

Analyzing the effects of short-term fasting on transcriptome and epigenome signatures compared with food consumption in healthy subjects

Submission date	Recruitment status	<input checked="" type="checkbox"/> Prospectively registered
06/12/2021	No longer recruiting	<input type="checkbox"/> Protocol
Registration date	Overall study status	<input type="checkbox"/> Statistical analysis plan
09/12/2021	Completed	<input type="checkbox"/> Results
Last Edited	Condition category	<input type="checkbox"/> Individual participant data
03/11/2022	Nutritional, Metabolic, Endocrine	<input type="checkbox"/> Record updated in last year

Plain English summary of protocol

Background and study aims

Several studies in animals and some in humans have reported that dietary interventions based on intermittent fasting or on time-restricted feeding lead to promising improvements in the cardiometabolic risk profile (decreasing body weight, reducing blood sugar and fat, etc). However, although some investigations in humans have associated these beneficial effects with epigenetic modifications (changes that modify the activation of certain genes, but not the genetic code sequence of DNA), mainly with DNA methylation and changes in gene expression in key genes related to metabolism, very few studies have analyzed the changes in the transcriptome (gene expression) and in the epigenome (mainly focusing on DNA methylation) at the genome-wide level. Therefore the main aim of this study is to explore the changes in the transcriptome at the genome-wide level in healthy subjects from a Mediterranean population after a short-term fasting period (24 hours) in comparison with frequent food consumption in the same period, and to establish the corresponding transcriptome and methylome signatures that identify the main differentially expressed and or methylated genes depending on the fasting or on the frequent food consumption status in the short-term intervention.

Who can participate?

Men and women (white European) from the general Mediterranean Spanish population (aged 18-50 years)

What does the study involve?

Each participant receives both interventions in a random order. One intervention will consist of a period of 24 hours fasting (only water will be allowed) and the other intervention will consist of a frequent meal pattern (6 times a day, every 3 hours) for the equivalent 24-hour period. In this food consumption intervention, the participant will be provided with typical foods of the Mediterranean diet: fruits, vegetables, legumes, extra virgin olive oil, fish, white meat and nuts). There will be a 2-week break between the interventions.

What are the possible benefits and risks of participating?

There are no direct medical benefits of participating in the study. Participants will be informed that there are no benefits and risks expected.

Where is the study run from?

University of Valencia (Spain)

When is the study starting and how long is it expected to run for?

January 2020 to December 2022

Who is funding the study?

Generalitat Valenciana and University of Valencia (Spain)

Who is the main contact?

Prof. Carolina Ortega-Azorín

Carolina.Ortega@uv.es

Contact information

Type(s)

Scientific

Contact name

Dr Carolina Ortega-Azorin

ORCID ID

<https://orcid.org/0000-0001-6719-9358>

Contact details

School of Medicine. University of Valencia

Department of Preventive Medicine

Avda Blasco Ibanez, 15

Valencia

Spain

46010

+34 (0)963864800

carolina.ortega@uv.es

Additional identifiers

Clinical Trials Information System (CTIS)

Nil known

ClinicalTrials.gov (NCT)

Nil known

Protocol serial number

PTRC_1521410

Study information

Scientific Title

Effect of fasting for 24 hours compared to frequent food consumption on anthropometric, biochemical and gene expression and methylation variations in the general population (AYUGEN)

Acronym

AYUGEN

Study objectives

The hypothesis is that fasting for 24 hours can change gene expression and DNA-methylation profiles at the genome-wide level in comparison with a pattern of food consumption, allowing us to identify the transcriptome and methylome signatures associated with fasting and frequent food consumption in a Mediterranean population.

Ethics approval required

Old ethics approval format

Ethics approval(s)

Approved 15/01/2021, Ethics Committee on Human Research at the University of Valencia (Avda. Blasco Ibanez 13, Valencia, 46010, Spain; +34 (0) 963864109; vicerec.investigacio@uv.es), ref: CR_1521410

Study design

Interventional randomized cross over trial

Primary study design

Interventional

Study type(s)

Other

Health condition(s) or problem(s) studied

Short-term fasting

Interventions

This is a short-term cross-over intervention trial in 25 participants (including men and women). Two interventions related to food consumption will be carried out. All participants will receive both interventions. Participants will be randomly assigned 1:1 to the order of the two interventions by a computer-generated randomization procedure. The interventions will consist of:

1. Fasting for 24 hours (water allowed)
2. Consumption of foods for the equivalent 24-hour period

The frequent food consumption intervention pattern includes eating 6 times a day every 3 hours. In this food consumption intervention, participants will be provided with typical foods of the Mediterranean diet: fruits, vegetables, legumes, extra virgin olive oil, fish, white meat and nuts). A wash-out period of 2 weeks between interventions will be carried out.

Intervention Type

Behavioural

Primary outcome(s)

Comparative genome-wide gene expression and methylation profiles in leukocytes (using DNA and RNA isolated from blood) to identify transcriptome and methylome signatures (detection of differentially expressed genes and DNA methylation sites) of 24-h fasting versus frequent food consumption after both interventions in healthy subjects from a Mediterranean population. Blood samples will be obtained at baseline and after 24 h of the corresponding intervention (fasting or food consumption). RNA and DNA will be isolated by standard procedures and quality control measures will be carried out. Transcriptome-wide human arrays will be used to analyze differential gene expression and RT-PCR will be used for validation. Methylation will be analyzed by arrays and for selected CpG sites by Massarray according to the standard protocols. Differential gene expression and CpG methylation sites will be statistically analyzed and the combination of the most relevant differentially expressed genes and DNA-methylation sites will be combined by computational analyses to build specific signatures for each intervention.

Key secondary outcome(s)

1. Plasma glucose and biochemical parameters (lipids) measured by standard procedures at baseline, at 6 h and after the 24 h interventions in both groups
2. Height, weight, waist circumference and body composition by bioimpedance measured by validated methods at baseline and after the interventions
3. Blood pressure and heart rate measured by standard protocols at baseline and after 24 h intervention in both groups
4. Selected gene expression (from the genome-wide transcriptome array) measured by RT-PCR at baseline, at 6 h and after the 24 h interventions
5. Selected DNA methylation measured by MASSARRAY at baseline, 6 h and 24 h after interventions
6. Analysis of differentially expressed genes, methylation sites and pathways (GO and KEEG) at baseline and after interventions in the whole sample and by sex/gender
7. Food intake (assessed with food frequency questionnaires) and adherence to the Mediterranean diet (measured by the 14-item score for adherence) at baseline
8. Physical activity measured using the short form of the Minnesota physical activity questionnaire at baseline
9. Sleep characteristics measured using the Pittsburgh Sleep Quality Index questionnaire and the Epworth questionnaires at baseline
10. Chronotype measured using the Horne and Östberg questionnaire at baseline
11. Additional analysis (depending on the budget) of plasma/urine metabolites (lipids, amino acids, inflammatory markers measured using a standard high-throughput nuclear magnetic resonance metabolomics platform) at baseline and after the interventions

Completion date

31/12/2022

Eligibility

Key inclusion criteria

Current inclusion criteria as of 25/07/2022:

1. White European subjects (men and women) recruited from the Mediterranean population
2. Age ranging from 18 to 50 years old
3. BMI between 21 and 34 kg/m²

Previous inclusion criteria:

1. White European subjects (men and women) recruited from the Mediterranean population
2. Age ranging from 18 to 50 years old
3. BMI between 23 and 33 kg/m²

Participant type(s)

Healthy volunteer

Healthy volunteers allowed

No

Age group

Adult

Lower age limit

18 years

Sex

All

Total final enrolment

28

Key exclusion criteria

1. Diabetic subjects
2. Other chronic diseases (cardiovascular, cancer, respiratory diseases, liver diseases, kidney diseases, etc)
3. Subjects with food allergies or food intolerances
4. Alcohol abuse or addiction
5. Immunodeficiency, HIV-positive status, COVID-19 positive status or other acute infections
6. Serious psychiatric disorders: schizophrenia, bipolar disease, eating disorders, depression, etc
7. Any severe co-morbid condition
8. History of major organ transplantation
9. Concurrent therapy with immunosuppressive drugs or cytotoxic agents
10. Current treatment with systemic corticosteroids
11. Current use of weight loss medication
12. Any other condition that may interfere with the completion of the study protocol

Date of first enrolment

20/12/2021

Date of final enrolment

31/10/2022

Locations

Countries of recruitment

Spain

Study participating centre

University of Valencia

School of Medicine

Avda. Blasco Ibanez 15

Valencia

Spain

46010

Study participating centre

CIBEROBN

Av. Monforte de Lemos, 3-5. Pabellón 11.

Madrid

Spain

28029

Sponsor information

Organisation

University of Valencia

ROR

<https://ror.org/043nxc105>

Funder(s)

Funder type

Government

Funder Name

Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital, Generalitat Valenciana

Alternative Name(s)

Conselleria of Innovation, Universities, Science and Digital Society, Valencia, Conselleria d'Innovació, Universitats, Ciència i Societat Digital, CIUCSD

Funding Body Type

Government organisation

Funding Body Subtype

Local government

Location

Spain

Funder Name

Universitat de València

Alternative Name(s)

University of Valencia, UV

Funding Body Type

Private sector organisation

Funding Body Subtype

Universities (academic only)

Location

Spain

Results and Publications

Individual participant data (IPD) sharing plan

Data will not be available outside the core research group as the informed consent form signed by participants stated that individual-level data will not be publicly available. Researchers who are interested in this study can contact the main investigator (Dr Carolina Ortega; carolina.ortega@uv.es) if they have any questions regarding the data or are interested in further collaborations.

IPD sharing plan summary

Not expected to be made available

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Participant information sheet	Participant information sheet	11/11/2025	11/11/2025	No	Yes