocytes and specialized cells. Beyond this, the intestinal immune system must balance tolerance toward symbiotic microbes with protection against pathogens *(12)*. Impairment of the mucus and epithelial layers increases gut permeability, triggering immune activation and potential bacterial translocation into the systemic circulation*(13, 14)*. These bacterial products reach the liver, a key organ known to develop fat accumulation and inflammation in obesity. Importantly, gut microbiota-derived metabolites are thought to contribute to MASLD progression *(15–18)*. This points to a close relationship between the microbiome, gut barrier dysfunction, and systemic and hepatic inflammation in obesity-associated diseases*(19–21)*.

Ultra-processed diets and sedentary behavior worsen these obesity-related conditions*(22)*. However, the mechanisms behind this remain largely unknown. Previous studies from our research group and others demonstrate acute and reversible changes in gut barrier integrity and inflammatory markers during short-term dietary interventions. For instance, hypercaloric diets rapidly induce metabolic

impairments and increase intestinal permeability markers such as zonulin, while weight loss interventions, both lifestyle-based and surgical, show a marked improvement in gut barrier integrity and inflammation (preliminary findings). These findings suggest a dynamic and responsive interplay between diet, microbiome, and host physiology. However, important gaps remain in our understanding of how short-term fast-food overfeeding interacts with microbiota composition and function.

Study design

This is a single group follow-up intervention study with measurements at the beginning of the intervention, at the end of the intervention and again after 20 weeks of recovery.

Outcomes

All primary and secondary outcomes are expected to exhibit skewness due to the underlying distribution of clinical measurements.

No.	**Primary outcome**	**Evaluation method**	**Measurement (before, after and follow-up)**
1	Intestinal permeability	Sugar probe test	Concentration, bsAUC, ratio
	Secondary outcomes		
2	Serum zonulin	Blood samples	Concentration
3	Liver steatosis	MRS	Tissue water and fat content
4	Plasma IgA-antiLPS	Blood samples	Concentration
5	Plasma Hs-CRP	Blood samples	Concentration
	Exploratory outcomes		

6	Local gut inflammation	Gut tissue biopsy	Histology, immunohistochemistry
7	Bacterial mucosa-perturbation	Gut tissue biopsy	Immunohistochemistry
8	Adipose tissue adaptation	Abdominal fat tissue biopsy	Histology, immunohistochemistry
9	Organ function markers	Blood samples	Concentration
10	Systemic and tissue specific inflammatory markers	Blood samples	Concentration
11	Gut integrity markers	Blood samples	Concentration
12	Leukocytes and immunological microbial translocation markers	Blood samples	Cell count/mL, concentration
13	Neuronal damage markers	Blood samples	Concentration
14	Markers of glucose metabolism	Blood samples	Concentration
15	Markers of lipid metabolism	Blood samples	Concentration
16	Markers of amino acid metabolism	Blood samples	Concentration
17	Metabolic hormones	Blood samples	Concentration
18	Reproductive hormones and related markers	Blood samples	Concentration
19	Human and bacterial metabolites	Blood samples	Concentration, proteomics, metabolomics
20	Steroid hormones and related markers	Urine samples	Concentration

21	Large intestine microbiome	Fecal sample	Bacterial DNA sequencing, reporter cell lines, metabolomics, transplantation to germ-free mice
22	Small intestine microbiome	Smart capsule sample	Bacterial DNA sequencing, metatranscriptomics, metaproteomics, metabolomics
23	Sperm markers	Sperm sample	Additional measurement after five weeks. Volume of semen sample, sperm concentration, motility, morphology and sperm DNA fragmentation (DFI)
24	Tissue fat infiltration/distribution	MRS	Tissue water and fat content
25	Liver stiffness	MRE	Tissue stiffness
26	Liver steatosis and/or fibrosis	Ultrasound	FibroScan
27	Oral microbiome	Saliva sample	Bacterial DNA sequencing, metatranscriptomics, metaproteomics, metabolomics

Table 1. Overview of study outcomes. bsAUC: baseline-subtracted area under the curve, IgA-antiLPS: Anti-lipopolysaccharide immunoglobulin A, Hs-CRP: High sensitivity C-reactive protein, MRS: Magnetic resonance spectroscopy, MRE: Magnetic resonance Elastography, DFI: DNA fragmentation index.

Participants

20 healthy male individuals will be included according to the following criteria:

Inclusion criteria:

- Normal fasting plasma glucose and glycated hemoglobin (HbA1c) <42 mmol/mol
- Body mass index (BMI) between 18.5 and 25 kg/m2
- Hemoglobin >8.3 mmol/l
- Participants must consume a ordinary mixed diet and not follow restrictive or specialized dietary regimens
- Age between 20 and 40 years
- Oral and written informed consent

Exclusion criteria:

- Diabetes
- Known gastrointestinal disease, major or recent abdominal surgery
- First degree relatives with inflammatory bowel disease
- History of BMI > 30.
- Current or history of psychiatric eating disorder
- Regular use of nicotine products in any form
- Regular alcohol intake above 18 units per week
- Fasting plasma triacylglycerol indicating dyslipidemia (≥2 mmol/l)
- Nephropathy (estimated glomerular filtration rate (eGFR) <60 ml/min and/or albuminuria with an albumin to creatinine ratio of >30 µg/mg)
- Known liver disease and/or alanine aminotransferase (ALT) >2 × upper reference limits

- Signs of liver fibrosis and/or steatosis evaluated by FibroScan (CAP value >238 dB/m and/or kPa >5.0) and/or FIB-4 score (>1.45)
- Regular use of prescription medication or treatment with systemic antibiotics less than three weeks before the screening visit.
- Use of over-the-counter medication or any dietary supplements known to alter gastrointestinal function, including probiotics, for the period of the study and three weeks prior to screening
- More than two weekly strength and/or aerobic training sessions on a regular basis
- Physical or mental conditions incompatible with magnetic resonance scanning modalities, including implanted metal objects.
- Not willing to agree to use effective contraception during and for 90 days after end of the diet intervention. Acceptable contraception includes condom use and/or ensuring that the female partner(s) uses highly effective contraception.
- Any condition that the investigator feels would interfere with study completion

Methods

The participants will be invited to an information meeting before an eventual screening visit. After the initial screening visit the volunteers will undergo a two-week diet intervention designed to induce changes in gut barrier integrity, microbiota dysbiosis and hepatic steatosis. There will be three study blocks: one before the intervention period (study block 1), another immediately after (study block 2), and a follow-up 20 weeks after the intervention period (study block 3). All study blocks contain the same samples and procedures, except for Study Block 3, which will include two sperm samples, but neither the smart capsule nor the colonoscopy (see fig. 1).

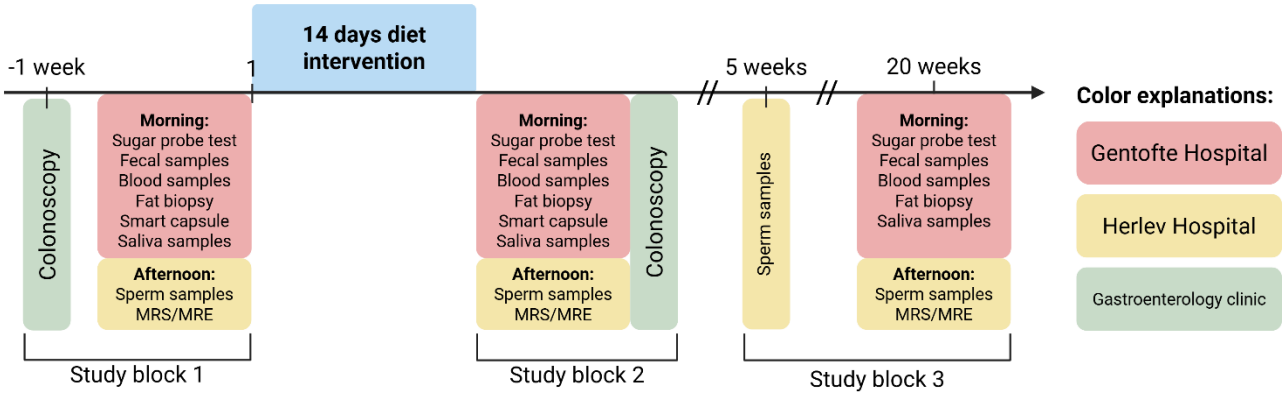

Figure 1: Timeline for study. MRS/MRE: Magnetic resonance spectrometry/elastography.

Information meeting

Prior to any protocol-related procedures, an information meeting will be arranged at Center for Clinical Metabolic Research, Gentofte Hospital. Subjects will be informed that the screening visit may be conducted on the same day, provided they choose to fast on the morning prior to the information meeting. During the information meeting, a member of the research group will explain the purpose, procedures and potential risks of the study, and it will be possible for subjects to ask questions (for details, see *Informed consent*). If informed consent is obtained the screening visit can take place.

Screening

Before the study days, the participants will come for a screening visit after an overnight fast including coffee. They will be instructed to avoid alcohol for at least 24 hour. The participant's anthropometrics, bioimpedance, medication and medical history will be recorded, and a routine physical examination will be performed. Standard blood samples will be taken for measurements of organ markers, hematology, plasma metabolites, HbA1c and plasma lipid status, and a urine sample will be used to assess kidney status using standard analysis. The total blood loss at the screening visit will amount to approximately 20 ml. FibroScan will be used to screen for steatosis and/or fibrosis prior to inclusion.

An MR safety checklist will also be completed (see enclosed *MR kontrolskema*). Subjects will fill out a short questionnaire concerning their diet habits and consumption of fast foods (see enclosed *Spørgsmål om madvaner*). If the subjects can be included in the project based on the screening results, the intervention period and dates for the following study days will be planned.

Study days

Participants will prepare for the study days by an overnight fast of 10-14 hours. Participants must abstain from alcohol and products containing artificial sweeteners (including beverages and chewing gum) for at least 24 hours prior to the visit. The study days consist of a morning session at Gentofte Hospital and an afternoon session at Herlev Hospital. Due to limited capacity for colonoscopies and MRS/MRE scans, the two sessions may be completed on the same or on adjacent days.

<u>Morning</u>

- Arrival: Participants will arrive in the morning at the Center for Clinical Metabolic Research, Gentofte Hospital
- Sample handling: Labeling and freezing of the fecal and smart capsule sample brought by the participant (see *methods – fecal samples* and *smart capsule*)
- Baseline urine collection
- Sugar probe test: Administration of the premeasured sugar solution (see *methods – Sugar probe test*)
- Anthropometrics, bioimpedance, blood and oral microbiome sampling, abdominal fat biopsy, questionnaires, and FibroScan (see *methods – FibroScan*)

<u>Afternoon</u>

- Arrival: Participants will arrive after lunch at The Division of Translational Endocrinology at Herlev Hospital
- Sperm samples: Samples are collected, analyzed and frozen (see *methods – Sperm samples*)

- MRS/MRE: Department of Radiology, Herlev Hospital

All urine is collected at predefined time frames (self-sampling) for 26 hours after ingestion of sugar solutions. Participants are instructed not to eat breakfast the following day until after the final urine sample has been collected.

Administered pre-prepared meals will be eaten by the participants for lunch and dinner during the study days.

Diet intervention

The diet intervention will last for two weeks. During these two weeks, all meals, including snacks, will be supplied and covered by the study. Meals will consist mainly of ultra processed fast food from major franchise restaurants, convenience stores and bakeries, but may be supplemented with snacks, pre-prepared meals or meals prepared by the participants. Food and drinks supplied in the diet intervention period will amount to approximately 5000kcal per day and must be consumed by participants in order to complete the study. Three meals per day are ordered through food delivery services and delivery is an option for all meals. Participants will be allowed to drink water, tea and coffee freely, unless explicitly asked not to in relation to study days. Alcohol intake in the intervention period should reflect the participant's normal consumption. Alcohol consumption in the diet intervention period must however not exceed eighteen units per week to minimize risk of alcohol-induced steatosis *(23)*, and only up to five units per occasion. To ensure the intended adaptations in metabolism, gut barrier function and microbiota, participants will be instructed to walk no more than 5000 steps per day and to refrain from any physical exercise.

Participants will be encouraged to report if they choose to consume additional food or drinks in the intervention period. Receipts from purchased meals are filed to document adherence to the diet.

Inability to adhere to diet regimen or activity restrictions will be documented and evaluated be investigators and may lead to termination of study participation or exclusion from analyses.
In the recovery period between the diet intervention and the follow-up study day, participants are instructed to return to their normal lifestyle and to avoid any restrictive or specialized diet or lifestyle regimens.

Blood samples

Blood samples will be collected at the screening visit and on all study days before and after lunch. Blood samples will be collected in appropriate tubes depending on the analyses to be performed. Samples intended for peptide and hormone analysis will be drawn into chilled tubes with anticoagulant. After collection, all samples will undergo centrifugation, and the resulting plasma or serum will be stored at -20 or -80°C until further analysis. Whole blood will be collected for the analysis of circulating bacterial DNA, with particular attention to maintaining sterility throughout the procedure. The intravenous access will be introduced in near-sterile conditions: the skin will be disinfected twice, and a sterile swap will be used to collect the skin microbiota at the place of puncture. Sterile drapes and gloves will be used throughout. All samples are snap frozen and stored in labelled aliquot tubes at -80°C until analysis. In addition to laboratory analyses performed immediately in relation to a study visit, blood samples will be drawn for biobank storage and later analysis. The total amount of blood drawn will be maximally 250 mL (all visits combined). The following markers will be analyzed:

- Inflammatory markers
- Gut barrier function integrity markers
- Leukocytes and immunological microbial translocation markers
- Neuronal damage markers

- Lipid markers
- Islet markers and incretin markers
- Metabolic hormones
- Human and bacterial metabolites, including proteomics and lipidomics.

Samples with significant variation due to inadequate sampling, technical problems, or deviations from instructions specified in the protocol, will be assessed by the investigators and may be excluded from analyses.

Saliva samples for collection of oral microbiome

After a minimum of two hours without eating or drinking, participants will chew approximately one gram of GUM-BASE, a non-flavoured chewing gum, to loosen bacteria from dental surfaces and gums, in order to standardize oral microbiome sampling. After one minute of chewing, the chewing gum is disposed of, and the partipants will collect up to 5 mL of saliva in a clean cup. The sample is then prepared for bacterial RNA-sequencing and analysis of microbiota composition and function. All samples are stored at -80°C.

Samples will be assessed individually by the investigators and samples with significant variation due to inadequate sampling/storage, technical problems, or deviations from instructions specified in the protocol, will be assessed by the investigators and may be excluded from analyses.

Fecal samples

Participants will be asked to collect a fecal sample at home. They will be carefully instructed in the procedure and kits containing the necessary collecting equipment (cooler bags, sampling tubes, plastic gloves, etc.) will be provided. Participants will store the fecal samples in their own freezer

prior to transporting them to the study site and transporting the samples in pre-delivered cooler bags. Fecal samples will be stored at -80°C at the hospital until analysis and will be analyzed for microbiota composition and function. These analyses will be performed at the Jensen Group, Department of Biomedical Sciences, University of Copenhagen and at the Microbiome-Host Interactions lab, Institut Pasteur, Paris, France. It will be ensured that the transfer of biological material abroad will adhere to the General Data Protection Regulation (GDPR) and the Danish Data Protection Act, safeguarding all personal data involved in the study.

Samples will be assessed individually by the investigators and samples with significant variation due to inadequate sampling/storage, technical problems, or deviations from instructions specified in the protocol, will be assessed by the investigators and may be excluded from analyses.

FibroScan

Liver fibrosis levels will be assessed by placement of an ultrasound probe over the liver region. Stiffness will be quantified in kiloPascals, while controlled attenuation parameter values, quantified as decibels per meter, are a measurement for steatosis levels.

Sugar probe test

Intestinal permeability will be assessed using a carbohydrate absorption test, including sucralose, saccharose, lactulose, and mannitol. The carbohydrate analytes will be orally administered after overnight fasting, and urine will be collected for 26 hours.

	Ingested dose (g)	Molar mass (g/mol)	Ingested dose in mol
Mannitol	5	182.172	0.027446589
Laktulose	10	342.296	0.029214481
Saccharose	20	342.2965	0.058428877
Sucralose	2	397.64	0.005029675

Mannitol, lactulose and saccharose will be dissolved in 100 ml water (drinking solution is prepared by pharmacy of university hospital Charité, Berlin. Sucralose is formulated as capsules (too sweet to drink). The urine will be further analyzed at the Department of Hepatology and Gastroenterology, Charité university hospital Berlin, Germany Concentrations of each carbohydrate in urine samples will be quantified by HPLC analysis using an electrochemical detector. The recovery rate of each carbohydrate will be calculated using standard curves of sucralose, saccharose, lactulose, and mannitol.

As other sugars ingested during the urine collection period may affect the test result, participants will be given identical, standardized meals on the study days.

Sperm samples

Participants will be instructed to abstain from ejaculation for 72 hours before each semen sample. The semen samples will be evaluated according to WHO guidelines i.e. sperm count, sperm motility, and sperm morphology within one hour. The samples will be frozen at -80°C and stored for later metabolomics and proteomics, as well as quantification of immunoglobulins and epigenetic changes. For each semen sample, an aliquot will be diluted and prepared for test of sperm DNA fragmentation (DFI) according to the protocol by SPZ Lab. These samples will be frozen and shipped to SPZ Lab.

DNA fragmentation index (DFI) will be analyzed by SPZ Lab (Copenhagen, Denmark) using the SDI®-test (improved version of the Sperm Chromatin Structure Assay).

Semen samples with significant variation (for instance due to fever, lost material during sample collection (spillage), prolonged illness, or short abstinence period (< 3 days)), will be assessed by the investigators and may be excluded from analyses.

Abdominal fat biopsies

These biopsies will be obtained from the percutaneous periumbilical subcutaneous adipose tissue by a modified Bergström needle with suction under local anesthesia (2 ml of xylocaine 2%). The biopsies will immediately be rinsed in ice-cold sterile saline and then separated to two fixation modalities: One for snap-frozen in nitrogen and subsequently stored at -80°C degrees until analysis for transcriptomics and proteomics and one in formalin which can be transferred the subsequent day to 70% ethanol for histology.

Samples with significant variation due to inadequate sampling or technical problems will be assessed by the investigators and may be excluded from analyses.

Colonoscopy

Colonoscopies will be performed by a gastroenterologist at 'Mavespecialisten', Jægersborg Allé 16, 2nd floor, 2920 Gentofte.

24 hours prior to the colonoscopy, participants will be carefully instructed to only consume clear liquids. "Clear" means water, clear soup, juice without pulp, coffee without milk, tea, juice, etc. At 1 PM and 8 PM the participant will take one dose laxative, Picoprep (magnesium oxide and sodium picosulfate), dissolved in 150 mL of tap water. During the procedure participants will be offered a

combination of 1 mg fentanyl and 1 mg midazolam to ease any discomfort or pain. If a participant is given medication during the procedure, he will be warned against driving the rest of the day both orally and in writing.

The colonoscope is lubricated with a water-based gel containing xylocaine 20mg/g, and is then inserted into the rectum, up through the colon, and if possible, into the terminal section of ileum. Puffs of air are pumped into the colon to ensure that the folds are open and that the physician can visualize the colon wall. Using small pincers inserted through the colonoscope, the physician will take samples from the mucous membrane. A total of 12 biopsies will be collected (9 from colon and 3 from ileum).

The analysis of biopsies will include a combination of full- and single cell transcriptomics, microbiome, proteomics, and metabolomics analyses. Histological analysis, including villus height, crypt depth and villus height:crypt depth ratio, as well as relevant immunohistochemistry will also be performed.

Magnetic resonance spectroscopy (MRS) and magnetic resonance elastography (MRE)

MRS/MRE will be performed at the Department of Radiology, Herlev Hospital with a Philips MR7700 scanner. The participant will be placed in a horizontal position during the scan (approximately 50 min). In addition to hepatic steatosis and elastography measurements, the MRS will be used for an estimation of fat infiltration in skeletal muscle and pancreas, as well as estimation of visceral and subcutaneous adipose tissue volumes.

Questionnaires

On study days participants will be asked to fill out questionnaires concerning general physical and mental wellbeing (see enclosed *PHQ-9 questionnaire*), and food cravings and eating behavior (see enclosed *COeQ questionnaire*). On the final visit, participants will answer a short questionnaire about recent dietary habits (see enclosed *Spørgsmål om madvaner*)

Activity tracking

Participants will be asked to carry an accelerometer-type smartwatch for one week before study block 1 and throughout the entire diet intervention period. It will monitor step count, sleep duration and quality, as well as overall activity level. All data will be collected in pseudonymized form and subsequently stored in accordance with the rules of The Danish Data Protection Agency.

Smart capsule

An ingestible sampling capsule will be used to collect luminal fluid from the gastrointestinal tract. Participants will be provided with the capsule and stool collection kits and will be instructed to ingest the capsule with breakfast on the day prior to the study visit. Participants will be instructed to resume normal eating four hours after capsule ingestion.

Participants will be asked to monitor their stool for passage of the capsule. The expected gastrointestinal transit time will be approximately 24–48 hours. Upon excretion, they will be instructed to promptly collect the capsule using the provided materials, place it in a supplied cooling container (ice box), and bring it to the study days for subsequent analysis.

Biobank

From each participant the following biological material will be colleceted: Up to 249 mL of blood, ejaculate from four ejaculations (up to 20 mL of seminal fluid), 42 mL of urine, three fat tissue biopsies, 24 intestinal biopsies, 15 mL of saliva, and up to 30 grams of feces. A specific research biobank will be established at the Center for Clinical Metabolic Research, Gentofte Hospital. The biobank will store full blood/plasma/serum/urine/feces/sperm samples as well as intestinal and adipose tissue samples at -80°C in pseudonymized form (all participants are assigned a participant number and will on datasheets and samples only appear with initials of responsible investigator and participant number) from the study days until analysis. After the project is completed, the specific research biobank will be terminated. Excess full blood/plasma/serum/urine/feces/sperm samples as well as intestinal and adipose tissue samples will be stored in another biobank for future research in pseudonymized form for up to 10 years after the completion of the study (no later than June 2037) until destruction, re-analyses, further analyses (assay development, validation, other relevant measurements), or new research projects. Samples will be stored in accordance with the rules of The Danish Data Protection Agency. If the contents of the biobank are to be used for future analysis and/or future research projects a new approval from the Regional Health Research Ethics Committee will be sought. At all times, and without further explanation, participants can get their samples retracted from the research biobank.

Urine and feces are analyzed in Department of Hepatology and Gastroenterology, Charité university hospital Berlin, Germany and Microbiome-Host Interactions lab, Institut Pasteur, Paris, France, respectively. It will be ensured that the transfer of biological material abroad will adhere to the General Data Protection Regulation (GDPR) and the Danish Data Protection Act, safeguarding all personal data involved in the study.

Calculations and statistics

Sample size

A previous study by Vanuytsel et al. including 21 healthy volunteers found that mean±SD of LMR increased from 0.030±0.022 under the control condition to 0.059±0.042 under public speech stress with individual LMRs showing a skewed distribution with a coefficient of variation of approximately 0.71 under both conditions*(24)*. From this we estimate that LMR is log-normally distributed with a standard deviation of 0.64 (95% CI 0.48 to 0.92) on log-scale. Vanuytsel et al further reports that the p-value for the mean change in LMR is between 0.001 and 0.01, implying that the correlation between the paired measurements must be between 0.45 and 0.75.

Assuming that our study participants will be similar to those in Vanuytsel et al, figure 2 shows the sample size required to demonstrate a significant increases in LMR at the 5% level for target effects between +20% and +100% for best, worst, and most likely case SDs of 0.48, 0.92, and 0.64 and for best, worst, and most likely case correlations of 0.45, 0.75, and 0.60 between the paired measurements.

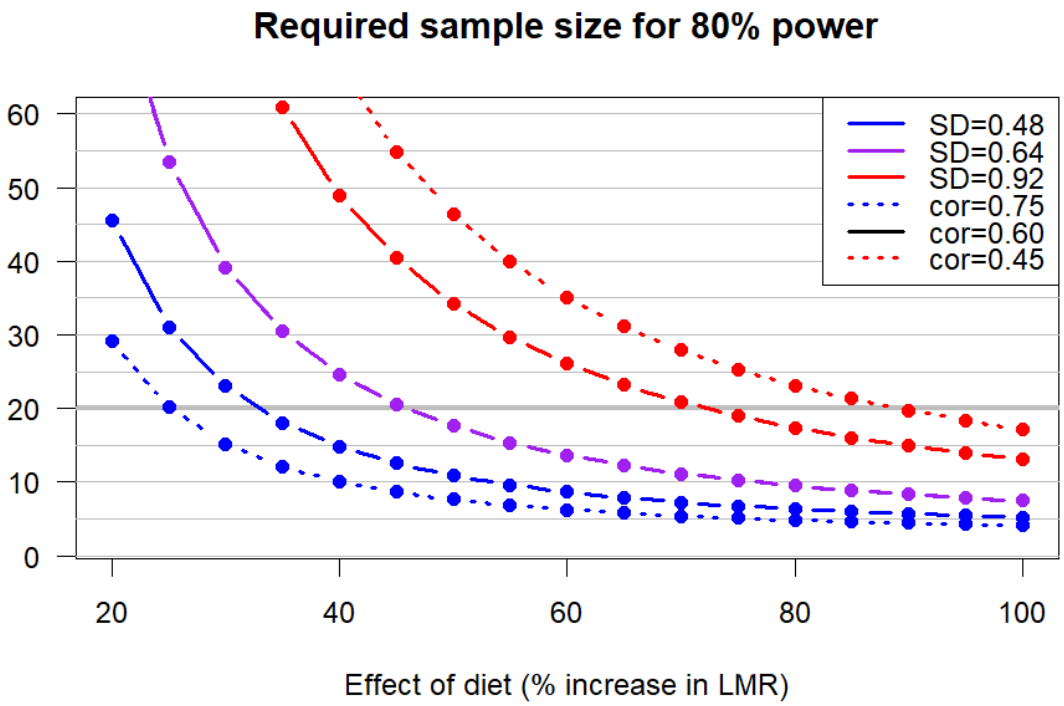

Figure 2: Sample size calculation.

In the most likely scenario, a 50% increase in LMR can be demonstrated with 80% power if 20 participants complete the baseline and end-of-diet follow-up without major study violations, while a 30% increase can be demonstrated with 80% power in the best case scenario, and a 90% increase (somewhat less than the 100% increase found by Vanuytsel et al.) can be demonstrated with 80% power in the worst case scenario. We therefore plan to continue enrollment until at least 20 participants have completed end-of-diet follow-up without major study violations.

Statistical methods used

Populations: The primary and secondary outcomes will be evaluated in the included study population including all study participants who adhere to at least 95% of the planned intervention by ordering at least 40 out of 42 meals (assessed using receipts for meals) during the intervention period and who

completes study blocks 1 and 2. Harms will be evaluated in the 'modified intention to treat population' including all participants enrolled in the study who order at least one meal during the intervention period regardless of further adherence and study completion.

Primary analyses: The primary outcome, LMR, will be log-transformed and analyzed using a linear mixed model including visit (baseline, end-of-intervention, end-of-recovery) as fixed effect and with an unstructured covariance pattern to account for repeated measurements on each participant. The estimated effects of intervention and recovery will be reported as ratios in geometric means for end-of-intervention vs baseline and end-of-recovery vs end of intervention with 95% confidence intervals.

Secondary analyses: The secondary outcomes will be analyzed using a linear mixed model as the one used for the primary outcome. Estimated effects of intervention and recovery will be reported as either differences in means with 95% confidence interval or ratios in geometric means with 95% confidence intervals depending on whether the distribution of the outcome is anticipated to be skewed or not as indicated in the outcomes section.

Harms analyses: Occurrence of harms will be reported with numbers and percentages and compared between the intervention and recovery period with McNemar's test for paired binary data. Cumulated numbers of harms will be reported with medians and quartiles and compared between the intervention and recovery period with a negative binomial generalized linear mixed model including a random effect of study participant.

Missing data: Missing data in the primary and secondary analyses will be handled implicitly by maximum likelihood estimation in the linear mixed model which handles missing data optimally

under a *missing at random assumption*. Due to the exclusion of non-adherent participants, missing data should occur only due to technical problems and study discontinuation during the recovery period, why the *missing at random assumption* seems reasonable. Missing data on harms should not occur as participants are required to report on these if they wish to discontinue the study. In case of substantial loss to follow-up, appropriate sensitivity analyses will be performed, e.g. using best case - worst case scenarios.

Multiplicity: The effects of intervention and recovery in the primary analysis will be tested hierarchically to control the family-wise error rate at the 5% significance level. The p-value for the effect of recovery will only be reported if the effect of the intervention is significant. The p-values from the secondary analyses will be adjusted for multiple testing using the method of Benjamini and Hochberg which controls the false discovery rate with p-values for the effects of the intervention and the effects of recovery adjusted separately. The p-values for the effects on harms will not be adjusted for multiple testing.

Side effects, risks and disadvantages for subjects

The overnight fast prior to screening visit and study days is a part of the natural nocturnal fast (at 23-09) and is not associated with discomfort or danger.

Blood samples will be taken at the screening visit and on study days. Investigators will ensure that the total blood loss for participants does not exceed 250mL, which is considerably less than a normal blood donation of 450 mL and is considered safe. Only individuals with hemoglobin >8.3 mmol/L can participate. Abdominal fat biopsies are safe, although subjects may experience limited bleeding and local soreness immediately after the biopsy extraction. Both fat biopsy and intravenous access are associated with a small risk of local infection in the site of the skin puncture, but the risk will be

minimized by following the clinical standards for sterile procedures including double wiping of the involved skin with disinfectant alcohol. In the event of an infection, participants will be treated with local antibiotics and if necessary be referred to adequate treatment.

Molecular genetic analyses are restricted to DNA/RNA analyses of fecal and tissue (intestinal and adipose) samples only. Somatic DNA analyses, expressively, blood (leucocyte, germline) genetic analyses are not performed, hereditary genetic disorders cannot be diagnosed.

Ingestion of smart capsules carry no particular risk – cases of capsule retention are very rare and usually caused by preexisting undiagnosed obstruction or stenosis of the GI-tract. Capsule retention is treated with laxatives, or in extremely rare cases with endoscopic retrieval.

Prior to the colonoscopy, subjects will undergo a bowel preparation routine involving clear liquid meals and laxatives, which may induce transient mild abdominal discomfort. Colonoscopy is the safest and most common method for obtaining tissue biopsies from the colon and ileum. Rare complications include bleeding, infections, perforation and reaction to sedation. The rate of serious complications in asymptomatic screening populations is reported to be 2.8/1000*(25)*, and an estimated 85% of serious complications are related to *ad hoc* polypectomies*(25),* which is not part of this study protocol. Inflation of the colon with air may cause discomfort and sense of bloating. Participants will be offered sedatives and analgesics to alleviate any pain or distress during the procedure. The colonoscopy will be performed for research purposes only and will not be evaluated diagnostically. Participants will only be informed about incidental findings if they have opted for this in the informed consent form.

The hypercaloric diet is expected to induce weight gain. In a previous overfeeding study performed in our group 20 young male participants on average gained 3.5 kilograms (95% CI: [2,8;4,2]) after 14 days*(26)*. The diet contained 4,641 kcal per day (caloric intake in the current study is 5,000 kcal/day), had a similar composition of macronutrients compared to the diet intervention in this

protocol*(26)*, and activity was also limited to 5,000 steps/day. In a study at Linköping University, Sweden, eighteen participants consumed on average 5,753 kcal/day solely from fast food in four weeks, and were also limited to 5,000 steps/day, resulting in an average weight gain of 6.4 kilograms *(27)*. Based on these previous studies, we expect participants to have gained 4-5 kilograms after the diet intervention in the present protocol. We expect the excess weight to be lost after 20 weeks of recovery. However, there is a risk of a persisting increase in weight. Therefore, we have excluded participants with a history of obesity and only include participants with normal weight at baseline. In the event of persisting weight gain after 20 weeks, participants will be offered counseling by a registered clinical dietician free of charge. However, despite having offered similar service in previous our overfeeding study, we have yet to receive feedback or complaints about residual weight gain from participant, and interpret this as a clear indication that participants have returned to their desired body weight.

The previous overfeeding study performed in our group led to increases in biomarkers for glucose and lipid metabolism, inflammation, bacterial translocation and increased intestinal permeability (*manuscript submitted*). All of these biomarkers had returned to normal after an 8-week washout period. We are therefore confident that the diet intervention will not have lasting impact on metabolism, inflammatory status and gut barrier integrity.

MRS/MRE is safe and not associated with discomfort or pain. A *Philips MR7700* scanner will be used for the MRS/MRE. Technical service is provided by *Philips Healthcare* quarterly to ensure the settings and quality of the scanner. Service reports are stored at the Department of Radiology, Herlev Hospital. The scanner will be making loud noises while scanning and the participants will wear proper ear protection provided by the staff at Herlev Hospital. MRS is the gold standard for evaluating hepatic steatosis and the method is safe. The scan is performed without the use of ionizing radiation or contrast agent. Contraindications to MRS/MRE will be checked using an MR safety checklist at

the screening visit (see enclosed *MR kontrolskema*). The MRS/MRE scanning protocol used in this study consists of highly specialized scanning techniques, that either do not produce images, or produce images unfit for radiologic image evaluation. Besides the designated outcomes of the study, no clinically meaningful diagnostic information can be obtained from the data generated by this scanning protocol.

FibroScan is a non-invasive ultrasound procedure and is not associated with discomfort or risk.

We expect the high-calorie, high-carbohydrate diet to induce simple hepatic steatosis. This is considered a common and benign condition with a negligible risk of progression to advanced liver disease*(28)*. The estimated prevalence among Europeans is 24%*(29)*. Simple hepatic steatosis is fully reversible and can be corrected by lifestyle modifications including physical activity and dietary intervention*(30)*, which will occur spontaneously as subjects return to their normal diet and exercise routines after the diet intervention has ended. In the beforementioned overfeeding study performed in our group almost all participants developed increases in liver fat content after two weeks overfeeding. All participants had normal liver fat content after an eight week washout period*(26)*.

Investigators will ensure the necessary privacy for sperm sample collection. Semen analyses included in this study cannot be used for the diagnosis of genetic disease or risk thereof. Results indicating severely impaired reproductive function will be considered a significant information about health status (*væsentlig oplysning om helbredstilstand*) and will be disclosed to participant if they have opted for this in signed consent form. We hypothesize that the diet intervention may lead to temporarily increased sperm DNA-fragmentation (DFI). Increased DFI has been shown to correlate with an increased risk of miscarriage and lower birth weight of the offspring*(31)*. Therefor participants must agree to the use of effective contraceptives for the entire diet intervention period and the following 90 days (see *exclusion criteria*).

The low-fiber diet may induce constipation. Participants will be warned of symptoms and receive information about non-pharmacological approaches to alleviate discomfort. Ultimately participants may be advised by the responsible physician to use over-the-counter laxatives.

Participants of the research group

Johan Platz, MD; Assoc. Prof. Asger Bach Lund, MD PhD; Assoc. Prof. Benjamin Anderschou Holbech Jensen, MSc PhD; Postdoc Malte P. Suppli, MD PhD; Postdoc Hannah Gilliam Vigh, MD PhD.

Funding and duality of interests

The study is initiated by the research group (see *participants of the research group*), and no member of the group has economic interests in the conduct or outcome of the study. The principal investigator for the project is associate professor, Asger Bach Lund MD, PhD. PhD student Johan Platz, Center for Clinical Metabolic Research will be responsible for study conduct, coordination of data collection, overseeing the analysis, and ensuring the integrity of the research methodology throughout the project.

Johan Platz has been granted a PhD scholarship from Danish Diabetes and Endocrinology Academy (1,100,000 DKK) covering Johan Platz' salary. The remaining expenses (2,600,000 DKK) will be funded by the Center for Clinical Metabolic Research as will additional funding be sought. When further funding is achieved, the Regional Health Research Ethics Committee will be notified and the document "Deltagerinformation" will be updated. Granted funds are administered by the finance department of Gentofte Hospital and received funding will be allocated to the department Center for Clinical Metabolic Research.

Remuneration and reimbursement of costs for participants

Any travel expenses the participants may have in relation to the project will be reimbursed. Due to the comprehensive timeframe of the project (three study days with a duration of approximately nine hours each, an additional sperm sample, two colonoscopies and the two-week intervention period with a dictated lifestyle), the subjects will each be remunerated 12,000 DKK (taxed as B income). If the participants choose to withdraw prematurely from the study, they will be reimbursed according to the time spent (4,000DKK pr each study block completed).

Recruitment of participants

Volunteers will be recruited from individuals who previously participated in studies at the Center for Clinical Metabolic Research and who, at that time, agreed to be contacted about future research projects. Recruitment will also take place by advertising in national and local newspapers and recruitment platforms (such as Forsoegspersoner.dk and Trialtree.com), by sharing the advertisement on social media and/or by distribution of flyers about the project. For this, the enclosed ad text will be used.

All inquiries about the study will be handled by the investigators by telephone or email and if an individual is deemed eligible for inclusion they will be invited to an information visit. Written participant information (“Deltagerinformation”) will be sent to all potentially eligible participants along with the brochure "Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt".

Informed consent

The investigators and study-related personnel will ensure that the subject is adequately informed about the study background, methods, design, risks and benefits, both orally and in writing. In

particular, the section of *Deltagerinformation* concerning side effects, risks, and disadvantages (*Bivirkninger, risici og ulemper*) will be reviewed thoroughly, item by item, with each subject until investigators are certain that subject fully understands the expected consequences and potential risks of study participation.

Investigators will also ensure that the conversation takes place in undisturbed and confidential surroundings. Subjects will be informed about the possibility of bringing a person of their own choice to the visit. Subjects are offered a consideration period of at least 24 hours, after which they can decide if they wish to participate in the study. If a subject declines the offered consideration period and wishes to participate, written and oral consent will be obtained, and screening will be performed immediately following the information visit. If a subject accepts the offered consideration period, the subject is contacted via telephone or e-mail within one week and offered an additional meeting. If the subject then wishes to participate in the study, written and oral consent will be obtained, and screening will be performed. The subjects will be informed that it is possible to withdraw their consent at any time prior to or during the study.

Participants' integrity and privacy

Information about participants in the project is protected in accordance with the applicable laws of Denmark, e.g. the General Data Protection Regulation (*Databeskyttelsesforordningen*) and the Data Protection Act (*Databeskyttelsesloven*). The protocol will be registered according to the data protection policies of Capital Region of Denmark.

Information obtained from medical record with consent

No information will be obtained from medical records of potential participants without consent.

Only when written and oral consent is obtained, investigators and authorized study personnel will directly access the medical record of the participant (through "*Sundhedsplatformen*" and other clinical systems directly related to "*Sundhedsplatformen*"). This access is strictly limited to data either generated within the framework of the study—such as results from blood ordered during the screening procedures—or information directly necessary to assess eligibility. Access may include laboratory test results, current medication lists, and entries in medical records, provided such data are directly relevant to the study. Oral and written consent applies for the investigators and authorized study personnel as part of the inclusion but also for regulatory authorities as part of regulation, self-monitoring, and quality control.

Publication of study results

The results of this project or elements of the project will be compiled into one or more manuscripts for publication in one or more international scientific journals. The results will also be presented at international and national scientific meetings and will be published in relation to the submission of Johan Platz' PhD thesis. Negative, inconclusive and positive results will be published in accordance with the law concerning processing of personal data. Co-authors shall fulfil criteria for co-authorship according to International Committee of Medical Journal Editors. By default, the authors will include all members in the research group (see *Participants of the research group*). Dr. Johan Platz, MD, will by default be first author on publications resulting from the project, while Asger Bach Lund, MD PhD, will be corresponding and last author.

Ethical perspectives

Apart from the free meals during the intervention period and the general medical examination at the screening visit, subjects will not benefit from participating in this study. They will contribute to the

overall objective of the study, which is to elucidate the clinical and mechanistic pathways through which diet-induced obesity disrupts gut barrier integrity and drives systemic inflammation, with the ultimate aim of identifying novel targets for prevention and treatment of obesity-associated diseases. It is essential that we investigate the effects of obesogenic factors in normal-weight individuals to avoid confounding and delineate causality. In the section *Side effects, risks and disadvantages for subjects* we believe to have established that the effects of two weeks overfeeding on metabolism, inflammation, gut barrier integrity and hepatic fat content are transient and completely reversible within eight weeks*(26)*. There is a potential risk of residual weight gain after the 20-week washout period, although weight loss has been obtained simply by returning to normal lifestyle in previous overfeeding studies. Participants will be clearly informed about this risk both orally and in writing, and will be offered counselling by a registered clinical dietician in the event that sufficient weight loss is not achieved after 20 weeks. In total, the investigators are convinced that the potential scientific benefits of the study outweigh the hazards and burdens of the study participants.For all study-related procedures, participants are covered by the mandatory patient insurance (Patient Compensation Association – *Patienterstatningen*).

The clinical applicability of study results is limited by selecting only male participants. Intestinal permeability is however affected by the menstrual cycle and use of oral contraceptives *(32, 33)*. To include women the study should either 1) prolong the diet intervention for all participants to the length of a full menstrual cycle or 2) synchronize study days with the female participants' menstrual cycle. Option 1) was considered too disadvantageous for participants in terms of weight gain and overall discomfort, and option 2) was not feasible due to MRS/MRE and colonoscopies only being available on specific weekdays.

Furthermore, an exploratory outcome is the causal effects of a hypercaloric fast food diet intervention on semen concentration, motility and DNA fragmentation. Including only men will allow a reasonable sample size for these analyses.

Availability of data for subjects

All subjects are guaranteed access to more information about the project. Contact: Dr. Johan Platz MD, Center for Clinical Metabolic Research, Gentofte Hospital, Gentofte Hospitalsvej 7, 3rd floor, DK-2900 Hellerup; e-mail: johan.platz.01@regionh.dk; Phone: +45 29933611.

Adjournment of study

The participants can withdraw from the study at any time without further explanation. The experiment is ended for participants in case exceptional circumstances make it impossible to complete the study. Likewise, any extraordinary event that makes it impossible to finish the project will result in the termination of ongoing experiments. In all cases the participants will be informed about the decision and the reason why.

References

1. NCD Risk Factor Collaboration (NCD-RisC), Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *The Lancet* **387**, 1377–1396 (2016).

2. E. Di Angelantonio, S. N. Bhupathiraju, D. Wormser, P. Gao, S. Kaptoge, A. B. de Gonzalez, B. J. Cairns, R. Huxley, C. L. Jackson, G. Joshy, S. Lewington, J. E. Manson, N. Murphy, A. V Patel, J. M. Samet, M. Woodward, W. Zheng, M. Zhou, N. Bansal, A. Barricarte, B. Carter, J. R. Cerhan, R. Collins, G. D. Smith, X. Fang, O. H. Franco, J. Green, J. Halsey, J. S. Hildebrand, K. J. Jung, R. J.

Korda, D. F. McLerran, S. C. Moore, L. M. O'Keeffe, E. Paige, A. Ramond, G. K. Reeves, B. Rolland, C. Sacerdote, N. Sattar, E. Sofianopoulou, J. Stevens, M. Thun, H. Ueshima, L. Yang, Y. D. Yun, P. Willeit, E. Banks, V. Beral, Z. Chen, S. M. Gapstur, M. J. Gunter, P. Hartge, S. H. Jee, T.-H. Lam, R. Peto, J. D. Potter, W. C. Willett, S. G. Thompson, J. Danesh, F. B. Hu, Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *The Lancet* **388**, 776–786 (2016).

3. K. Bhaskaran, I. Douglas, H. Forbes, I. dos-Santos-Silva, D. A. Leon, L. Smeeth, Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5·24 million UK adults. *The Lancet* **384**, 755–765 (2014).

4. A. Must, J. Spadano, E. H. Coakley, A. E. Field, G. Colditz, W. H. Dietz, *The Disease Burden Associated With Overweight and Obesity* (; www.jama.com).

5. D. Furman, J. Campisi, E. Verdin, P. Carrera-Bastos, S. Targ, C. Franceschi, L. Ferrucci, D. W. Gilroy, A. Fasano, G. W. Miller, A. H. Miller, A. Mantovani, C. M. Weyand, N. Barzilai, J. J. Goronzy, T. A. Rando, R. B. Effros, A. Lucia, N. Kleinstreuer, G. M. Slavich, Chronic inflammation in the etiology of disease across the life span. *Nature Medicine 2019 25:12* **25**, 1822–1832 (2019).

6. D. Khanna, S. Khanna, P. Khanna, P. Kahar, B. M. Patel, Obesity: A Chronic Low-Grade Inflammation and Its Markers. *Cureus* (2022), doi:10.7759/cureus.22711.

7. J. Choi, L. Joseph, L. Pilote, Obesity and C-reactive protein in various populations: A systematic review and meta-analysis*Obesity Reviews* **14** (2013), doi:10.1111/obr.12003.

8. V. K. Ridaura, J. J. Faith, F. E. Rey, J. Cheng, A. E. Duncan, A. L. Kau, N. W. Griffin, V. Lombard, B. Henrissat, J. R. Bain, M. J. Muehlbauer, O. Ilkayeva, C. F. Semenkovich, K. Funai, D. K. Hayashi, B. J. Lyle, M. C. Martini, L. K. Ursell, J. C. Clemente, W. Van Treuren, W. A. Walters, R. Knight, C. B. Newgard, A. C. Heath, J. I. Gordon, Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice. *Science (1979).* **341** (2013), doi:10.1126/science.1241214.

9. S. Bikel, G. López-Leal, F. Cornejo-Granados, L. Gallardo-Becerra, R. García-López, F. Sánchez, E. Equihua-Medina, J. P. Ochoa-Romo, B. E. López-Contreras, S. Canizales-Quinteros, A. Hernández-Reyna, A. Mendoza-Vargas, A. Ochoa-Leyva, Gut dsDNA virome shows diversity and richness alterations associated with childhood obesity and metabolic syndrome. *iScience* **24**, 102900 (2021).

10. C. Menni, M. A. Jackson, T. Pallister, C. J. Steves, T. D. Spector, A. M. Valdes, Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. *Int. J. Obes.* **41**, 1099–1105 (2017).

11. W. A. Walters, Z. Xu, R. Knight, Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett.* **588** (2014), doi:10.1016/j.febslet.2014.09.039.

12. M. Iacucci, G. Santacroce, S. Majumder, J. Morael, I. Zammarchi, Y. Maeda, D. Ryan, A. Di Sabatino, M. Rescigno, M. R. Aburto, J. F. Cryan, S. Ghosh, Opening the doors of precision medicine: novel tools to assess intestinal barrier in inflammatory bowel disease and colitis-associated neoplasia. *Gut* **73**, e331579 (2024).

13. J. Sawaed, L. Zelik, Y. Levin, R. Feeney, M. Naama, A. Gordon, M. Zigdon, E. Rubin, S. Telpaz, S. Modilevsky, S. Ben-Simon, A. Awad, S. Harshuk-Shabso, M. Nuriel-Ohayon, M. Werbner, B. O. Schroeder, A. Erez, S. Bel, *Antibiotics damage the colonic mucus barrier in a microbiota-independent manner* (2024; https://www.science.org).

14. K. L. Krigul, R. H. Feeney, S. Wongkuna, O. Aasmets, S. M. Holmberg, R. Andreson, F. Puértolas-Balint, K. Pantiukh, L. Sootak, T. Org, T. Tenson, E. Org, B. O. Schroeder, A history of repeated antibiotic usage leads to microbiota-dependent mucus defects. *Gut Microbes* **16** (2024), doi:10.1080/19490976.2024.2377570.

15. S. L. Friedman, B. A. Neuschwander-Tetri, M. Rinella, A. J. Sanyal, Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* **24**, 908–922 (2018).

16. L. Zhu, S. S. Baker, C. Gill, W. Liu, R. Alkhouri, R. D. Baker, S. R. Gill, Characterization of Gut Microbiomes in Nonalcoholic Steatohepatitis (NASH) Patients: A Connection Between Endogenous Alcohol and NASH. (2012), doi:10.1002/hep.26093.

17. M. P. Suppli, K. T. G. Rigbolt, S. S. Veidal, S. Heebøll, X. Peter, L. Eriksen, M. Demant, J. I. Bagger, J. C. Nielsen, D. Oró, S. W. Thrane, A. Lund, C. Strandberg, M. J. Kønig, T. Vilsbøll, N. Vrang, K. L. Thomsen, H. Grønbaek, J. Jelsing, H. H. Hansen, F. K. Knop, Hepatic transcriptome signatures in patients with varying degrees of nonalcoholic fatty liver disease compared with healthy normal-weight individuals. *Am J Physiol Gastrointest Liver Physiol* **316**, 462–472 (2019).

18. H. Tilg, T. E. Adolph, M. Trauner, Gut-liver axis: Pathophysiological concepts and clinical implications. *Cell Metab.* **34**, 1700–1718 (2022).

19. J. R. Enriquez, H. A. McCauley, K. X. Zhang, J. Guillermo Sanchez, G. T. Kalin, R. A. Lang, J. M. Wells, A dietary change to a high-fat diet initiates a rapid adaptation of the intestine. *Cell Rep* **41**, 111641 (2022).

20. B. Zhou, […], J. Z. Cisneros, Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4·4 million participants. *The Lancet* **387**, 1513–1530 (2016).

21. O. Stojanović, J. Altirriba, D. Rigo, M. Spiljar, E. Evrard, B. Roska, S. Fabbiano, N. Zamboni, P. Maechler, F. Rohner-Jeanrenaud, M. Trajkovski, Dietary excess regulates absorption and surface of gut epithelium through intestinal PPARα. *Nat Commun* **12**, 7031 (2021).

22. G. Pagliai, M. Dinu, M. P. Madarena, M. Bonaccio, L. Iacoviello, F. Sofi, Consumption of ultra-processed foods and health status: A systematic review and meta-Analysis*British Journal of Nutrition* **125**, 308–318 (2021).

23. G. Marchesini, C. P. Day, J. F. Dufour, A. Canbay, V. Nobili, V. Ratziu, H. Tilg, M. Roden, A. Gastaldelli, H. Yki-Jarvinen, F. Schick, R. Vettor, G. Fruhbeck, L. Mathus-Vliegen, EASL–EASD–

EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J. Hepatol.* **64**, 1388–1402 (2016).

24. T. Vanuytsel, S. van Wanrooy, H. Vanheel, C. Vanormelingen, S. Verschueren, E. Houben, S. Salim Rasoel, J. Tóth, L. Holvoet, R. Farré, L. Van Oudenhove, G. Boeckxstaens, K. Verbeke, J. Tack, Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* **63**, 1293–1299 (2014).

25. Screening for Colorectal Cancer: A Targeted, Updated Systematic Review for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* **149**, 638–658 (2008).

26. M. P. Suppli, A. Høgedal, J. I. Bagger, E. Chabanova, G. van Hall, J. L. Forman, M. B. Christensen, N. J. W. Albrechtsen, J. J. Holst, F. K. Knop, Signs of Glucagon Resistance After a 2-Week Hypercaloric Diet Intervention. *Journal of Clinical Endocrinology and Metabolism* **109**, 955–967 (2024).

27. Å. Ernersson, F. H. Nystrom, T. Lindström, *Long-term increase of fat mass after a four week intervention with fast food based hyper-alimentation and limitation of physical activity* (2010; http://www.nutritionandmetabolism.com/content/7/1/68).

28. S. Singh, A. M. Allen, Z. Wang, L. J. Prokop, M. H. Murad, R. Loomba, Fibrosis Progression in Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis: A Systematic Review and Meta-analysis of Paired-Biopsy Studies. *Clinical Gastroenterology and Hepatology* **13**, 643-654.e9 (2015).

29. T. Marjot, A. Moolla, J. F. Cobbold, L. Hodson, J. W. Tomlinson, Nonalcoholic Fatty Liver Disease in Adults: Current Concepts in Etiology, Outcomes, and Management. *Endocr. Rev.* **41** (2020), doi:10.1210/endrev/bnz009.

30. F. Nassir, R. Scott Rector, G. M. Hammoud, J. A. Ibdah, *Pathogenesis and Prevention of Hepatic Steatosis* (2015).

31. F. Li, X. Duan, M. Li, X. Ma, Sperm DNA fragmentation index affect pregnancy outcomes and offspring safety in assisted reproductive technology. *Sci. Rep.* **14** (2024), doi:10.1038/s41598-023-45091-6.

32. L. Leao, S. Miri, R. Hammami, Gut feeling: Exploring the intertwined trilateral nexus of gut microbiota, sex hormones, and mental health. *Front. Neuroendocrinol.* **76**, 101173 (2025).

33. T. R. Flood, M. R. Kuennen, S. D. Blacker, S. D. Myers, E. F. Walker, B. J. Lee, The effect of sex, menstrual cycle phase and oral contraceptive use on intestinal permeability and ex-vivo monocyte TNFα release following treatment with lipopolysaccharide and hyperthermia. *Cytokine* **158**, 155991 (2022).