6. Annexes

6.1. Annex A: Informed Consent Form of the ALASKA Study

"**Appendix 1**" of the ALASKA Study protocol: https://static-content.springer.com/esm/art%3A10.1186%2Fs13063-024-08307-2/MediaObjects/13063_2024_8307_MOESM3_ESM.pdf

Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, López-Seoane J, Wusterhausen P, Matthias T, Urrialde R, González-Gross M. **The multifactorial approach and the food allergen-specific substitutive diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: study protocol for a randomized controlled trial-the ALASKA Study**. Trials. 2024 Jul 20;25(1):494. doi: 10.1186/s13063-024-08307-2. PMID: 39033266; PMCID: PMC11264907.

POLITÉCNICA

PARTICIPANT CODE: A L

RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

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ALASKA Study. ALLERGIES AND FOOD INTOLERANCES IN ADULTS AND ATHLETES

APPENDIX 1. INFORMED CONSENT MODEL

INSTRUCTIONS:
Welcome to the ALASKA study on Food Allergies and Intolerances in sedentary and athletic adults.
Please read carefully and sign the following Informed Consent.

Participant code: _____ (If you haven't receive your participant code, please contact the research staff at l.pantoja@upm.es, marcela.gonzalez.gross@upm.es, or gi.imfine@upm.es and request the unique participant code that you will use throughout the entire study as identification. If possible, memorize this code)

Today's date: _____ (DD/MM/YYYY format)

Sex (biological):
- ○ (1) Man
- ○ (2) Woman

Age: _____

Date of birth: _____ (DD/MM/YYYY format)

INFORMED CONSENT

Dear participant:

The ImFINE Research Group, of the Universidad Politécnica de Madrid (UPM) is carrying out a research project that aims to determine the impact of the adverse reactions to foodstuffs (ARFS), such as food allergies and food intolerances, over the quality of life of adults of the Mediterranean population with different levels of physical activity.
The study is carried out following current legislation on research activities in humans, the guidelines of the Declaration of Helsinki on ethical principles in research and has been approved by the Committee of Ethics of the Polytechnic University of Madrid (UPM) dated 07-20-2020 and reference number 20200602. The personal data of all participants will be treated in accordance with Regulation (EU) 2016/679, of 27 April 2016 and Royal Decree 1720/2007, of December 21, which approves the Development Regulation of Organic Law 15/1999, of December 13, Protection of Personal Data and Law 14/2007 of Biomedical Research.

You have been invited to participate in this study. Please find below the information regarding your decision to voluntarily participate. Use as much time as you want to study the content of this document before deciding whether to participate in this study or not.

ALASKA STUDY INFORMATION

The ALASKA study lasts approximately 5 to 6 months, and your participation will consist of:
- Responding to validated questionnaires related to the objective of the study.
- Clinical measurements, including: venous blood analysis and breath test. Physical tests, including: measurement of body composition, body perimeters, blood pressure, heart rate and a battery of physical exercises.
- Accomplishment of dietary recommendations as requested by the researchers of this study.

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CLINICAL MEASUREMENTS

- Blood test: venous blood extraction under fasting conditions to carry out hematological, biochemical and immunological analysis (and subsequent genetic tests if authorized by the participant).
- Breath test: Analysis of the concentration of exhaled gases of Hydrogen (H_2) and Methane (CH_4) through the collection of expired gas samples (blow into a disposable air collection device) for subsequent determination of food intolerance to lactose and/or fructose.

PHYSICAL TESTS

- Blood pressure and heart rate: measurement of the blood circulation intensity in the bloodstream and the number of times the heart beats per minute using a medical blood pressure monitor.
- Electrical bioimpedance: body composition analysis using an electrical bioimpedance scale. Note: If you have a pacemaker, metal prosthesis or are pregnant, please notify the researcher through gi.imfine@upm.es . Or instead, answer carefully the questions in the next section.
- Muscular function: determination of muscle strength, balance and speed of getting up of a chair (assessment of equilibrium) using a calibrated platform.
- Dynamometry: monitoring of physical condition using a hand-dynamometer that measures hand grip strength, assessing the contraction of the intrinsic and extrinsic muscles of the hand.
- Aerobic capacity: estimation of maximum oxygen consumption, VO_2 max, using heart rate measurement after performing aerobic exercise for a period of 5 minutes (Åstrand-Ryhming Step Test, stÅ-R).

RISKS

- The collection of blood and breath samples will be carried out by a qualified professional for clinical practice, so it signifies minimal to no risk for the participant.
- Your diet and exercise habits could moderately change. However, these changes will not represent dietary or physical alterations that could threaten against your health and will be monitored by a qualified professional for nutritional practice.
- In case of participants presenting metals allergies with potential adverse effects such as atopic dermatitis due to contact with metals (nickel, cobalt, chromium, among others), please notify the researcher through gi.imfine@upm.es. Or instead, answer carefully the questions in the next section.

GUARANTEE

- Active communication by the researcher with the participant.
- The physical tests will be carried out by a qualified professional in physical activity and sports sciences practice.
- Pseudonymization and safeguarding of data on a secure platform called REDCap. REDCap is a secure web platform and electronic data capture tool hosted at the Supercomputing and Visualization Center of Madrid of the Universidad Politécnica de Madrid (CESVIMA-UPM).
- Clinical samples obtained from the participant will be stored under frozen conditions (-20ºC) to subsequent genetic tests and ultra-freezing (-80ºC) for storage in the Faculty of Physical Activity and Sports Sciences (INEF-UPM) and the health center of the Spanish Health Protection Agency in Sports (AEPSAD) for academic and research purposes.
- The processed results will be published in scientific journals always in an anonymized format. The pseudonymized data files will be destroyed after being kept for a period of 10 years.
- At the end of the study we will confidentially provide you with a digital report containing the personal results obtained in the clinical and physical tests that you have done.

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ALASKA Study

ALASKA Study. ALLERGIES AND FOOD INTOLERANCES IN ADULTS AND ATHLETES

Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, *et al*. The multifactorial approach and the Food-Allergen Specific Substitutive Diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: Study Protocol for a Randomized Controlled Trial. The ALASKA study. Appendix 1. *Trials*. 2024.

Pantoja-Arévalo, L et al.

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RESEARCHERS: _____
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Answer the following questions according to your current status and physiological condition:

Are you currently pregnant?:
- ○ (1) Yes
- ○ (2) No

Number of weeks of pregnancy: _____ (current week of pregnancy)

Do you have a pacemaker, prosthesis or metal implants?:
- ○ (1) I have a pacemaker
- ○ (2) I have metal prostheses or implants
- ○ (3) I have a pacemaker and prosthetics or metal implants
- ○ (4) I wear something similar
- ○ (5) I do not have a pacemaker or prosthesis or metal implants or anything similar.

You have selected "I wear something similar", write:
What similar device do you wear?: _____

Do you have a metal allergy (nickel, cobalt, chromium, among others)?:
- ○ (1) Yes
- ○ (2) No

You have selected that you have a metal allergy, write:
The type of metal you are allergic to: _____ (e.g. nickel, cobalt, chromium)

Are you advised against physical exercise by your doctor?:
- ○ (1) Yes
- ○ (2) No

Do you have any limitations that prevent you from performing physical exercise safely?:
- ○ (1) Yes
- ○ (2) No

Have you ever suffered cardiac arrest, angina pectoris or heart failure?:
- ○ (1) Yes
- ○ (2) No

When you exercise, have you had chest pain, chest tightness, or dizziness?:
- ○ (1) Yes
- ○ (2) No

SUMMARY OF THE ALASKA STUDY CHARACTERISTICS

Your data will be anonymously used for research purposes only. Even if you agree to voluntarily participate in this study, you can abandon this study at any time, without having to justify yourself, and of course, without any fear of being penalized. Likewise, the researcher reserves the right to terminate your participation if the

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RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

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researcher consider that it is for the benefit of the study and/or the participant. You will not get any financial benefits for participating in this study, although you will receive benefits in terms of dietary healthy advice, exercise and lifestyle based on your metabolic status. The participation in this study requires no cost for you.

Once you have carefully read the above information, please sign the following document:

INFORMED CONSENT ACT

I _____ (name) _____ (lastname), agree to voluntarily and anonymously participate in the Research Project study entitled: RELATIONSHIP BETWEEN ADVERSE REACTIONS TO FOOD, PHYSICAL PERFORMANCE AND HEALTH IN MEDITERRANEAN POPULATION (ALASKA study), developed by BSc. Eng. MSc. LISSET PANTOJA-ARÉVALO, predoctoral researcher of the Doctorate program in PHYSICAL ACTIVITY AND SPORTS SCIENCES at the Universidad Politécnica de Madrid, supervised by Prof. Dr. MARCELA GONZÁLEZ-GROSS, Director of the ImFine Research Group (Improvement of Health by Fitness, Nutrition and Exercise Research Group) of the Faculty of Physical Activity and Sports Sciences-INEF of the Universidad Politécnica de Madrid and co-directed by Dr. PharmD. EVA GESTEIRO ALEJOS, Permanent Professor of the Faculty of Physical Activity and Sports Sciences-INEF of the Universidad Politécnica de Madrid.

_____
(Identity Document Number (without letter))

I declare that:

- I consent to perform all physical and clinical tests, including those requiring venous blood extraction and the collection of breath samples and answer all necessary questionnaires to carry out the present investigation.
- I know that the provided information will be confidential and anonymous.
- I understand that the obtained information will be saved and safeguarded by the researcher in a secure digital platform and in the facilities of the Faculty of Physical Activity Sports Sciences-INEF of the Universidad Politécnica de Madrid.
- I have been informed that my participation does not involve any harm or danger to my physical or mental health.
- I have understood the purpose of the tests and their possible associated risks.
- I have received sufficient oral and written information about the research protocol and have been able to make the necessary questions resolving all my doubts.
- I have been informed that there is no financial compensation for participating in this study.

About the research protocol, I have spoken with:

_____
(name of the researcher who provided you information about the study)

I understand that:

- My participation is free, voluntary and anonymous.
- For this study, I have to provide clinical samples: blood and exhaled air (breath sample).
- My diet and exercise habits may change moderately by participating in the study.
- I may refuse to participate or stop participating at any time without giving reasons or receiving any sanction.

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ALASKA Study

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RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

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I authorize the use of my partial data collected by the research group if I decide to withdraw from the study:

- ○ (1) Yes
- ○ (2) No

I give my consent freely to participate in the study:

- ○ (1) Yes
- ○ (2) No

If you do not agree, describe the reason: _____

I wish to know the results of the tests that are done on me:

- ○ (1) Yes
- ○ (2) No

I authorize the use of a part of my anonymized blood sample for further genetic research:

- ○ (1) Yes
- ○ (2) No

I authorize the sample storage of my anonymized blood samples for further studies. Otherwise, the leftover samples will be destroyed.:

- ○ (1) Yes
- ○ (2) No

This document is signed in two copies, leaving one in the possession of each of the parties.

Today's date: _____ (DD/MM/YYYY format)

_____
Participant's signature

_____
Principal researcher's signature

Contact information and principal researchers

Eng MSc Lisset Pantoja- Arévalo: l.pantoja@upm.es
Predoctoral researcher and trainee professor

Dr Eva Gesteiro Alejos: eva.gesteiro@upm.es
Researcher, professor and Co-director of the thesis project

Prof Dr Marcela González-Gross: marcela.gonzalez.gross@upm.es
Principal researcher and Director of the thesis project

Ethics Committee of the Universidad Politécnica de Madrid: secretaria.adjunto.vinvestigacion@upm.es

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RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

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END OF THE INFORMED CONSENT FORM

THANK YOU VERY MUCH

HOW TO CITE THIS DOCUMENT:
Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, *et al*. The multifactorial approach and the Food-Allergen Specific Substitutive Diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: Study Protocol for a Randomized Controlled Trial. The ALASKA study. Appendix 1 Informed Consent Form. *Trials*. 2024.

6.2. Annex B: Demographics form

The demographics form is shown in its original language, which is Spanish, as distributed during the ALASKA Study.

The current Demographics form was retrieved from the ALASKA Study RedCap® profile. Properly citing the current manuscript and its accompanying articles (Article 1 [27], Article 2 [34], and Article 3 [5]) is essential for the design, creation, management, and use of questionnaires.

For more details about how to use this questionnaire or any other issues related to this study, please contact:

Lisset Pantoja Arévalo, MSc, l.pantoja@upm.es

Doctoral Thesis researcher and coordinator of the study

Dr. Eva Gesteiro Alejos, eva.gesteiro@upm.es

Doctoral Thesis Co-director and coordinator of the study

Prof. Dr. Marcela González-Gross, marcela.gonzalez.gross@upm.es

Doctoral Thesis Director and PI of the study

DATOS DEMOGRÁFICOS, DATOS PERSONALES E HISTORIA CLÍNICA

Complete el siguiente Formulario de Datos Personales e Historia Clínica luego de leer las instrucciones generales.

Código de participante: _____

INSTRUCCIONES GENERALES

Responda de forma clara y concisa. No utilice frases incompletas ni acrónimos. Si cumplimenta el cuestionario a mano, utilice tinta de color azul y utilice letra clara, legible y preferiblemente MAYÚSCULAS. Si es cumplimentado de manera telemática y tiene cualquier duda, comuníquelo al investigador enviando un correo electrónico a: gi.imfine@upm.es

DATOS PERSONALES

Fecha de hoy: _____

(Pulse el botón "Hoy" y luego modifique con los datos correspondientes a su respuesta)

Iniciales de su nombre completo: _____

(P.e. María López Sánchez, respuesta = MLS)

Código postal de su domicilio:

_____

(Número de 5 cifras)

Teléfono de contacto:

_____

(Número de 9 cifras)

Profesión:

_____

(Describa con una palabra a qué se dedica)

Seleccione el nivel de educación más alto que usted haya obtenido hasta este momento:

- ◯ Sin educación
- ◯ Primaria
- ◯ Secundaria (ESO, BUP)
- ◯ Formación profesional medio
- ◯ Formación profesional superior
- ◯ Universitario(a) (graduado(a), licenciado(a), ingeniero(a))
- ◯ Máster
- ◯ Doctor(a)

¿Cómo definiría su situación laboral actual?

◯ Empleado por cuenta propia o ajena
◯ Estudiante en periodo de aprendizaje retribuido
◯ Estudiante en periodo de aprendizaje no retribuido
◯ Desempleado
◯ Jubilado o prejubilado
◯ Discapacitado permanente
◯ Realizo las tareas domésticas de mi casa
◯ Otra situación laboral

Escriba, qué otra situación laboral:

_____

¿Con cuántas personas convive en el hogar actualmente? (sin contar con usted)

◯ 0
◯ 1
◯ 2
◯ 3
◯ 4
◯ 5
◯ Más de 5

¿Cuál es su país de nacimiento?

◯ España ◯ Otro

Si usted no nació en España, indique el país:

_____

HISTORIA CLÍNICA

Grupo sanguíneo:

◯ A+
◯ A-
◯ B+
◯ B-
◯ O+
◯ O-
◯ AB+
◯ AB-
◯ No lo sé/ No estoy seguro(a)

¿Se ha hecho usted alguna prueba clínica para la identificación de coronavirus SARS-CoV-2 (p.e. PCR, anticuerpos, antígenos) durante los últimos 3 meses?

◯ Si
◯ No

Tipo de prueba (coronavirus SARS-CoV-2):

_____
(p.e. PCR, anticuerpos, antígenos)

Resultado de prueba (coronavirus SARS-CoV-2):

◯ Positivo
◯ Negativo

¿Se ha vacunado usted contra coronavirus SARS-CoV-2 hace menos de 3 meses?

◯ Si
◯ No

Número de dosis que se ha puesto de la vacuna del coronavirus SARS-CoV-2:	◯ 0 ◯ 1 ◯ 2 ◯ 3
Señale la marca de su última vacuna contra el coronavirus SARS-CoV-2:	◯ Pfizer/BioNTech ◯ Moderna ◯ AstraZeneca ◯ Janssen ◯ Novavax ◯ CureVac ◯ Sanofi Pasteur/GSK ◯ Valneva ◯ Sputnik V (Rusia) ◯ Sinovac (China) ◯ Otra marca ◯ No lo sé
Escriba, ¿qué otra marca?	_____

NOTAS ADICIONALES

Escriba aquí cualquier dato o información adicional que usted desee comunicar al investigador:
(Dejar vacío si usted no tiene información adicional que comunicar al investigador)

FIN DEL FORMULARIO DE DATOS PERSONALES E HISTORIA CLÍNICA

MUCHAS GRACIAS

6.3. Annex C: Inclusion and exclusion criteria form

"**Appendix 2**" of the ALASKA Study protocol: https://static-content.springer.com/esm/art%3A10.1186%2Fs13063-024-08307-2/MediaObjects/13063_2024_8307_MOESM4_ESM.pdf

Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, López-Seoane J, Wusterhausen P, Matthias T, Urrialde R, González-Gross M. **The multifactorial approach and the food allergen-specific substitutive diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: Study protocol for a randomized controlled trial- The ALASKA Study**. Trials. 2024 Jul 20;25(1):494. doi: 10.1186/s13063-024-08307-2. PMID: 39033266; PMCID: PMC11264907.

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PARTICIPANT CODE: A L _ _ _ _

RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

ALASKA Study. ALLERGIES AND FOOD INTOLERANCES IN ADULTS AND ATHLETES

APPENDIX 2. INCLUSION AND EXCLUSION CRITERIA FORM

INSTRUCTIONS:
Complete the following Inclusion and Exclusion Criteria Form.

Participant code: _____ (If you haven't receive your participant code, please contact the research staff at l.pantoja@upm.es, marcela.gonzalez.gross@upm.es, or gi.imfine@upm.es and request the unique participant code that you will use throughout the entire study as identification. If possible, memorize this code)

Today's date: _____ (DD/MM/YYYY format)

Are you participating in another research study, simultaneously?:
- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

Are you smoker?:
- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

If your answer is "Yes", then write down the number of cigarettes which you smoke and choose the frequency (/day, /week, /month, or /year):

Number of cigarettes:

_____

Frequency
- ○ (1) per day
- ○ (2) per week
- ○ (3) per month
- ○ (4) per year

Are you taking any type of antibiotics currently?:
- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

You have selected that you are taking some type of antibiotic...
Explain: the name, the concentration, the reason, the medical prescription time and the remaining time to end the antibiotic treatment:

_____ (e.g. Amoxicillin, 500mg, tooth extraction, 15 days, I have 3 days of treatment left)

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Pantoja-Arévalo, L et al.

PARTICIPANT CODE: A L

RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

Have you or have you had a Helicobacter pylori bacteria infection (during last year) (with medical diagnosis)?:

- (1) Yes
- (2) No
- (3) I don't know/ I am not sure

Do you have any type of food allergy (with medical diagnosis)?:

- (1) Yes
- (2) No
- (3) I don't know/ I am not sure

Write down the used method or test for your diagnosis of food allergy:

- (1) Skin prick test
- (2) Blood test
- (3) Feces or urine test
- (4) Other

What other method was it used for your diagnosis of food allergy?:

_____

Write down the allergens to which you tested positive after taking the food allergy test:

_____ (e.g. milk, sulfites.)

Do you have any type of food intolerance or food hypersensitivity (with medical diagnosis)?:

- (1) Yes
- (2) No
- (3) I don't know/ I am not sure

Write down the used method or test for your diagnosis of food intolerance or food hypersensitivity:

- (1) Breath test
- (2) Blood test
- (3) Feces or urine test
- (4) Genetic analysis
- (5) Electrodermal test
- (6) Other

What other method was it used for your diagnosis of food intolerance or food hypersensitivity?:

_____

Write down the allergens to which you tested positive after taking the food intolerance or food hypersensitivity test:

(e.g. lactose, fructose, glucose, etc.)

Do you have coeliac disease (with medical diagnosis)?:

- (1) Yes
- (2) No
- (3) I don't know/ I am not sure

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Write down the used method or test for your diagnosis of coeliac disease:

- ○ (1) Breath test
- ○ (2) Blood test
- ○ (3) Feces or urine test
- ○ (4) Biopsy
- ○ (5) Other

What other method was it used for your diagnosis of coeliac disease?:

_____

Are you taking any type of antidepressant, sleeping pill, or anxiolytic?:

- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

You have selected that you take some type of antidepressant, sleeping pill, or anxiolytic…… Explain: the name, the concentration, the reason, the medical prescription time and the remaining time to end the treatment:

_____ (e.g. lorazepam, 5mg, for sleep, since 2005 and indefinite prescription)

Do you have a job or lifestyle that may potentially interfere with your regular sleep schedule (e.g. shift night, guards, etc.)?:

- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

You have selected that you have a job or lifestyle that potentially interferes with your regular sleep schedule (e.g. night shift, guards, etc.)... Explain: the type of work, your weekday work schedule and weekend work schedule: Type of job:

_____ (e.g. civil guard, nurse, etc.)

Weekday work schedule:

_____ (e.g. 11:30 p.m.-6:30 a.m.)

Weekend work schedule:

_____ (e.g. 9:30 p.m.-4:30 a.m.)

Do you have or have you had some type of eating disorder? (anorexia, bulimia, orthorexia, binge eating, etc.)?:

- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

You have selected that you have had some type of eating disorder (anorexia, bulimia, orthorexia, binge eating, etc.)... Explain: type of ED, year of diagnosis and current treatment:

_____ (e.g. bulimia, diagnosed in 2005, discharged)

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ALASKA Study

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Do you have or have you had any type of major surgery that have involved having been hospitalized for one or several days (during the last 5 years)?:

- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

You have selected that you have or have had any type of major surgery that involves hospitalization of one or several days (during the last 5 years)...
Explain: type of major surgery, year of intervention and current treatment/status:

_____ (e.g. maxillofacial surgery, year 2019, currently fully recovered)

Do you have or have had some type of cancer (during the last 5 years)?:

- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

You have selected that you have or have had some type of cancer (within the last 5 years)...
Explain: type of cancer, year of diagnosis and current treatment:

_____ (e.g. prostate cancer, diagnosed 2020, cancer remission)

Have you or have you had another illness (during the last 5 years)?:

- ○ (1) Yes
- ○ (2) No
- ○ (3) I don't know/ I am not sure

Write, what other type of illness have you had (during the last 5 years):

_____

In general, how many hours of uninterrupted night sleep do you have?:

- ○ (1) 1
- ○ (2) 2
- ○ (3) 3
- ○ (4) 4
- ○ (5) 5
- ○ (6) 6
- ○ (7) 7
- ○ (8) 9
- ○ (9) 9
- ○ (10) 10
- ○ (11) 11
- ○ (12) 12
- ○ (13) more than 12 hours of sleep per night

(number of hours/night)

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ALASKA Study

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PARTICIPANT CODE: A L
RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

How many hours of general night sleep do you have?:

- (1) 1
- (2) 2
- (3) 3
- (4) 4
- (5) 5
- (6) 6
- (7) 7
- (8) 9
- (9) 9
- (10) 10
- (11) 11
- (12) 12
- (13) more than 12 hours of sleep per night

(number of hours/night)

AVAILABILITY AND POSSIBILITIES OF PARTICIPANTS

Do you have time availability to attend 2 venous blood sample extractions now and in 5 to 6 months?

- (1) Yes
- (2) No

Do you have the possibility of fasting for at least 8 hours (for blood sample collection purposes)?
Note: If you have any negative recommendations from your doctor related to fasting or blood sample collection procedures, select "No"

- (1) Yes
- (2) No

Are you able to dedicate 30 minutes now and in 5 to 6 months to complete online questionnaires related to this study?

- (1) Yes
- (2) No

POLITÉCNICA

PARTICIPANT CODE: A L

RESEARCHERS: _____
(INITIALS OF THE RESEARCHERS)

INEF

END OF THE INCLUSION AND EXCLUSION CRITERIA FORM

THANK YOU VERY MUCH

HOW TO CITE THIS DOCUMENT:
Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, *et al*. The multifactorial approach and the Food-Allergen Specific Substitutive Diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: Study Protocol for a Randomized Controlled Trial. The ALASKA study. Appendix 2 Inclusion and exclusion criteria form. *Trials*. 2024.

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ALASKA Study

6.4. **Annex D: Blood bank registration sheet**

This annex is displayed in Spanish language, which was the originally used language during the ALASKA Study project.



ALASKA Study
Registro Seroteca

Proyecto: ☐ ALASKA Study ☐ EXERNET ☐ HELENA ☐ otro_____
Investigador:_____
Investigador responsable:_____
Seleccione lugar: ☐ Laboratorio de Bioquímica ☐ CTB ☐ AEPSAD ☐ otro_____
Tº de congelador: ☐ -80ºC ☐ -20ºC ☐ 4ºC ☐ otro_____
Número o nombre del congelador: _____
Ubicación dentro del congelador:_____
Número de caja:_____
ml de las alícuotas en esta caja: ☐ 1ml ☐ 0.5ml ☐ varios. ☐ otro_____

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

6.5. Annex E: Article 1

Title: "Association between food-specific Immunoglobulin G_4 antibodies in adults with self-reported signs and symptoms attributed to adverse reactions to foodstuffs".

Reference: [27]

(Published) Pantoja-Arévalo L, Gesteiro E, Matthias T, Urrialde R, González-Gross M. **Association between food-specific Immunoglobulin G_4 antibodies in adults with self-reported signs and symptoms attributed to adverse reactions to foodstuffs**. *Biomedicines*. 2023 Mar 17;11(12):3335. doi: 10.3390/biomedicines11123335.

biomedicines

MDPI

Article

Association between Food-Specific Immunoglobulin G_4 Antibodies in Adults with Self-Reported Signs and Symptoms Attributed to Adverse Reactions to Foodstuffs

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Abstract: Signs and symptoms attributed to adverse reactions to foodstuffs (ARFS) need tools for research and evaluation in clinical practice. The objectives of this study were (a) to evaluate the most frequent self-reported signs and symptoms attributed to ARFS in Spanish adults, (b) to determine the prevalence of food-specific IgG_4 antibody reactions (AbRs), and (c) to investigate the association between self-reported ARFS symptomatology and food-specific IgG_4 AbRs. Food-specific IgG_4 AbRs against 57 common food and beverages (AESKUCARE-T2FA® in vitro point-of-care test kit, Aesku.Diagnostics GmbH, Germany) were determined in capillary blood samples of 205 volunteers living in the Region of Madrid (Spain). The most frequent self-reported signs and symptoms were related to skin (43%), digestive (41%), and nervous system (NS, 33%) problems. The prevalence of food-specific IgG_4 AbRs was cow's milk (73%), sheep's milk (70%), casein (66%), and goat's milk (56.10%). Positive IgG_4 AbRs against tomato had a profile consisting of 3/4 of skin problems, more than half of digestive, and 2/5 of NS self-reported signs and symptoms. In conclusion, at least 1/3 of the studied sample reported skin, digestive, and NS signs and symptoms. The most frequent food-specific IgG_4 AbRs were related to dairy. Skin problems were more frequent in positive tomato IgG_4 AbRs.

Keywords: adverse effects; allergens; antibodies; food hypersensitivity; immunoglobulin G; point-of-care testing; signs and symptoms

check for updates

Citation: Pantoja-Arévalo, L.; Gesteiro, E.; Matthias, T.; Urrialde, R.; González-Gross, M. Association between Food-Specific Immunoglobulin G_4 Antibodies in Adults with Self-Reported Signs and Symptoms Attributed to Adverse Reactions to Foodstuffs. *Biomedicines* **2023**, *11*, 3335. https://doi.org/10.3390/biomedicines11123335

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1. Introduction

Immune reactions to food-specific allergens can be indicative of adverse reactions to foodstuffs (ARFS), a significant cause of morbidity worldwide and a considerable burden on current public health in both developed countries and emerging economies [1–3]. ARFS refers to any abnormal reaction to the ingestion of food products and their byproducts (e.g., casein, lactose). These food reactions include, among others, food allergy (FA) and food intolerance (FI) reactions [4]. The scarcity of precise data makes it difficult to estimate the prevalence of FA and FI among the world population. Some studies have indicated 2%, raising to more than 30%, respectively [5–7]. Different diagnostic tools have been used over the years: immunology-based tests and other clinical or blood tests, skin prick tests (SPTs), self-reported questionnaires, and double-blind placebo-controlled food challenges

(DBPCFCs), which are currently considered the gold standard method to determine FA and FI [8].

Food antigens can enhance gastric acid secretion and alter the gastrointestinal (GI) mucosa, which subsequently increases its permeability to food antigens. The production of large quantities (>3.4 kU_A/L) of positive food-specific immunoglobulin E (IgE) antibodies may not be sufficient for a good model of GI allergy, as certain IgG subclasses (IgG_4 in humans) can also bind to and activate mast cells [9]. Clinical observations have suggested that food-specific IgG antibody subclasses involved in type III reactions may initiate some ARFS, permeability, and chronic intestinal inflammations [10]. Meanwhile, some other studies suggest that IgG_4 could be related to improving the prediction of DBPCFC outcomes [5].

Signs and symptoms of FA can start within minutes of exposure (commonly type I or IgE-mediated responses) to a trigger food and any combination of local oral, dermatological, GI, or respiratory signs and symptoms can occur [6]. Meanwhile, the symptomatology of some FI (IgG, IgG_4, or other classes and non-immune-mediated responses) cannot be easily explained by the currently understood biological processes, except for fructose and lactose intolerance, which have been correlated with signs and symptoms of different functional GI disorders (FGIDs) [6,11]. IgG_4-mediated responses have been associated with a wide range of specific and non-specific symptomatology [12] and implicated in signs and symptoms related to allergies such as rashes, urticaria, and asthma, but also signs and symptoms of the gastrointestinal tract (mostly suggestive of irritable bowel syndrome (IBS), including abdominal cramps, diarrhea, and constipation [12]. Manifestations such as migraines, chronic fatigue, and hair loss have also been reported [12,13]. Therefore, a detailed study of the most frequent self-reported signs and symptoms attributed to ARFS and the association with IgG_4 antibody reactions (AbRs) may contribute and provide a wider vision of the role of IgG_4.

Today, it is recommended to analyze an open panel of food and beverages' allergens and allergen mix in the Mediterranean zone. A continuous change in dietary patterns toward a more Western diet has been observed in Mediterranean areas. Due to the current globalization of crops, there are Mediterranean areas in which typical foods from other regions are grown, and which are also part of the current frequent consumption of the Mediterranean diet (MD). As a consequence, the influence of other cultures (Asian, e.g., soja, kiwi; African, e.g., pineapple; and American, e.g., chocolate) has been responsible for some of the recent changes in the food intake in the Spanish population when choosing food [14,15].

The aims of this study were (a) to evaluate the most frequent self-reported signs and symptoms attributed to ARFS in Spanish adults, (b) to determine the prevalence of food-specific IgG_4 antibody reactions (AbRs), and (c) to investigate the association between self-reported ARFS symptomatology and food-specific IgG_4 AbRs.

2. Materials and Methods

This is a cross-sectional descriptive study conducted from October 2017 to October 2019 in point-of-care (POC) health centers, such as medical health centers and pharmacies in the city of Madrid and surrounding villages of the Region of Madrid (Spain). Informed consent was obtained from all subjects involved in the study. All subjects were informed about the goals of the study and agreed that their clinical data could be used for research purposes [16,17]. Data were managed using a participant code to preserve the anonymity of all subjects. This study was carried out according to the principles of the Declaration of Helsinki and following the Spanish and European regulations regarding data protection [18]. The protocol has been approved by the Ethics Committee of the Universidad Politécnica de Madrid (reference number 20200602) and registered on ClinicalTrials.gov (Clinical Trials ID NCT05681975 and protocol ID 1720IL0389).

Subjects were enrolled in their nearest POC health center during working hours from 9 a.m. to 9 p.m. The inclusion criteria were adults (>18 years old) who presented self-

reported signs and symptoms related to ARFS. The exclusion criteria were adults with previous medical diagnoses of a positive FA and/or FI, adults receiving antibiotic treatment, and those living outside of the region of Madrid, Spain.

Subjects registered their self-reported signs and symptoms through a generally adapted anamnesis form, which included a list of the main signs and symptoms of a FA recommended in the 'Guidelines for the use and interpretation of diagnostic methods in adult food allergy' [19]. A blank space was also provided for participants to register any additional signs or symptoms not included in the provided list. Self-reported signs and symptoms were classified into the 3 most reported categories: skin and subcutaneous tissue (or dermatological, CIE-10: L00-L99), digestive (or GI, CIE-10: K00-K93), and nervous system (NS, CIE-10: G00-G99) problems using the ICD-10 or CIE-10 classification to establish the human body systems involved in the studied sample [20].

Food-specific IgG_4 AbRs against 57 common food antigens of the current Spanish eating patterns were analyzed in 50 µL of capillary blood obtained from the fingertip using a 7157 safety lancet (HTL-Strefa Inc., Lodz, Poland). Subjects were asked to avoid all 57 foodstuffs from the complete panel for a minimum of 24 to 72 h before the blood sampling. Immunoassay kits, previously NutriSMART® and currently commercialized under AESKUCARE-T2FA® in vitro diagnostic POC test kits (Aesku.Diagnostics GmbH, Wendelsheim, Germany), were used with 3 semiquantitative levels of detection of IgG_4 Ab (levels 1, 2, and 3 being low, moderate, and high levels, respectively). All levels were compared to the standard control of the manufacturer (IgG_4). IgG_4 values ranged from 0.08 to 1259.7 U/mL; values higher than 3.50 U/mL were considered higher than the standard control; 1 U = 1.47 ng. Food-specific IgG_4 AbRs were considered as Level 1 (low): equal or below the standard control of (IgG_4); Level 2 (moderate): slightly above the standard control of (IgG_4); and Level 3 (high): above the standard control of (IgG_4). According to the manufacturer, for food-specific IgG_4 AbR determinations using the AESKUCARE-T2FA® test kit, it is not recommended to consider strictly defined positive population limits, as it is currently established for IgE AbR levels, and because IgG_4 is a biomarker that is still under research when related to food and beverages' allergens. Thus, for this study, a positive population response was considered when Levels (2 + 3) were summed up equal to or greater than 50% of the prevalence of the same food, beverage, or food allergen mix (≥50%). Individual positive responses were Level 3 of IgG_4 AbRs.

The 57 studied food antigens were classified into 40 different wells of the AESKUCARE-T2FA® kit and 40 variables were considered: wheat, rye, barley, oat, grain mix A (buckwheat/amaranth/quinoa mix), grain mix B (corn/rice mix), gluten, peanut, hazelnut, almond, banana, fruit mix A (lemon/orange mix), fruit mix B (strawberry/grape/peach mix), apple, pineapple, kiwi fruit, egg white, egg yolk, casein, cow's milk, goat's milk, sheep's milk, cod, fish mix (salmon/trout mix), tuna, seafood mix (shrimp/squid/octopus mix), tomato, legume mix (peas/green beans mix), vegetable mix A (carrot/celery mix), vegetable mix B (cabbage/broccoli mix), tuber mix (garlic/onion/leek mix), lamb or mutton, meat mix A (pork/beef mix), meat mix B (chicken/turkey mix), potato, soy, yeast mix (baker's/brewer's yeast mix), cocoa, coffee, and mustard.

Body composition was measured using a bioelectrical impedance analysis (BIA) scale, Renpho ES-26M-W (RENPHO, Hong Kong, China), and height was assessed using a standardized stadiometer, InBody BSM170, 35–210 m measure range (InBody Co. Ltd., Seoul, Korea). Self-reported PA was assessed using a modified and adapted version of the International Physical Activity Questionnaire Short Form (IPAQ-SF) and categorized according to the recommendations of the World Health Organization (WHO) [21] and the American College of Sports Medicine (ACSM) guidelines [22,23].

Statistical analysis was performed using SPSS Statistics software (version 25.0, IBM Corp., Armonk, NY, USA). All variables followed the non-normal distribution. A descriptive analysis was performed. Quantitative variables were expressed as mean (M) and standard deviation (SD) and qualitative variables as a percentage (%). Food-specific IgG_4 AbR levels for 57 different allergens were analyzed as 40 variables, including the

allergen mix variables, which were analyzed as one single variable, e.g., legume mix (peas/green beans), fish mix (shrimp, squid, and octopus), etc. The Spearman rank correlation coefficient (rho) was considered to obtain the correlation and association between the food-specific IgG_4 AbR variables with symptomatology, number of signs and symptoms, body composition, and PA. The non-parametric Kruskal–Wallis test was used to establish if symptomatology was different towards different levels of BMI (underweight, normal weight, overweight, class I obesity, class II obesity, and class III obesity), and also to measure the differences between the 17 most prevalent food-specific IgG_4 AbRs and the studied age ranges (20–34, 35–49, 50–64, and 65–79-year-old groups). Cut points were established according to quartiles to determine associations with food and beverages' allergens and their level of IgG_4 AbRs. The non-parametric Mann–Whitney U test was used for the analysis of the effect of food-specific IgG_4 AbRs and the number of signs and symptoms over sex. The eta-squared index (η^2) was used as the effect size and was classified according to Cohen [24] as small significant effect sizes from $\geq$0.01 to <0.06; medium and significant, from $\geq$0.06 to <0.14; and large and significant, from $\geq$0.14. A Chi-squared test was used to analyze whether the clinical profile expressed in the self-reported symptomatology was independent of the level of food-specific IgG_4 AbRs following the statistical guidelines of the Life Cycle Committee [25]. The level of statistical significance was set at 0.05.

3. Results

Two-hundred eleven individuals volunteered for this study; three subjects from outside the Region of Madrid and three subjects under antibiotic treatment were excluded. A total sample of 205 Spanish adults was analyzed: 143 women and 62 men with a mean (±SD) age of 45.5 years old (±14.9 y.o., from 20 to 79 y.o.).

3.1. Body Composition

Descriptive data from the sample are shown in Table 1. More than half of the participants were over normal weight (56.59%) and did not meet the WHO recommendations for physical activity (PA) (52.7%).

Table 1. Sample characteristics.

	M ± SD or %			
	Total (n = 205)	**Men (n = 62)**	**Women (n = 143)**	**Min–Max**
Age	45.46 ± 14.91	47.45 ± 13.27	44.59 ± 15.53	20–79
Body composition				
Height (m)	1.67 ± 0.09	1.76 ± 0.06	1.63 ± 0.07	1.50–1.92
Weight (kg)	73.39 ± 16.12	82.92 ± 15.63	69.26 ± 14.54	42.30–130.60
Fat-free mass (kg)	47.72 ± 10.12	52.93 ± 10.65	45.46 ± 9.03	30.71–74.75
Muscular mass (%)	59.28 ± 12.20	56.96 ± 13.29	60.28 ± 11.60	26.91–97.12
Bone mass (kg)	2.77 ± 0.76	3.08 ± 1.23	2.63 ± 0.34	1.90–12.11
Fat mass (%)	33.17 ± 9.07	32.46 ± 9.43	33.47 ± 8.92	10.80–60.30
Body mass index	26.32 ± 4.99	28.05 ± 4.93	25.56 ± 4.84	16.90–43.30
Body mass index				
<18.5 underweight (%)	1.95	0	2.80	--
18.5–24.9 normal weight (%)	41.46	29.03	46.85	--
25.0–29.9 overweight (%)	31.71	30.65	32.17	--
30.0–34.9 class I obesity (%)	20.00	30.65	15.38	--
35.0–39.9 class II obesity (%)	4.39	9.68	2.10	--
>40 class III obesity (%)	0.49	0	0.70	--
Physical activity (IPAQ-SF [1])				
Low (%)	87.30	95.20	83.90	--

Table 1. *Cont.*

	M ± SD or %			
	Total (n = 205)	**Men (n = 62)**	**Women (n = 143)**	**Min–Max**
Moderate (%)	12.70	4.80	16.10	--
High (%)	0	0	0	--
Sitting time (min/week)	2577.37 ± 943.82	2499.68 ± 978.15	2611.05 ± 930.03	1260–5040
Sitting time (h/week)	42.96 ± 15.73	41.66 ± 16.30	43.52 ± 15.50	21–84
Symptomatology				
Dermatological (%)	43.27	37.40	45.50	--
Digestive or GI [2] (%)	40.74	47.90	38.60	--
NS [3] (%)	33.33	52.40	34.50	--

[1] IPAQ-SF: International Physical Activity Questionnaire Short Form; [2] GI: gastrointestinal; [3] NS: nervous system.

3.2. Signs and Symptoms

The most frequent self-reported signs and symptoms attributed to ARFS in the studied sample were skin and subcutaneous tissue (43.27%), digestive (40.74%), and those related to NS (33.33%) problems. When comparing these signs and symptoms by sex (Table 1), in women, the most common were skin and subcutaneous tissue problems (eczema, psoriasis, dermatitis, and/or acne), followed by digestive self-reported signs and symptoms (abdominal bloating, heartburn, nausea, and/or gastritis), followed by NS problems (body pain and heaviness (back, legs, arms, lower back, facial, jaw and/or joint)). In contrast, the most frequent self-reported signs and symptoms in men were NS problems (depression, anxiety, fatigue, and/or lack of energy), followed by digestive self-reported signs and symptoms (abdominal bloating, heartburn, nausea, and/or gastritis), and lastly, skin and subcutaneous tissue problems (eczema, psoriasis, dermatitis and/or acne).

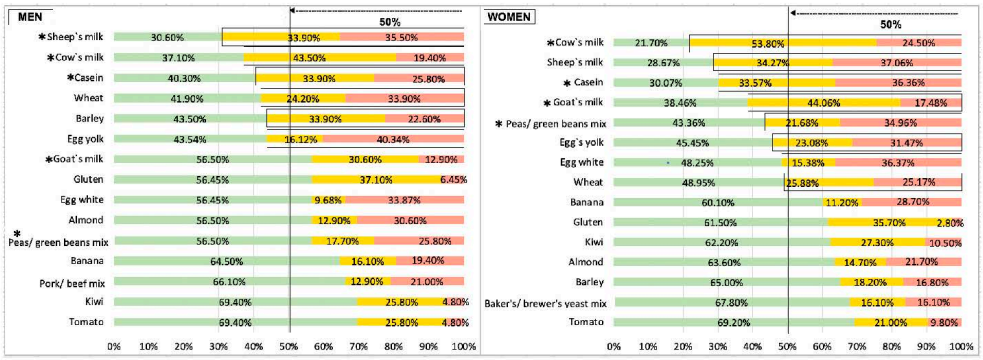
Significant differences were observed in the frequency of the reported subcategories of signs and symptoms according to BMI values. There was a significantly higher frequency of subjects who reported a mixture of two or more subcategories of skin and subcutaneous tissue problems and one respiratory symptom (eczema, dermatitis, acne, psoriasis, atopic skin, dry skin, skin spots, itching, tickly, burning throat, back, legs, nose, eyes, arms, and/or coughing) when the BMI was 30 to 34.9 (class I obesity) (25%) than when the BMI was 18.5 to 24.9 (normal weight) (20.8%) (χ^2 (4) = 41.11; $p < 0.001$; $\eta^2 = 0.93$). Class II obesity subjects reported mostly a single category (and not a mixture) of skin and subcutaneous tissue (eczema, dermatitis, acne, psoriasis, atopic skin, dry skin) (66.7%); making it not suitable enough to merge class I and class II obesity for statistical analysis. Similarly, there was a significantly higher frequency of subjects who reported signs and symptoms of the subcategory of abdominal boating and/or abdominal pain (18%) in overweight than in subjects of normal weight (15.4%) (χ^2 (4) = 14; $p = 0.007$; $\eta^2 = 0.58$). Furthermore, there was a significantly higher frequency of subjects who reported signs and symptoms of the subcategory of pain and heaviness (back, legs, arms, lower back, facial, jaw, and/or joint) (37.5%) in overweight than in subjects of normal weight (28.3%) (χ^2 (5) = 24.49; $p < 0.001$; $\eta^2 = 0.72$).

Regarding the different reported 'number of signs and symptoms' in men and women, 22% of women reported a mean of four self-reported signs and symptoms versus 14.5% of men. A total of 21% of men reported a mean of one self-reported sign or symptom.

Additionally, signs and symptoms of four different groups of age ranges were analyzed (20–34, 35–49, 50–64, and 65–79-year-old groups) and significant differences were found between age ranges and NS problems. The 50–64-year-old group reported mostly pain and heaviness (back, legs, arms, lower back, facial, jaw, and/or joint). Meanwhile, the 20–34-year-old group mostly reported migraines, dizziness, headache, and/or stress (χ^2 (5) = 17.40; $p = 0.004$; $\eta^2 = 0.17$).

3.3. Food-Specific Immunoglobulin G_4 Antibody Reactions

The most prevalent food-specific IgG_4 AbRs are shown in Figure 1. The positive population response (≥50%, equal or greater than 50% of levels 2 + 3 IgG_4 AbRs) in men was sheep's milk (69.40%), cow's milk (62.90%), casein (59.70%), wheat (58.10%), barley (56.50%), and egg yolk (56.46%). However, in women, the positive population response was cow's milk (78.30%), sheep's milk (71.33%), casein (69.93%), goat's milk (61.54%), legume mix (peas/green beans mix) (56.64%), egg yolk (54.55%), egg white (51.75%), and wheat (51.05%). The positive response had more food, beverages, and allergen mix involved in women than in men, eight vs. six foodstuffs.



(a) (b)

Figure 1. Food-specific IgG_4 AbRs in Spanish men and women. (**a**) Prevalence of food-specific IgG_4 AbRs in men, (**b**) prevalence of food-specific IgG_4 AbRs in women. Green color: Level 1, low, food-specific IgG_4 AbRs. Yellow color: Level 2, moderate, food-specific IgG_4 AbRs. Red color: Level 3, high, food-specific IgG_4 AbRs. * $p < 0.05$.

When comparing sex in Level 3 of IgG_4 AbRs, the following were significantly higher for women than men: casein ($Z = 1.68$; $p = 0.047$; $\eta^2 = 0.014$); cow's milk ($Z = 1.94$; $p = 0.026$; $\eta^2 = 0.018$); goat´s milk ($Z = 2.18$; $p = 0.015$; $\eta^2 = 0.023$); and legume mix (peas/green beans mix) ($Z = 1.68$; $p = 0.046$; $\eta^2 = 0.014$). Similarly, when comparing sex along Level 2 of IgG_4 AbRs, goat's milk IgG_4 AbRs was also significantly higher for women than men ($Z = 2.18$; $p = 0.029$; $\eta^2 = 0.023$) (Figure 1).

There were some foodstuffs that directly correlated with each other. As expected, gluten allergen correlated with the level of IgG_4 AbRs against wheat, rye, barley (all $p < 0.001$), and oats ($p = 0.037$). The variable 'number of signs and symptoms' had different correlations with food-specific IgG_4 AbRs: positive correlations were found with grain mix A (buckwheat/amaranth/quinoa mix) ($p = 0.035$), as well as, with soy ($p = 0.007$) and pineapple ($p = 0.04$), but a negative correlation was found with fruit mix A (lemon/orange mix) ($p = 0.007$).

Multiple comparison tests (MCTs) were performed and revealed that the level of IgG_4 AbRs against pineapple was higher (Levels 2 and 3) when subjects presented from 3 to 5 self-reported 'signs and symptoms' related to skin and subcutaneous tissue, digestive, or NS problems (all $p = 0.036$). For the rest of the possible comparisons, no significant differences were found ($p > 0.05$).

3.3.1. Food-Specific IgG_4 AbRs and Age

Food-specific IgG_4 AbRs between the four studied age range groups are shown in Table 2. Seventeen most prevalent foodstuff IgG_4 AbRs were analyzed. When evaluating

Level 3 positive responses, subjects belonging to the 20–34-year-old group showed a higher prevalence of casein IgG_4 AbRs than subjects from all the other age range groups. Furthermore, significant differences were found between subjects from the 20–34 and the 35–49-year-old group (χ^2 (3) = 12.99; p = 0.005; η^2 = 0.06). Similarly, subjects from the 35–49-year-old group had a higher prevalence of egg white IgG_4 AbRs compared to the subjects from all the other age range groups, but significant differences were found between subjects from the 35–49 and the 50–64-year-old group (χ^2 (3) = 8.32; p = 0.040; η^2 = 0.04).

3.3.2. Food-Specific IgG_4 AbRs and Symptomatology

Figure 2 shows the frequency of self-reported signs and symptoms of subjects when presenting positive Level 3 of food-specific IgG_4 AbRs against the most prevalent foodstuffs in the studied sample.

Skin and subcutaneous tissue self-reported signs and symptoms (eczema, dermatitis, acne, psoriasis, atopic skin, and/or dry skin) were the most frequent when subjects had Level 3 of food-specific IgG_4 AbRs against tomato (75%), almond (56%), egg white (55%), and egg yolk (54.10%). Likewise, all digestive self-reported signs and symptoms (abdominal bloating, and/or abdominal pain, acidity and/or burning sensation, nausea, vomiting and/or reflux, gas, and/or gastritis) were the most frequent when subjects had Level 3 of food-specific IgG_4 AbRs against tomato (55.60%), egg yolk (44.40%), and meat mix A (pork/beef mix, 44.40%). Finally, NS self-reported signs and symptoms (anxiety, fatigue, depression, tiredness, and/or sleepiness) were the most frequent when subjects had Level 3 of food-specific IgG_4 AbRs against tomato (40%), followed by pain and heaviness (back, legs, arms, lower back, facial, jaw, and/or joint) frequent signs and symptoms when subjects had Level 3 of food-specific IgG_4 AbRs against legume mix (peas/green beans mix) (38.20%).

Positive (Level 3) food-specific IgG_4 AbRs against barley had a symptomatology profile consisting of mostly 50% of skin and subcutaneous tissue self-reported signs and symptoms (eczema, dermatitis, acne, psoriasis, atopic skin, and/or dry skin); 35.50% of digestive signs and symptoms (abdominal bloating and/or abdominal pain, acidity, and/or burning sensation, nausea, vomiting and/or reflux, gas, and/or gastritis over the other types of GI signs and symptoms); and 38.90% of NS self-reported signs and symptoms (pain and heaviness (back, legs, arms, lower back, facial, jaw, and/or joint)).

Positive (Level 3) food-specific IgG_4 AbRs against tomato had a symptomatology profile covering mostly 75% of skin and subcutaneous tissue (eczema, dermatitis, acne, psoriasis, atopic skin, and/or dry skin); 55.60% of digestive (abdominal bloating, and/or abdominal pain, acidity, and/or burning sensation, nausea, vomiting, and/or reflux, gas and/or gastritis); and 40% of NS self-reported signs and symptoms (anxiety, fatigue, depression, tiredness, and/or sleepiness).

The skin and subcutaneous tissue, and the NS self-reported signs and symptoms explained 25% of the variance of total IgG_4 Ab reaction count. However, when the age range variable was adjusted as a covariate, both symptomatology variables explained 30% of the variance of the total IgG_4 Ab reaction count. Significance improved from 0.239 to 0.149 but no statistical differences were found between symptomatology and the total IgG_4 Ab reaction count.

Table 2. Food-specific IgG_4 AbRs levels in Spanish adults of different age range groups.

Distribution of Food-Specific IgG_4 AbRs in Men and Women in Different Age Range Groups										
Age range (20–34)										
	Total				**Men**			**Women**		
	***n* = 57**				***n* = 13**			***n* = 44**		
Level of IgG_4 AbRs	**1**	**2**	**3**	*p* Values	**1**	**2**	**3**	**1**	**2**	**3**
Casein	24.60%	24.60%	50.80%	NS	15.40%	30.80%	53.80%	27.30%	22.70%	50.00%
Sheep's milk	19.30%	33.30%	47.40%	NS	0.00%	46.20%	53.80%	25.00%	29.50%	45.50%
Egg white	52.60%	15.80%	31.60%	NS	53.80%	23.10%	23.10%	52.30%	13.60%	34.10%
Peas/green beans mix	45.60%	22.80%	31.60%	NS	53.80%	23.10%	23.10%	43.20%	22.70%	34.10%
Wheat	45.60%	22.80%	31.60%	NS	23.00%	38.50%	38.50%	52.30%	18.20%	29.50%
Banana	59.60%	12.30%	28.10%	NS	69.20%	7.70%	23.10%	56.90%	13.60%	29.50%
Cow's milk	15.80%	56.10%	28.10%	NS	7.70%	61.50%	30.80%	18.20%	54.50%	27.30%
Egg yolk	43.90%	29.80%	26.30%	NS	30.70%	38.50%	30.80%	47.70%	27.30%	25.00%
Pork/beef mix	61.40%	14.00%	24.60%	NS	61.50%	0.00%	38.50%	61.40%	18.20%	20.50%
Lamb or mutton	68.40%	8.80%	22.80%	NS	61.50%	7.70%	30.80%	70.50%	9.10%	20.50%
Almond	64.90%	15.80%	19.30%	NS	76.90%	7.70%	15.40%	61.40%	18.10%	20.50%
Barley	57.90% a	24.60% b	17.50% ab	0.06	23.00%	46.20%	30.80%	68.20%	18.20%	13.60%
Goat's milk	36.80%	49.20%	14.00%	NS	38.50%	53.80%	7.70%	36.40%	47.70%	15.90%
Baker's/brewer's yeast mix	73.70%	15.80%	10.50%	NS	84.60%	0.00%	15.40%	70.50%	20.50%	9.00%
Tomato	73.70%	19.30%	7.00%	NS	53.80%	38.50%	7.70%	79.60%	13.60%	6.80%
Kiwi	77.20%	17.50%	5.30%	NS	84.60%	15.40%	0.00%	75.00%	18.20%	6.80%
Gluten	57.90%	40.40%	1.70%	NS	38.50%	61.50%	0.00%	63.60%	34.10%	2.30%
Age range (35–49)										
	Total				**Men**			**Women**		
	***n* = 67**				***n* = 22**			***n* = 45**		
Level of IgG_4 AbRs	**1**	**2**	**3**	*p* Values	**1**	**2**	**3**	**1**	**2**	**3**
Casein	35.80%	43.30%	20.90%	NS	45.50%	40.90%	13.60%	31.20%	44.40%	24.40%
Sheep's milk	44.80%	26.90%	28.30%	NS	50.00%	31.80%	18.20%	42.20%	24.50%	33.30%
Egg white	38.80%	11.90%	49.30%	NS	40.90%	9.10%	50.00%	37.80%	13.30%	48.90%
Peas/green beans mix	53.70%	19.40%	26.90%	NS	68.20%	18.20%	13.60%	46.70%	20.00%	33.30%
Wheat	43.30%	31.30%	25.40%	NS	36.40%	22.70%	40.90%	46.70%	35.50%	17.80%
Banana	56.70%	16.40%	26.90%	NS	72.70%	9.10%	18.20%	48.90%	20.00%	31.10%

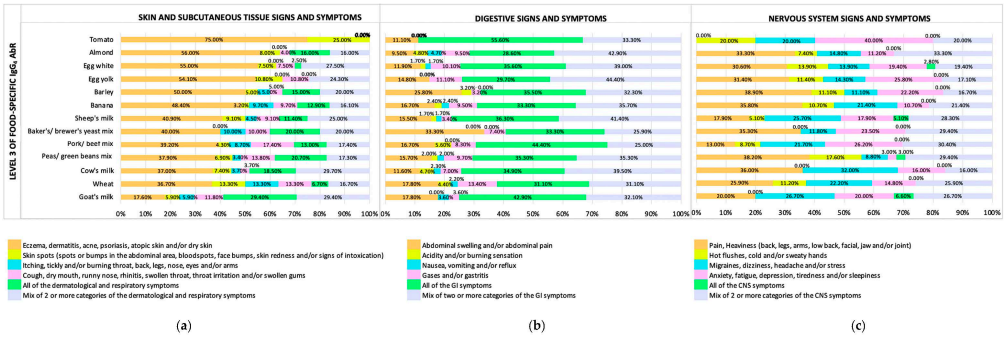
Table 2. *Cont.*

Distribution of Food-Specific IgG_4 AbRs in Men and Women in Different Age Range Groups										
Cow's milk	29.90%	47.80%	22.30%	NS	40.90%	50.00%	9.10%	24.40%	46.70%	28.90%
Egg yolk	34.30%	17.90%	47.80%	NS	31.80%	4.60%	63.60%	35.60%	24.40%	40.00%
Pork/beef mix	76.10%	7.50%	16.40%	NS	77.30%	9.10%	13.60%	75.60%	6.70%	17.70%
Lamb or mutton	80.60%	6.00%	13.40%	NS	86.40%	4.50%	9.10%	77.80%	6.70%	15.50%
Almond	65.70%	11.90%	22.40%	NS	72.70%	4.50%	22.70%	62.20%	15.60%	22.20%
Barley	49.20%	23.90%	26.90%	NS	45.50%	27.30%	27.20%	51.10%	22.20%	26.70%
Goat's milk	55.20%	26.90%	17.90%	NS	68.20%	22.70%	9.10%	48.90%	28.90%	22.20%
Baker's/brewer's yeast mix	71.60% a	11.90% ab	16.50% b	0.01	90.90%	9.10%	0.00%	62.30%	13.30%	24.40%
Tomato	65.70%	23.90%	10.40%	NS	72.70%	18.20%	9.10%	62.20%	26.70%	11.10%
Kiwi	64.10% a	29.90% b	6.00% ab	0.001	90.90%	9.10%	0.00%	51.10%	40.00%	8.90%
Gluten	61.20%	34.30%	4.50%	NS	45.50%	45.50%	9.00%	68.90%	28.90%	2.20%
Age range (50–64)										
	Total				**Men**			**Women**		
	***n* = 59**				***n* = 20**			***n* = 39**		
Level of IgG_4 AbRs	**1**	**2**	**3**	*p* Values	**1**	**2**	**3**	**1**	**2**	**3**
Casein	35.60%	32.20%	32.20%	NS	45.00%	30.00%	25.00%	30.80%	33.30%	35.90%
Sheep's milk	25.40%	39.00%	35.60%	NS	40.00%	25.00%	35.00%	17.90%	46.20%	35.90%
Egg white	61.00%	13.60%	25.40%	NS	70.00%	5.00%	25.00%	56.50%	17.90%	25.60%
Peas/green beans mix	44.10%	27.10%	28.80%	NS	50.00%	20.00%	30.00%	41.00%	30.80%	28.20%
Wheat	52.60%	23.70%	23.70%	NS	55.00%	25.00%	20.00%	51.30%	23.10%	25.60%
Banana	67.80%	13.60%	18.60%	NS	60.00%	35.00%	5.00%	71.80%	2.60%	25.60%
Cow's milk	28.80%	50.80%	20.40%	NS	45.00%	35.00%	20.00%	20.50%	59.00%	20.50%
Egg yolk	54.30%	16.90%	28.80%	NS	55.00%	15.00%	30.00%	53.80%	17.90%	28.30%
Pork/beef mix	54.30%	23.70%	22.00%	NS	65.00%	20.00%	15.00%	48.80%	25.60%	25.60%
Lamb or mutton	61.00%	20.40%	18.60%	NS	70.00%	20.00%	10.00%	56.40%	20.50%	23.10%
Almond	57.70%	16.90%	25.40%	NS	40.00%	30.00%	30.00%	66.70%	10.30%	23.00%
Barley	61.00%	27.10%	11.90%	NS	50.00%	40.00%	10.00%	66.70%	20.50%	12.80%
Goat's milk	39.00%	45.80%	15.20%	NS	55.00%	30.00%	15.00%	30.80%	53.80%	15.40%
Baker's/brewer's yeast mix	72.90%	11.90%	15.20%	NS	65.00%	10.00%	25.00%	76.90%	12.80%	10.30%
Tomato	71.20%	20.30%	8.50%	NS	75.00%	25.00%	0.00%	69.30%	17.90%	12.80%
Kiwi	55.90%	28.80%	15.30%	NS	84.60%	15.40%	0.00%	64.20%	17.90%	17.90%
Gluten	57.60%	35.60%	6.80%	NS	75.00%	15.00%	10.00%	48.70%	46.20%	5.10%

Table 2. *Cont.*

Distribution of Food-Specific IgG$_4$ AbRs in Men and Women in Different Age Range Groups										
Age range (65–79)										
	Total				**Men**			**Women**		
	***n* = 22**				***n* = 7**			***n* = 15**		
Level of IgG$_4$ AbRs	**1**	**2**	**3**	*p* Values	**1**	**2**	**3**	**1**	**2**	**3**
Casein	40.90%	31.80%	27.30%	NS	57.10%	28.60%	14.30%	33.30%	33.40%	33.30%
Sheep's milk	18.20%	45.50%	36.30%	NS	0.00%	42.90%	57.10%	26.70%	46.60%	26.70%
Egg white	54.50%	13.70%	31.80%	NS	71.40%	0.00%	28.60%	46.70%	20.00%	33.30%
Peas/green beans mix	40.90%	0.00%	59.10%	NS	42.90%	0.00%	57.10%	40.00%	0.00%	60.00%
Wheat	45.50%	18.20%	36.30%	NS	57.10%	0.00%	42.90%	40.00%	26.70%	33.30%
Banana	63.60%	0.00%	36.40%	NS	42.90%	0.00%	57.10%	73.30%	0.00%	26.70%
Cow's milk	36.30%	45.50%	18.20%	NS	57.10%	14.30%	28.60%	26.70%	60.00%	13.30%
Egg yolk	54.50%	18.20%	27.30%	NS	71.40%	14.30%	14.30%	46.70%	20.00%	33.30%
Pork/beef mix	50.00%	22.70%	27.30%	NS	42.80%	28.60%	28.60%	53.30%	20.00%	26.70%
Lamb or mutton	59.10%	22.70%	18.20%	NS	42.80%	28.60%	28.60%	66.70%	20.00%	13.30%
Almond	50.00% a	9.10% ab	40.90% b	0.009	14.30%	0.00%	85.70%	66.70%	13.30%	20.00%
Barley	81.80%	4.50%	13.70%	NS	57.10%	14.30%	28.60%	93.30%	0.00%	6.70%
Goat's milk	40.90%	40.90%	18.20%	NS	57.10%	14.30%	28.60%	33.30%	53.40%	13.30%
Baker's/brewer's yeast mix	68.20%	13.60%	18.20%	0.036	100.00%	0.00%	0.00%	53.30%	20.00%	26.70%
Tomato	63.70%	31.80%	4.50%	NS	71.40%	28.60%	0.00%	60.00%	33.30%	6.70%
Kiwi	54.50%	36.40%	9.10%	NS	57.10%	28.60%	14.30%	53.30%	40.00%	6.70%
Gluten	68.20%	31.80%	0.00%	NS	71.40%	28.60%	0.00%	66.70%	33.30%	0.00%

Values bearing different letters were significantly different (a ≠ b). NS: not statistically significant.



(**a**) (**b**) (**c**)

Figure 2. Symptomatology profile of subjects with Level 3 of food-specific IgG$_4$ AbRs. (**a**) Skin and subcutaneous tissue profile of subjects with Level 3 of food-specific IgG$_4$ AbRs. (**b**) Digestive profile of subjects with Level 3 of food-specific IgG$_4$ AbRs. (**c**) Nervous system profile of subjects with Level 3 of food-specific IgG$_4$ AbRs.

4. Discussion

ARFS currently represent a growing global health concern. Thus, their attributable signs and symptoms, as well as possible related biomarkers, are of crucial interest for the development of research and evaluation. The study of which foods are causal of specific novel immunoglobulins is challenging since foods can be a trigger, but there are many additional triggers, including other variables, such as infection, presence of other diseases, food frequency consumption, dietary intake, crosslinking reactions with other foodstuffs, or even environmental allergens [26]. In the present study, IgG_4 markers have been investigated as a supporting tool for the current IgE, oral challenges, and other currently used tests for the evaluation and research of ARFS. This marker has been suggested as a complementary marker when measuring IgE, as well as during FA treatment [27]. The present study analyzed a sample of 205 Spanish adults with self-reported signs and symptoms attributed to FA or FI. The most frequent self-reported signs and symptoms were related to skin and subcutaneous tissue (eczema, psoriasis, dermatitis, acne). The skin is the most frequently affected target organ in ARFS and has been reported in the past two decades to be around 40 to 60% for type I allergy (food-specific IgE AbRs) and a generalized eczematous rash or dyshidrosiform reactions of the fingers, palms, and soles for delayed-type reactions (IgG_4 AbRs) [28]. The second most common type of self-reported signs and symptoms in this study was related to the digestive system. Previous research in participants who claimed to have a FI has reported high levels of IgG_4 AbRs with signs and symptoms related to the GI tract, such as abdominal inconvenience, GI discomfort, and other mainly characteristic features of gut permeability and their participation in intestinal inflammatory and metabolic reactions [29]. The last prevalent type of self-reported signs and symptoms of the present study was related to the NS (depression, anxiety, fatigue, and lack of energy). According to a related published research study, [30] participants with suspicion of ARFS have also pointed out lack of energy as one of the most reported signs and symptoms.

The study of the prevalence of food-specific IgG_4 AbRs could contribute to a clearer idea of some delayed-type ARFS and diseases related to immunoglobulin G_4, such as IBS, ulcerative colitis (UC), or Crohn's disease (CD). A European study investigating the presence of food-specific IgG_4 AbRs in participants with IBS has reported a high prevalence of reactions against milk, egg, wheat, beef, pork, and lamb [31], which are among the 17 most prevalent foodstuffs in the present study.

Another study conducted in Saudi Arabia with participants presenting allergy signs and symptoms also determined similar prevalent food-specific IgG AbRs, even though the evaluated AbRs were not specifically Subclass 4 (IgG_4), as in this present study [12]. Among 11 prevalent food-specific IgG AbRs, 6 of them were against similar food allergens: brewer's yeast, wheat, pea, egg white, barley, and cow's milk when compared to the present study. Except for brewer's yeast, prevalence in men was lower for the rest of similar prevalent food-specific IgG AbRs than those found in the present study. However, in women, food-specific IgG AbRs were higher against brewer's yeast, wheat, pea, egg white, and barley, and lower against cow's milk. The fact that the prevalence between women is slightly close, except for brewer's yeast, considering different types of samples and diet but similar symptomatology related to ARFS, supports the promising clinical reproducibility of IgG_4-mediated ARFS symptomatology [12,29,31–33] and reinforces the link between a symptomatology profile with likely specific foodstuffs [5,34,35].

Regarding the variable of age, a Saudi Arabian study was analyzed using 2 groups: 20–39-year-olds and 40–80-year-olds [12], showing that food-specific IgG AbRs against all allergens decreased in prevalence according to age. Compared to the present study, age was analyzed using a wider distribution of ages: 20–34, 35–49, 50–64, and 65–79-year-old groups observing that IgG_4 AbRs against dairy-related allergens such as goat's milk might change depending on the age range, showing a similar prevalence in all age ranges except for the 35–49-year-old group, which had the lowest prevalence. Similarly, for IgG_4 AbRs against egg white, the prevalence was similar in all age ranges, except for the 35–49-year-

old group, which had the highest prevalence. In the present study, the age range that marked the most difference was the 35–49-year-old group and not the youngest age range as in the Saudi Arabian study. Age distribution and the specific characteristics of the test (sensitivity, specificity, antigen concentration, and levels of detection) might be one of the main reasons for this contrast. Using the AESKUCARE-T2FA® POC test kit in the present study, steps such as incubation, washing, and staining were performed using a ready-to-use main reaction plate and reagents, decreasing the risk of practitioner/technician error. Usually, young adults (35–49 y.o.) tend to be the most common age range affected in Europe regarding FA and ARFS-related symptoms [36]. In the older group (65–79-year-old group), there were no cases of moderate Level 2 of IgG_4 AbRs against legume mix (peas/green beans mix) and banana.

Comparably, a South Korean study that evaluated food-specific IgE/IgG_4 AbRs against milk, egg white, wheat, and soybean, explained the importance of allergen-specific IgE/IgG_4 as a tool to investigate the mechanism of FA in atopic dermatitis [32]. The underlying mechanism of ARFS involves a combination of multiple paths, including immediate reactions (type I, IgE mediated) and delayed-type reactions (IgE, IgM, IgA, IgG antibodies and subclasses) [37].

The present study had a defined sample with the body composition characteristics of an individual with self-reported signs and symptoms attributed to ARFS. In other words, subjects with diet and lifestyle struggles usually lead to sedentarism, obesity, inflammation, and even difficulty digesting some of the most common food products and components of the MD diet (e.g., milk, legume, tomato, gluten). Gluten and IgG reactions might be associated with systemic inflammation [34] and contribute to the pathophysiology of IBS [38]. Furthermore, the literature suggests that overweight and obese subjects tend to have a higher frequency of GI comorbidity [39]. Barley, and its components, also appear to play an important role in obesity [40,41].

According to an article on precision medicine in FA [42], no participants with the same FA are expected to show the same reported signs and symptoms. In the case of a similar positive FA test (e.g., food-specific IgE AbRs), the natural history may be different. In another study, wheat sensitivity is mainly attributed only to GI reported signs and symptoms [43]. However, in the present study, it was also related to a dermatological and NS profile.

The close relationship between PA and the immune system is exposed in the present study when analyzing whether participants meet the WHO recommendations for PA [44] and correlating it with the most prevalent food-specific IgG_4 AbRs. Negative associations were observed between the achievement of the minimum WHO requirements of PA and IgG_4 AbRs against barley and between low PA and IgG_4 AbRs against gluten and sheep's milk. The fact that PA might increase gut and colon permeability and inflammation could explain the amount of positive correlations between PA and food-specific IgG_4 AbRs [45].

Legume mix (peas/green beans mix) was the only food allergen missing from the EU list of mandatory declaration of all prevalent allergens in the present study. Peas have been closely related to FA in the Spanish population for several decades [46] and green beans have been associated with frequent signs and symptoms of FA and FI such as urticaria, asthma, or rhinitis [47,48]. European regulatory agencies usually base decisions on the annual reported cases of ARFS, which are mostly type I reactions (anaphylactic or other immediate reactions) [49], leaving out delayed-type reactions that might also affect the Spanish population in terms of skin and subcutaneous tissue, digestive and NS problems.

4.1. Main Findings

There are self-reported signs and symptoms of the skin and subcutaneous tissue, digestive, and NS in a Spanish sample of adults aged 20 to 79 years who attribute their symptomatology to ARFS (FA and FI) and live in the Region of Madrid. Low PA and overweight are common parameters among the studied sample. The most common positive food-specific IgG_4 AbRs among men were mainly related to milk (cow's milk, sheep's milk,

and casein), followed by wheat, barley, and egg yolk. And the most common among women were also mainly related to milk (cow's milk, sheep's milk, goat's milk, and casein); followed by wheat, egg white, egg yolk, and legume mix (peas/green beans mix).

4.2. Strengths and Limitations

This study has been performed using a AESKUCARE-T2FA® in vitro POC and ready-to-use test kit that decreases the technician and practitioners' error during immunological detection practices. Data collection and clinical analysis were performed before the COVID-19 pandemic. This condition benefited the uniformity of the sample. The self-reported signs and symptoms might be less biased avoiding possible post-COVID sequelae that are still being studied. Current evidence reports that individuals who have recovered from COVID-19 may still have persistent signs and symptoms, radiological abnormalities, and compromised respiratory function [50]. Food-specific IgG_4 AbRs have been studied to expand the scientific knowledge of its role in ARFS since there is a current unclear studied path.

As a limitation, data collection started as a regular clinical appointment without research purposes; however, the results were adjusted according to validated scales when the answers allowed it. Furthermore, there is a lack of similar published data using the same food-specific IgG_4 AbRs panel together with symptomatology profiles for a more accurate comparison with the results of the present study.

4.3. Future Research

A strong protocol containing more variables of interest is needed to maximize statistical power based on these preliminary observations. The following validated tools will be used for future ARFS research to assess the subject's diseases, signs, symptoms, and food and beverage intake: Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs (PSIMP-ARFSQ-10); and Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs (FBFC-ARFSQ-18) [51]. Obtained results suggest interesting trends of food-specific IgG_4 AbRs toward body composition, PA, sex, age, and symptomatology profiles that lead to continuing research in the field. Additional health parameters and variables will be considered for future similar research, such as clinical analysis (e.g., hematobiochemical) and food intake evaluations (e.g., food frequency consumption and dietary intake). Follow-up studies using validated tools will be performed to establish a consistent clinical picture of ARFS and more data investigating the role of IgG_4 AbRs in ARFS. Food-specific IgE AbRs evaluations will be also considered for future research due to the tight relationship that they currently have with food allergies.

5. Conclusions

The most frequent self-reported signs and symptoms in the studied Spanish sample of adults with suspicion of ARFS included skin and subcutaneous tissue, digestive, and NS problems. Spanish adults aged 35 to 49 years may be of potential interest regarding the frequency of the symptomatology attributed to ARFS. The higher prevalence of IgG_4 AbRs found in the studied sample was related to milk (cow's milk, goat's milk, sheep's milk, and casein), followed by cereal (wheat and barley), egg (egg white and egg yolk), and legume (peas/green beans mix). Food-specific IgG_4 AbRs are similar among men and women in terms of the type of food and beverage allergen or allergen mix (milk, egg, cereal, and legume mix), but vary in terms of its prevalence, except for legume mix (peas/green beans mix), which is only prevalent in women. Women had positive responses to a greater number of food and beverage allergens/allergen mix than men (eight versus six). Moreover, positive Level 3 of IgG_4 AbRs against tomato had a symptomatology profile consisting of 3/4 of dermatological, more than half of GI, and 2/5 of self-reported signs and symptoms of the NS.

Author Contributions: Conceptualization, L.P.-A., R.U. and M.G.-G.; data curation, L.P.-A. and M.G.-G.; formal analysis, L.P.-A.; funding acquisition, L.P.-A.; investigation, L.P.-A., E.G., T.M., R.U. and M.G.-G.; methodology, L.P.-A., E.G. and T.M.; project administration, L.P.-A. and T.M.; resources, L.P.-A. and T.M.; software, L.P.-A., T.M. and M.G.-G.; supervision, T.M., R.U. and M.G.-G.; visualization, L.P.-A., E.G., T.M., R.U. and M.G.-G.; writing—original draft, L.P.-A.; writing—review and editing, L.P.-A., E.G., T.M., R.U. and M.G.-G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Universidad Politécnica de Madrid (reference number 20200602) and registered on ClinicalTrials.gov (Clinical Trials ID NCT05681975 and protocol ID 1720IL0389) for studies involving humans.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. All subjects were informed about the goals of the study and agreed that their data could be used for research purposes.

Data Availability Statement: The supporting reported dataset of this study can be found in The Multidisciplinary Data Repository of the Madroño Consortium, 'e-cienciaDatos' at (https://doi.org/10.21950/NA8QKC), accessed on 16 October 2023, reference: (10.21950/NA8QKC) [52].

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Conflicts of Interest: T.M. is part of the Aesku.Diagnostics staff. T.M. did not participate in the design of the protocol or in the analysis and interpretation of the data outcomes. The rest of the authors have nothing to report.

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6.6. Annex F: Article 2

Title: "Design and validity of the Spanish version of two questionnaires related to adverse reactions to foodstuffs".

Reference: [34]

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Nutrición Hospitalaria



Trabajo Original

Epidemiología y dietética

Design and validity of the Spanish version of two questionnaires related to adverse reactions to foodstuffs

Diseño y validación de la versión española de dos cuestionarios relacionados con reacciones adversas a alimentos

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Abstract

Introduction: there is an emerging current necessity of valid questionnaires, encompassing most of food, beverages, diseases, signs and symptoms currently related to the pathogenesis of adverse reactions to foodstuffs (ARFS) in the Spanish population.

Objectives: this study aimed to design and validate two questionnaires to assess ARFS in the Spanish population, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs (FBFC-ARFSQ-18); and Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs (PSIMP-ARFSQ-10).

Methods: both questionnaires were designed adapting questionnaires from the literature; and validated, using the expert judgment method, in five phases: questionnaires development, pilot test and reliability, content validity, face validity, and ethical considerations. Questionnaires were developed using the REDCap™ tool hosted at the Universidad Politécnica de Madrid. A total of 20 Spanish experts evaluated the questionnaires. Cronbach's alpha reliability coefficients were calculated using SPSS version 25.0 (IBM Corp., Armonk, NY-USA) and Aiken's V coefficient values were calculated using ICaiken.exe (Visual Basic 6.0, Lima-Perú).

Results: a final construct of questions was designed, ensuring no overlap, for FBFC-ARFSQ-18 and PSIMP-ARFSQ-10. Cronbach's alpha reliability coefficients were 0.93 and 0.94; and Aiken's V coefficient values were 0.90 (0.78-0.96 CI) and 0.93 (0.81-0.98 CI) for FBFC-ARFSQ-18 and PSIMP-ARFSQ-10, respectively.

Conclusions: both validated questionnaires could be used to analyze the association between certain food and beverages consumption with ARFS, such as food allergies and food intolerances; also, to investigate the link between some specific diseases, signs and symptoms with ARFS.

Keywords:

Adverse effects. Disease management. Food and beverages. Reliability and validity. Surveys and questionnaires. Symptom assessment. Validation study.

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Resumen

Introducción: actualmente, existe una necesidad emergente de cuestionarios validados que abarquen la mayor parte de los alimentos, bebidas, enfermedades, signos y síntomas relacionados con la patogénesis de las reacciones adversas a los alimentos (RAA).

Objetivos: diseñar y validar dos cuestionarios para evaluar las RAA en población española, el Cuestionario de Frecuencia de Consumo de Alimentos y Bebidas para Identificar Reacciones Adversas de Origen Alimentario (CFCAB-RAA-18); y el Cuestionario de Patologías y Sintomatología Asociadas a Reacciones Adversas a Alimentos (PSIMP-RAA-10).

Métodos: ambos cuestionarios se diseñaron adaptando cuestionarios de la literatura y se validaron, utilizando el método de juicio de expertos, en cinco fases: desarrollo de cuestionarios, prueba piloto y confiabilidad, validez de contenido, validez aparente y consideraciones éticas. Los cuestionarios se desarrollaron utilizando la herramienta REDCap™. Un total de 20 expertos evaluaron los cuestionarios. Se calcularon coeficientes de confiabilidad alfa de Cronbach con SPSS versión 25.0 (IBM Corp., Armonk, NY-Estados Unidos) y valores del coeficiente V de Aiken con ICaiken.exe (Visual Basic 6.0, Lima-Perú).

Resultados: se diseñó una construcción final de preguntas, evitando solapamiento entre ambas herramientas. Los coeficientes de confiabilidad alfa de Cronbach fueron 0,93 y 0,94, y los valores del coeficiente V de Aiken fueron 0,90 (IC: 0,78-0,96) y 0,93 (IC: 0,81-0,98) (CFCAB-RAA-18 y PSIMP-RAA-10, respectivamente).

Conclusiones: ambos cuestionarios fueron validados y podrían utilizarse para analizar la asociación entre el consumo de determinados alimentos y bebidas con las RAA, como alergias e intolerancias alimentarias, así como para investigar el vínculo entre algunas enfermedades, signos y síntomas específicos con las RAA.

Palabras clave:

Alimentos y bebidas. Cuestionarios. Fiabilidad. Reacciones adversas. Síntomas y signos. Validez.

INTRODUCTION

The global prevalence of adverse reactions to foodstuffs (ARFS), including components of food and beverages allergy (CFBA) and components of food and beverages intolerance (CFBI), in developed countries is around 30 %, with a female dominance in adults of 60 %, representing an emerging public health concern (1,2). CFBA and CFBI can start at any age; in fact, there is an increased reported incidence of new-onset CFBA in adults (one adult for every four children) (1-3). A detailed record of the consumption of any substance that is used as food and beverages or to make food and beverages (foodstuffs) and a clinical history are essential keys for the diagnosis, management, analysis and the study of ARFS (2,4). The consumption of specific foodstuffs can induce a wide range of adverse reactions ranging from abdominal swelling to life-threatening anaphylaxis (5). The relationship of foodstuffs intake, that may cause ARFS, and the analogous diseases and symptomatology make it challenging for researchers and physicians to establish a proper analysis of ARFS.

Several approaches of tools have been launched to identify food and beverages frequency consumption in the adult population with ARFS, focusing on CFBA and celiac disease (CD): Gluten Food Frequency Questionnaire (G-FFQ) (6), the Global Allergy and Asthma European Network (GA2LEN) FFQ (7) and the Pregnancy FFQ (8). Similarly, various tools have been designed to screen a CFBA such as the CFBA Screening Questionnaire (9), the Food Allergy Questionnaire (FAQ) (10), the Allergy Questionnaire for Athletes (AQUA©) (11), the Lahey Health Allergy Questionnaire (12) and the Weill Cornell Medicine Adult Allergy Questionnaire (13). There are also separate tools available to identify specific groups of symptoms by system, such as the Gastrointestinal Symptom Rating Scale (GSRS), the self-reported Skin Complaints Questionnaire (14), the Sensitive Skin Questionnaire (15) or the UCLA Dizziness Questionnaire (UCLA-DQ) (16).

Health professionals can frequently confuse non-adverse reactions to food and beverages with ARFS, especially when using only one of the tools available without considering a group of appropriate questionnaires, or their adaptation, for the population or patient of interest (17). The diagnosis of CFBA can be confirmed with a physical exploration, clinical history, oral food challenge (OFC) or by evidence of sensitization to the culprit using, for example, skin prick test (SPT) or serologic testing measuring food-specific immunoglobulin E (IgE) by a physician. However, it has been highly suggested by previous studies (18,19), specially by the European Academy of Allergy and Clinical Immunology (EAACI) (20), to support any of the clinical tests using adequate questionnaires, to report both, foodstuffs consumption and symptomatology, as the strongest predictors of a probable CFBA (20). Nowadays, food behavior, the expansion and management of diseases and the relationship between them is evolving in developing countries (21,22). There are extensive new foodstuffs consumed in the Mediterranean Spanish area besides those belonging to the Mediterranean diet (MD); diets are becoming highly processed and several fad diets are appearing (22,23). In addition, various pathologies and diseases are currently being investigated for their potential to have a direct or indirect relationship to ARFS: atopic dermatitis (24), irritable bowel syndrome (IBS) (25), and other diseases (26,27). In this sense, to identify all the main food consumption, pathologies and symptomatology that influence ARFS, focusing on CFBA and CFBI characteristics, designing and validating a specific tool should be a priority for an efficient diagnosis, analysis and study of ARFS.

However, there is no consensus or validated questionnaire to measure, in the Spanish population, up-to-date food and beverages consumption, including current available food groups, diets, new foodstuffs and all types of possible diseases, symptoms and signs that may have a current, potential and direct relation to ARFS (not only to CFBA). Therefore, as part of a broader investigation, the objectives of this study were to design and validate, through the expert judgement method, two questionnaires to assess ARFS for the Spanish population: the Food and Beverages Frequency Consumption Questionnaire to identify Adverse Reactions to Foodstuffs (FBFC-ARFSQ-18) (*Cuestionario de Frecuencia de Consumo de Alimentos y Bebidas para Identificar Reacciones Adversas de Origen Alimentario*, CFCAB-RAA-18);

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and the Pathologies and Symptomatology Questionnaire Associated with Adverse Reactions to Foodstuffs (PSIMP-ARFSQ-10) (*Cuestionario de Patologías y Sintomatología Asociadas a Reacciones Adversas a Alimentos*, PSIMP-RAA-10). Both objectives, as a first step to facilitate the identification of a population with a high probability of having ARFS, are under the umbrella of the subsequent ARFS analysis initiative through a follow-up study.

MATERIALS AND METHODS

This study developed and validated the FBFC-ARFSQ-18 and the PSIMP-ARFSQ-10 questionnaires using the expert judgement method over the following phases: a) questionnaires development; b) pilot test and reliability; c) content validity; d) face validity; and e) ethical considerations (Fig. 1).

PHASE 1: QUESTIONNAIRES DEVELOPMENT

Both questionnaires were previously adapted with available tools found in the literature to the particularities of ARFS. The FBFC-ARFSQ-18 questionnaire used a basis-format of the semiquantitative Fernández-Ballart JD et al. FFQ (28) and the International Statistical Classification of Diseases and Related Health Problems (ICD-10) was followed for the PSIMP-ARFSQ-10 questionnaire (29). Tools were designed and structured according to the researchers' insights (authors of this study). Items of FBFC-ARFSQ-18 were intended as food groups and items of PSIMP-ARFSQ-10 were designed as diseases and symptomatology of a human body system. Food groups of the FBFC-ARFSQ-18 consisted in foodstuffs with possible causative food allergens proposed by Lyons et al. (20) (Annex I; https://www.nutricionhospitalaria.org/anexos/04631-01.pdf). Initially, there were 21 items for the FBFC-ARFSQ-18 tool, however, "vegetables, garden vegetables and legume" were consolidated as a single item. Similarly, olive oil was added to item 15 "other food groups", acquiring 18 items. Likewise, there were initially five items as five human body systems as a starting point for the PSIMP-ARFSQ-10 (each item was separated into two categories, "diseases or pathologies" and "symptoms and signs", acquiring ten items in accordance with the researchers' insights). The items of FBFC-ARFSQ-18 were classified into three sections (eating habits; frequency of consumption of food and beverages; and frequency of consumption of food supplements) to identify the eating patterns of interest in diets "free of" or "containing" allergens, components and/or foodstuffs related to ARFS that can possibly cause CFBA or CFBI. The items of PSIMP-ARFSQ-10 followed the ICD-10 classification (29) to stablish the human body systems

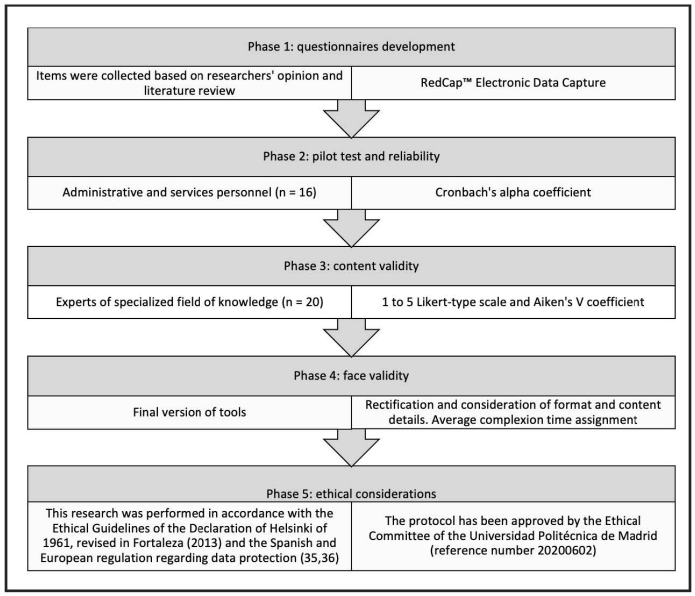


Figure 1.

Flow diagram of the phases involved in the design and validity using the expert judgement method of the FBFC-ARFSQ-18 and PSIMP-ARFSQ-10 questionnaires to assess ARFS.

and the EAACI guidelines (30) for the identification of the specific diseases, signs and symptoms related to ARFS (five sections: digestive system; skin and subcutaneous tissue; nervous system [NS]; respiratory system [RS]; and other human body systems [other diseases/pathologies, symptoms and signs, not elsewhere classified]). Both tools were hosted using RedCap™ (Research Electronic Data Capture), an electronic data capture program specifically designed for research and a secure web platform hosted at the Supercomputing and Visualization Center of Madrid (CESVIMA) of the Universidad Politécnica de Madrid (UPM). RedCap™ was also used to build and manage the online databases for both questionnaires.



Annex I.

PHASE 2: PILOT TEST AND RELIABILITY

Tools were evaluated by a pilot sample with similar characteristics to the target population to obtain qualitative assessments such as the identification of semantic errors, writing and comprehension. Inclusion criteria were: to be Spanish, living in the Region of Madrid, with age over or equal to 18 years (≥ 18 years), with non-scientific qualification (including students and retired) and presenting at least five diseases or subjective symptoms related to ARFS (Table I). Subjects with scientific background were excluded. Sampling was applied to the administrative and service personnel (ASP) of the Faculty of Physical Activity and Sport Sciences (INEF) of the UPM, to ensure evaluations by individuals who were not familiar with science. The complexion time of both questionnaires was recorded and evaluations of the approach of the items to their ARFS were calculated using the Cronbach's alpha reliability coefficient. Values greater than or equal to 0.80 (≥ 0.80) were considered as acceptable. Statistical analysis was carried out using SPSS Statistics software version 25.0 (31). At the end of the pilot test, the revised version of the tool was then reviewed by the expert judges for validation.

PHASE 3: CONTENT VALIDITY

Experts were recruited from different national research groups, hospitals, scientific institutions and universities using the contacts of the research group conducting this study (academics and practitioners of the intended field of knowledge). The inclusion criteria for being considered as an expert were: with age over or equal to 35 years old (≥ 35 years); over or equal to 15 years of career experience (≥ 15 years); academic background related to food science and/or nutrition (food science and technology professionals, dietitians, nutritionists, pharmacists, nurses and fam-

Table I. Descriptive data of the pilot sample for the adaptation of FBFC-ARFSQ-18 and PSIMP-ARFSQ-10

Evaluators	Age (years)	M/F	Country of birth	Residence	Qualification	Number of diseases, symptoms and signs related to ARFS
1	43	M	Spain	Region of Madrid	IT support	14
2	55	M	Spain	Region of Madrid	Administrative assistant	8
3	24	M	Spain	Region of Madrid	Student	16
4	24	F	Spain	Region of Madrid	Student	9
5	57	F	Spain	Region of Madrid	Administrative assistant	14
6	43	M	Spain	Region of Madrid	Audio-visual technician	7
7	44	F	Spain	Region of Madrid	Concierge	26
8	47	F	Spain	Region of Madrid	Technical assistant	7
9	55	M	Spain	Region of Madrid	Stationer	27
10	62	M	Spain	Region of Madrid	Concierge	19
11	58	F	Spain	Region of Madrid	Administrative assistant	19
12	64	F	Spain	Region of Madrid	Marketing assistant	11
13	33	M	Spain	Region of Madrid	Telecommunications technician	12
14	35	F	Spain	Region of Madrid	Security guard	6
15	64	F	Spain	Region of Madrid	Retired	8
16	66	M	Spain	Region of Madrid	Retired	13

ARFS: adverse reactions to foodstuffs; F: female; IT: information technology; M: male.

ily physicians) for the evaluation of FBFC-ARFSQ-18; and related to medicine and/or nursing (nurses and family physicians) for the evaluation of PSIMP-ARFSQ-10. A group of 33 experts were contacted, 15 experts with a wide experience in food science and/or nutrition for the FBFC-ARFSQ-18 questionnaire; and 18 experts with extensive experience in the field of medicine and/or nursing for the PSIMP-ARFSQ-10 questionnaire. After they received an e-mail invitation or on-line correspondence using a RedCap™ unique Uniform Resource Locator (URL) link, 28 experts consented to participate (15 experts for the FBFC-ARFSQ-18 and 13 experts for the PSIMP-ARFSQ-10). The final number of experts was 20 (eight experts were excluded for not accomplishing the inclusion criteria, not answering the evaluation questionnaire, or sending an incomplete evaluation).

Experts were asked to indicate below each item their opinion using an open box of comments and their degree of agreement, using a 1 to 5 Likert-type scale, where five points indicated the highest agreement and one, the lowest agreement. The standard deviations (SD) of the differences between scores have been established as a viable option for quantifying validity (32); thus, when an expert's evaluation was greater than or equal to 3 (≥ 3) SD different from the mean of the other nine experts in two or more questions (≥ 2), these values were not considered as valid because of the discordance with the rest of experts (33). The content validation coefficient for the final ten experts was calculated using Aiken's V coefficient (95 % confidence interval [CI]) and a minimum Aiken's V coefficient score of ≥ 0.75 was required for each question to be validated (33,34). Aiken's V coefficient and the lower and upper limits of confidence intervals were calculated using the software ICaiken.exe (Visual Basic 6.0, Lima, Perú) (34). Descriptive data of the experts are shown in tables III and IV.

Table II. Descriptive data of experts for the evaluation of FBFC-ARFSQ-18

Expert	Age (years)	M/F	Qualification	Highest degree	Career completion (year)	Career experience (years)
1	47	M	Human Nutrition and Dietetics	Ph.D.	2001	21
2	41	M	Pharmacy and Biochemistry	Ph.D.	2002	20
3	66	F	Nursing	MSc.	1977	45
4	65	F	Nursing	MSc.	1979	43
5	63	M	Medicine	Ph.D.	1982	40
6	71	M	Pharmacy and Nutrition	Ph.D.	1972	50
7	40	F	Human Nutrition and Dietetics, Food Science and Technology	Ph.D.	2004	18
8	49	F	Pharmacy	Ph.D.	1995	27
9	50	F	Pharmacy	Ph.D.	1999	23
10	64	M	Medicine	Ph.D.	1982	40

F: female; M: male; MSc: Master of Science; Ph.D.: Doctor of Philosophy.

Table III. Descriptive data of experts for the evaluation of PSIMP-ARFSQ-10

Expert	Age (years)	M/F	Qualification	Highest degree	Career completion (year)	Career experience (years)
1	66	F	Nursing	MSc.	1977	45
2	60	F	Medicine	Ph.D.	1992	30
3	65	F	Nursing	MSc.	1979	43
4	64	F	Nursing	MSc.	1977	45
5	65	M	Medicine	Ph.D.	1979	43
6	58	F	Medicine	Ph.D.	1985	37
7	57	F	Medicine	BSc.	1988	34
8	62	F	Medicine	Ph.D.	1983	39
9	50	M	Medicine	Ph.D.	1996	26
10	68	M	Medicine	Ph.D.	1979	43

BSc: Bachelor of Science; F: female; M: male; MSc: Master of Science; Ph.D.: Doctor of Philosophy.

Table IV. Likert-type scale values offered by the panel of experts in all FBFC-ARFSQ-18 items for validation

	FBFC-ARFSQ-18 validation									
	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Expert 6	Expert 7	Expert 8	Expert 9	Expert 10
Item 1	5	5	5	2	5	5	3	5	4	5
Item 2	5	5	5	2	5	5	2	5	4	5
Item 3	5	5	5	5	5	5	4	5	5	5
Item 4	5	5	5	5	5	5	5	5	5	4
Item 5	5	5	5	5	4	5	5	5	5	5
Item 6	5	5	5	5	5	5	4	5	5	5
Item 7	5	5	5	5	5	4	4	5	5	5
Item 8	5	5	5	5	5	4	4	5	3	5
Item 9	5	5	5	4	5	5	4	5	5	5
Item 10	5	5	5	2	5	4	4	5	5	5
Item 11	5	5	5	2	5	3	5	5	3	5
Item 12	5	5	5	5	5	5	5	5	3	4
Item 13	5	5	5	3	5	4	4	5	4	5
Item 14	5	5	5	5	5	3	5	5	3	5
Item 15	5	5	5	3	5	4	5	5	5	5
Item 16	4	5	5	5	5	4	5	5	5	5
Item 17	4	5	5	5	5	4	4	5	1*	4
Item 18	5	5	5	5	5	3	4	5	2	4

*FBFC-ARFSQ-18: Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs. *Values ≥ 3 SD of the mean of the rest of the nine experts.*

PHASE 4: FACE VALIDITY

Open questions after each domain or section of each questionnaire were placed for experts to analyze if the categories of each questionnaire captured the intended concept; and also, to specify what experts thought the questionnaire measured, possible missing items and what they thought in general means. The percentage of experts who commented at least one section was recorded, as well as the mean complexion time of each questionnaire (data described in the Results section, Phase 4 of this document).

PHASE 5: ETHICAL CONSIDERATIONS

This research was performed in accordance with the Ethical Guidelines of the Declaration of Helsinki of 1961, revised in Fortaleza (2013) (35) and following the Spanish and European regulations on data protection (36). The protocol has been approved by the Ethics Committee of the UPM (reference number 20200602).

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RESULTS

PHASE 1: QUESTIONNAIRES DEVELOPMENT

The final pool of items was categorized into three sections for the FBFC-ARFSQ-18 (eating habits, food and beverages frequency consumption and food supplement frequency consumption) and five sections for the PSIMP-ARFSQ-10 (digestive system, skin and subcutaneous tissue, nervous system, respiratory system and other human body systems [other diseases/pathologies, symptoms and signs, not elsewhere classified]). Both validated tools followed the recommendations of previous studies (9,14,20,37).

Items 1 and 2 of the FBFC-ARFSQ-18 were based on the timing of food and beverages intake, an aggregation of the number of the individual foods, main meals and food and beverages intake between meals, both to represent more closely the required food and beverages consumption patterns recommended in a previous structural validation European FFQ (37). Items 3 and 4 describe the most reported aspects of concern when it comes to ARFS: type of diet (vegan, gluten-free, fermentable oligosac-

charides, disaccharides, monosaccharides and polyols [FODMAP], biogenic amines, etc.) and type of foodstuff (processed, non-processed and type of preserved products), especially when comparing regular population and athletes (5,26,38). Items 16, 17 and 18 were added to more deeply investigate the whole food intake of the population with possible ARFS including food supplements (probiotics, vitamin D), which are not very common in a FFQ but of high significance in the pathogenesis of ARFS (25,39). Items 5 to 15 corresponded to eleven food groups designed to be answered based on the consumption of a participant or patient in the last six months with a scale describing the monthly, weekly or daily intake of each foodstuff. There is plenty of information on paediatric oral immunotherapies (OIT) and the required time interval, for an ARFS observation, after changing a specific food intake. However, some case studies with adults suggest a minimum observation of three to six months (18,40). Trying to fill in the gaps, the present study proposes the FBFC-ARFSQ-18, describing the food and beverages intake during the last six months, for the Spanish population to support the current clinical tools of diagnosis and to obtain a meaningful approach from a population with possible ARFS.

All items (from 1 to 10) of the PSIMP-ARFSQ-10 included four body categories of diseases and symptomatology (digestive, skin and subcutaneous tissue, nervous system, respiratory system) and one category of other human body systems (other diseases/pathologies, symptoms and signs, not elsewhere classified). All of these items were designed to be answered with a yes or no question with a special annotation for the questions about pathologies and diseases that if the answer is "yes", the pathology may have medical diagnosis. This annotation was made to assess the awareness of a valid relationship with ARFS. The experts agreed with the relevance of this nature of the question and considered it as a reductor of bias in self-perception of discomfort towards ARFS. Finally all pathologies and symptomatology sections have an ending question to select other diseases or symptomatology related to the same body system. There could still be some case studies approaching pathologies such as mononucleosis and CFBA (41). A final construct of questions was designed for both tools, ensuring no overlap (Annex I; https://www.nutricionhospitalaria.org/anexos/04631-01.pdf).

PHASE 2: PILOT TEST AND RELIABILITY

The pilot test was completed by 16 INEF-UPM ASP evaluators, aged 24 to 66 years (50 % women, 48.38 ± 13.96 years), during December 2021 (Table I). Three observations regarding clarity and comprehension were considered for the final version: another level for the frequency scale for food and beverages in the FBFC-ARFSQ-18 tool, "< 1 time per month", between "never" and "1 to 3 times per month"; notification of the approximate complexion time to fill each questionnaire in the instructions section of both tools; and the addition of "with medical diagnostic" after the option "yes" in the PSIMP-ARFSQ-10 tool. The average complexion time for both questionnaires, reported by ASP, was 15:05 and 6:34 minutes for the FBFC-ARFSQ-18 and PSIMP-ARFSQ-10 tools, respectively. Cronbach's alpha reliability coefficient was greater than 0.80 in both cases: 0.93 for FBFC-ARFSQ-18 and 0.94 for PSIMP-ARFSQ-10.

PHASE 3: CONTENT VALIDITY

A total of 20 Spanish experts accomplished the inclusion criteria. Five experts of the FBFC-ARFSQ-18 and three experts of the PSIMP-ARFSQ-10 were excluded; two experts did not accomplish one of the inclusion criteria and the other six, for various reasons, as not answering the evaluation questionnaire, or sending an incomplete evaluation. All experts were different and none of the experts evaluated both questionnaires (ten food science and/or nutrition professionals aged mean ± SD 55.6 ± 11.37 years evaluated the FBFC-ARFSQ-18; and ten medicine and/or nursing professionals aged mean ± SD 61.5 ± 5.38 years evaluated the PSIMP-ARFSQ-10) (Tables II and III).

Resulting values of the Likert-type scale by the ten selected experts for both questionnaires are shown in tables IV and V. Aiken's V coefficient values were greater than 0.75 in both cases: 0.90 (0.78-0.96 CI) for the FBFC-ARFSQ-18 and 0.93 (0.81-0.98 CI) for the PSIMP-ARFSQ-10 (Table VI).

PHASE 4: FACE VALIDITY

Food groups of the FBFC-ARFSQ-18 were organized in ten food groups in section 2 of the FBFC-ARFSQ-18 (Annex I https://www.nutricionhospitalaria.org/anexos/04631-01.pdf). In the FBFC-ARFSQ-18, due to the Spanish diet habits, sunflower (item 6) and olive oil (item 15) were separated, according to the recommendations of expert 7, into independent questions inside their corresponding food group. Each foodstuff represented a single question, and all their common commercial formats or types of packaging were merged in the same question. Foodstuffs that were not listed into each food group (e.g., currant, passion fruit, or any other less common food in the Mediterranean Spanish area) could be described and reported by selecting the option "Other food or beverages of this Group. Specify". Analogously, the diseases recommended by experts 1 and 3 (otitis, short bowel syndrome [SBS] and small-intestinal bacterial overgrowth [SIBO]) and the symptoms recommended by experts 1 and 6 (muscle cramps, sleep apnea, dyspnea and snoring) were included in the PSIMP-ARFSQ-10 questionnaire. Peripheral edema was relocated from item 4 ("Symptoms and signs of the skin and subcutaneous tissue") to item 10 ("Other symptoms and signs"). Nine out of ten experts commented at least one section of the FBFC-ARFSQ-18 and the PSIMP-ARFSQ-10 tool (except for experts number 9 and 5, correspondingly of each questionnaire). The average of complexion time, reported by experts, to complete both questionnaires was 14:18 and 5:52 minutes

for FBFC-ARFSQ-18 and PSIMP-ARFSQ-10 tools, respectively (Annex II and Annex III; https://www.nutricionhospitalaria.org/anexos/04631-02.pdf).

Annex II
Annex III

DISCUSSION

Both RedCap™-designed questionnaires, FBFC-ARFSQ-18 and PSIMP-ARFSQ-10, for the Spanish population with possible ARFS were validated after a previous rigorous investigation about the current causative foodstuffs of ARFS (42), as well as the associated diseases and symptomatology recently reported (30,43). To make progress on science and in response to the demands of the conclusions of the articles of Garcia-Larsen et al. (7) and Cade et al. (44), the FBFC-ARFSQ-18 tool included the evaluation of not only a single food, such as milk or egg consumption to analyze allergy, but instead, it included most of the current investigated causative foodstuffs of ARFS, such as CFBA or CFBI (42). Similarly, it contributed to the improvement of the evaluation of associated diseases and symptomatology related to ARFS; apart from following the recommendations of previous studies by Makatsori et al. (45) and Schafer et al. (46), the PSIMP-ARFSQ-10 tool not only included the assessment of a single disease and its symptoms, but instead conveniently collected the current diseases, signs and symptoms related to ARFS.

Regarding the construction and design of the FBFC-ARFSQ-18, the scientific literature provides clear evidence about the possible causative food and beverages of a CFBA or CFBI. In self-reported studies (42,47), foodstuffs such as milk, apple, tomato, egg, kiwi,

Table V. Likert-type scale values offered by the panel of experts in all PSIMP-ARFSQ-10 items for validation

	PSIMP-ARFSQ-10 validation									
	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Expert 6	Expert 7	Expert 8	Expert 9	Expert 10
Item 1	5	5	5	5	3	4	4	5	5	5
Item 2	5	5	5	5	3	5	5	5	5	5
Item 3	5	5	5	5	2	5	4	5	5	5
Item 4	5	5	5	5	3	5	5	5	5	5
Item 5	5	5	5	5	4	5	5	5	5	5
Item 6	5	5	5	5	4	5	4	5	5	5
Item 7	5	5	5	5	2	4	4	5	5	5
Item 8	5	5	5	5	4	4	5	5	5	4
Item 9	5	5	4	5	4	4	5	5	5	5
Item 10	5	5	5	5	4	5	4	5	5	5

PSIMP-ARFSQ-10: Pathologies and Symptomatology Questionnaire Associated with Adverse Reactions to Foodstuffs.

Table VI. Number of experts pointing to each Likert-type scale value, mean score and Aiken's V coefficient for all items evaluated by experts

	FBFC-ARFSQ-18							PSIMP-ARFSQ-10						
	Likert-type scale*					Mean	Aiken's V (95 % CI) value (lower-upper limit)	Likert-type scale					Mean	Aiken's V (95 % CI) value (lower-upper limit)
	1	2	3	4	5			1	2	3	4	5		
	Number of experts							Number of experts						
Item 1	0	1	1	1	7	4.40	0.85 (0.71-0.93)	0	0	1	2	7	4.60	0.90 (0.77-0.96)
Item 2	0	2	0	1	7	4.30	0.83 (0.68-0.91)	0	0	1	0	9	4.80	0.95 (0.84-0.99)
Item 3	0	0	0	1	9	4.90	0.98 (0.87-0.99)	0	1	0	1	8	4.60	0.90 (0.77-0.96)
Item 4	0	0	0	1	9	4.90	0.98 (0.87-0.99)	0	0	1	0	9	4.80	0.95 (0.84-0.99)

(Continues on next page)

Table VI (Cont.). Number of experts pointing to each Likert-type scale value, mean score and Aiken's V coefficient for all items evaluated by experts

	FBFC-ARFSQ-18							PSIMP-ARFSQ-10						
	Likert-type scale*					Mean	Aiken's V (95 % CI) value (lower-upper limit)	Likert-type scale					Mean	Aiken's V (95 % CI) value (lower-upper limit)
	1	2	3	4	5			1	2	3	4	5		
	Number of experts							Number of experts						
Item 5	0	0	0	1	9	4.90	0.98 (0.87-0.99)	0	0	0	1	9	4.90	0.98 (0.87-0.99)
Item 6	0	0	0	1	9	4.90	0.98 (0.87-0.99)	0	0	0	2	8	4.80	0.95 (0.84-0.99)
Item 7	0	0	0	2	8	4.80	0.95 (0.84-0.99)	0	1	0	2	7	4.50	0.88 (0.74-0.95)
Item 8	0	0	1	2	7	4.60	0.90 (0.77-0.96)	0	0	0	3	7	4.70	0.93 (0.80-0.97)
Item 9	0	0	0	2	8	4.80	0.95 (0.84-0.99)	0	0	0	3	7	4.70	0.93 (0.80-0.97)
Item 10	0	1	0	2	7	4.50	0.88 (0.74-0.95)	0	0	0	2	8	4.80	0.95 (0.84-0.99)
Item 11	0	1	2	0	7	4.30	0.83 (0.68-0.91)	-	-	-	-	-	-	-
Item 12	0	0	1	1	8	4.70	0.93 (0.80-0.97)	-	-	-	-	-	-	-
Item 13	0	0	1	3	6	4.50	0.88 (0.74-0.95)	-	-	-	-	-	-	-
Item 14	0	0	2	0	8	4.60	0.90 (0.77-0.96)	-	-	-	-	-	-	-
Item 15	0	0	1	1	8	4.70	0.93 (0.80-0.97)	-	-	-	-	-	-	-
Item 16	0	0	0	2	8	4.80	0.95 (0.84-0.99)	-	-	-	-	-	-	-
Item 17	1	0	0	4	5	4.20	0.80 (0.65-0.90)	-	-	-	-	-	-	-
Item 18	0	1	1	2	6	4.30	0.83 (0.68-0.91)	-	-	-	-	-	-	-
Total Aiken's V Mean (± SD)							0.90 (± 0.06)							0.93 (± 0.03)

*CI: confidence interval; FBFC-ARFSQ-18: Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; PSIMP-ARFSQ-10: Pathologies and Symptomatology Questionnaire Associated with Adverse Reactions to Foodstuffs. *Likert-type scale ranged from 1 to 5, where the minimum (1) value was based on very poor relevance and the maximum (5) on the highest degree of relevance.*

shrimp, fish, hazelnut, walnut, wheat and peanut were the most reported, although kiwi is not strictly considered as part of a strict MD. However, Telleria-Aramburu et al. (48) proposed an approach of a short FFQ for a Spanish sample of the Basque Country region, located in the north of Spain, with common Spanish food and also differencing proccessed and non-processed food, but still not very detailed regarding types of nuts, seeds, cereals, beverages, food supplements and other food groups of interest for the analysis of ARFS (48). Similarly, a European FFQ (7) has been proposed to assess the relation to allergy and asthma, not specifically CFBA; the Gluten FFQ (6), specifically focused only on cereal allergens; and the Pregnancy FFQ (8), with questions oriented to the gestation period. They are not yet available in the Spanish language and with particular characterisitics of the population of interest and/or allergy in general and not specifically ARFS, CFBA or CFBI.

EAACI and Ogulur et al. already proposed in 2017 and 2021 several diseases and symptomatology associated with CFBA (30,43). In order to improve the range of possibilities and precision of the tool (PSIMP-ARFSQ-10), specific diseases of recent investigations related to ARFS were taken into account (e.g., food protein-induced enterocolitis syndrome [FPIES], SIBO, IBS, atopic dermatitis, dizziness, etc.) (16,24-26,49). Experts who validated the questionnaire agreed with the authors of the present study about this, and other groups of diseases (SBS, hiatal hernia, pytiriasis, alopecia, panic attack) and symptomatology (xerostomia, erythema, sleep apnea, dyspnea, muscle cramps) were added from phase 1 and also following the experience and observations of 20 experts in phase 4.

This study could offer the key point to establish two validated questionnaires to analyze the main aspects of both, food consumption and diseases related to ARFS in a Spanish population under their self-perception, with the intention of being used together with a clinical diagnosis tool of the physician's choice. Furthermore, these tools could be used together with other health promotion studies and future ARFS local research projects.

This study has several strengths: the validation procedure is based on a rigorous qualitative research process, the use of a robust secure web platform for design and hosting, and the difference in the type of expertise that each professional may have towards the Spanish ARFS field, avoiding bias through multidisciplinary knowledge. The main limitation of this study is that questionnaires are designed and validated only for adults.

CONCLUSIONS

The findings of this study allow to conclude that both questionnaires designed to assess food consumption, diseases and

symptomatology in a Spanish population with possible ARFS could be used for clinical applications to analyze the association between certain food and beverages consumption with ARFS, such as, CFBA and CFBI. In addition, they could be used to investigate the link between some specific diseases, signs and symptoms with ARFS due to their optimal validity values. The FB-FC-ARFSQ-18 and the PSIMP-ARFSQ-10 questionnaires should facilitate research projects and be used together (recommended) with the clinical diagnostic tools of CFBA and CFBI.

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6.7. Annex G: Article 3

Title: "The multifactorial approach and the food allergen-specific substitutive diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: Study protocol for a randomized controlled trial—The ALASKA Study".

Reference: [5]

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Trials

STUDY PROTOCOL Open Access

The multifactorial approach and the food allergen-specific substitutive diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: study protocol for a randomized controlled trial—the ALASKA study

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Abstract

Background Adverse reactions to foodstuffs (ARFS), specifically food allergy (FA) and food intolerance (FI), are increasing worldwide and represent a major public health concern. Thus, ARFS management, its identification, evaluation, and intervention, must provide a comprehensive solution.

Objectives (a) To develop a multifactorial strategy for ARFS management in adults with FA and/or FI; (b) to describe the multiple influential variables in ARFS within the realm of ARFS management; and (c) to design a personalized food allergen-specific substitutive diet (FASSD), as a 6-month dietary treatment option for adults with ARFS and as a component of ARFS management.

Methods The ALASKA study will consider the following main variables as part of the ARFS management: (1) demographics and clinical information; (2) symptomatology, food and beverages intake and physical activity; (3) hematobiochemical study; (4) immunology; (5) enzymatic activity; (6) anthropometry, body composition, and physical fitness; (7) QoL; (8) 6-month intervention; (9) end of the study; and (10) other assessments. The FASSD will be designed with special emphasis on the commonly lacking micronutrients in the ARFS population: niacin, Mg, K, P, Ca, Zn, B12, folate, Fe, and fiber.

Discussion The ALASKA study protocol has been developed as a global strategy to manage and evaluate ARFS in Spanish adults older than 18 years of age. Approaching ARFS with multiple assessments, as influencing factors, will lead to a novel strategy for ARFS management. The FASSD has been designed as a personalized tool to avoid crucial micronutrient deficiencies that a current strict food allergen avoidance or elimination diet may provoke.

Trial registration The protocol has been approved by the Ethics Committee of the UPM (REF.20200602) and registered on ClinicalTrials.gov (NCT05802017).

Keywords Clinical trial, Diet, Disease management, Food hypersensitivity, Food intolerance, Nutrition therapy

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Introduction

Adverse reactions to foodstuffs (ARFS) are based on an individual's specific response to food components and have been generally classified by pathogenic mechanism of action and attributed mostly to food allergy (FA) and/or food intolerance (FI) (Fig. 1) [1]. Currently, more than 150 million Europeans suffer from chronic allergic disease, including about 20 million suffering from FA and more than 80 million from FI [2, 3]. Prevalence of ARFS, especially the ones related to FA and FI, has been increasing over the past decades in developed and developing countries becoming an important public health concern [4]. Given that the reactions to foodstuffs are often regarded as pediatric problems, these reactions have not been studied in-depth in adults and no accurate strategies focusing on this population have been established. It has been estimated that by 2050, 50% of the world's population will be affected by at least one type of allergy; and as one in every five people globally will be aged 60 years or over by that time, the general health and quality of life (QoL) of the adult population emerges as an essential object of study for the coming years [5].

The *gold standard* to diagnose immune-mediated and non-immune-mediated reactions to foodstuffs is the oral food challenge (OFC) [12]. However, to avoid procedures that may imply a prolonged regular practice, there are short breath tests available, such as the Cerascreen® Lactose/Fructose hydrogen H_2 and methane CH_4 breath test kit (Cerascreen GmbH, Schwerin, Germany). Similarly, there are clinically relevant blood biomarkers, such as specific immunoglobulins (mostly food-specific IgE antibody (Ab) reactions for type I hypersensitivity or type I food allergy (T1FA)), that together with a clinical history of symptoms can help in diagnosis [13, 14]. In addition, other physical and clinical tests might also contribute to the clarification of the study of specific populations, such as athletes, who may present different physical activity levels (PAL). The presence of ARFS in individuals with particular symptomatology could be linked to gastrointestinal, skin, and/or nervous system (NS) conditions, specifically when having type II hypersensitivity or type II food allergy (T2FA) [15, 16].

In fact, T2FA has been one of the most recently and newly explored hypersensitivities as a tool in the development of ARFS research. Figure 1 outlines the ARFS classification according to the pathogenic mechanism of action. The clinical relevance of food-specific IgG_4 Ab in food allergy is currently unclear and not recommended to be used alone as a food allergy predictor. It has been observed that IgE/IgG_4 ratios can be used to enhance the diagnostic and prognostic performance of sIgE [17].

Current treatments of ARFS are not optimal nor ideal from the nutritional and physical condition point of view. For T1FA, treatment consists of strict food allergen avoidance and the use of adrenaline (for allergen-induced systemic reactions), epinephrine, and/or a 24-h dietary recall tool [18]. The application of treatments such as strict food allergen avoidance may cause nutritional deficiencies in certain cases of multiple food allergen responses due to the avoidance of important food groups. For instance, milk avoidance in adults may result in calcium deficiency [19] and lower intakes of zinc and vitamin B2. Likewise, prolonged gluten-free diet make individuals prone to the development of, most notably folate deficiency and lower intakes of calcium, iron, zinc, folate, and fiber; affecting their nutritional status and altering the intestinal microbiota [20]. Substitutive diets suggesting the replacement of allergy-related food for allergen-free food have shown symptoms' score improvement. Hence, the 6-month intervention of the ALASKA study, to manage and ameliorate ARFS in adulthood, consists in the implementation of a general nutritional advice with dietary recommendations for a healthy lifestyle for subjects in the control group; and a 6-month food allergen-specific substitutive diet (FASSD) for subjects in the intervention group. These effects are greater in subjects with specific pathologies: atopic dermatitis [21], urticaria [22], and gastrointestinal disorders [23].

Consequently, a novel strategy involving the assessment of multiple variables and a FASSD to manage and ameliorate ARFS is proposed in this study. The aim of this study is (a) to develop a multifactorial strategy for ARFS management in adults with FA and/or FI; (b) to describe the multiple influential variables in ARFS within the realm of ARFS management; and (c) to design a personalized FASSD, as a 6-month dietary treatment option for adults with ARFS and as a component of ARFS management.

Materials and method

Study design

This will be a randomized controlled trial (RCT), the Allergies and Food Intolerances in Adults and Athletes study (ALASKA study), with matched pair trial design, exploratory framework, and a 1:2 allocation ratio between the control (Group A) and the intervention group (Group B) respectively, conducted in the Universidad Politécnica de Madrid (UPM) located in the Region of Madrid, Spain. The flow diagram of this study is shown in Fig. 2. The following chart meets The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [24].

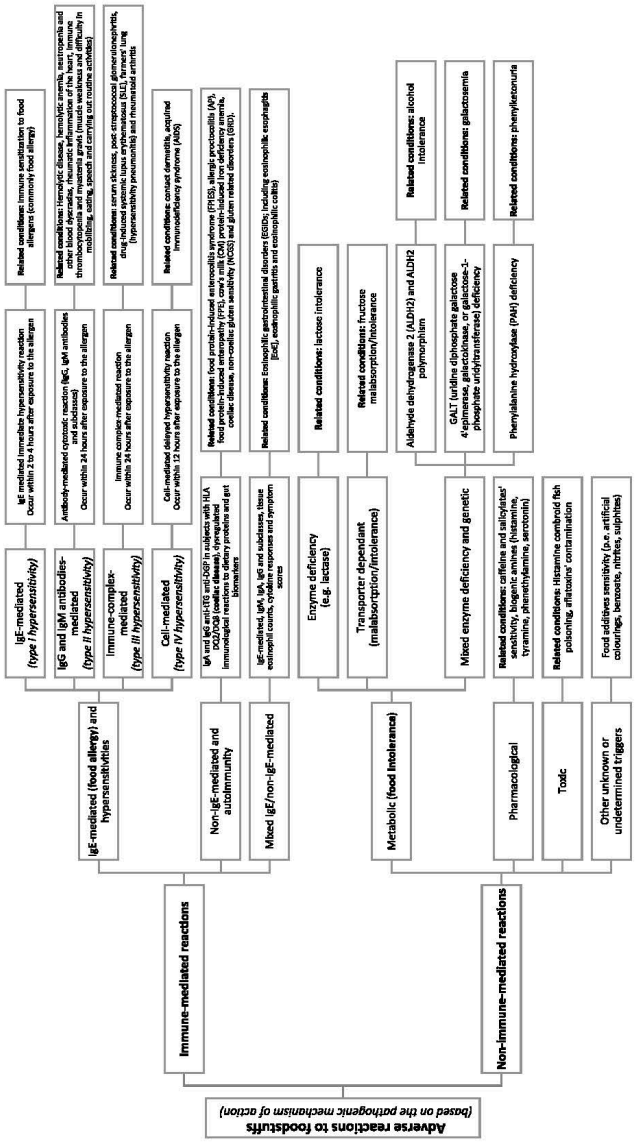


Fig. 1 Adverse reactions to foodstuffs classification updated and adapted from a selected literature (described below). Adapted from: Immune-mediated reactions: [6–9]; Non-immune-mediated reactions: [7, 10, 11]

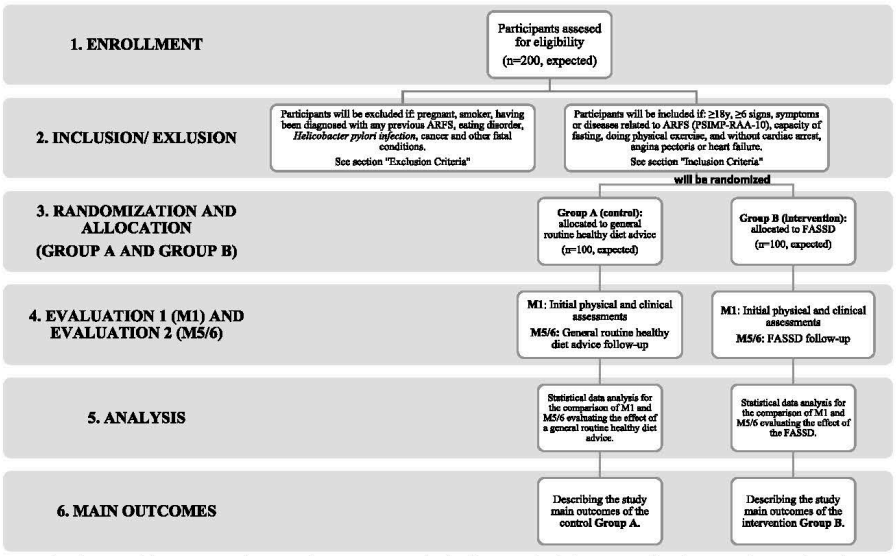


Fig. 2 Flow diagram of the ALASKA study. ARFS: adverse reactions to foodstuffs; FASSD: food allergen-specific substitutive diet; M1: physical and clinical assessments at month 1; M5/6: physical and clinical assessments at month 5 or 6; PSIMP-RAA-10: Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; y: years of age

Participants

Recruitment

Volunteers with signs, symptoms, and/or diagnosed diseases related to ARFS and older than 18 years, including men and women, will be recruited. The recruitment period will be active for approximately 1 to 3 months and according to the enrollment response. Enrollment mass e-mail campaigns, social media, digital display screens in public areas of the UPM, and the local online contact flyer/website (https://short.upm.es/kwgpo) of the ImFINE Research Group (ImFINE-RG) will be used to recruit interested adults to participate in the ALASKA study. The first approach with possible participants will take place with the use of phone informative conversations between the researcher and the possible participant to share a wider vision about the trial. Engaged participants will be registered in the study and an anonymous participant code will be assigned (see the " Data storage" section). Participants will initially complete and sign the Informed consent form (Appendix 1), the Inclusion and exclusion criteria form (Appendix 2), the Demographics and clinical characteristics form (Table 3), and the Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs (PSIMP-RAA-10). After completing these initial questionnaires, participants will be enrolled and their data will be checked for eligibility by a researcher of the ALASKA study. If extra measures are needed to recruit a sufficient number of participants, the recruitment period will be expanded and all the steps of the recruitment phase will be repeated (expected enrollment rate of approximately 150%). Participants will not receive any economic compensation for volunteering in this study, but they will receive personalized nutritional advice and individual reports of all the performed clinical and physical measurements.

The Research Electronic Data Capture Platform (RedCap®), hosted in the Supercomputing and Visualization Center of Madrid Community of the UPM (CESVIMA-UPM) will be used to digitally secure and perform all types of communication, whether massive or individual. RedCap® platform is a specialized tool to build and manage clinical trials and their corresponding databases. All questionnaires and documents (including Appendix 1, Appendix 2, Table 3, the PSIMP-RAA-10 questionnaires, and any other) that will be used during the ALASKA study will be uploaded and will be shared to participants, when applicable, via the RedCap® platform.

Sample size and missing data

The required sample size with 95% of power was calculated using the G*Power software v. 3.1 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) [25] and previously related-published data [15] and datasets [16]. The cross-sectional study requires a sample size of $n=154$ ($d=0.3$, $\alpha=0.05$). Meanwhile, the longitudinal study requires a sample size of $n=33$ for the control group and $n=65$ for the intervention group ($d=0.8$, $\alpha=0.05$).

Even if the participants have agreed to join the ALASKA study voluntarily and signed the informed consent form, they will be allowed to abandon the study at any time, without having to provide a specific argument or being penalized (loss to follow-up, missing data, drop-out, or similar). Likewise, the researchers of this study reserve the right to end any participation, according to each researcher's criteria, for the benefit of the research study (e.g., injuries, long trips, low commitment, outbreak of severe diseases, or similar). Due to the nature of the dietary interventions, modifying allocated interventions is not foreseen in this study. However, if a subject chooses to discontinue the intervention on their own or if the researcher decides to stop a participant's intervention for any reason, it will be documented in each participant's individual case report and in the End of Study Form (Annex VIII). Subjects will be able to explain, or not, the reason for voluntarily abandoning the study.

Data storage

Participants' alphanumeric codification will be assigned in RedCap® adopting the format "ALXXXX" (two static letters, pointing the study name, ALASKA study, and four random-digit unique code). Complete records of each participant will be stored in RedCap® platform, locally hosted in the CESVIMA-UPM. Digital databases of each assessment and instrument will be created promoting the data quality and avoiding the need of duplicating questionnaire measurements. Databases will be locked using a secure password and with access to the coordinator and principal investigator (PI) of the ALASKA study (L.P.-A., M.G.-G). Additional researchers, after the authorization of the PI (M.G.-G), will have also access to the password and the study databases. M.G.-G will make final decisions regarding the RedCap® platform management, trial supervision, and the trial start/termination point. RedCap® platform provides the "Data Exports, Reports, and Stats" module, which allows to view reports, inspect plots and descriptive statistics of the data, at any point/time of the trial, as well as export data to Microsoft Excel, SAS, Stata, R, or SPSS for analysis (if access provided by M.G.-G to the researchers of the Alaska study). Complete individual records will be documented using the unique and anonymous participant code and will not contain identifiable participants' information. Routine day-to-day audits; weekly back-ups of the datasets; and monthly internal audits of the available services (water and electricity) and resources (laboratory equipment) will be performed to support data quality.

Subjects of the study will be selected according to the following described inclusion and exclusion criteria and if they have completed the required preliminary questionnaires: Informed consent model (Appendix 1), Inclusion and exclusion criteria form (Appendix 2), and the Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs (PSIMP-RAA-10) [14].

Inclusion criteria

Enrolled participants who will accomplish the following inclusion criteria will be eligible subjects of study: (1) participants without a pacemaker, prosthetic devices, or metal implants; (2) participants with availability of time to attend two venous blood sample extractions and to dedicate at least 30 min to complete online questionnaires related to this study; (3) participants with the possibility, and not restricted by medical advice, of fasting for at least 8 h (for the extraction of blood samples) and doing physical exercise (including the physical capacity of going up and down stairs); (4) participants that, during physical exercise, have not experimented any chest pain, chest stiffness-tightness, or dizziness; (5) participants who have not had a cardiac arrest, angina pectoris or heart failure; and (6) participants with equal or greater than 6-score signs, symptoms or diseases using the previously validated PSIMP-RAA-10 tool [14].

Exclusion criteria

Participants with the following conditions will be excluded from the study: (1) being simultaneously involved in another research study; (2) heavy smokers (>25 cigarettes/day), according to The Aspect Consortium of the European Commission classification scales [26]; (3) pregnant women; (4) receiving drug therapies with any type of antibiotic, antidepressant, sleeping pill, or anti-anxiety medication, or participants who will need to change their drug treatment protocol during the study; (5) previous diagnosis of coeliac disease (CeD), FA and/or FI, eating disorders, or infection due to the *Helicobacter pylori* bacteria during the last year; (6) participants with a job or lifestyle that potentially interferes with their regular sleep schedule (e.g., night shift and on-call); (7) participants with some type of major surgery that involves having been hospitalized during the last 5 years, including current muscle injuries and muscle cramps; (8) suffering from any type of cancer or

other fatal illness within the last 5 years; (9) participants who are not willing to continue and finish the study for any reason; and (10) participants who, according to the researchers' criteria, cannot continue the study (e.g., injuries, long trips, low commitment, outbreak of severe diseases, or similar).

Randomization

The ALASKA study will follow a sample randomization using the RedCap® randomization automated tool (RRAT) for the study allocation of subjects. RRAT assigns subjects by chance (rather than by choice) into specific groups (Group A and Group B) and monitors the overall allocation progress and assignment of randomized subjects. It is not possible to blind subjects or staff in this study from the tested treatment due to nutritional interventions which will be used as dietary treatments for the management of ARFS. Nevertheless, the outcome assessors will be blinded to the group assignment given the use of anonymized participant codes among all the steps of the study.

Allocation

Subjects will be randomly placed in Group A (control), or Group B (intervention) in a 1:2 allocation ratio using the RRAT tool. RRAT tool access will be granted to the outcome researchers of the Alaska study. The duration of the study for both control and intervention groups will be between 5 and 6 months. Individuals assigned to the control group will receive general nutritional advice with dietary recommendations for a healthy lifestyle (Annex I), while subjects assigned to the intervention group will receive a personalized food allergen-specific substitutive diet or FASSD (Annex II).

Intervention

The 6-month intervention of the ALASKA study consists of the implementation of a general nutritional advice with dietary recommendations for a healthy lifestyle (Annex I) during 5 to 6 months for subjects in the control group (Group A); and a 5/6-month FASSD (Annex II) for subjects in the intervention group (Group B) (Fig. 2). Nutrition and dietetics experts will elucidate the corresponding Annexes to the study participants during a one-to-one oral training session.

Annex I is based on the dietary guidelines for the Spanish population provided by the Spanish Society of Community Nutrition (SENC) and includes the following food groups: whole grain cereals, fruits, vegetables, oils and fats, meat, fish, legumes, nuts and seeds, eggs and dairy [27]. On the other hand, Annex II is based on substitutive foodstuffs according to national Spanish (Spanish Food Composition Database, BEDCA) [28] and international food databases (United States Department of Agriculture Food Composition Database, USDA-FCD and Nutrition Coordinating Center Food and Nutrient Database, NCCDB) [29, 30]. Foodstuffs with equal or higher predominant nutrients than the foodstuff to replace are chosen as equivalent foodstuffs.

Subjects in both groups (A and B), will be encouraged to follow the recommendations of Annex I. However, only Group B will be instructed to follow the personalized FASSD described in Annex II. Subjects in both groups will be followed up monthly, using an adherence monitoring tool (Annex IV. Dietary Adherence Questionnaire, DAQ, adapted to the 6-month intervention) during the ALASKA study (month 1 to month 5/6).

Regarding physical activity and sport habits, Annex I and Annex II request both groups (A and B) to maintain their usual physical activity and sport habits during the ALASKA study. Subjects will be instructed to make sure they accomplish the described conditions of the Inclusion and exclusion criteria form (Appendix 2) throughout this study.

Food allergen-specific substitutive diet

The FASSD (Annex II) is designed considering seven principal characteristics explained in Table 1. Substitutive foodstuffs of the FASSD are intended to be mainly a nutritional optimal replacement (concerning micronutrients of interest in the ARFS population); and only, when possible, a gastronomic substitutive belonging to the Mediterranean diet (MD). Instructions of the FASSD will be given as a printed catalog (Annex II). All the sections of this document will be explained to the corresponding subjects of study (Group B) verbally in a one-to-one oral training session.

Ethical considerations and clinical trial registration

This research will be performed in accordance with the Ethical Guidelines of the Declaration of Helsinki of 1964, revised in Fortaleza (2013) [31], and following the Spanish and European regulations on data protection [32]. The protocol has been approved by the Ethics Committee of the UPM (reference number 20200602, date: 17/07/2020) and registered on ClinicalTrials.gov (Clinical Trials ID NCT05802017, name: "Relation Between Adverse Reactions to Food, Physical Performance and Health in a Mediterranean Population"). A signed informed consent model will be obtained from all study participants before their participation commencement. As part of the information

Table 1 Design of a healthy food allergen-specific substitutive diet

Characteristic	Source	FASSD
(1) Essential food groups for a balanced diet	SENC Spanish nutritional pyramid [27]	whole grain cereals, fruits, vegetables, oils and fats, meat, fish, legume, nuts and seeds, eggs, and dairy
(2) Rich in lacking micronutrients of the ARFS population	literature [19, 20]	B2, B3, Mg, K, P, Ca, Zn, B12, B9, Fe, and fiber
(3) Foodstuffs with equal or greater micronutrients than the replaced foodstuff	BEDCA [28], USDA-FCD [29] and NCCDB [30] food composition databases	substitutive foodstuffs from the MD
(4) Positive immunological and enzymatic results	ALASKA study	Immunological: ≥ 3.5 kU_A/l Enzymatic: $H_2 \geq 20$, $CH_4 \geq 12$ ppm changes
(5) Written session	ALASKA study	Printed report of the substitutive foodstuffs (Annex II) and the immunological and enzymatic results
(6) Oral session	ALASKA study	One-to-one personalized explanation of Annex II and the immunological and enzymatic results
(7) Monthly follow-up	DAQ (Annex IV)	Evaluation of the FASSD adherence

ARFS, adverse reactions to foodstuffs; *BEDCA*, Spanish Food Composition Database; *FASSD*, food allergen-specific substitutive diet; *MD*, Mediterranean Diet; *NCCDB*, Nutrition Coordinating Center Food and Nutrient Database; *USDA-FCD*, United States Department of Agriculture Food Composition Database. The FASSD was constructed according to the following references: SENC Spanish nutritional pyramid [27], micronutrients deficiency in ARFS population [19, 20], and national and international food composition databases [28–30]

provided in the Informed consent model (oral and written information), participants will be informed about the researchers who will perform the study. The ALASKA study will be carried out by qualified professionals for medical, clinical, nutritional, and physical activity and sport sciences practice of the ImFINE-RG of the Department of Health and Human Performance of the Faculty of Physical Activity and Sport Sciences of the UPM (INEF-UPM). Besides, participants will be asked whether they authorize the researchers of this study to use their samples for subsequent genetic tests and/or future studies (see the " Other variables" section).

In case of modification of the ALASKA study protocol (e.g., changes to eligibility criteria, outcomes, analyses, trial deviations, trial violations), the PI of the study, according to the favorable reports of the Ethics Committee of the UPM, will have to request a new protocol authorization, using the corresponding UPM official forms. After authorization and after receiving the new favorable reports, protocol modifications will be updated in the Clinical Trials registry. Protocol modifications will be communicated to the internal and external researchers involved in the ALASKA study (including the funding institution, in this case, the UPM) using the 'File Repository' section in RedCap®.

Study variables

The following Table 2 displays a checklist example of a participant's timeline with the assessments and instruments that will be considered during the ALASKA study. The following table meets The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [24].

Main variables

The ALASKA study, as a proposed global strategy to manage ARFS in the Spanish adult population, is organized in the following main variables described as 10 main assessments (Fig. 3).

Demographics and clinical information

Demographics and clinical characteristics presented in Table 3 will be collected from all the selected subjects of the study. Information about the latest SARS-CoV-2 vaccine (last 3 months), brand, number of received doses, and the result of the most recently performed coronavirus disease 2019 (COVID-19) test will be gathered. Additional clinical information will be possible to gather using an optional open text box of other relevant clinical information as part of the demographics and clinical characteristics form.

Symptomatology, food and beverages intake, and physical activity

The validated Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs (PSIMP-RAA-10); and Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs (FBFC-ARFSQ-18) tools will be used to record the clinical picture (diseases, signs and symptoms) and the food frequency consumption of the subjects of study [14]. Additionally, hydration status will be assessed using the Hydration Questionnaire of the ImFINE Research Group (IMFINE-HYD) to record water and other beverage intake [33]. Besides, food and beverages record will be measured through a 24-h dietary recall interview (24HDRI) (Annex III). Medication and

Table 2 Checklist example of a participant's timeline with assessments and instruments during the ALASKA study

ALASKA STUDY TIMELINE											
DATA COLLECTION		**Month -1**	**Month 0**	**Month 1**		**Month 2**	**Month 3**	**Month 4**	**Month 5/6**		**Month 6**
		M-1	**M0**	**M1 (visit 1: M1A)**	**M1 (visit 2: M1B)**	**M2**	**M3**	**M4**	**M5/6 (visit 1: M5A)**	**M5/6 (visit 2: M5B)**	**M6 End of Study**
Enrollment (mass e-mail campaigns, social media and website contact form)		✔	–	–	–	–	–	–	--	–	--
1. DEMOGRAPHICS AND CLINICAL INFORMATION											
ASSESSMENT	**INSTRUMENTS**	–	–	–	–	–	–	–	--	–	--
Participant consent	Informed consent form (RedCap® platform)	✔	–	–	–	–	–	–	--	–	--
Eligibility screening	Inclusion and exclusion criteria form (RedCap® platform)	✔	–	–	–	–	–	–	--	–	--
Allocation **INTERVENTIONS** **GROUP A:** General routine healthy diet advice (CONTROL) **GROUP B:** Food-allergen specific substitutive diet (FASSD)	RedCap® randomization automated tool (RRAT)	– – –	✔	–	–	–	–	–	--	–	--
Demographics and clinical information	Demographics and clinical characteristics form (RedCap® platform)	–	✔	–	–	–	–	–	--	–	--
2. SYMPTOMATOLOGY, FOOD AND BEVERAGES INTAKE, AND PHYSICAL ACTIVITY											
Diseases, signs and symptoms	PSIMP-ARFSQ-10 Questionnaire (RedCap® platform)	✔	–	–	–	–	–	–	✔	–	--
Food and beverages frequency consumption	FBFC-ARFSQ-18 Questionnaire (RedCap® platform)	–	–	✔	–	–	–	–	✔	–	--
	Hydration Questionnaire (IMFINI-HYD)	–	–	–	–	–	–	–	--	✔	--
Food and beverages record	24-h Dietary Recall Interview (24HDRI)	–	–	–	✔	–	–	–	--	✔	--
Physical activity	The International Physical Activity Questionnaire Long Form (IPAQ-LF) (RedCap® platform)	–	–	–	✔	–	–	–	--	✔	--
	The Rapid Assessment of Physical Activity (RAPA) questionnaire (RedCap® platform)	–	–	–	✔	–	–	–	--	✔	--
3. HEMATOBIOCHEMICAL STUDY											
Participant requirements prior to blood collection	Blood Sample Questionnaire (BSQ)	–	–	✔	–	–	–	–	✔	–	--
Hemogram	Hematology analyzer (Beckman Coulter, Inc., UniCel DxH 800 Coulter Cellular Analysis System, Brea, CA, USA)	–	–	✔	–	–	–	–	✔	–	--
Biochemical analysis	Biochemistry analyzer (Beckman Coulter Diagnostics Inc., Beckman Coulter® AU5800, Brea, California, USA)	–	–	✔	–	–	–	–	✔	–	--
	Biochemistry analyzer (Beckman Coulter Diagnostics Inc., Beckman Coulter® DxC 700 AU, Brea, California, USA)	–	–	✔	–	–	–	–	✔	–	--
Vitamin analysis	Electrochemiluminescence analyzer (Roche Diagnostics Ltd., Cobas e411, Rotkreuz, Switzerland)	–	–	✔	–	–	–	–	✔	–	--
4. IMMUNOLOGY											
Immunoglobulin E and G_4 analysis	In-line immunoassay analyzer (Aesku.Diagnostics GmbH, Helin® Helmed, Wendelsheim, Germany)	–	–	✔	–	–	–	–	✔	–	--

Table 2 (continued)

5. ENZYMATIC ACTIVITY												
Lactose intolerance		Lactose Hydrogen H_2 and Methane CH_4 breath test kit (Cerascreen GmbH, Cerascreen® Lactose kit, Schwerin, Germany)	–	–	✔	–	–	–	–	--	–	--
Fructose intolerance		Fructose Hydrogen H_2 and Methane CH_4 breath test kit (Cerascreen GmbH, Cerascreen® Fructose kit, Schwerin, Germany)	–	–	✔	–	–	–	–	--	–	--
6. ANTHROPOMETRY, BODY COMPOSITION AND PHYSICAL FITNESS												
Blood pressure		Tensiometer (Omron Healthcare Co. Ltd., model M3 Intellisense HEM-7051-E, Kyoto, Japan)	–	–	✔	–	–	–	–	✔	–	--
		Tensiometer (Omron Healthcare Co. Ltd., model M3 Comfort HEM-7155-E(C), Kyoto, Japan)	–	–	✔	–	–	–	–	✔	–	--
Height		Stadiometer (Seca GmbH & Co. KG., model 213 1721009, Hamburg, Germany)	–	–	–	✔	–	–	–	--	✔	--
Bioelectrical impedance analysis (BIA) with segmental multifrequency		BIA bascule (Tanita, model MC-780MA-N, Tokyo, Japan)	–	–	–	✔	–	–	–	--	✔	--
Hand grip strength (upper body physical fitness)		Grip strength dinamometer (Takei, model T.K.K. 5401 Grip-D, Tokyo, Japan)	–	–	–	✔	–	–	–	--	✔	--
Balance and strength function (lower body physical fitness)		Force platform (Tanita, model BM-220 7341(0), Tokyo, Japan)	–	–	–	✔	–	–	–	--	✔	--
Waist-hip ratio (ISAK criteria)		Metallic measuring tape (Cescorf Ltda., 2-meter Innovare measuring tape, Porto Alegre City, Brasil)	–	–	–	✔	–	–	–	--	✔	--
Aerobic capacity	Estimated VO_{2max}	Astrand-Ryhming Step Test (mA-R) method	–	–	–	✔	–	–	–	--	✔	--
	Pulse rate	Pulse oximeter (OI Healthcare, Ohmeda Tuffest, Boston, Massachusetts, USA)	–	–	–	✔	–	–	–	--	✔	--
	Pace/cycles	Metronome (KORG Inc., model MA-1, Tokyo, Japan)	–	–	–	✔	–	–	–	--	✔	--
7. QUALITY OF LIFE (QoL)												
General health status		12-Item Short Form Health Survey (SF-12) (RedCap® platform)	–	–	–	✔	–	–	–	--	✔	--
Impact of food allergy in the QoL		Food Allergy Quality of Life Questionnaire-Adult Form (S-FAQLQ-AF) (RedCap® platform)	–	–	–	✔	–	–	–	--	✔	--
8. SIX-MONTH INTERVENTION												
Group A or group B written and oral one-to-one training session		Control diet advice or FASSD file format	–	–	–	✔	–	–	–	--	–	--
Adherence to the group A or group B diet		Dietary Adherence Questionnaire adapted to the group A and group B diets (RedCap® platform)	–	–	–	–	✔	✔	✔	--	–	--
9. END OF STUDY												
Adverse events report		Adverse events form (RedCap® platform)	–	–	–	–	–	–	–	--	–	✔
End of study report		End of study form (RedCap® platform)	–	–	–	–	–	–	–	--	–	✔
10. (OPTIONAL) REPETITION OF THE ASSESSMENTS 2 TO 8 AND INTERVENTION ADJUSTMENT												
(Optional) Repetition of the assessments 2 to 8 (if needed by the participant/patient)		Repetition of the instruments from assessment 2 to 8	–	–	–	–	–	–	–	--	–	✔
(Optional) Group A or group B diet adjustment (if needed by the participant/patient)		Control diet advice or FASSD file format	–	–	–	–	–	–	–	--	–	✔

BIA, bioelectrical impedance analysis; *FASSD*, food allergen-specific substitutive diet; *FBFC-ARFSQ-18*, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; *IMFINE-HYD*, ImFINE Research Group Hydration Questionnaire; *IPAQ-LF*, The International Physical Activity Questionnaire Long Form; *ISAK*, International Society for the Advancement of Kinanthropometry; H_2, hydrogen; CH_4, methane; *M – 1*, month 1; *M0*, month 0; *M1*, month 1; *M2*, month 2; *M3*, month 3; *M4*, month 4; *M5/6*, month 5 or month 6; *M5*, month 5; *M6*, month 6; *M1A*, visit 1 at month 1; *M1B*, visit 2 at month 2; *M5A*, visit 1 at month 5 or month 6; *M5B*, visit 2 at month 5 or month 6; *RAPA*, The Rapid Assessment of Physical Activity Questionnaire; *RRAT*, RedCap® randomization automated tool; *RedCap®*, Research Data Capture Platform; *PSIMP-ARFSQ-10*, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; *QoL*, quality of life; *S-FAQLQ-AF*, Food Allergy Quality of Life Questionnaire-Adult Form; *SF-12*, 12-Item Short Form Health Survey; VO_{2max} maximal oxygen consumption

food supplement intake will be also assessed in this interview. The daily energy and nutrient intake of each participant will be calculated by using DIAL software [34], which works with food composition databases such as the Spanish Food Composition Database (BEDCA) and the United States Department of Agriculture Food Composition Database (USDA-FCD), among others. Additionally, to encourage subjects to follow the assigned 6-month

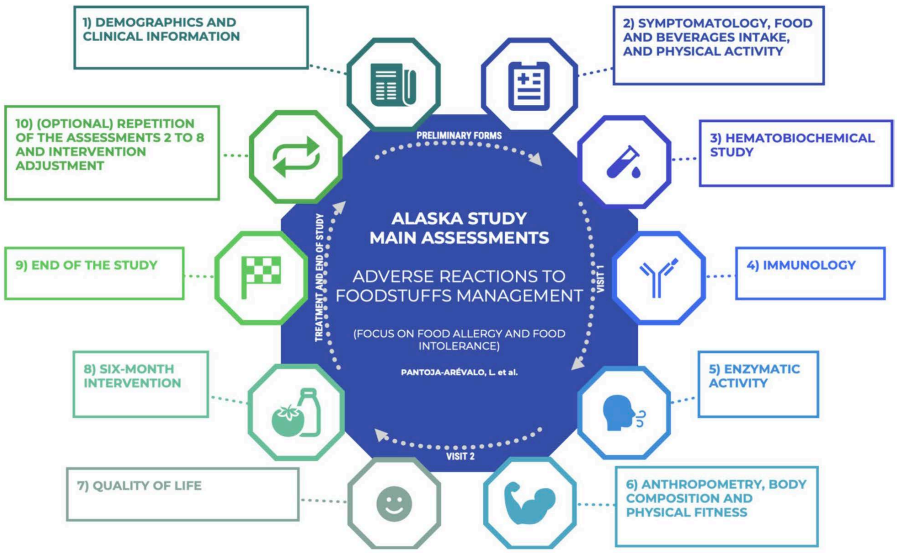


Fig. 3 ALASKA study main variables organized as 10 main assessments for the adverse reaction to foodstuffs management

Table 3 Demographics and clinical information

Instrument	Variable	Description
Demographics and clinical characteristics form	Age	Years of age
	Sex	Men, women
	Country of birth	Country of origin
	Home city	Place of residence
	Level of education	Without education, school, high school, professional training medium/superior level, university, master and doctorate levels
	Employment	Self-employed or employed, student paid/unpaid, unemployed, retired or pre-retired, permanently disabled, houseworker
	Occupation	Profession
	Number of people living together	Number of people living together, in the same residence, with the subject of study
	Blood type	A+, A−, B+, B−, O+, O−, AB+, AB−
	SARS-CoV-2 test	Type, brand, and result (p/n)
	SARS-CoV-2 vaccine	y/n, doses and brand
	(Optional) additional relevant clinical information	Open text box

p/n, positive or negative; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2; *y/n*, yes or no

intervention and promote the dietary treatment adherence, all subjects will be contacted monthly during the study using a Dietary Adherence Questionnaire (Annex IV, authors' own creation, based on questions of previously published validated questionnaires [35] and adapted to the 6-month intervention). Subjects will be asked to maintain their normal physical activity habits during the time of the study, from M1 to M5/6.

The International Physical Activity Questionnaire Long Form (IPAQ-LF) [36] and the Rapid Assessment

of Physical Activity (RAPA) [37] will be administered to determine the minutes per week of self-reported physical activity and the physical activity scores of the study sample, respectively. Using the IPAQ-LF, evaluations are made according to the types of physical activities that people do as a part of their daily lives (walking, moderate to severe information about time spent in activities, and time spent sitting). The energy required will be calculated with the MET (metabolic equivalent)-minute scores.

Hematobiochemical study

Venous blood samples will be collected in fasting conditions (at least 8 h), and after completing the Blood Sample Questionnaire (BSQ) (Annex V. BSQ adapted for the ALASKA study). Blood samples will be collected into one 3-ml tube containing dipotassium (K2) ethylene diamine tetraacetic acid (EDTA) (K2-EDTA Vacutainer® BD tube, REF367838) (BD Becton, Dickinson and Company NYSE: BDX, Sunnyvale, CA, USA); two 5-ml tubes containing separating gel (Vacutainer® BD serum-separating tube SST™ II Advance, REF368968) (BDX, Sunnyvale, CA, USA); and one 4-ml tube containing lithium heparin (LH Vacutainer® BD tube, REF368884) (BDX, Sunnyvale, CA, USA).

Complete blood count (CBC) will be performed using a hematology analyzer, UniCel DxH 800 Coulter Cellular Analysis System (Beckman Coulter, Inc., Brea, CA, USA), with included impedance, radio frequency (RF), flow cytometric light scatter, spectrophotometry, and supravital staining techniques to be applied in the subjects' whole blood K2-EDTA tubes. Quantitative determination of the following hematological parameters will be tested: erythrocyte series (red blood cell count), leukocyte series (white blood cell count), and platelets.

After separating the serum by centrifugation, the samples will be measured for biochemical analysis of glucose, cholesterol (total, HDL, and LDL), triglycerides, serum albumin, urate, creatinine, urea, sodium, potassium, chloride, bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, creatine kinase, iron, calcium, magnesium, lactate dehydrogenase, amylase, and lipase. Biochemical analysis will be measured using two clinical chemistry analyzers through the spectrophotometric method, Beckman Coulter® AU5800 (Beckman Coulter Diagnostics Inc., Brea, California, USA) and Beckman Coulter® DxC 700 AU (Beckman Coulter Diagnostics Inc., Brea, California, USA).

Lastly, the vitamin profile will be determined through electrochemiluminescence of the Cobas e411 (Roche Diagnostics Ltd., Rotkreuz, Switzerland). Remaining samples from subjects who offer their consent (serum, plasma, and whole blood) will be stored in 1 ml aliquots at − 80 °C (ultrafreezing conditions). Colored 2-ml microtubes will be used for the ultrafreezing storage of 1 ml aliquots of serum (yellow, REF05-408–140), plasma (green, REF05-408–142) and whole blood (red, REF05-408–139) (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

Immunology

Quantitative detection of food-specific IgE and IgG_4 antibody (Ab) reactions, in kilounits of allergen-specific IgE or IgG_4 per liter (kU_A/l), against coated food antigens will be determined in human serum samples (Table 4). Ready-to-use AESKUBLOTS® kits will be performed using the immunoblot multiplex technology of the Helia® Helmed Line Immunoassay analyzer (Aesku. Diagnostics GmbH, Wendelsheim, Germany). Positive Ab reactions will be considered as ≥ 3.5 kU_A/l. In vitro tests, specifically molecular-based allergy diagnostics with multiplexed technology, allow researchers to define the immunoglobulin profile of interest of the study sample. This approach is in line with the Precision Medicine Statements [38].

Enzymatic activity

Hydrogen (H_2) and methane (CH_4) concentrations will be measured in the participant's exhaled air for the assessment of lactose intolerance and fructose malabsorption. Ready-to-use Cerascreen® lactose and fructose substrate kits will be used (Cerascreen GmbH, Schwerin, Germany). Cerascreen® kits consist of a single-use blower device, 5 breath-sample collector glass tubes, and 20 g of the substrate. Before taking the breath test, subjects will need to meet specific test requirements (Annex VI. Breath test instructions), such as fasting conditions (at least 8 h) and a restrictive diet consisting of white rice, meat (fish, beef, chicken, or egg), extra virgin olive oil and water (water, tea or black coffee). Subjects will be able to perform the breath tests at home following the steps of Annex VI.

In order to collect the breath samples, subjects will drink a solution of 20 g of the substrate (lactose or fructose). Then, breath samples will be collected every 30 min up to 120 min (5 glass tubes). The first tube will be saved for a blank breath sample collection before drinking the solution (control tube). The concentration of exhaled gases (H_2/CH_4) will be quantified using the BreathTraker® gas breath analyzer (QuinTron Instrument Company Inc., Milwaukee, WI, USA). Results will be read according to the following criteria: changes of $H_2 \geq 20$ ppm and $CH_4 \geq 12$ ppm as positive results.

Table 4 Food allergen panels in type I and type II food allergy for the detection of IgE and IgG_4 Ab reactions in human serum samples

ARFS	Marker	AESKUBLOTS® kit (reference/name)	Food allergen panel
T1FA	IgE	421,001/ E-5	Egg white (f1), egg yolk (f75), milk (f199), cod (f3), wheat (f4), soybean (f14), peanut (f13), cashew nut (f204), crab (f23), shrimp (f24), beef (f27), lamb (f88), tomato (f25), pineapple (f52), mango (f91), and total IgE (IgE)
		421,011/ E-11	Tomato (f25), carrot (f31), celery (f85), mustard (f89), garlic (f47), onion (f48), banana (f29), orange (f33), strawberry (f44), apple (f49), peach (f53), kiwi fruit (f84), pineapple (f52), mango (f91), egg white (f1), milk (f199), cod (f3), salmon (f41), tuna (f40), shrimp (f24), beef (f27), lamb (f88), chicken (f83), wheat (f4), barley (f6), oat (f7), maize (f8), rice (f9), sesame (f10), soybean (f14), peanut (f13), bean, green (f950), potato (f35), ginger (s15, walnut (f16), hazelnut (f17), almond (f20), cashew nut (f204), CCD (CCD), and total IgE (IgE)
T2FA	IgG_4	431,401/ G4-1	Salmon (f41), cod (f3), herring (f21), shrimp (f24), blue mussel (f37), pork (f26), beef (f27), chicken (f83), wheat (f4), rye (f5), oat (f7), maize (f8), rice (f9), buckwheat (f11), amaranth (f811), carrot (f31), celery (f85), cabbage (f39), garlic (f47), onion (f48), pea, green (f12), bean, green (f950), soybean (f14), peanut (f13), tomato (f25), cucumber (f120), potatoe (f35), mustard (f89), egg white (f1), egg yolk (f75), milk (f199), goat´s milk (f300), sheep´s milk (f325), orange (f33), banana (f29), apple (f49), kiwi fruit (f84), hazelnut (f17), walnut (f16), and almond (f20)
		431,402/ G4-2	Salmon (f41), cod (f3), plaice (f152), squid (f176), blue mussel (f37), octopus (f819), pork (f26), beef (f27), lamb (f88), wheat (f4), oat (f7), sesame (f10), spelt (f158), amaranth (f811), carrot (f31), cucumber (f120), broccoli (f182), garlic (f47), maize (f8), cabbage (f39), celery (f85), sweet lupine (f827), egg white (f1), egg yolk (f75), cheese, gouda (f200), casein (f78), apple (f49), orange (f33), grape (f50), peach (f53), mango (f91), almond (f20), hazelnut (f17), peanut (f13), walnut (f16), pistachio nut (f818), cashew nut (f204), potato (f35), baker's yeast (f45),and soybean (f14)
		431,403/ G4-3	Tuna (f40), trout (f930), pollock (f802), herring (f21), oyster (f177), shrimp (f24), duck (f58), chicken (f83), turkey (f143), rye (f5), buckwheat (f11), barley (f6), durum wheat (f159), millet (f164), quinoa (f832), gluten (f79), tomato (f25), onion (f48), zucchini (f197), olive, green (f812), lentil (f65), pea, green (f12), bean, green (f950), milk (f199), sheep´s milk (f325), goat´s milk (f300), banana (f29), kiwi fruit (f84), lemon (f32), strawberry (f44), pineapple (f52), sunflower seed (f156), pumpkin seed (f157), mustard (f89), sweet basil (s11), ginger (s15), coffee (f955), cacao (f97), button mushroom (f141), and rice (f9)

ARFS, adverse reactions to foodstuffs; *CCD*, cross-reactive carbohydrate determinants; *IgE*, immunoglobulin E; *IgG_4*, immunoglobulin G_4; *T1FA*, type I food allergy; *T2FA*, type II food allergy

Anthropometry, body composition, and physical fitness

Anthropometry

Body weight (BW) will be measured in fasting conditions with an electronic calibrated MC-780MA-N Tanita bascule device (Tanita, Tokyo, Japan). Height will be assessed using a 2-m stadiometer Seca 213 1,721,009 (Seca GmbH & Co. KG., Hamburg, Germany) in a standing position and vertical plane. Subjects will be without shoes and with heels against the stadiometer base wall. Feet will be together, and knees will be straight and without bending. Subjects will be looking straight ahead with head in the Frankfort horizontal plane position (the lower margin of the eye bony socket aligned with the upper margin of the external auditory meatus). Subjects will be asked to breathe slowly during height measurements. Height will be recorded twice with an accuracy of 0.1 cm. Body mass index (BMI) will be calculated using body weight in kilograms (kg) divided by height in meters squared (m^2), kg/m^2 [39].

Waist-hip ratio will be calculated according to The International Society for the Advancement of Kinanthropometry (ISAK) using the 2-m Innovare metallic measuring tape (Cescorf Ltda., Porto Alegre City, Brazil).

Body composition

Multi-frequency segmental body composition will be performed using bioelectrical impedance analysis (BIA) using the MC-780MA-N Tanita device (Tanita, Tokyo, Japan). The analysis will include body fat percentage (BFP), body fat mass (BFM), fat-free mass (FFM), basal metabolic rate (BMR), total muscle mass (MM), mineral bone mass (MBM), skeletal muscle mass (SMM) and total body water (TBW). Before the test, subjects will have to accomplish particular requirements: 8-h fasting condition, wear comfortable clothes avoiding adjusted fabrics, avoid body unguents and having wet skin or hair, avoid alcoholic beverages during the previous 24 h, avoid difficult to digest foodstuffs (dairy, bakery, vinegar, or soy sauce) during the previous 12 h, urinate 30 min before the test; and avoid physical activity that may provoke cramps during the previous 12 h. Subjects will place their feet on top of the electrodes so that the heel is central to the smaller posterior electrode and the forefoot is central to the larger anterior electrode. Hands will touch the electrodes so that the electrode separator is positioned between the middle and ring finger. Duplicate measurements will be saved and stored in a secured digital (SD) memory card.

Physical fitness: cardiorespiratory fitness test, equilibrium test and handgrip strength

Cardiorespiratory fitness test

Cardiorespiratory fitness (CRF) will be determined by estimating the maximal oxygen consumption (VO_{2max}) using the Åstrand-Ryhming Step Test (stÅ-R) [40, 41]. VO_{2max} will be predicted according to Å-R's nomograms by the determination of submaximal heart rate (HR) values using a professional oximeter device (General Electric, GE, Ohmeda Tuffsat) (GE Healthcare, Boston, Massachusetts, USA). HR will be recorded every minute during a total test time of 5 min. Final HR will be recorded as the maximal value during the last 30 s of the submaximal fifth minute. For the final reading of the estimated VO_{2max}, Å-R's nomograms will consider the participant's final HR, sex, age, and weight.

The stÅ-R will be performed using a 40-cm and 33-cm height step for men and women, respectively. A MA-1 metronome (KORG Inc., Tokyo, Japan) will be set up at 22.5 cycles per minute to help subjects maintain a standard stepping pace. All measurements will start with a two-step warm-up to check the ability of the participant using the assigned height and step pace. The American Heart Association (AHA) classification [42] will be used to determine the categories of the estimated VO_{2max} and to subsequently organize the sample in low, normal, and excellent aerobic capacity.

Equilibrium test

Power, speed, and balance (chair sit-to-stand test) will be measured using the Tanita BM-220 force platform and the Zaritz software (Tanita, Tokyo, Japan). Sarcopenia assessment, lower extremity function, and physical fitness (PF) will be evaluated. Subjects will sit on a 40-cm-height standard chair with their barefoot, legs shoulder-width apart, the trunk stretched vertically in a straight line, and their ankles held at 90°. Following the instructions of the Zaritz software, subjects will have to sit and stand 3 consecutive times from the chair. Values of a single try of 3 times will be recorded.

Handgrip strength

Handgrip strength (HGS) will be measured, using a digital 5401 Grip-D dynamometer (Takei Scientific Instruments Co. Ltd, Tokyo, Japan). Muscle strength, upper extremity function and PF will be evaluated. Before the performance of the test, the dynamometer regulation will be set at 3.5 and 4 cm according to the participant's size between their phalanges. HGS will be performed in both hands, right and left, while sitting (40-cm-height standard chair) and standing (supine position). Subjects will rest for 30 s between each measurement and the highest values will be recorded after duplicate testing.

Quality of life

The self-perceived health-related QoL of the subjects of study will be assessed using the validated tools: Short-Form Health Survey 12 (SF-12) and the Spanish validated version of the Food Allergy Quality of Life Questionnaire—Adult Form (S-FAQLQ-AF).

Six-month intervention

Once the results of the blood samples are acquired, the expert-participant meetings will be arranged. During a one-to-one oral training session of approximately 15 min, Annexes I and II will be discussed and explained to the corresponding group of subjects. Annex I will be explained to the control group (Group A) and Annexes I and II, to the intervention group (Group B). Positive (> 3.5 kU_A/l) foodstuffs from serum (sIgE) and $sIgG_4$ AbR blood tests will be replaced with alternative food items listed in Annex II (FASSD). Adherence to the FAASD will be checked on a monthly basis (Annex IV. Dietary Adherence Questionnaire, DAQ) and any adverse event, missing data, or dropouts will be documented during the 6-month intervention (Annex VII. Adverse Event Form and Annex VIII End of Study Form).

End of the study and adverse events

Any potential negative outcomes linked to the dietary changes (minimal to zero risk probability) of the ALASKA study intervention, if any, will be documented using Annexes VII and VIII. The UPM will assist with an available medical physician, part of the ALASKA study researchers (M.P.-R.), to cover for any emergency associated with any step of this study protocol and will be present during all the clinical and physical assessments.

(Optional) Repetition of the assessments 2 to 8 and intervention adjustment.

The ALASKA study focuses mainly on a 6-month intervention/follow-up. However, the influence of ARFS and the effect of a FASSD in health and QoL may be long term. At the end of the trial, if the study budget allows, patients will have the opportunity to undergo a re-evaluation for all main variables from 1 to 8. Nevertheless, they will always be able to visit the INEF-UPM and get evaluated for variables with non-consumable measurement instruments, such as variables from 1 to 2 and 6 to 8 (Fig. 3).

Therefore, as an optional step, assessments will be repeated at M5/6 according to the established ALASKA study timeline described in Table 2. Food-specific sIgE and $sIgG_4$ Ab reactions, as well as its corresponding substitutive foodstuffs, may change according to the new results of the immunological food profile and/or enzymatic tests. Following another consecutive 6-month

intervention, using the recommendations of Annex I and Annex II instructions and based on the new results, will be suggested as an optional step. If no optional step will be taken by the subjects of study, they will be advised by a qualified professional of the nutritional practice on the adequate steps to be followed to retake a common healthy balanced diet (Dietary Guidelines for the Spanish population provided by the Spanish Society of Community Nutrition (SENC) [27].

Other variables

When analyzing other types of ARFS, apart from FA or FI, such as CeD, there could be other recommended relevant variables of interest. Some of these variables include the characterization of the microbiota, genetic testing (HLA DQ-2, DQ-8), and serum biomarkers such as type 2 transglutaminase (TG2), deamidated gliadin peptide (DGP), gliadins, microbial transglutaminase (mTG), and mTG neo epitopes [43, 44].

Statistical analysis

The sample size required for the current study was calculated using G*Power software v.3.1. (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) [25]. All subjects of study with complete data will be analyzed. First, the normal distribution of the data will be checked using the Kolmogorov–Smirnov test. In case of the non-normal distribution of a variable, a logarithmic transformation will be conducted. Independent sample's *t*-test (or its non-parametric Mann–Whitney *U* test) will be used to compare the baseline values of continuous variables between the two groups (A and B). Moreover, the chi-square test will be conducted to compare categorical variables. To examine the effect of the FASSD on the outcome variables, we will apply repeated measures analysis of variance (RM-ANOVA), in which the effect of time, intervention, and time*intervention interaction will be explored. Changes in outcome variables will also be examined between the two groups using an analysis of covariance (ANCOVA), in which the baseline values will be considered as covariates. A strong exclusion criteria list will be used in the ALASKA study to reduce confounding variables from interfering in the effect analysis. However, to analyze the multifactorial approach of multiple influencing variables over the ARFS together with the effect of the FASSD, the selection of confounding variables (dietary habits, QoL, sampling time and trial season, medication or food supplement intake), other than baseline characteristics, will be also considered. Corresponding Odds ratio (OR), or when justified Mantel–Haenszel (M-H) estimators will be used together with logistic or liner regressions, or ANOVA and ANCOVA tests, as it may apply for the corresponding variable.

Valid data values will be considered of subjects of study accomplishing at least main variables from 1 to 4 and from 6 to 7 (see the " Main variables" section), the rest of the data values will be carefully either cleaned or imputed as missing values. Data will be analyzed using IBM-SPSS® Statistics software, version 26.0, and statistical significance will be set at 0.05.

Discussion

ARFS is a current prevalent complication and chronic disease, which is continuously raising specially in patients with specific related-diseases and symptomatology. ARFS management approaching a multivariable model, including most influencing factors of FA and FI, can be used to evaluate ARFS from a wider perspective.

Demographic variables are considered a key predictor of literacy, QoL, and health in the clinical research and practice of chronic diseases. Age and sex may lead to different clinical outcomes, such as signs and symptoms, depending on specific pathologies and chronic diseases [14]. The country of birth, home city, or district may signify an essential determinant of health given that mortality, healthcare, culture, politics, socio-economics, and environment will be directly related to the participant's place of birth and living [45]. Education and occupation, as skill predictors, may signify key factors in chronic diseases involving the NS (e.g., memory, aptitude, ability, vitality) [46]. Employment has been also considered an influencing factor of the QoL in several chronic diseases involving the NS [47]. The number of people living together, in the same residence, can be optimal to measure the dynamics of relationships, as relevant sources of support, at different age stages of adulthood [48]. The identification of clinical variables is essential when investigating chronic diseases because, combined with demographic variables, they represent a consistent measurement of health and QoL [49].

ARFS may have a significant influence in the QoL and health, especially in individuals with FA or FI [50]. Lactose intolerance and fructose malabsorption (FIs) have been associated with poorer perceived QoL and health [51, 52]. Furthermore, studies have agreed that there is a lack of information and relatively little is known regarding the impact on QoL and health of individuals with FI. On the other hand, despite recent consensus on the FA detection procedures (IgE Ab reactions) and treatment (strict allergen avoidance); the necessity to expand the potential biomarkers for ARFS research and the need of novel proposals of nutritional treatments that could avoid micronutrient deficiencies is still latent [4]. IgG subclass 4 Ab reactions might be of interest when studying food and environmental allergens and its significance to specific types of diseases and symptoms (e.g., irritable bowel syndrome [53], allergic rhinitis [54], and chronic pain [55]).

Non-strict and partial avoidance diets (encouraging the substitutive dietary treatment proposal) have suggested possible improved hematobiochemical and immunological profiles, anthropometry, body composition, and muscle strength [56]. Review studies have observed that a complete exclusion of dairy products may favor the development of bone diseases such as osteopenia, osteoporosis, and muscle sarcopenia [57]. In clinical trials, the strict avoidance of positive food-specific IgG_4/IgE Ab reactions against food allergen mixtures has improved irritable bowel disease (IBD) symptomatology [58], atopic dermatitis [59], and mental illnesses [60]. Weight and blood dyscrasias seem to be other key features in ARFS, weight loss, and abdominal pain seem to be tightly related in FI-positive profiles [61].

To our knowledge, there is no further clinical trial examining the effect of the FASSD, or similar, on symptomatology, nutritional status, hematobiochemical and immunological makers, enzymatic activity, anthropometry, body composition, and QoL of patients with ARFS. Considering that muscle mass and strength, nutritional status, and QoL of patients with ARFS are being influenced due to strict food avoidance diets, it is expected that this type of dietary treatment, the FASSD, might improve the nutritional status of patients with ARFS. The logic behind such hypothesis was the particular main characteristic of the FASSD which includes the micronutrients that are generally deficient in individuals with FA or FI, and through which it may affect QoL and health.

Strengths and limitations

This study proposes a novel methodological tool for the evaluation and management of ARFS considering a variety of influencing factors such as the symptomatology, food and beverages intake, hematobiochemical and immunological studies, enzymatic activity, physical fitness, anthropometry and body composition. This study proposes the first RCT on the effect of a substitutive diet on symptomatology, nutritional status, hematobiochemical and immunological makers, enzymatic activity, anthropometry, body composition, and QoL among patients with ARFS. The findings of this study will provide evidence on the protocol.

Conclusions

The ALASKA study protocol has been developed as a global strategy to manage and evaluate ARFS (particularly FA and FI) in Spanish adults older than 18 years of age. Approaching ARFS with multiple assessments, as influencing factors, such as symptomatology, food and beverages intake, hematobiochemical and immunological studies, enzymatic activity, physical fitness, anthropometry, and body composition, will lead to a novel strategy for ARFS management and the evaluation of its impact on QoL and health. The FASSD has been designed as a personalized tool to avoid crucial micronutrient deficiencies that a current strict food allergen avoidance or elimination diet may provoke. The FASSD considers essential food groups for a balanced diet and includes special attention to B2, B3, Mg, K, P, Ca, Zn, B12, B9, Fe, and fiber. The ALASKA study protocol can be used, in the research and clinical practice, for the investigation and evaluation of general ARFS and to seek its relationship with related diseases, symptomatology, food and beverages intake, physical fitness, anthropometry, body composition and novel potential food-specific biomarkers.

Trial status

Recruitment of participants started in April 2022 and finished May 2023. Earlier study protocol submission was not possible because of protocol adaptations due to COVID-19 pandemic. The ALASKA study is still ongoing now and fieldwork is foreseen to be finished by July 2024.

Abbreviations

Ab	Antibody
AHA	American Heart Association
ALASKA study	Allergies and Food Intolerances in Adults and Athletes. UPM study project: Relation between adverse reactions to food, physical performance and health in a Mediterranean population. The ALASKA study
ARFS	Adverse reactions to foodstuffs
BD	Becton Dickinson
BEDCA	Spanish Food Composition Database
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BSQ	Blood Sample Questionnaire
CeD	Coeliac disease
CESVIMA	Supercomputing and Visualization Center of Madrid Community
CBC	Complete blood count
COVID-19	Coronavirus disease 2019
CRC	Clinical research coordinator
CRF	Cardiorespiratory fitness
DAQ	Dietary Adherence Questionnaire
DGP	Deamidated gliadin peptide
EAACI	European Academy of Allergy & Clinical Immunology
EDTA	Ethylene diamine tetraacetic acid
e.g.	Exempli gratia (latin), for example
FA	Food allergy
FASSD	Food allergen-specific substitutive diet
FBFC-ARFSQ-18	Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs
FI	Food intolerance
HGS	Handgrip strength
HLA	Human leukocyte antigen
HR	Heart rate
i.e.	Id est (latin), that is, in other words
IgE	Immunoglobulin E
IgG4	Immunoglobulin G4
IHR	Immune-mediated immediate hypersensitivity reactions
INEF-UPM	Faculty of Physical Activity and Sport Sciences of the UPM
ImFINE-RG	ImFINE Research Group
IMFINE-HYD	Hydration Questionnaire of the ImFINE Research Group
IPAQ-LF	The International Physical Activity Questionnaire Long Form
ISAK	International Society for the Advancement of Kinanthropometry
kUA/l	Kilounits of allergen-specific IgE or IgG4 per liter
K2	Dipotassium

LH	Lithium heparin
mTG	Microbial transglutaminase
M1	Month 1 of the ALASKA study
M5/6	Month 5 or 6 of the ALASKA study
NCCDB	Nutrition Coordinating Center Food and Nutrient Database
NS	Nervous system
OFC	Oral food challenge
PAL	Physical activity level
PF	Physical fitness
PPM	Parts per million
PSIMP-RAA-10	Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs
QoL	Quality of life
RAPA	The Rapid Assessment of Physical Activity Questionnaire
RCT	Randomized controlled trial
RedCap®	Research Electronic Data Capture Platform
RF	Radio frequency
RRAT	RedCap® randomization automated tool
SENC	Spanish Society of Community Nutrition
SF-12	Short-Form Health Survey 12
S-FAQLQ-AF	Spanish validated version of the Food Allergy Quality of Life Questionnaire-Adult Form
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
stÅ-R	Åstrand-Ryhming Step Test
T1FA	Type I hypersensitivity/ food allergy
T2FA	Type II hypersensitivity/ food allergy
TG2	Type 2 transglutaminase
UPM	Universidad Politécnica de Madrid
USDA-FCD	United States Department of Agriculture (USDA) Food Composition Database
VO_{2max}	Maximal oxygen consumption
WHO	World Health Organization
24HDRI	24-h dietary recall interview

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-024-08307-2.

Additional file 1: Supplementary Information (Annex I to VIII). Annex I. General nutritional advice with dietary recommendations for a healthy lifestyle. Annex II. Instructions of the food-allergen specific substitutive diet (FASSD). Annex III. 24-hour dietary recall interview (24HDRI). Annex IV. Dietary Adherence Questionnaire (DAQ, adapted to the ALASKA study). Annex V. Blood Sample Questionnaire (BSQ) adapted to the ALASKA study. Annex VI. Breath test instructions. Annex VII. Adverse Event Form. Annex VIII. End of Study Form.

Additional file 2. SPIRIT checklist.

Additional file 3: Appendix 1. Informed consent model for the ALASKA study.

Additional file 4: Appendix 2. Inclusion and exclusion criteria form.

Trial team

Institution: Universidad Politécnica de Madrid (UPM).
Faculty: Faculty of Physical Activity and Sport Sciences (INEF-UPM).
Department: Department of Health and Human Performance.
Director of the Department: M.G.-G.
Research group: ImFINE Research Group (ImFINE-RG).
Director of the ImFINE-RG: M.G.-G.
Principal or chief investigator (PI): M.G.-G. Role: Oversee the overall clinical trial (protocol design, recruitment, informed consents collection, data collection, analysis, interpretation and publication of results), responsible for all aspects of local organization and ensure that all rules and clinical trial regulations are followed.
Clinical research coordinators (CRC): L.P.-A., E.G., M.P.-R, R.U., M.G.-G.. Roles: organization of tasks or activities and conduct of the trial clinical procedures; delivery of the intervention; reporting of safety events (reviewed by the PI); and maintenance of the trial database file. The coordinator researchers are involved in running the clinical trial (ALASKA study). The CRC prepare and carry out the protocol (plan) for the study, monitor the safety of the study, monitor conduct and progress, collect and analyze the data, and report the results of the study. The CRC will organize monthly meetings to oversee the mentioned aspects and report to the PI.
Professional fields and other supporting staff: L.P.-A. (Food Engineer, researcher), E.G. (PhD in Pharmacy, Professor), M.P.-R (Medical Doctor, Professor), J.L.-S (Nutritionist, researcher), A.N.S. (Laboratory Staff, Technician), C.Q. (PhD in Mathematics, Professor), P.W. (Biochemist, external staff), T.M. (PhD in Physical Biochemistry, external staff), R.U. (PhD in Biology, researcher), M.G.-G (PhD in Pharmacy, Professor and PI researcher), other members of the ImFINE-RG including laboratory technician, nurse and master/doctoral students from the INEF-UPM. Roles: Conduct clinical and physical assessments in accordance with the ALASKA study protocol, assist investigators throughout the process, from the set-up to the completion of clinical trials.
Ethics Committee: Ethics Committee of the UPM approved this study, reference number 20200602, date: 17/07/2020. The Ethics Committee for R&D + i works at UPM, was established by the Governing Board on 26 January 2006- Its main purpose of guaranteeing scientific rigor, compliance with national, European and international laws related to research and ethics, and vigilance of all research performed at UPM.
The Ethics Committee of the UPM is divided into different areas. Therefore, the ALASKA study received several applicable favorable reports:
- General Data Protection Regulation Report: The PI confirms knowledge, specifically, of Parliament Regulation (EU) 2016/679. Regulation from the European Parliament and Council dated April 27, 2016 regarding safeguarding individuals, the processing of personal data and the free circulation of these data repealing Directive 95/46/EC (General Data Protection Regulation), and commits to complying with it.
- Human Research Report: The PI declares to know the ethical principles and legal regulations that govern the research activities and undertakes to respect these principles and standards in the development of the ALASKA study. In particular, the PI declares that she knows and abides by the provisions of the Declaration of Helsinki of the World Medical Association (64th General Assembly, Fortaleza, Brazil, October 2013); the Oviedo Convention on Human Rights and Biomedicine (Council of Europe, 1997); and the Basic Law 41/2002 regulating patient autonomy and rights and obligations in terms of clinical information and documentation.

Data sharing statement

No later than 3 years after the collection of 1-year post-randomization processes, publications (articles, abstracts, and other manuscripts) explaining and evaluating the study outcomes will be delivered. Public access to the present protocol publication and its subsequent publications will be available at the local projects section of the ImFINE Research Group website (https://short.upm.es/kwgpo).

Authors' contributions

Conceptualization, L.P.-A., E.G., M.P.-R., and M.G.-G.; methodology, L.P.-A., E.G., M.P.-R, T.M., R.U. and M.G.-G.; software, L.P.-A.; validation, L.P.-A., M.P.-R., R.U. and M.G.-G.; formal analysis, L.P.-A., M.P.-R., and M.G.-G.; investigation, L.P.-A., E.G., M.P.-R., J.L.-S. and P.W.; resources, L.P.-A., P.W., T.M. and M.G.-G.; data curation, L.P.-A., E.G., M.P.-R., and M.G.-G.; writing—original draft preparation, L.P.-A., E.G., M.P.-R. and P.W.; writing—review and editing, L.P.-A., E.G., M.P.-R., J.L.-S., P.W., T.M., R.U. and M.G.-G.; visualization, L.P.-A. and M.P.-R.; supervision, E.G., M.P.-R., R.U., T.M. and M.G.-G.; project administration, M.G.-G.; funding acquisition, L.P.-A. and M.G.-G. All authors have read and agreed to the final version of the manuscript.

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AESKUBLOTS® kits will be manufactured by Aesku.Diagnostics GmbH (Aesku. Diagnostics GmbH, Wendelsheim, Germany). The design, management, analysis, and reporting of the study are entirely independent of the manufacturers of AESKUBLOTS® kits (Aesku.Diagnostics GmbH, Wendelsheim, Germany).
The ALASKA study RCT is conducted in the ImFINE Research Group (gi.imfine@upm.es) of the Universidad Politécnica de Madrid. Industries and/or private institutions have no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Declarations

Competing interests
PW and TM are part of Aesku.Diagnostics GmbH staff. Aesku.Diagnostics GmbH did not participate in the design of the protocol, nor in the analysis and interpretation of the data outcomes. The rest of the authors have nothing to report.

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6.8. **Annex H: Article 4**

Title: “Is there a relationship between physical performance factors and adverse reactions to foodstuffs? - The ALASKA Study”.

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Article

Is there a relationship between physical performance factors and adverse reactions to foodstuffs? - The ALASKA study.

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Abstract: The aims of this study were a) to investigate the prevalence of adverse reactions to foodstuffs (ARFS) according to age; b) to analyze physical performance (PP) variables according to the type of ARFS; and c) to determine the probability of suffering any of the studied types of ARFS according to PP. A cross-sectional study with 254 Spanish adults (61% women; mean age 43.7±13y) scoring ≥6 in PSIMP-ARFSQ-10 questionnaire was conducted in the Region of Madrid, Spain following the published ALASKA study protocol. ARFS were sIgE-mediated (type 1 food hypersensitivity, T1FH), $sIgG_4$-mediated (T2FH) antibody reactions (AbR), and lactose and fructose intolerance (LCI and FCI, respectively). Statistical significance was set at 0.05. The most prevalent sIgE and $sIgG_4$-mediated ARFS were against legume (53% and 46%; 60% and 68% in subjects with ≤45y and >45y, respectively). Handgrip strength (HGS) was significantly lower when positive LCI compared to negative LCI subjects ($p<0.05$). Both positive mean sIgE and IgG_4 AbR were significantly associated to high PP ($p<0.05$). Subjects with high PP had 1.5-fold risk to experience a positive mean sIgE and a positive legume T2FH. In conclusion, subjects with 45y or younger had higher prevalence of total T1FH and T2FH, than subjects older than 45y. Positive LCI was linked to lower values of HGS. Significant risk of experiencing nuts T1FH was observed in subjects with high PP who were either male, with ≤45y or presenting ≥25 of BMI.

Keywords: Cardiorespiratory fitness; Exercise test; Food hypersensitivity, Immunoglobulin E, Immunoglobulin G, Physical fitness, Physical functional performance.

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1. Introduction

Food allergies (FA), together with other related adverse reactions to foodstuffs (ARFS), is currently considered a global public health concern and it is among the five most important chronic diseases of the world according to the World Health Organization (WHO) due to its increasing prevalence in the past decade (1). The most recent available European FA prevalence data based on food allergen-specific serum IgE (sIgE) antibody reactions (AbR) was published by the European Academy of Allergy and Clinical Immunology (EAACI) in 2023, reporting a prevalence of 16.6% for FA based on sIgE AbR in European individuals; specifically, 18.4% and 11.2% for children and adults, respectively (2).

According to the recently published ARFS classification by Pantoja-Arévalo et al. (2024), ARFS refers to any immune-mediated and non-immune-mediated food reaction, including FA or food hypersensitivity (FH), food malabsorption and food intolerance (FI), among other ARFS (3). Type 1 FH (T1FH, mostly sIgE-mediated ARFS) and type 2 FH (T2FH, mostly sIgG4-mediated ARFS) have been linked to the FA pathogenesis identification and to self-reported symptoms related to ARFS, respectively (4, 5). Additionally, individuals with lactose and fructose intolerance (LCI and FCI, respectively) presenting symptoms related to the gastrointestinal (GI) system have been observed to have higher levels of FH and physical commotions, than those without LCI (6).

ARFS encompasses a range of related aspects that can be affected by intrinsic and extrinsic factors (3). Optimal cardiorespiratory fitness (CRF) and other physical performance (PP) variables have been shown to have a positive impact on the health of adults with chronic diseases in developed countries. However, there is a lack of robust data regarding the direct relationship between ARFS and PP related variables.

Mechanisms of FA may vary among different populations worldwide. It has been observed that individuals experience variations in the positivity of ARFS when exposed to the same amount or even type of food allergen at different stages of life (7). These variations have been tried to be explained describing several general risk factors in ARFS such as family history, dietary habits, environmental and food allergen exposure, and other related factors (8). ARFS in the Physical Activity (PA) and Sport Sciences context is essential due to the "dual allergen exposure hypothesis" which suggests that changes in environmental conditions during sports can contribute to the food-related ARFS disease progression (9). Therefore, for the present study it is considered that factors, such as, sex, age, body mass index (BMI), specific symptomatology, CRF and PP variables, could help in comprehending the nature of ARFS in this scenario. In addition, understanding the potential risk factors related to ARFS would significantly help improve intervention and treatment studies in the future.

The aims of this study were a) to investigate the prevalence of adverse reactions to foodstuffs according to age; b) to analyze physical performance variables according to the type of adverse reaction to foodstuffs; and c) to determine the probability of suffering any of the studied types of adverse reactions to foodstuffs according to physical performance variables in a sample of Spanish adults.

2. Materials and Methods

The methodology of this study was carried out following the published ALASKA study protocol previously described elsewhere (3).

2.1. Study design and participants

The present study analyzed data collected from the ALASKA study. This is a cross-sectional study conducted from April 2022 to May 2023 in the Region of Madrid, Spain. A total of 295 Spanish adults were eligible to participate. Individuals aged ≥18y, with no medical restrictions on PA, maintaining a regular sleep schedule, not heavy smokers (<25 cigarettes/day), not pregnant, with no prior diagnosis of ARFS, and a PSIMP-ARFSQ-10 score of ≥6, volunteered to participate in this study. Twenty-one participants did not accomplish the inclusion criteria, ten participants did not sign the consent follow-up section, 6 participants were excluded for attrition and loss to follow-up and 4 participants for missing data. A total sample of 254 Spanish adults with complete and plausible data on the assessments of Table 1 were evaluated and considered as a valid sample.

2.2. Study variables

The variables and instruments used in this study are summarized in Table 1. Questionnaires and forms were administered online using the Research Electronic Data Capture Platform (RedCap®), hosted in the Supercomputing and Visualization Center of

Madrid Community of the Universidad Politécnica de Madrid (UPM) (CESVIMA-UPM). Diseases, signs and symptoms related to any type of ARFS were assessed using the validated PSIMP-ARFSQ-10 tool (10). Participants supplied information on their date of birth, age, sex, level of education, employment situation, occupation, living situation (number of people living together) and home city.

Table 1. Summary of the variables and research instruments of the cross-sectional analysis of the ALASKA study

	Variables/ Assessments	Questionnaires/ Instruments	References
Sample characteristics	Participant consent	Informed consent form	(3)
	Eligibility screening	Inclusion and exclusion criteria form	(3)
	Demographics and clinical information	Demographics and clinical characteristics form	(3)
	Diseases, signs and symptoms	PSIMP-ARFSQ-10	(10)
	Height (cm)	Stadiometer	(Seca GmbH & Co. KG., model 213 1721009, Hamburg, Germany)
	Waist-hip ratio (ISAK criteria)	Anthropometric tape	(Cescorf Ltd., 2-meter Innovare measuring tape, Porto Alegre City, Brazil)
	Weight (kg) and BIA with segmental multifrequency	BIA bascule	(Tanita, model MC-780MA-N, Tokyo, Japan)
	Body mass index (kg/m^2, mass in kilograms divided by height in meters squared)	Height and weight instruments	(11, 12)
	Physical activity	IPAQ-LF	(13, 14)
		RAPA	(15)
Clinical analysis	Recommended practices and requirements for participants before blood collection	Blood Sample Questionnaire	(3)
	T1FH, food allergen-specific sIgE AbR T2FH, food allergen-specific sIgG$_4$ AbR	In-line immunoassay analyzer	(Aesku.Diagnostics GmbH, Helia® Helmed, Wendelsheim, Germany)
	Lactose intolerance	Lactose hydrogen and methane breath test kit	(Cerascreen GmbH, Cerascreen® Lactose kit, Schwerin, Germany)
	Fructose intolerance	Fructose hydrogen and methane breath test kit	(Cerascreen GmbH, Cerascreen® Fructose kit, Schwerin, Germany)
Physical performance	Resting heart rate	Tensiometer	(Omron Healthcare Co. Ltd., model M3 Comfort HEM-7155-E(C), Kyoto, Japan)

Handgrip (hand/ upper extremity strength)		Dynamometer	(Takei, model T.K.K. 5401 Grip-D, Tokyo, Japan)
Power (leg/ lower extremity strength)		Force platform	(Tanita, model BM-220 7341(0), Tokyo, Japan)
Sit-to-stand speed			
Body balance			
Cardiorespiratory fitness	Estimated VO_2 max	Åstrand-Ryhming Step Test	(16)
	Pulse rate	Pulse oximeter	(GE Healthcare, Ohmeda Tuffsat, Boston, Massachusetts, USA)
	Pace/cycles	Metronome	(KORG Inc., model MA-1, Tokyo, Japan)

BIA, bioelectrical impedance analysis; *IPAQ-LF*, The International Physical Activity Questionnaire Long Form; *ISAK*, International Society for the Advancement of Kinanthropometry; *PSIMP-RAA-10*, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; *RAPA*, The Rapid Assessment of Physical Activity Questionnaire; *T1FH*, type 1 food hypersensitivity; *T2FH*, type 2 food hypersensitivity; *VO_2 max*, maximal oxygen consumption.

Subjects were organized by age groups as young adults (YA, ≤45y) and older adults (OA, >45y). BMI was evaluated according to the classification used by the National Institute of Health (NIH) and the WHO (12). PA was arranged in accordance with the WHO 2020 guidelines on PA and sedentary behavior (17). ARFS were categorized based on the ALASKA study protocol classification into immune-mediated reactions (sIgE AbR and $sIgG_4$ AbR) and non-immune mediated reactions (LCI and FCI) (3). Each ARFS subcategory was also evaluated as sIgE-only, $sIgG_4$-only, LCI-only and FCI-only indicating individuals with just a single positive subcategory of either immune or non-immune mediated reactions. CRF levels were classified according to the 95% of the good age-sex-estimated VO_2max value below which there is risk of developing major health problems (mainly cardiovascular diseases, diabetes and/or hypertension) (18). Moreover, maximal isometric HGS was recorded after at least two attempts from each hand. The handle width of the dynamometer was adjusted to subjects' hand size; and subjects were instructed to maintain neutral standing position while encouraged to squeeze the dynamometer and exert maximum force.

2.3. *Statistical analysis*

Descriptive statistics consisted of the mean and standard deviation (SD) for continuous variables, as well as frequency and percentage for categorical variables. A model utilizing linear regression and hierarchical multiple regression analysis was used to create different regression equations predicting the correlation between sex, age, living situation, PA, BMI, symptomatology, and PP variables (VO_2max values, maximal HGS, leg power, sit-to-stand (STS) speed, body balance and resting heart rate (RHR)). Age-sex adjustments were applied to all PP variables and median cut off values of maximal HGS and CRF were determined to classify the sample into low and high PP groups. Binary logistic regression was performed using dichotomic datasets of PP variables. Odds ratios (OR) were used as measurement of association and random-effects risk model. Statistical significance was set at 0.05.

2.4. *Ethical considerations*

This research was performed in accordance with the Ethical Guidelines of the Declaration of Helsinki of 1964, revised in Fortaleza (2013) (19) and following the Spanish and European regulations on data protection (20). The protocol was approved by the

Ethics Committee of the UPM (reference number 20200602) and registered on ClinicalTrials.gov (Clinical Trials ID NCT05802017).

The ALASKA study was carried out by qualified professionals for medical, clinical, food science, nutritional and PA and Sport Sciences practice. These experts were part of the ImFINE Research Group (ImFINE-RG) of the Department of Health and Human Performance of the Faculty of Physical Activity and Sport Sciences of the UPM (INEF-UPM). Signed informed consent forms were obtained from all participants before their participation commencement.

3. Results

3.1. Sample characteristics

A total sample of 254 Spanish adults participated in this study (61% women) with a mean age of 43.71±12.61 years. Data regarding demographics and descriptive characteristics of the ALASKA study are presented in Table 2. Men was significantly older than women (Table 3); however, there were no significant differences of socio-demographic characteristics (education level, job and living situation) between men and women.

More than 60% of the studied sample presented normal weight with no significant differences between men and women's categories of BMI. Furthermore, the results of Table 2 showed expected statistically significant anthropometric differences between men and women's BMI, height, weight, waist circumference, waist-to-hip ratio and body composition variables (all $p<0.05$).

Table 2. Sample characteristics of the ALASKA study compared by sex

	Mean±SD or *n* (%)				
	Total (*n*=254)	**Men (*n*=99)**	**Women (*n*=155)**	**Min-Max**	***p*-values$_{sex}$**
Education level					
Highschool	27 (10.6)	10 (10.1)	17 (11.0)	--	
Intermediate level professional training	11 (4.3)	2 (2.0)	9 (5.8)	--	
Higher level professional training	36 (14.2)	16 (16.2)	20 (12.9)	--	*NS* 0.275
University education (college graduate, engineer, high degree)	105 (41.3)	41 (41.4)	64 (41.3)	--	
Master degree	60 (23.7)	23 (23.2)	37 (23.9)	--	
PhD/doctorate degree	15 (5.9)	7 (7.1)	8 (5.2)	--	
Job situation					
Employed or freelance	209 (82.3)	86 (86.9)	123 (79.4)	--	
Learning period paid student	5 (2.0)	1 (1.0)	4 (2.6)	--	
Learning period non-paid student	12 (4.7)	3 (3.0)	9 (5.8)	--	
Unemployed	7 (2.8)	2 (2.0)	5 (3.2)	--	
Retired or on early retirement	9 (3.5)	4 (4.0)	5 (3.2)	--	*NS* 0.140
Permanent disability individual	1 (0.4)	1 (0.4)	1 (0.6)	--	
Homemaker or houseworker (housewife/husband)	5 (2.0)	0 (0.0)	5 (3.2)	--	
Other	6 (2.4)	3 (3.0)	3 (1.9)	--	
Living situation					
Number of people living with (NPLW)	2.05±1.27	2.03±1.31	2.06±1.24	0.00-6.00	*NS* 0.433
≤2 NPLW	162 (63.8)	65 (65.7)	97 (62.6)	--	*NS* 0.620
>2 NPLW	92 (36.2)	34 (34.3)	58 (37.4)	--	

Anthropometry					
BMI (kg/m²)	24.40±4.54	25.17±3.80	23.90±4.89	17.10-41.00	<0.05
Height (cm)	168.70±9.08	176.67±6.68	163.60±6.32	149.00-196.00	<0.001
Weight (kg)	69.59±14.64	78.65±13.18	63.80±12.46	41.80-124.80	<0.001
Waist circumference (cm)	79.62±12.74	87.19±11.04	74.78±11.35	38.00-126.80	<0.001
Hip circumference (cm)	97.02±12.06	97.95±8.25	96.42±13.96	63.00-192.00	*NS* 0.163
Waist-to-hip ratio	0.82±0.10	0.89±0.07	0.78±0.09	0.32-1.36	<0.001
Body mass index					
<18.5 underweight (%)	5 (2.0)	1 (1.0)	4 (2.6)	--	
18.5 – 24.9 normal weight (%)	163 (64.2)	57 (57.6)	106 (68.4)	--	
25.0 – 29.9 overweight (%)	63 (24.8)	34 (34.3)	29 (18.7)	--	*NS* 0.220
30.0 – 34.9 class I obesity (%)	9 (3.5)	3 (3.0)	6 (3.9)	--	
35.0 – 39.9 class II obesity (%)	14 (5.5)	4 (4.0)	10 (6.5)	--	
Body composition					
Fat mass (%)	25.08±8.63	20.48±6.62	28.02±8.50	5.60-51.60	<0.001
Fat mass (kg)	17.93±9.07	16.74±8.07	18.68±9.61	3.90-51.00	<0.05
Fat free mass (kg)	51.66±10.12	61.91±6.97	45.12±5.18	31.20-79.30	<0.001
Muscle mass (kg)	49.06±9.64	58.82±6.64	42.82±4.92	29.60-75.40	<0.001
Bone mass (kg)	2.60±0.48	3.09±0.33	2.29±0.26	1.60-3.90	<0.001
Body water (%)	51.89±5.95	56.27±4.13	49.10±5.21	35.90-66.60	<0.001
Body water (kg)	35.85±7.38	43.80±4.56	30.77±3.10	24.30-56.90	<0.001
Visceral fat rating (VFR [1])	5.96±4.18	8.27±4.43	4.48±3.25	1.00-23.00	<0.001

[1] VFR, score ranges from 1 to 59 using the Tanita, model MC-780MA-N, Tokyo, Japan.

Women revealed significantly higher total PSIMP-ARFSQ-10 score associated with ARFS pathogenesis (p<0.05); and particularly higher nervous system symptomatology PSIMP-ARFSQ-10 score (p<0.001) than men (Table 3). Estimated VO_2max, maximal HGS and leg power distribution values were significantly higher in men than women (all p<0.001), as expected. However, non-significant sex differences were found between other PP variables, specifically, STS speed and body balance. In contrast, RHR distribution values were significantly higher in women than men (p<0.05) (Table 3).

Mean ages for YA and OA were 33.77±7.55y and 54.99±5.90y, respectively. Both mean GI and nervous system symptomatology PSIMP-ARFSQ-10 scores were higher in YA than OA (p<0.05). GI PSIMP-ARFSQ-10 scores were still higher for the group of YA *vs* OA in women (p<0.05). Self-reported PA in men was worse in OA than in YA, with twice as many OA not meeting WHO recommendations compared to YA (20% vs. 10%). Regarding maximal HGS, there were no significant differences by age groups in men, while YA women showed higher maximal HGS than OA ones (p<0.05). As expected, leg power and STS speed were significantly higher in YA than OA, in both, men and women (both p<0.001). Estimated VO_2max was significantly higher in YA, maintaining its significance when analyzing the specific CRF levels between YA and OA. The proportion of subjects showing good CRF was 70% of YA and 50% of OA (Table 3).

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Table 3. Symptomatology, physical activity and physical performance compared by sex and age

	Mean±SD or *n* (%)													
	Total (n=254)	Men (n=99)	Women (n=155)	Min-Max	p-values$_{sex}$	≤45y (n=135)			>45y (n=119)			p-values$_{age}$	p-values$_{age-sex}$	p-values$_{age-women}$
						Total ≤45y	Men (n=43)	Women (n=92)	Total >45y	Men (n=56)	Women (n=63)			
Age (years)	43.71±12.61	46.23±13.04	42.10±12.10	18.00-77.00	<0.05	33.77±7.55	33.91±7.94	33.71±7.40	54.99±5.90	55.70±6.68	54.37±5.08	<0.001	<0.001	<0.001
Symptomatology (PSIMP-ARFSQ-10 score) [1]														
GI system	4.87±2.77	4.77±2.71	4.94±2.81	0.00-15.00	NS 0.319	5.29±2.56	4.98±2.56	5.43±2.56	4.40±2.93	4.61±2.83	4.21±3.02	<0.01	*NS* 0.252	<0.05
Skin and subcutaneous tissue	2.92±2.65	2.64±2.54	3.10±2.71	0.00-14.00	NS 0.089	2.84±2.48	2.70±2.19	2.91±2.62	3.00±2.84	2.59±2.79	3.37±2.85	*NS* 0.321	*NS* 0.417	*NS* 0.195
Nervous system	3.04±2.75	2.07±2.34	3.66±2.83	0.00-16.00	<0.001	3.36±2.76	2.44±2.35	3.79±2.85	2.67±2.71	1.79±2.31	3.46±2.82	<0.05	*NS* 0.084	*NS* 0.236
Respiratory system	1.56±1.86	1.70±1.84	1.47±1.87	0.00-11.00	NS 0.173	1.47±1.69	1.77±1.48	1.33±1.77	1.66±2.04	1.64±2.08	1.68±2.01	*NS* 0.200	*NS* 0.370	*NS* 0.123
Other pathologies/ diseases	2.49±2.34	2.14±2.13	2.72±2.45	0.00-10.00	<0.05	1.81±2.00	1.42±1.79	1.99±2.07	3.27±2.47	2.70±2.22	3.78±2.58	<0.001	=0.001	<0.001
Total PSIMP-ARFSQ-10 score	14.88±7.97	13.31±7.33	15.88±8.21	6.00-44.00	<0.05	14.77±7.46	13.30±6.67	15.46±7.74	15.00±8.53	13.32±7.86	16.49±8.88	*NS* 0.410	*NS* 0.495	*NS* 0.221
Physical activity (IPAQ-SF)														
Accomplish WHO recommendatrions	219 (86.2)	85 (85.9)	134 (86.5)	--	NS 0.447	119 (88.1)	39 (90.7)	80 (87.0)	100 (84.0)	46 (82.1)	54 (85.7)	*NS* 0.172	*NS* 0.115	*NS* 0.413
Do not Accomplish WHO recommendations	35 (13.8)	14 (14.1)	21 (13.5)	--		16 (11.9)	4 (9.3)	12 (13.0)	19 (16.0)	10 (17.9)	9 (14.3)			
Physical Performance														
Handgrip strength (HGS)														
Total maximal HGS (kg)	34.46±9.91	44.41±6.30	28.11±5.66	17.30-66.00	<0.001	34.18±9.96	45.36±6.39	28.95±6.36	34.78±9.90	43.68±6.18	26.88±4.20	*NS* 0.314	*NS* 0.095	<0.05
Power, speed and balance														
Leg power (l/w)	1.55±0.24	1.61±0.26	1.51±0.23	1.10-2.39	<0.001	1.65±0.25	1.78±0.22	1.58±0.23	1.44±1.19	1.47±0.20	1.41±0.17	<0.001	<0.001	<0.001
Sit-to-stand speed (RFD/w)	12.61±3.75	12.79±4.10	12.49±3.51	0.01-23.60	NS 0.271	13.85±3.58	15.27±3.23	13.18±3.55	11.20±3.43	10.88±3.66	11.49±3.23	<0.001	<0.001	=0.001
Body balance (BP T-score)	50.12±9.42	49.43±10.46	50.55±8.69	1.00-63.00	NS 0.178	50.43±8.61	50.33±8.33	50.48±8.77	49.76±10.28	48.75±11.86	50.67±8.64	*NS* 0.575	*NS* 0.230	*NS* 0.448
Cardiorespiratory fitness (CRF)														
Estimated VO2max (ml/kg*min)	39.25±9.92	42.79±10.06	36.98±9.17	21.45-68.96	<0.001	43.28±9.39	48.80±8.98	40.70±8.44	34.67±8.45	38.18±8.29	31.55±7.34	<0.001	<0.001	<0.001
Low CRF (%)	46 (18.1)	15 (15.2)	31 (20.0)	--	NS 0.139	15 (11.1)	2 (4.7)	13 (14.1)	31 (26.1)	13 (23.2)	18 (28.6)	<0.05	<0.05	*NS* 0.092
Normal CRF (%)	68 (26.8)	26 (26.3)	42 (27.1)	--		39 (28.9)	11 (25.6)	28 (30.4)	29 (24.4)	15 (26.8)	14 (22.2)			
Good CRF (%)	115 (45.3)	47 (47.5)	68 (43.9)	--		66 (48.9)	23 (53.5)	43 (46.7)	49 (41.2)	24 (42.9)	25 (39.7)			
Excellent CRF (%)	25 (9.8)	11 (11.1)	14 (9.0)	--		15 (11.1)	7 (16.3)	8 (8.7)	10 (8.4)	4 (7.1)	6 (9.5)			

[1] Number of pathologies/diseases, signs and symptoms related to ARFS (21). *BP*, body power; *f*, force; *GI*, gastrointestinal; *HGS*, hand grip strength; *IPAQ*, The International Physical Activity Questionnaire; *NS*, statistically non-significant; *PAL*, physical activity level; *RAPA*, Rapid Assessment of Physical Activity; *RFD*, Rate of Force Development in N/s; *T-score*, bone health indicator; *VFR*, visceral fat ratio; *w*, weight; *WHO*, World Health Organization; *y*, years old.

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3.2. Prevalence of adverse reactions to foodstuffs according to age groups

Figure 1 presents the prevalence of positive T1FH and T2FH across different age groups. The most prevalent positive sIgG4-mediated or T2FH and sIgE-mediated or T1FH were legumes (64% and 50%), nuts (61% and 40%), and egg (63% and 5%, $p<0.05$), respectively. The most prevalent T1FH in YA adults were against legume (53%) and seeds (45%), while against legume (46%) and nuts (42%) in OA. Analogously, the most prevalent T2FH in YA adults were against egg (66%) and nuts (63%), while against legume (68%) and egg (60%) in OA adults.

3.2.1. Type 1 food hypersensitivity

Mean total sIgE AbR was 4.22±4.9kUA/l, meaning almost 40% of sIgE AbR positivity in the studied sample. Nearly half of the subjects showed positive food-specific sIgE AbR against legume, seeds and nuts, while nearly one third presented positive sIgE AbR against cereal and gluten-free cereal (Figure 1). Shellfish food group was the only group showing significant differences by sex, with a higher prevalence of positive sIgE AbR in women compared to men (20.6% *vs* 11.1%, respectively) ($p<0.05$). Mean total food allergen-specific sIgE AbR for YA *vs* OA were 4.6±5.6 and 3.8±3.9kU$_A$/l, respectively ($p>0.05$). However, cereal and meat food groups showed statistically higher prevalence of positive sIgE AbR in YA compared to the OA group (both $p<0.05$). Comparing food allergen-specific sIgE AbR between YA *vs* OA by sex, higher prevalence of positive sIgE AbR against seeds, fish and meat was found among young men compared to older men; while higher prevalence of positive sIgE AbR against meat and egg, among young women compared to older women (all $p<0.05$).

3.2.2. Type 2 food hypersensitivity

Mean total sIgG4 AbR was 13.65±13.93kU$_A$/l, meaning around 70% of sIgG4 AbR positivity in the studied sample. Approximately two thirds of the subjects showed a positive food-specific sIgG4 AbR against legume, nuts, and egg- specific sIgG4 AbR; followed by around one third of subjects with positive specific AbR against seeds, cereal, gluten-free cereal, vegetable, fruits and milk (Figure 1). Women showed higher prevalence of positive sIgG4 AbR against legume food group compared to men (68.4% *vs* 56.6%, respectively) ($p<0.05$); while men showed higher prevalence of positive sIgG4 AbR against gluten-free cereal, seeds, fish, and meat food groups compared to women (39.4% *vs* 21.9%, 45.5% *vs* 30.3%, 20.2% *vs* 5.2%, and 26.3% *vs* 15.5%, respectively) (all $p<0.05$). Mean total food allergen-specific sIgG4 AbR for YA *vs* OA were 14.37±14.12 and 12.84±13.71kU$_A$/l, respectively ($p>0.05$). However, higher prevalence of positive sIgG4 AbR against cereal and milk food groups were found in YA compared to the OA age group (both $p<0.05$). Comparing food allergen-specific sIgG4 AbR between YA *vs* OA by sex, higher prevalence of positive sIgG4 AbR against cereal was found among young men compared to older men ($p<0.05$). Similarly, higher prevalence of positive sIgG4 AbR against cereal and fish was found among young women compared to older women (all $p<0.05$). On the other hand, legume food group, showed a higher prevalence of positive sIgG4 AbR in OA compared to YA.

Further information about T1FH and T2FH comparing groups by sex and age are referred in Annex I, Table IA.

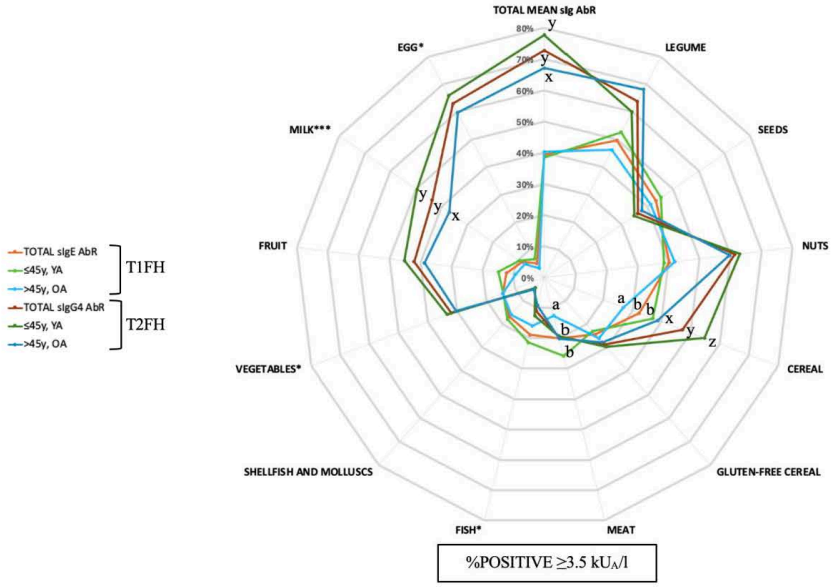


Figure 1. Prevalence of type I and type II hypersensitivity reactions according to age groups. *p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001. Asterisks " * " beside the food groups show statistical differences between the overall orange color curves, mean sIgE AbR compared to total mean sIgG4 AbR among the 12 established food groups. Values bearing different letters were significantly different (a≠b≠c; x≠y≠z). kUA/l, kilounits of allergen per liter; sIg, serum immunoglobulins; sIgE, serum immunoglobulin E; sIgG4, serum immunoglobulin G4; T1FH, type 1 food hypersensitivity; T2FH, type 2 food hypersensitivity.

3.2.3. Lactose and fructose intolerance

Around one third of the studied subjects showed LCI or FCI, and around one out of ten subjects presented both LCI and FCI. The highest prevalence of intolerance in the studied sample was found in the OA group, although there were no significant differences among the evaluated age and sex groups (Figure 2).

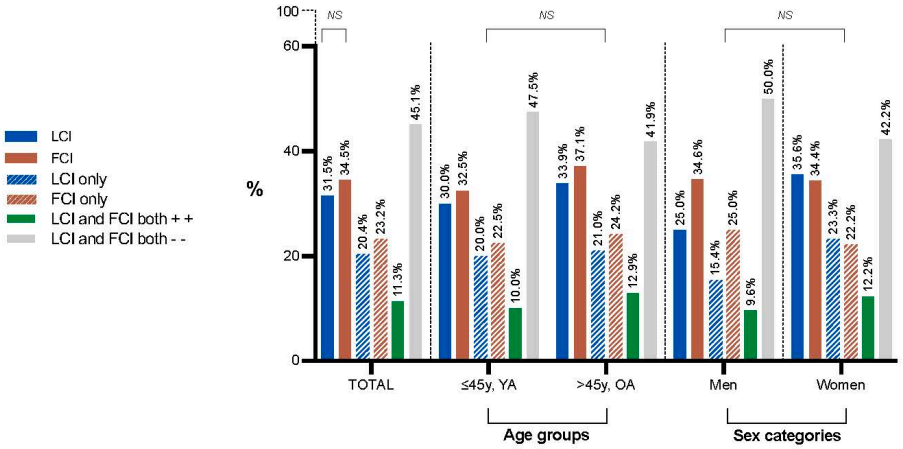


Figure 2. Prevalence of lactose and fructose intolerance according to age and sex groups. *OA*, older adults; *YA*, younger adults; *LCI*, lactose intolerance; *FCI*, fructose intolerance; *NS*, statistically non-significant; *y*, years of age.

3.3. Physical performance

3.3.1. Physical performance variables and sample characteristics

Significant associations were found between the PP variables (CRF as estimated VO_2max, maximal HGS, leg power, STS speed, body balance and RHR distributions) and the studied categories of sex, age, BMI and symptomatology (Figure 3). There were no significant differences between PP variables and PA; or between PP variables and the subjects' living situation (number of people living with) (data not shown). As expected, significant associations, endorsing men, were observed between estimated VO_2max, maximal HGS, and leg power, with sex categories (all $p<0.001$). Besides, women exhibited signifficantly higher RHR values. There were no significant associations between STS speed and body balance, with sex categories. YA significantly outperformed OA in CRF, leg power, and STS speed (all $p<0.001$). CRF and leg power were significantly associated with BMI and it was observed that greater distribution values of estimated VO_2max and leg power were significantly related to BMI values of <25 ($p<0.01$). Furthermore, greater distribution values of maximal HGS, leg power, and RHR were significantly associated with lower PSIMP-ARFSQ-10 scores (scores from 6 to 12) in the total sample of studied subjects (all $p<0.05$). Sex, age, BMI and symptomatology PSIMP-ARFSQ-10 scores were all contributing factors in leg power distribution values (all $p<0.05$).

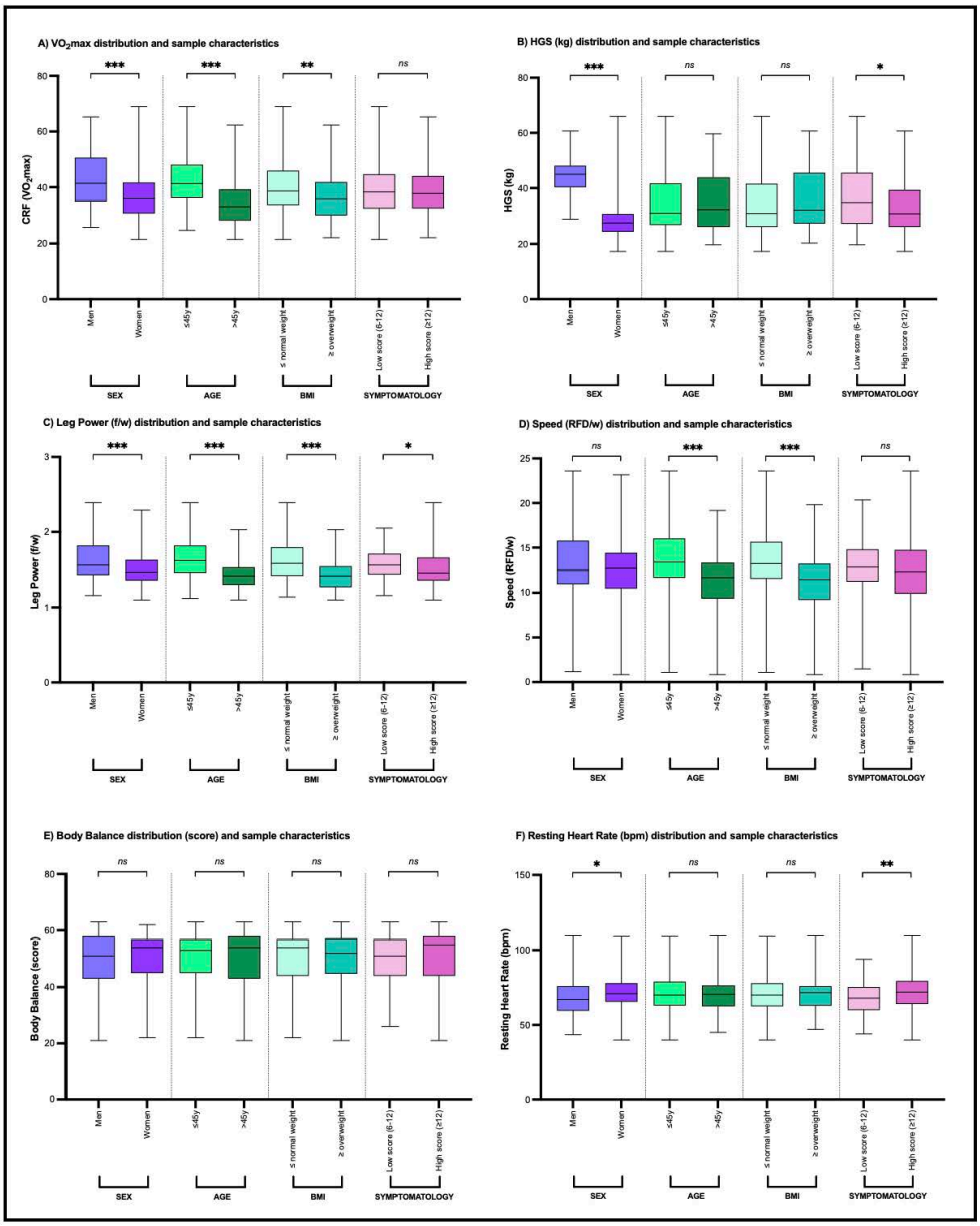


Figure 3. Physical Performance variables and sample characteristics. *p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001. *ARFS*, adverse reactions to foodstuffs; *bpm*, beats per minute; *CRF*, cardiorespiratory fitness; *f/w*, force/weight; *HGS*, handgrip strength; *RFD/w*, rate of force development/ weight (sit-to-stand speed); *VO2max*, maximal oxygen consumption; *y*, years of age.

3.3.1. Physical performance variables and adverse reactions to foodstuffs

The prevalence of subjects testing positive for both T1FH and T2FH was greater in those with high PP compared to those with low PP. Meanwhile, positive LCI subjects were significantly associated with low PP compared to high PP (Figure 4). Maximal HGS showed a significant decrease with positive LCI compared to negative LCI (p<0.05). Nonetheless, there were no significant variations in CRF between the established positive groups of FA and FI. Nevertheless, some associations have been observed while studying the 12 food groups and PP variables (Annex I, Table IB).

Positive food-specific sIgG4 AbR were associated to several PP variables. Food-specific sIgG4 AbR were positively related to higher CRF values for the cereal, fish, nuts and shellfish food groups; higher maximal HGS values for milk and meat food groups; higher STS speed values for legume food group; and higher balance values for fish food group. In contrast, food-specific sIgG4 AbR were related to lower STS speed values for cereal food group, and RHR values for egg food group (all p<0.05). No significant differences in higher leg power were found, or in higher or lower PP values for food-specific sIgE AbR (Annex I, Figure IC).

OA subjects had more significant positive food-specific sIgE and sIgG4 AbR against food groups when higher levels of the analyzed PP variables were observed. OA subjects showed significant higher values of CRF when positive cereal, fish and seeds sIgG4 AbR, or when positive egg sIgE AbR; besides, higher maximal HGS when positive fish sIgG4 AbR, or when positive meat sIgE AbR; moreover, higher leg power when positive vegetables sIgE AbR; furthermore, higher STS speed when positive cereal, fish and legume sIgG4 AbR; and finally, higher body balance when positive fish, shellfish and mollusks sIgG4 AbR, or when positive meat, seeds, shellfish and mollusks sIgE AbR. There were no significant associations between positive food-specific AbR and RHR (Annex I, Figure ID).

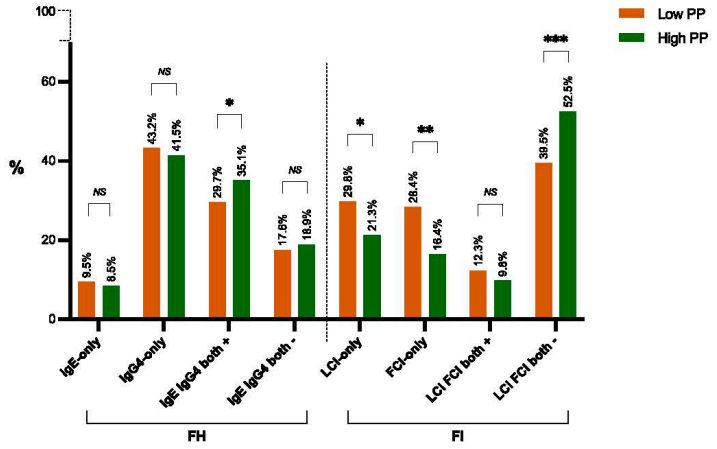


Figure 4. Physical performance according to the types of ARFS. *FCI*, fructose intolerance; *FCI-only*, positive fructose intolerance and negative lactose intolerance subjects; *FH*, food hypersensitivity; *FI*, food intolerance; *IgE*, immunoglobulin E; *IgE-only*, positive sIgE AbR and negative sIgG4 AbR subjects; *IgG4*, immunoglobulin G4; *IgG4-only*, positive sIgG4 AbR and negative sIgE AbR subjects; *LCI*, lactose intolerance; *LCI-only*, positive lactose intolerance and negative fructose intolerance subjects.

3.4. Adverse reactions to foodstuffs as a risk factor of physical performance

In order to comprehend the connection between PP and ARFS, logistic regression models were constructed, adjusting the data for demographic factors and sample characteristics (age, sex and BMI). Odds ratio (OR) were determined to measure how strongly having a better PP was associated with having any of the analyzed categories of ARFS (Figure 5).

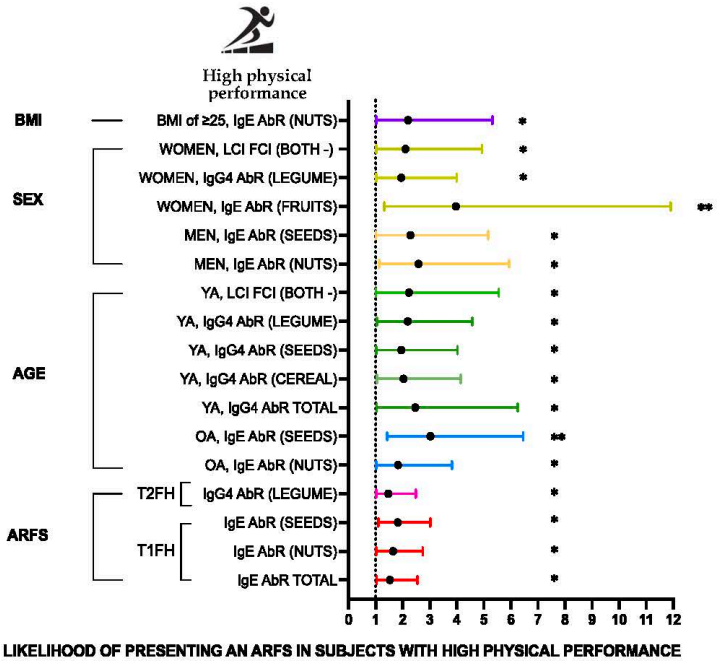


Figure 5. Odds ratio associations between PP and ARFS food groups. *LCI*, lactose intolerance; *FCI*, fructose intolerance; *OA*, older adults (>45y); *OW*, overweight; *T1FH*, type 1 food hypersensitivity; *T2FH*, type 2 food hypersensitivity; *YA*, young adults (≤45y); Both -, both negative.

Subjects with high PP were over 1.5 times as likely to have experienced a T1FA compared to low PP subjects (45.3% *vs* 35.1%, respectively; OR = 1.53, 95% CI (1.00, 2.54); $p<0.05$). Significant risk correlations to experience a positive nuts sIgE-mediated FH were found among subjects with high PP compared to low PP in those who were men (52.4% *vs* 29.8%, respectively; OR = 2.59, 95% CI (1.13, 5.94); $p<0.05$), aged >45 years (50.0% *vs* 35.4%, respectively; OR = 1.83, 95% CI (1.10, 3.82); $p<0.05$), or had overweight or a higher BMI (50.0% *vs* 31.3%, respectively; OR = 2.27, 95% CI (1.02, 5.31); $p<0.05$). In contrast, high PP subjects compared to those with low PP who were women (76.6% *vs* 62.6%, respectively; OR = 1.95, 95% CI (1.03, 3.99); $p<0.05$); and subjects aged ≤45y (71.2% *vs*

53.0%, respectively; OR = 2.19, 95% CI (1.04, 4.58); $p<0.05$), appeared to be more prone to develop legume IgG_4-mediated FH.

No significant associations were observed for the calculated OR within the LCI and FCI categories. However, the age categories analysis of LCI and FCI showed that YA with high PP were associated with a 2.23-fold increase in odds of testing both negative LCI and FCI (59.4% *vs* 36.9%, respectively; OR = 2.23, 95% CI (1.04, 5.56); $p<0.05$).

4. Discussion

This study utilized a well-defined group of Spanish adults self-reporting symptoms related to ARFS to determine potential factors analyzing novel relationships between PP and ARFS. ARFS prevalences were determined, associations between PP variables and food allergen-specific AbR were evaluated; and risk factors associating high PP with the likelihood of presenting an ARFS were explored.

It remains unclear at this point whether $sIgG_4$ AbR is associated with the disease improvement, involved directly in the allergic response; or else, serves as an indicator of disease severity and food hypersensitivity. Nevertheless, the mechanisms of $sIgG_4$ AbR in the FA cascade have not been analyzed in present study. Food allergen-specific $sIgG_4$ AbR was classified as T2FH associated with ARFS symptoms based on descriptive studies (4, 22). Among the studied food groups, prevalence of T2FH was significantly higher than T1FH. Participants with T1FH had a higher prevalence of positive sIgE AbR against legumes and seeds; while those with T2FH showed more prevalence of positive $sIgG_4$ AbR against legumes and nuts.

In a previous study conducted in Sweden, it was observed that sensitization to nuts is highly prevalent in adults (23). In this Swedish cohort, it was found that the prevalence for sIgE AbR against nuts was 21.2% in YA. However, the present research found that YA had a prevalence of 38.5% for sIgE AbR against nuts. It was also stated that tree nut FH is linked to the development of early, long-lasting, and severe allergic conditions in adulthood. Overall, a European systematic review and meta-analysis have revealed that FH to certain food groups, previously considered unimportant in adulthood, are now acknowledged as important FH, particularly in T1FA (fruits, vegetables, seeds, legumes, grains, and cereal). (24).

Associations were identified among various physical fitness tests, age groups, sociodemographic descriptions, general sample characteristics, FH and FI biomarker values. Generally, the CRF has been measured together with asthma allergic incidence, but data regarding the direct relationship to FH is still lacking (25). The direct and novel relationship between physical fitness tests and the prevalence of certain positive FH reactions is still unexplored. However, the role that any FH may have over PP is clearly different depending on the type of FH and the food group involved in the disease (Annex IB). A study of allergy in English runners, showed that the prevalence of allergy (response to environmental allergens) in recreational marathon runners was higher than in the general population (26). Considering the "dual allergen exposure hypothesis", changes in environmental conditions during sports may also contribute to the ARFS disease progression, and the discipline and environment around the exercise development might also be of great influence (9).

The food allergen-specific biomarkers of T1FH and T2FH, sIgE AbR and $sIgG_4$ AbR, examined in 12 specific food groups and associated to PP variables, showed significant differences across age groups and the fitness tests measured as PP variables. Compared to OA, the YA group was positive for less food groups when comparing each PP variable to the analyzed 12 food groups. CRF, in OA, was found to have significant associations in half of the food groups when compared with YA. CRF is currently acknowledged as a key factor in studies involving health assessment (27). CRF has also been recognized as an indicator of inflammation, whether it is acute (infectious illness) or chronic (long-term from other conditions like atherosclerotic heart disease), both of which can affect physical abilities (28). Previous studies have also found direct connections between osteoarthritis,

allergies, and metrics of physical health (29). Previous research has shown associations between FA and osteoarthritis, particularly because patients with milk, meat and vegetables FA often follow restrictive diets low in calcium, magnesium and other key nutrients which can impact their musculoskeletal and bone health, worsening their physical condition in the long term (30).

In both children and adults, LCI is frequently associated with GI discomfort. Studies in the adult population were focused on the impact of LCI on athletes and physically active individuals. Lactose appears to have a significant role in muscle recovery and function, as a vehicle for the delivery of glucose and galactose (31). In this current research, adults with positive LCI showed significant reduced HGS values. These results are aligned with previous findings about the strong correlation between HGS and GI symptoms, enzymatic FI like LCI, and other chronic diseases (32).

Risk factors related to FH include such aspects as clinical history and the type of allergen exposure. In developed countries, physically active adults with limited allergen exposure since childhood may be greatly influenced by the transition from a protected environment to engaging in physical exercise in adulthood when it comes to FH. Some food allergen extracts in certain age groups have a bigger impact than others; such as nuts, with higher prevalence in European adults than children, or legumes with significant prevalence in Spanish women older than 14 years of age (33).

4.1. Strengths and Limitations

Significant disparities in levels of physical fitness and exercise have been documented among adults based on race and income. The present paper involves a clearly defined sample of Caucasian Spanish adults, most of them employed (>80%) and having received a university education or higher (>70%).

4.2. Future research

Dietary patterns and the type of exercise or sport are important aspects to be considered for future research studies regarding ARFS. Further investigation is needed to determine the potential influence of focused ARFS interventions on the connections between PP and overall sample characteristics.

5. Conclusions

The most common ARFS in the overall sample of the studied Spanish adults was against legume (50% and 64%, respectively for type 1 and type 2 food hypersensitivities). Adults having 45y or younger had the most prevalent sIgE-mediated or type 1 food hypersensitivities against legume (53%). Analogously, adults older than 45y of age presented the most prevalent sIgG$_4$-mediated or type 2 food hypersensitivities against legume (68%). Total positive sIgG$_4$ AbR was associated with higher values of physical performance, being significantly related with higher values of body balance, compared to negative sIgG$_4$ AbR. Non-immune-mediated reactions or food intolerances were linked to lower values of physical performance; specifically, lactose intolerance was inversely associated with maximal hand grip strength. The probability of developing total positive sIgE immune-mediated reactions or type 1 food hypersensitivity is higher in subjects presenting better physical performance. Age, sex and specific involved food groups (nuts, seeds, legume, cereal and fruits) might be factors of crucial influence when evaluating the risk of developing ARFS in high physical performance adults. Nevertheless, there is no strict definite risk or protective factor distinguishing between ARFS and physical performance. Further research is needed to clarify the relationship between physical performance-related variables and ARFS.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1:

Table S1: Food-specific Immunoglobulin E antibody reactions compared by sex and age title; **Table S2:** Food-specific Immunoglobulin G4 antibody reactions compared by sex and age; **Figure S1:** Physical Performance variables and adverse reactions to foodstuff against 12 food groups of the total studied sample of Spanish adults; **Figure S2:** Physical Performance variables and adverse reactions to foodstuff against 12 food groups of Spanish adults older than 45y of age.

Author Contributions: Conceptualization, L.P.-A., E.G., M.P.-R., and M.G.-G.; methodology, L.P.-A., E.G., M.P.-R., S.T., R.U. and M.G.-G.; software, L.P.-A. and M.G.-G.; validation, L.P.-A., E.G., R.U. and M.G.-G.; formal analysis, L.P.-A., E.G., M.P.-R., and M.G.-G.; investigation, L.P.-A., E.G., M.P.-R., S.T. and M.G.-G.; resources, L.P.-A., E.G., S.T. and M.G.-G.; data curation, L.P.-A., E.G., M.P.-R., and M.G.-G.; writing—original draft preparation, L.P.-A., E.G., and M.G.-G.; writing—review and editing, L.P.-A., E.G., M.P.-R., S.T., R.U. and M.G.-G.; visualization, L.P.-A., E.G., and M.P.-R.; supervision, E.G., M.P.-R., R.U. and M.G.-G.; project administration, M.G.-G.; funding acquisition, L.P.-A. and M.G.-G. All authors have read and agreed to the final version of the manuscript.

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The ALASKA study RCT was conducted in the ImFINE Research Group (gi.imfine@upm.es) of the Universidad Politécnica de Madrid. Industries and/or private institutions had no role in the design, execution, analyses, interpretation of the data, or decision to submit results.

Institutional Review Board Statement: This research was conducted in accordance with the Ethical Guidelines of the Declaration of Helsinki of 1964, revised in Fortaleza (2013) (19) and following the Spanish and European regulations on data protection (20). The protocol was approved by the Ethics Committee of the UPM (reference number 20200602, date of approval 17/07/2020.) for studies involving humans and registered on ClinicalTrials.gov (Clinical Trials NCT05802017).

The ALASKA study was carried out by qualified professionals for medical, clinical, food science, nutritional and PA and Sport Sciences practice. These experts were part of the ImFINE Research Group (ImFINE-RG) of the Department of Health and Human Performance of the Faculty of Physical Activity and Sport Sciences of the UPM (INEF-UPM). Signed informed consent forms were obtained from all participants before their participation commencement.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in this article and supplementary material, further inquiries can be directed to the corresponding author: Lisset Pantoja-Arévalo, l.pantoja@upm.es (L.P.-A), and to the principal investigator (PI) of this study: Prof. Dr. Marcela González-Gross, marcela.gonzalez.gross@upm.es (M.G.-G.).

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6.9. Annex I: Article 5

Title: “Effect of the food allergen-specific substitutive diet on health, quality of life and physical performance in adults with adverse reactions to foodstuffs: A Randomised Controlled Trial - The ALASKA Study”.

Reference: [48]

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Effect of the food allergen-specific substitutive diet on health, quality of life and physical performance in adults with adverse reactions to foodstuffs : A Randomised Controlled Trial - The ALASKA study.

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Effect of the food allergen-specific substitutive diet on health, quality of life and physical performance in adults with adverse reactions to foodstuffs : A Randomised Controlled Trial - The ALASKA study.

ABSTRACT

Individuals with any adverse reaction to foodstuffs (ARFS), such as type 1 and type 2 food hypersensitivity (T1FH and T2FH), following a food allergen-specific substitutive diet (FASSD) may be less likely to experience nutrient deficiencies or decreased physical performance than strict elimination diets. The aims of this study were a) to evaluate the body composition (BC) and physical performace (PP) variables before and after the FASSD; b) to analyze the impact of the FASSD on blood biomarkers and symptomatology; and c) to determine the influence of the FASSD on quality of life (QoL) of Spanish adults scoring ≥6 in PSIMP-ARFSQ-10. A randomized controlled trial with 101 volunteers (62% women; mean age 45.35±11.76) was conducted in the Region of Madrid, Spain. The methodology of this study followed the published ALASKA study protocol. The control group (CG) was instructed to follow a standard healthy Mediterranean Diet; meanwhile, the intervention group (IG) followed the FASSD. Statistical significance was set at 0.05. Mean anthropometric, BC and PP values before and after the FASSD of the IG were: 78.97±12.80 and 73.89±11.33cm of waist circumference; 68.60±13.25 and 65.87±13.72kg of body weight; 25.56±8.59 and 21.27±8.42% of body fat; besides, 39.65±10.06 and 41.67±12.08 ml/kg*min of estimated VO_2max (all $p<0.05$). Mean general health values before and after the FASSD of the IG were: 15.85±9.71 and 11.24±7.97 of PSIMP-ARFSQ-10 symptomatology score. ($p<0.05$). Finally, there were no statistical differences between blood hematobiochemical biomarkers, SF-12 QoL punctuations, or ARFS QoL punctuation before and after the FASSD of the IG (all $p>0.05$). No adittional statistical differences were found before and after the FASSD among the rest of BC, PP, general health and QoL variables (all $p>0.05$). In conclusion, BC improved in the IG in terms od body fat% after the FASSD; specifically almost 5% decrease of body fat% and 3kg decrease of body weight were observed; similarly, general health values PSIMP-ARFSQ-10 score improved in the IG in almost 5 points. General QoL and specific ARFS QoL were apparently equal. Significant relative risk of experiencing both T1FH and T2FH after the FASSD, was observed in subjects following the FASSD dietary treatment.

Trial approval and registration: This study was approved by the Ethics Committee of the UPM (REF.20200602) and registered on ClinicalTrials.gov (NCT05802017).

Keywords (MeSH): Biomarkers, Body Composition, Cardiorespiratory fitness, Food hypersensitivity, Population Characteristics, Signs and Symptoms.

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Abbreviations

AbR: antibody reactions.

ALASKA study: Allergies and Food Intolerances in Adults and Athletes.

ANOVA: analysis of variance.

ARFS: adverse reactions to foodstuffs.

BB: body balance.

BC: body composition.

BIA: bioelectrical impedance analysis.

BMI: body mass index.

CeD: coeliac disease.

CESVIMA-UPM: Supercomputing and Visualization Center of Madrid Community of the UPM.

CRF: cardiorespiratory fitness.

FA: food allergy/ food allergies.

FASSD: food allergen-specific substitutive diet.

FH: food hypersensitivity.

FI: food intolerance.

GFD: gluten-free diet.

GI: gastrointestinal.

HGS: Handgrip strength.

HRQoL: health-related quality of life.

IBS: irritable bowel syndrome.

ImFINE-RG: ImFINE Research Group.

INEF-UPM: Faculty of Physical Activity and Sport Sciences of the Universidad Politécnica de Madrid.

ISAK: International Society for the Advancement of Kinanthropometry.

MCS: mental component score.

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NIH: National Institute of Health.

PA: physical activity.

PCS: physical component score.

PF: physical fitness.

PP: physical performance.

PSIMP-ARFSQ-10: Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs.

QoL: quality of life.

RCT: randomised controlled trial.

RedCap®: Research Electronic Data Capture Platform.

RHR: resting heart rate.

sIgE: serum immunoglobulin E.

sIgG4: serum immunoglobulin G4.

SF-12: 12-Item Short Form Health Survey.

SSTs: sit-to-stand test speed.

T1FH: type 1 food hypersensitivity.

T2FH: type 2 food hypersensitivity.

UPM: Universidad Politécnica de Madrid.

VO_2max: maximal oxygen consumption.

vs: *versus*, "against to", "opposed to" "compared to" or "in contrast to".

WHO: World Health Organization.

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Introduction

Type 1 (T1FH) and type 2 food hypersensitivities (T2FH) are one of the most common adverse reaction to foodstuffs (ARFS) with around 30 and 70% of prevalence in local Spanish adult population living in the region of Madrid, Spain, and scoring ≥6 in the Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs (PSIMP-ARFSQ-10) tool. Despite acceptable prognosis regarding current dietary treatments such as elimination diets, it may have negative long-term effects on the metabolic, emotional well-being and physical health of affected individuals (1, 2). Health problems such as relevant nutritional deficiencies, muscle weakness and atrophy (sarcopenia) are on the one hand significantly related issues to treatment adherence, quality of life (QoL) and overall well-being of the ARFS adult population (2-4). Thus, given that ARFS adult population is commonly characterized by compromised immunological processes, due to continuing environmental and/or digestive allergen exposure, and also by adopting elimination dietary treatments; worse macro and micronutrient absortion may occur causing a decreased physical functional performance of healthy muscle tissue, increased rates of fat oxidation, high total colesterol and, usually increased ARFS-related diseases, signs and symptoms (5).

ARFS population may encounter challenges to meet current recommended dietary guidelines to maintain good health, avoid body mass index (BMI) desregulation and lower the chance of experiencing the physical discomfort of the chronic disease (3). Analogically, focusing health in ARFS population by only improving body composition (BC) and physical fitness (PF) may have contrary outcomes on immunological metabolic processes; specifically, on the positivity of FH; whereas prioritizing ARFS management, focusing on the improvement of the associated symptomatology, may lead to disregarding BC and PF because of the restrictive and strict diets associated with ARFS. Interestingly, it has been studied in a mouse model that FH may affect not just skeletal muscle, but also the contractility of the jejunal circular muscle and the expression of local inflammatory cytokines (6). This present research proposes a dietary intervention that could prevent the need to eliminate

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essential nutrients, necessary for the optimal metabolic and physical well-being of ARFS population, and prevent the risk of muscle dysfunction.

Several diseases, signs and symptoms has been linked to FH (7); thus, a variety of hematological and biochemical markers profiles could be anticipated. Crohn's disease, irritable bowel syndrome (IBS), dermatitis herpetiformis, fatigue and other gastrointestinal (GI) problems are common pathologies among ARFS; furthermore, chronic pain, migraine, eczema and colitis, are also commonly present as common signs and symptoms in ARFS (7). However, it has been previously observed that individuals who have ≥25 of BMI, they may have more probability to have positive ARFS (8, 9). Consequently, following the appropriate dietary intervention, promising improvements in glucose, cholesterol, triglycerides, vitamins, and inflammation markers are foreseen (8). Nevertheless, this benefits have been mostly reported in individuals presenting a diagnosed ARFS-related disease. Specifically, individuals who do not test positive for gluten FH or coeliac disease (CeD), are not currently instructed to adopt a gluten-free diet (GFD) or any other elimination diet due to the lack of evidence of health improvements without a confirmed pathology (10). The food allergen-specific substitutive diet (FASSD) proposed in this study is intended for individuals with a PSIMP-ARFSQ-10 score of ≥6. In addition to a confirmed diagnosis, the mentioned score can be also met by signs and symptoms related to ARFS.

Evaluating how ARFS affects QoL is crucial and is considered a standard assessment when evaluating ARFS interventions. The analysis of the impact of ARFS on QoL ensures a comprehensive assessment and helps determine the effectiveness of interventions designed to ameliorate ARFS (11, 12). In the last decade, ARFS individuals with more FH have been generally linked to worse QoL, but there are also several other factors, other than the number of FH, that can influence health-related QoL (HRQoL) in ARFS population. Number and type of FH, self-perceived disease severity, self-reported symptomatology, type of treatment and socio-demographics are some of the key factors that have been studied for their potential impact on HRQoL in ARFS population (13, 14).

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Given that the common reported prevalence of FH is mostly of children than adults, information on the impact of dietary interventions in ARFS adult population is limited; however, multifactorial approaches for ARFS management, and suggestions for implementing a carefully chosen list of influencing factors of ARFS have been made over the past 5 years (1, 3, 11). Obesity and body mass index (BMI) could be potentially related to FH, as both have been steadily rising in the adult population (15). However, obesity treatment, would not necessary signify an improvement in ARFS without taking into account the mentioned multifactorial approach of influencing variables, such as, BC, PF, symptomatology, QoL and general metabolic health. Therefore, the aims of this study were a) to evaluate the BC and physical performance (PP) variables before and after the FASSD; b) to analyze the impact of the FASSD on blood biomarkers and symptomatology; and c) to determine the influence of the FASSD on quality of life (QoL) of ARFS adult population.

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Materials and Method

The methodology of this study followed the protocol of the Allergies and Food Intolerances in Adults and Athletes (the ALASKA study) previously published elsewhere (11). The present study analyzed data collected from the ALASKA study intervention.

Study design and participants

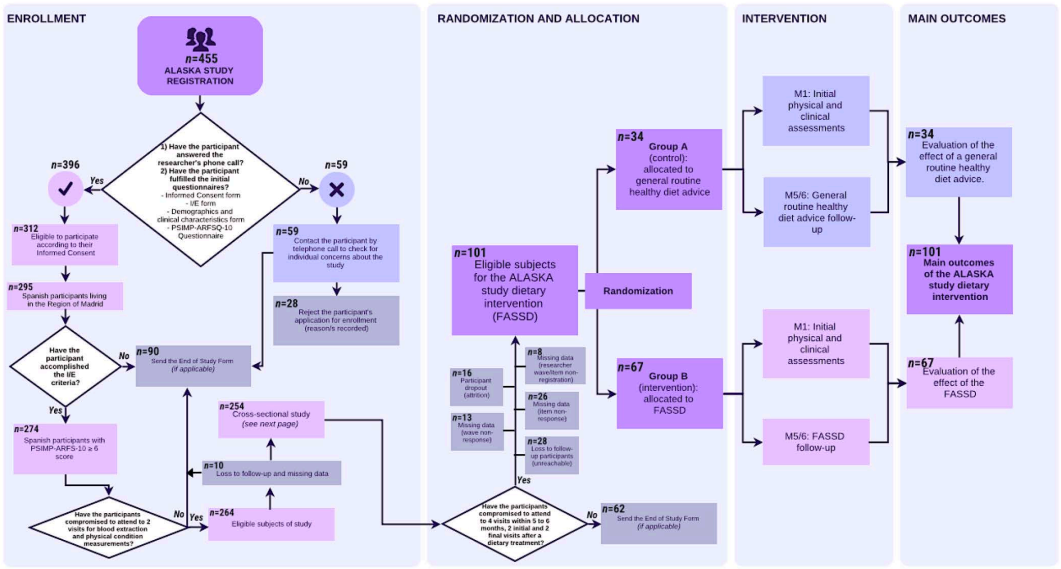
This study was a randomized controlled trial (RCT) with matched pair trial design (established by demographics), with selected inclusion and exclusion criteria, and a 1:2 allocation ratio between the control (Group A) and the intervention group (Group B) respectively, conducted between April 2022 and July 2024 in the Universidad Politécnica de Madrid (UPM) located in the Region of Madrid, Spain. The flow diagram of this study was created using the previously published chart of the ALASKA study design, and included the total number of participants at each stage (Figure 1).

Participants aged ≥18y accomplishing the following summary list of inclusion and exclusion criteria were included as subjects of study. Subjects who were not involved in any other research study at the same time, did not heavily smoke (<25 cigarettes/day), were not currently prescribed certain medication (antibiotic, antidepressant, sleeping pill, or anxiolytics), had not been diagnosed with specific conditions during the last year (*Helicobacter pylori* infection, anorexia, bulimia, orthorexia, binge eating), were not currently adopting a special diet (for food allergies (FA), food intolerances (FI), CeD, or any other FH), had no medical restrictions on physical activity (PA), had a regular sleep schedule, were not pregnant, had not dealt with major health issues in the last five years (cancer, terminal illness or undergone significant surgery requiring hospitalization), and had a PSIMP-ARFSQ-10 score of ≥6. The validated PSIMP-ARFSQ-10 tool was employed to assess diseases, signs, and symptoms associated with ARFS (16). Additional information regarding exclusion and exclusion criteria can be found in the published ALASKA study protocol.

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FASSD, Food allergen-specific substitutive diet; *M1*, physical and clinical assessments at month 1; *M5/6*, physical and clinical assessments at month 5 or 6.

Figure 1 Flow diagram of the ALASKA study intervention

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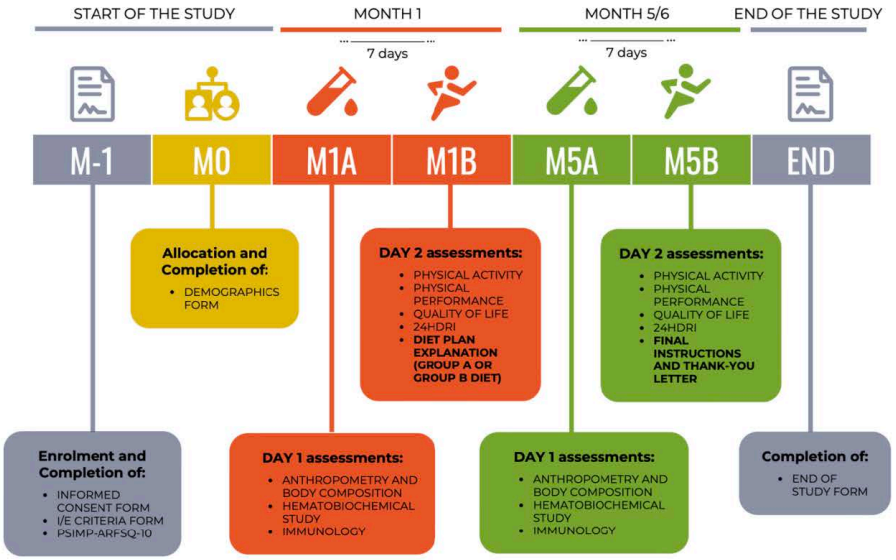
A total of 295 Spanish adults were eligible to participate. Twenty-one participants did not accomplish the inclusion criteria, ten participants did not sign the follow-up agreement section, ten participants were lost during the first follow-up period or had missing data after the fist appointment at month 1 (M1). Two-hundred and fifty four volunteers were successfully measured at M1, 62 subjects did not compromised to attend to two more visits at month 5 or month 6 (M5/6) and 91 subjects were excluded from the analysis for participant drop-out, non-response, item non-registered, missing data or participant loss to follow-up after the second appointment at M5/6. A final sample of 101 volunteers were eligible subjects for the ALASKA study intervention (Figure 1).

Intervention

Subjects were randomized and allocated in the control group or Group A and intervention group or Group B using the Research Electronic Data Capture Platform (RedCap®) which includes a randomization automated tool. Individuals assigned to the control group were instructed to follow a general nutritional advice with dietary recommendations for a healthy lifestyle, while subjects assigned to the intervention group were instructed to follow a personalized FASSD based on the FH results. In-depth information on both dietary treatments can be found in the published intervention description and in Annexes I and II of the published ALASKA study protocol. The duration of the study for both groups was between 5 and 6 months. Participants in the ALASKA study intervention were required to attend the research facilities in person four times. There were two in-person visits at month 1 (M1A and M1B) and two at month 5/6 (M5A and M5B), one at the beginning of the month and the other one approximately 7 days after getting their hematobiochemical and immunological test results. Subjects were assessed for anthropometry, BC, PP, PA, health-related biomarkers, and QoL at both points of the ALASKA study timeline (M1 and M5/6). All tests were performed in the same sequence and time intervals for all participants (Figure 2).

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PSIMP-ARFSQ-10, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; *24HDRI*, 24-hour dietary recall interview; *Group A*, control group; *Group B*, intervention group; *M-1*, one month before the start of the study; *M0*, fifteen days before the start of the study; *M1A*, visit 1 at month 1; *M1B*, visit 2 at month 1; *M5A*, visit 1 at month 5/6; *M5B*, visit 2 at month 5/6.

Figure 2 Timeline of the ALASKA study intervention

Study variables

The variables and instruments used in this intervention are summarized in Table 1. Subjects were assessed both clinically and physically at the M1 and M5/6 timeline points of the ALASKA study timeline.

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Table 1 Summary of the variables and instruments of the ALASKA study intervention

	VARIABLES/ ASSESSMENTS		QUESTIONNAIRES/ INSTRUMENTS	REFERENCES
Demographics	Date of birth, age, sex, level of education, employment situation, occupation, living situation (number of people living together) and home city		Demographics form	(11)
Anthropometry and Body Composition	Height (cm)		Stadiometer	(Seca GmbH & Co. KG., model 213 1721009, Hamburg, Germany)
	Waist-hip ratio (ISAK criteria)		Anthropometric tape	(Cescorf Ltd., 2-meter Innovare measuring tape, Porto Alegre City, Brazil)
	Weight (kg) and BIA with segmental multifrequency analysis		BIA bascule	(Tanita, model MC-780MA-N, Tokyo, Japan)
	Body mass index (kg/m^2, mass in kilograms divided by height in meters squared)		Height and weight instruments	(17, 18)
Physical performance	Resting heart rate		Tensiometer	(Omron Healthcare Co. Ltd., model M3 Comfort HEM-7155-E(C), Kyoto, Japan)
	Handgrip strength (hand/ upper extremity strength)		Dynamometer	(Takei, model T.K.K. 5401 Grip-D, Tokyo, Japan)
	Power strength (leg/ lower extremity strength)		Force platform	(Tanita, model BM-220 7341(0), Tokyo, Japan)
	Sit-to-stand speed			
	Body balance			
	Cardiorespiratory fitness	Estimated VO_2 max	Åstrand-Ryhming Step Test	(19)
		Pulse rate	Pulse oximeter	(GE Healthcare, Ohmeda Tuffsat, Boston, Massachusetts, USA)
		Pace/cycles	Metronome	(KORG Inc., model MA-1, Tokyo, Japan)
Physical Activity	PA habits and WHO accomplishment		IPAQ-LF	(20, 21)
			RAPA	(22)
Health	Diseases, signs and symptoms (PSIMP-ARFSQ-10 score)		PSIMP-ARFSQ-10	(11)
	Hematobiochemical study	Complete blood count	Hematology analyzer	(Beckman Coulter, Inc., UniCel DxH 800 Coulter Cellular Analysis System, Brea, CA, USA)
		Biochemical composition	Biochemistry analyzer	(Beckman Coulter Diagnostics Inc., Beckman Coulter® AU5800, Brea, California, USA)
		Vitamin profile	Electrochemiluminescence analyzer	(Roche Diagnostics Ltd., Cobas e411, Rotkreuz, Switzerland)
	Immunology	T1FH, food allergen-specific sIgE AbR	In-line immunoassay analyzer	(Aesku.Diagnostics GmbH, Helia® Helmed, Wendelsheim, Germany)
		T2FH, food allergen-specific sIgG$_4$ AbR		
Nutrition	Group A diet		General nutritional advice healthy diet	(11)
	Group B diet		FASSD	
	Food and beverages record		24-h Dietary Recall Interview (24HDRI)	

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	Dietary adherence	Dietary Adherence Questionnaire (DAQ)	
Quality of Life (QoL)	Health-related QoL	12-Item Short Form Health Survey (SF-12)	(23)
	FA-related QoL	Food Allergy Quality of Life Questionnaire-Adult Form (S-FAQLQ-AF)	(24)

AbR, antibody reactions; *DAQ*, Dietary adherence questionnaire; *BIA*, bioelectrical impedance analysis; *FA*, Food allergy; *FASSD*, Food Allergen-specific substitutive diet; *IPAQ-LF*, The International Physical Activity Questionnaire Long Form; *ISAK*, International Society for the Advancement of Kinanthropometry; *PSIMP-RAA-10*, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; *QoL*, quality of life; *RAPA*, The Rapid Assessment of Physical Activity Questionnaire; *S-FAQLQ-AF*, Food Allergy Quality of Life Questionnaire-Adult Form; *SF*-12, 12-Item Short Form Health Survey; *sIgE*, serum immunoglobulin E; *sIgG4*, serum immunoglobulin *G4*; *T1FH*, type 1 food hypersensitivity; *T2FH*, type 2 food hypersensitivity; VO_2 *max*, maximal oxygen consumption; *24HDRI*, 24-h Dietary Recall Interview.

All subjects of the ALASKA study completed the forms and questionnaires listed in Table 1 in an online format using RedCap® platform hosted in the Supercomputing and Visualization Center of Madrid Community of the UPM (CESVIMA-UPM). The standardized procedures of the International Society for the Advancement of Kinanthropometry (ISAK) were followed for quantifying the described anthropometric assessments. Body composition was assessed using bioelectrical impedance analysis (BIA) according to the ALASKA study protocol and to The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines for utilization of BIA in clinical practice (11, 25). BMI was evaluated according to the classification used by the National Institute of Health (NIH) and the World Health Organization (WHO) (18); and PA was arranged in accordance with the WHO 2020 guidelines on physical activity and sedentary behavior (26).

The PP-related variables considered in this research were resting heart rate (RHR), handgrip strength (HGS), leg power strength, sit-to-stand speed (SSTs), body balance (BB) and Cardiorrespiratory fitness (CRF). Muscle strength of the upper- and lower-extremity muscles was measured Maximal isometric HGS was recorded after at least two attempts from each hand while standing and sitting. Handle width of the dynamometer was adjusted to subjects' hand size; and subjects were instructed to maintain anthropometric neutral position while encouraged to squeeze the dynamometer and exert maximum force.

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CRF was classified into four groups (low, normal, good and excellent) according to the 95% of the good age-sex-estimated maximal oxygen consumption (VO_2max) value below which there is risk of developing major health problems (mainly cardiovascular diseases, diabetes and/or hypertension) (27).

In order to further comprehend the impact of the FASSD on HRQoL and food allergy (FA)-related QoL, two QoL questionnaires were assessed in both timeline points (M1 and M5/6). The 12-Item Short Form Health Survey (SF-12) was performed to evaluate subject's general health perception and the Food Allergy Quality of Life Questionnaire-Adult Form (S-FAQLQ-AF) was performed to evaluate emotions towards T1FH and T2FH.

Statistical analysis

Descriptive statistics were described as mean and standard deviation (SD) for continuous variables, and as frequency with percentage for categorical variables. Subjects were described and evaluated by sex and age groups, young adults (YA, ≤45y) and older adults (OA, >45y) age groups. Age-sex adjustments were applied to all PP variables. Median cut off values of maximal HGS and CRF were determined to classify the sample into low and high PP groups and to create the dichotomic datasets of PP variables.

Univariate repeated measures analysis of variance (ANOVA) was utilized to assess the differences in T1FH, T2FH, and QOL values between baseline (M1) and post-treatment (M5/6). Eta squared ($\eta 2$) was used as an indicator of effect size (28). $\eta 2$ was categorized as small for effect sizes ≥ 0.01 to <0.06, medium for $\eta 2 \geq 0.06$ to <0.14, and large for $\eta 2 \geq 0.14$ (29).

The SF-12 QoL questionnaire was used as a generic health assessment tool and it had two summary scores, one for mental health (mental component score, MCS-12) and one for physical health (physical component score, PCS-12). The results were presented in Z-scores (variation from the mean

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population value, in terms of standard deviations). The mean scores for PCS-12 and MCS-12 were both rated at 50 points. The standard deviation was 10 points on average. Each deviation of 10 points above or below 50 corresponded to one standard deviation from the mean. Scores above 50 signified a high HRQoL (better-than-average HRQoL), whereas scores lower or equal than 50 indicated low HRQoL (below-average HRQoL).

Binary logistic regression was performed using the dichotomic datasets of T1FH and T2FH variables. Relative risk (RR) or relative risk ratios were calculated to identify the relative risk factors for developing T1FH and T2FH under the FASSD against the control dietary treatment. RR was computed as measurement of association and random-effects risk model. Statistical significance was set at 0.05.

Ethical considerations

This research was performed in accordance with the Ethical Guidelines of the Declaration of Helsinki of 1964, revised in Fortaleza (2013) (30) and following the Spanish and European regulations on data protection (31). The protocol was approved by the Ethics Committee of the UPM (reference number 20200602) and registered on ClinicalTrials.gov (Clinical Trials ID NCT05802017). Signed informed consent forms were obtained from all study participants before their participation commencement.

The ALASKA study intervention was carried out by qualified professionals for medical, clinical, food science, nutritional and PA and Sport Sciences practice. Experts in their respective fields carried out clinical and physical evaluations for each specific measurement. These experts were part of the ImFINE Research Group (ImFINE-RG) of the Department of Health and Human Performance of the Faculty of Physical Activity and Sport Sciences of the UPM (INEF-UPM).

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Results

Sample characteristics

A total sample of 101 Spanish adults participated in the ALASKA study intervention (62% women) with a mean age of 45.35±11.76 years. Data regarding demographics and baseline characteristics of the ALASKA study are presented in Table 2. There were no significant differences of socio-demographic characteristics (education level, job and living situation) between control and intervention groups in the baseline timeline point. Around 60% of the studied sample presented normal weight with no significant difference between the BMI categories of the control and intervention groups. Expected significant differences were observed between men and women's anthropometric and body composition features (data published elsewhere, data not shown). No further statistical differences were observed between the results presented in Table 2 (all $p>0.05$).

Table 2 Baseline characteristics of the ALASKA study intervention

		Mean±SD or *n* (%)				
		Total (*n*=101)	**Control (*n*=34)**	**Intervention (*n*=67)**	**Min-Max**	***p*-values$_{control-intervention}$**
Age (years)		45.35±11.76	46.59±11.59	44.72±11.89	18 - 69	NS 0.453
Sex	Male	38 (37.6)	10 (29.4)	28 (41.8)	--	NS 0.225
	Female	63 (62.4)	24 (70.6)	39 (58.2)	--	
Height (cm)		168.48±8.34	167.59±9.78	168.93±7.55	150.00 - 186.00	NS 0.449
Weight (kg)		68.61±14.01	68.64±15.61	68.60±13.24	41.80 – 124.80	NS 0.989
BMI (kg/m^2)		24.17±4.67	24.44±5.13	24.04±4.45	17.10 – 38.50	NS 0.686
BMI						
<18.5 underweight (%)		3 (3.0)	2 (5.9)	1 (1.5)	--	
18.5 – 24.9 normal weight (%)		67 (66.3)	20 (58.8)	47 (70.1)	--	
25.0 – 29.9 overweight (%)		20 (19.8)	7 (20.6)	13 (19.4)	--	NS 0.475
30.0 – 34.9 class I obesity (%)		7 (6.9)	3 (8.8)	4 (6.0)	--	
35.0 – 39.9 class II obesity (%)		4 (4.0)	2 (5.9)	2 (3.0)	--	
EDUCATION LEVEL						
Highschool		15 (14.9)	4 (11.8)	11 (16.4)	--	
Intermediate and Higher professional training level		13 (12.9)	4 (11.8)	9 (13.4)	--	
University education (college graduate, engineer, high degree)		44 (43.6)	15 (44.2)	29 (43.3)	--	NS 0.723
Master degree		21 (20.8)	8 (23.5)	12 (17.9)	--	
PhD/doctorate degree		8 (7.8)	3 (8.7)	6 (9.0)	--	
JOB SITUATION						
Employed or freelance		84 (83.4)	27 (79.4)	54 (80.5)	--	NS 0.521
Paid and non-paid student		7 (6.9)	3 (8.8)	5 (7.5)	--	

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Unemployed (including houseworkers)	5 (4.8)	2 (5.9)	4 (6.0)	--	
Retired or on early retirement	5 (4.9)	2 (5.9)	4 (6.0)	--	
LIVING SITUATION					
Number of people living with (NPLW)					
≤2 NPLW	62 (61.4)	21 (61.8)	41 (61.2)	--	NS 0.956
>2 NPLW	39 (38.6)	13 (38.2)	26 (38.8)	--	

BMI, body mass index.

Table 3 presents the main variables measured before and after the ALASKA study intervention (anthropometry, body composition, physical performance, physical activity, symptomatology, immunology, hematobiochemical study and QoL) and their statistical significance for the control and FASSD groups (Group A and Group B, respectively). No PA or QoL significant effects were self-reported by subjects of the ALASKA study intervention.

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Table 3 ALASKA study assessments before and after the FASSD

	Mean±SD or *n* (%)						*p*-values		
	M1 (*n*=101)			M5/6 (*n*=101)			M1 *vs* M5/6		
	Total Baseline	Group A Control (*n*=34)	Group B FASSD (*n*=67)	Total Intervention	Group A Control (*n*=34)	Group B FASSD (*n*=67)	*p*-values BASELINE-INTERVENTION	Group A *p*-values CONTROL	Group B *p*-values FASSD
ANTHROPOMETRY									
BMI (kg/m²)	24.17±4.67	24.44±5.13	24.04±4.45	24.09±4.78	24.45±5.25	23.90±4.56	NS 0.601	NS 0.979	NS 0.458
Height (cm)	168.48±8.34	167.59±9.78	168.93±7.55	168.10±8.33	167.38±9.64	168.46±7.64	NS 0.366	NS 0.421	NS 0.452
Weight (kg)	68.61±14.01	68.64±15.61	68.60±13.25	66.80±14.66	68.63±16.42	65.87±13.72	<0.01	NS 0.991	<0.001
Waist circumference (cm)	79.40±12.77	80.25±12.86	78.97±12.80	76.02±12.45	80.20±13.62	73.89±11.33	<0.001	NS 0.933	<0.001
Hip circumference (cm)	94.94±10.92	95.70±10.67	94.56±11.10	95.37±8.17	100.33±9.96	92.85±5.69	NS 0.634	<0.001	NS 0.151
Waist-to-hip ratio	0.84±0.11	0.84±0.09	0.84±0.12	0.80±0.10	0.80±0.08	0.80±0.11	<0.001	<0.001	<0.01
BODY COMPOSITION									
Fat mass percentage (%)	25.04±8.55	24.12±8.64	25.56±8.59	23.35±8.71	27.44±7.87	21.27±8.42	NS 0.143	NS 0.053	<0.01
Fat mass (kg)	17.96±9.09	16.83±9.03	18.59±9.19	15.14±9.02	17.36±9.37	14.02±8.69	<0.05	NS 0.774	<0.01
Fat free mass (kg)	50.85±9.18	49.80±9.91	51.39±8.82	50.98±9.48	49.27±10.44	51.86±8.91	NS 0.757	NS 0.150	NS 0.447
Muscle mass (kg)	48.29±8.74	47.29±9.44	48.80±8.39	48.44±9.03	46.77±9.94	49.29±8.49	NS 0.711	NS 0.139	NS 0.403
Bone mass (kg)	2.56±0.44	2.51±0.47	2.59±0.42	2.55±0.45	2.50±0.50	2.57±0.43	NS 0.357	NS 0.524	NS 0.448
Body water (%)	52.04±6.25	50.70±6.23	52.72±6.20	51.88±6.11	50.70±5.75	52.48±6.24	NS 0.473	NS 0.987	NS 0.394
Body water (kg)	35.39±6.88	34.44±7.31	35.88±6.65	35.01±7.01	34.44±7.64	35.30±6.71	NS 0.225	NS 0.984	NS 0.202
Visceral fat ratio	6.00±3.98	6.15±4.17	5.93±3.91	6.00±3.96	6.41±4.23	5.79±3.84	NS 0.998	NS 0.239	NS 0.462
PHYSICAL PERFORMANCE									
Handgrip strength (HGS)									
Total maximal HGS (kg)	33.72±8.76	32.60±9.56	34.28±8.34	33.83±9.07	32.54±9.20	34.49±9.00	NS 0.734	NS 0.882	NS 0.661
Power, speed and balance									
Leg power (f/w)	1.59±0.24	1.56±0.22	1.60±0.25	1.50±0.18	1.55±0.22	1.47±0.15	<0.001	NS 0.076	<0.001
Sit-to-stand speed (RFD/w)	13.62±2.89	13.36±2.61	13.75±3.03	12.53±2.63	13.35±2.61	12.12±2.55	<0.001	NS 0.543	<0.001
Body balance (BP T-score)	49.08±10.05	49.35±9.17	48.94±10.53	54.91±7.87	54.03±6.66	55.36±8.43	<0.001	<0.01	<0.001
Cardiorespiratory fitness (CRF)									
Estimated VO_2max (ml/kg*min)	38.59±9.85	36.51±9.21	39.65±10.06	39.56±11.63	35.38±9.55	41.67±12.08	NS 0.356	NS 0.429	<0.05
Low CRF (%)	18 (17.8)	7 (20.6)	11 (16.4)	23 (22.8)	4 (11.8)	19 (28.4)			
Normal CRF (%)	27 (26.7)	11 (32.4)	16 (23.9)	25 (24.8)	12 (35.3)	13 (19.4)	NS 0.705	NS 0.432	NS 0.289
Good CRF (%)	44 (43.6)	11 (32.4)	33 (49.3)	37 (36.6)	13 (38.2)	24 (35.8)			
Excellent CRF (%)	12 (11.9)	5 (14.7)	7 (10.4)	16 (15.8)	5 (14.7)	11 (16.4)			
PHYSICAL ACTIVITY									

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(IPAQ-SF)									
Accomplish WHO recommendatrions	13 (12.9)	3 (8.8)	10 (14.9)	16 (15.8)	5 (14.7)	11 (16.4)	NS 0.083	NS 0.160	NS 0.321
Do not Accomplish WHO recommendations	88 (87.1)	31 (91.2)	57 (85.1)	85 (84.2)	29 (85.3)	56 (83.6)			
SYMPTOMATOLOGY									
(PSIMP-ARFSQ-10 score) ᵃ									
Digestive system	4.87±2.79	4.74±2.82	4.94±2.80	3.48±2.72	4.47±3.11	2.97±2.36	<0.001	NS 0.556	<0.001
Skin and subcutaneous tissue	3.19±2.97	2.82±2.08	3.37±3.33	2.63±2.74	2.76±2.73	2.57±2.77	<0.05	NS 0.892	<0.01
Nervous system	2.80±2.40	2.74±2.49	2.84±2.37	2.32±2.42	2.85±2.98	2.04±2.06	<0.05	NS 0.758	=0.001
Respiratory system	1.54±1.84	1.18±1.57	1.73±1.95	1.22±1.69	1.12±1.43	1.27±1.82	<0.05	NS 0.818	=0.01
Other pathologies/ diseases	2.90±2.48	2.76±2.18	2.97±2.63	2.53±2.27	2.82±2.30	2.39±2.25	NS 0.059	NS 0.870	<0.05
Total PSIMP-ARFSQ-10 score	15.31±8.66	14.24±6.10	15.85±9.71	12.18±7.61	14.03±6.56	11.24±7.97	<0.001	NS 0.743	<0.001
IMMUNOLOGY									
T1FH, Total sIgE$_{\text{CALCULATED}}$ AbR (kUA/l)	3.63±3.97	2.88±3.72	4.01±4.06	2.91±3.09	2.44±3.49	3.15±2.87	<0.01	NS 0.089	<0.05
T2FH, Total sIgG$_{\text{CALCULATED}}$ AbR (kUA/l)	13.77±14.37	8.06±10.07	16.67±15.39	9.47±11.52	7.14±10.63	10.66±11.85	<0.01	NS 0.133	<0.001
HEMATOBIOCHEMICAL STUDY									
Hemoglobin (g/dL)	14.51±1.24	14.09±1.30	14.72±1.16	14.37±1.27	14.04±1.46	14.54±1.13	<0.05	NS 0.557	<0.05
Hematocrit (%)	42.68±3.47	41.68±3.64	43.19±3.30	43.10±3.58	42.20±4.02	43.56±3.28	<0.05	NS 0.071	NS 0.086
Platelet (10^9/L)	235.62±54.86	229.50±56.45	238.73±54.19	230.86±53.89	227.35±54.96	232.64±53.67	NS 0.077	NS 0.597	NS 0.084
Leukocytes (10^9/L)	5.79±1.55	5.38±1.46	5.99±1.56	5.38±1.33	5.35±1.51	5.40±1.24	<0.01	NS 0.896	<0.001
Neutrophils (10^9/L)	3.24±1.13	2.85±0.81	3.44±1.22	2.96±0.95	2.95±1.06	2.96±0.89	=0.01	NS 0.457	<0.01
Lymphocytes (10^9/L)	1.86±0.54	1.84±0.54	1.87±0.54	1.78±0.53	1.78±0.56	1.79±0.52	<0.05	NS 0.189	NS 0.062
Monocytes (10^9/L)	0.46±0.15	0.44±0.14	0.47±0.16	0.42±0.15	0.42±0.12	0.43±0.16	=0.001	NS 0.109	<0.01
Basophils (10^9/L)	0.03±0.05	0.03±0.04	0.03±0.05	0.03±0.05	0.04±0.05	0.03±0.05	NS 0.495	NS 0.263	NS 0.849
Eosinophils (10^9/L)	0.19±0.32	0.22±0.52	0.17±0.12	0.18±0.14	0.18±0.17	0.18±0.12	NS 0.655	NS 0.542	NS 0.756
Glucose (mg/dL)	81.73±11.38	79.62±7.72	82.81±12.77	86.83±10.22	86.32±6.50	87.09±11.70	<0.001	<0.001	<0.001
Total Cholesterol (mg/dL)	198.29±34.98	205.94±35.54	194.40±34.30	197.27±37.84	202.06±31.63	194.84±40.64	NS 0.649	NS 0.277	NS 0.880
LDL (mg/dL)	134.78±28.90	141.70±28.50	131.26±28.67	134.68±30.57	138.06±25.86	132.96±32.75	NS 0.960	NS 0.198	NS 0.467
HDL (mg/dL)	62.38±12.36	62.91±12.33	62.10±12.45	61.91±12.59	63.09±13.21	61.31±12.32	NS 0.527	NS 0.868	NS 0.418
Triglycerides (mg/dL)	84.27±41.18	82.65±38.09	85.09±42.92	87.07±56.81	88.26±63.93	86.46±53.34	NS 0.457	NS 0.411	NS 0.763
ALT/GPT (U/L)	20.49±14.58	21.26±22.23	20.09±8.63	20.66±15.36	21.47±24.19	20.25±8.02	NS 0.815	NS 0.836	NS 0.874
AST/GOT (U/L)	22.97±7.00	22.50±8.08	23.21±6.44	23.06±9.86	21.59±7.11	23.81±10.97	NS 0.923	NS 0.371	NS 0.647
GGT (U/L)	21.50±12.77	23.21±17.94	20.63±9.15	20.98±12.27	23.00±16.66	19.96±9.27	NS 0.316	NS 0.791	NS 0.317
Albumin (g/dL)	44.48±2.44	43.62±2.12	44.91±2.48	43.25±2.37	42.41±2.35	43.67±2.29	<0.001	<0.01	<0.001
Creatinine (mg/dL)	0.80±0.19	0.75±0.18	0.83±0.18	0.80±0.17	0.78±0.17	0.82±0.17	NS 0.675	<0.05	NS 0.312
Creatine Kinase (CK)	141.47±170.96	144.41±178.58	139.97±168.31	145.55±242.98	120.68±80.92	158.18±292.74	NS 0.880	NS 0.366	NS 0.639
Amilase (U/L)	76.42±28.56	73.90±24.36	77.70±30.56	73.42±32.10	68.19±21.18	76.07±36.28	NS 0.145	<0.01	NS 0.576

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Lipase (U/L)	30.12±15.17	26.67±12.76	31.87±16.07	33.27±41.87	26.66±12.28	36.63±50.46	NS 0.376	NS 0.995	NS 0.372
LDH (U/L)	179.31±28.77	180.38±31.51	178.76±27.50	162.84±22.08	165.17±23.22	161.65±21.56	<0.001	<0.001	<0.001
Iron (µg/dL)	100.21±38.57	88.59±32.22	106.10±40.37	99.85±32.32	88.35±29.10	105.69±32.51	NS 0.925	NS 0.967	NS 0.933
Calcium (mg/dL)	9.73±0.33	9.63±0.32	9.78±0.33	9.67±0.34	9.56±0.34	9.73±0.34	<0.05	NS 0.113	NS 0.169
Sodium (mEq/L)	141.18±2.00	141.21±1.39	141.16±2.25	141.75±1.77	141.74±1.42	141.76±1.94	<0.05	NS 0.098	NS 0.061
Potassium (mEq/L)	4.47±0.38	4.49±0.38	4.46±0.38	4.53±0.35	4.50±0.32	4.55±0.36	NS 0.180	NS 0.825	NS 0.161
Chloride (mEq/L)	105.31±2.05	105.91±1.60	105.00±2.19	105.52±2.11	105.97±1.95	105.30±2.17	NS 0.300	NS 0.873	NS 0.248
Magnesium (mg/dL)	2.11±0.13	2.11±0.13	2.11±0.13	2.06±0.14	2.07±0.14	2.07±0.15	<0.01	NS 0.051	<0.05
B12 (pg/mL)	540.17±210.59	510.52±187.00	555.21±221.42	540.05±211.02	536.48±192.73	541.86±221.10	NS 0.991	NS 0.126	NS 0.353
Folic Acid (ng/mL)	9.53±4.38	9.03±3.65	9.78±4.70	9.95±5.52	9.15±4.52	10.36±5.95	NS 0.421	NS 0.847	NS 0.431
Vitamin D (ng/mL)	33.19±11.56	32.18±8.68	33.70±12.81	36.45±11.27	37.39±13.36	35.98±10.13	<0.01	<0.05	NS 0.063
QUALITY OF LIFE									
SF-12									
MCS	74.36±20.66	76.18±20.89	73.43±20.64	75.15±22.92	75.00±21.50	75.22±23.76	NS 0.806	NS 0.829	NS 0.657
High MCS	81 (80.2)	29 (85.3)	52 (77.6)	83 (82.2)	29 (85.3)	54 (80.6)	NS 0.657	NS 1.000	NS 0.621
PCS	87.62±18.66	84.71±21.07	89.10±17.30	88.91±20.29	87.35±26.66	89.70±16.33	NS 0.625	NS 0.659	NS 0.819
High PCS	94 (93.1)	31 (91.2)	63 (94.0)	94 (93.1)	30 (88.2)	64 (95.5)	NS 1.000	NS 0.661	NS 0.708
Total HRQoL score (scale of 100)	80.99±16.19	80.44±18.23	81.27±15.18	82.03±17.85	81.18±21.50	82.46±15.84	NS 0.666	NS 0.884	NS 0.647
Total High HRQoL score	96 (95.0)	31 (91.2)	65 (97.0)	92 (91.1)	30 (88.2)	62 (92.5)	NS 0.207	NS 0.661	NS 0.182
S-FAQLQ-AF									
Total FAQLQ score (scale of 7)	2.61±1.16	2.52±1.13	2.65±1.18	2.75±1.16	2.61±1.16	2.82±1.16	NS 0.284	NS 0.712	NS 0.301

*Number of pathologies/diseases, signs and symptoms related to ARFS (32).

AbR, antibody reactions; *BMI*, body mass index; *BP*, body power; *f*, force; *HGS*, hand grip strength; *HRQoL*, health-related quality of life; *IPAQ*, The International Physical Activity Questionnaire; *MCS*, mental component score; *PAL*, physical activity level; *PCS*, physical component score; *RAPA*, Rapid Assessment of Physical Activity; *RFD*, Rate of Force Development in N/s; *T-score*, bone health indicator; *T1FH*, type 1 food hypersensitivity; *T2FH*, type 2 food hypersensitivity; *VFR*, visceral fat ratio; *w*, weight; *WHO*, World Health Organization; *y*, years old.

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Anthropometry and body composition

Significant differences in group x time interaction were found in body composition variables. Group B revealed significant decreases in body weight (BW, $F_{1,66}$=13.61; p< 0.001; η2=0.17), body fat mass (BFM, $F_{1,66}$=9.27; p=0.003; η2=0.12) body fat mass percentage (BFMP, $F_{1,66}$=8.59; p=0.005; η2=0.12) and waist circumference (WC, $F_{1,66}$=42.04; p<0.001; η2=0.39) after the FASSD intervention (p< 0.05). However, no significant differences in group x time interaction were determined in body composition variables of Group A after the control intervention (p> 0.05).

Physical performance

No significant change in HGS was observed over time whereas the rest of PP tests changed over time (leg power, sit-to-stand speed, body balance). Accordingly, no significant effect of the ALASKA study intervention on either Group A or Group B were observed for HGS variable (p> 0.05). There were significant group x time interactions in CRF ($F_{1,66}$=2.13; p<0.034; η2=0.039) and BB ($F_{1,66}$=15.50; p<0.001; η2=0.190) for subjects belonging to Group B.

General Health

Symptomatology

The PSIMP-ARFSQ-10 symptomatology score significantly reduced in Group B after the FASSD dietary treatment. All body systems' PSIMP-ARFSQ-10 score significantly decreased in Group B after the FASSD intervention: digestive system or gastrointestinal (GI, $F_{1,66}$=36.37; p<0.001; η2=0.036), skin and subcutaneous tissue ($F_{1,66}$=8.59; p<0.005; η2=0.115), nervous ($F_{1,66}$=11.02; p<0.001; η2=0.143) and respiratory system ($F_{1,66}$=7.03; p<0.010; η2=0.096). Group A did not show any statistically significant changes in PSIMP-ARFSQ-10 scores before and after the control intervention.

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The amelioration of the symptomatology in subjects with FH showed positive results across all types of symptomatology PSIMP-ARFSQ-10 scores: GI, skin and subcutaneous tissue, nervous, and respiratory system. In addition, subjects of different PF levels in Group B showed positive symptomatology trends after the FASSD treatment (Figure 3).

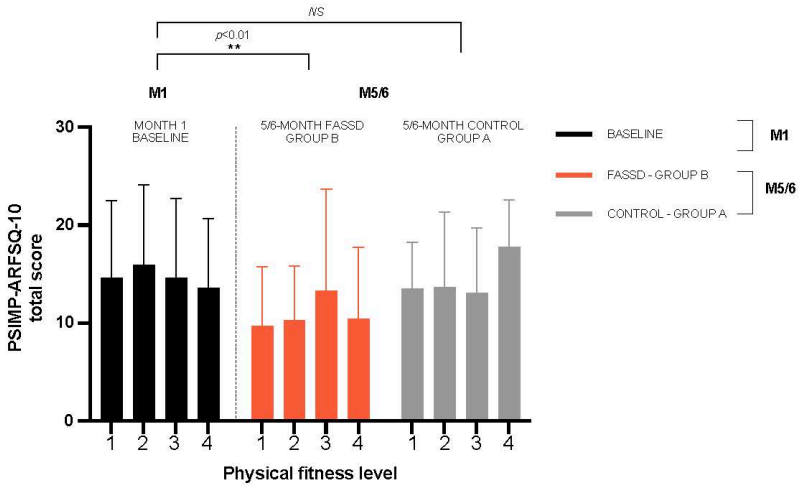


Figure 3 PSIMP-ARFSQ-10 symptomatology scores according to physical fitness level before and after the ALASKA study intervention

Adverse reactions to foodstuffs

Significant associations were found between the immunological biomarkers (total $sIgE_{CALCULATED}$ AbR for T1FH, and total $sIgG4_{CALCULATED}$ AbR) in participants of the intervention group before and after the FASSD. Improvements of 1 and 6 kUA/l were observed for total $sIgE_{CALCULATED}$ AbR ($F_{5.95}$=275.46; p=0.017; $\eta 2$=0.083) and total $sIgG4_{CALCULATED}$ AbR ($F_{60.85}$=1312.85; p<0.001; $\eta 2$=0.480), respectively after the FASSD and both yielded large effect sizes. There were no significant differences between the mentioned immunological biomarkers in participants of the control group before and after the ALASKA study intervention. Further information of analyzed food groups in T1FH and T2FH was referred in Annex I, Tables IA and IB.

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Hematobiochemical study

Despite all blood biomarkers were inside the normal and expected ranges pre-ALASKA study intervention, there were slight significant improvements in Group B for leucocytes, neutrophils and monocytes biomarkers post-intervention. However, the overall hematobiochemical profile of the subjects in the ALASKA study following the FASSD intervention is not physiologically different, as the values fall within the normal range.

Quality of life

No significant differences in mental, physical or FH-related QoL were observed among the FASSD group of participants before and after the ALASKA study intervention ($p>0.05$).

Relative risk ratios

In order to further comprehend the RR ratios between the FASSD and the control dietary treatment were calculated, logistic regression models were constructed, adjusting the data using treatment and control groups; and baseline and 5/6-month timepoints. RR were determined to measure how the probability of having positive FH was associated with being exposed to either the FASSD or control dietary treatments. Control cohorts had a significantly higher risk of developing both T1FH or T2FH (RR=2.55, 95% CI: 1.70-3.84, and RR=3.12, 95% CI: 2.16-4.51, respectively), $p<0.001$); and FASSD cohorts, significantly lower risk of T1FH or T2FH (RR=0.11, 95% CI: 0.04-0.28 and RR=0.13, 95% CI: 0.03-0.45, respectively), $p<0.01$).

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Discussion

A RCT was conducted in Spanish adults who had self-reported diseases, signs, and symptoms associated with T1FH and T2FH. This study examined the impact of the FASSD, proposed on the published ALASKA study protocol (11), on various variables including anthropometric measurements, body composition, physical performance, symptomatology, immunology, hematobiochemical study results, and QoL assessments.

Regarding anthropometry and body composition evaluation, statistically significant group x time interactions were observed in BW, BFM, BFMP, WC variables before and after the FASSD. However, there were no significant changes in anthropometric and body composition variables before and after the control intervention group. Mice models have shown that individuals with a positive FH generally have higher body weight and epididymal adipose tissue mass (33). Since T1FH and T2FH levels have dropped in Group B following the FASSD intervention, it is expected that BW, BFM, and WC will also decrease. Likewise, alterations in muscle mass (MM) are usually anticipated with high protein diets and/or exercise programs ; thus, as predicted, there was no significant change in MM throughout the duration of the ALASKA study intervention.

FH and other types of ARFS commonly manifest with GI symptoms in approximately half of diagnosed patients (34, 35). In this present study, we examined four distinct symptom scores (GI, skin and subcutaneous tissue, nervous and respiratory) and one miscellaneous score. It has been observed that the symptomatology related to ARFS can be improved after dietary exclusions(36); however, healthy blood biomarkers can be affected over time and nutritional deficiencies can affect the overall health of indivuals with FH (1). During long-term exclusion or substitutive diets lasting months, regular fluctuations in blood biomarkers such as glucose and LDH may occur periodically (37). While exclusion diets have been linked to nutritional deficiencies and negative nutrient blood markers, it was

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noted as a positive outcome that none of the measured blood markers worsened following the FASSD dietary intervention.

The main international societies in the field of FH management have released evidence-based guidelines covering mostly T1FH drug treatments and immunotherapies (38). Moreover, in terms of dietary treatments for T1FH, elimination diets have been the top recommended dietary approach due to their successful outcomes in managing T1FH (39). However, negative effects of elimination diets have been reported, particularly those related to nutrient deficiencies (40). In the present study, where a substitutive dietary intervention was applied, the FASSD, subjects decreased their sIgE AbR, which is a T1FH biomarker; similarly, subjects reduced their $sIgG_4$ AbR, which is the T2FH biomarker of this study.

Although SF-12 scores are typically expected to fluctuate over time, the ALASKA study intervention group did not showed significant enhancements before and after the FASSD. QoL improvement was also expected given that a specific FH tool was used to evaluate FH-related QoL. Dietary interventions are mostly expected to influence QoL of individuals when weight loss intention is involved (41). Subjects of the ALASKA study intervention were not strictly healthy since they had diseases, signs and symptoms related to ARFS with a minimum PSIMP-ARFSQ-10 score of 6. Therefore, their QoL did not significantly improved either worsen after the FASSD treatment. Their starting point might not be clearly different from their end point regarding emotions, mental o physical perceptions towards FH. A review study suggests that physical therapy treatments could have a significant impact on QoL, except when certain pathologies are present (42) (43). Further research is needed to identify the influencing factors that could have a significant effect on the QoL of individuals with FH.

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The RR of developing any of the studied FH was higher in Group A, for subjects following the healthy control dietary treatment; and statistically lower for subjects from Group B and following the FASSD dietary treatment.

Strengths and Limitations: The present study involves a robust defined sample of Caucasian Spanish adults, with no significant associations between the control and the intervention groups at the baseline starting timepoint. The ALASKA study intervention adhered to well-known standardized methods for accurately measuring body composition and physical performance factors. The ALASKA study research team has previously designed and validated specific tools for the accurate analysis of ARFS population.

Future research: Since FH are common in adults, there is a need to further explore the potential benefits of dietary treatments like the FASSD on physiological variables. Therefore, more in-depth analysis is needed to understand the positive effects of the FASSD treatment on BC, PP, and symptomatology aspects of ARFS population.

UNDER REVIEW

Conclusions

The ALASKA study intervention had positive effects in body weight, body fat mass, waist circumference, cardiorespiratory fitness and body balance of subjects following the food allergen-specific substitutive diet than the subjects under the healthy control dietary treatment. The food allergen-specific substitutive diet greatly influenced the PSIMP-ARFSQ-10 symptomatology score, improving all digestive, skin, nervous, and respiratory symptoms in individuals with type 1 and type 2 food hypersensitivity.

The food allergen-specific substitutive diet had a direct positive effect on the immunological biomarkers of IgE and IgG_4, leading to improvements in type 1 and type 2 food hypersensitivities in participants of the intervention group following the food allergen-specific substitutive diet. The ALASKA study intervention did not play a significant role in health-related quality of life or food hypersensitivity-related quality of life aspects of individuals with food hypersensitivities.

There is a significant high risk of developing higher either type 1 or type 2 food hypersensitivity in subjects not following a food allergen-specific substitutive diet. Further research studies are still needed to clarify the relationship between physical performance related variables and ARFS.

UNDER REVIEW

Funding

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The ALASKA study RCT is conducted in the ImFINE Research Group (gi.imfine@upm.es) of the Universidad Politécnica de Madrid. Industries and/or private institutions had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Declaration of Interests

None.

Authors' contributions

Supplementary information

Supplementary information is presented as Annex I containing:

UNDER REVIEW

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7. Appendixes

7.1. Appendix A: Funding Documentation

UNIVERSIDAD POLITÉCNICA DE MADRID

I+D+I R&D Projects and scholarships > Private project

RELATION BETWEEN ADVERSE REACTIONS TO FOOD (ARF), PHYSICAL PERFORMANCE AND HEALTH IN A MEDITERRANEAN POPULATION

Date: 2020-07-01 - 2024-06-30 | **ID:** P2011600273

PhD student, Researcher: Pantoja Arévalo, Lisset Shyrlenne [1]
Principal Researcher: González Gross, María Marcela [1]

AFFILIATIONS:

[1] ImFINE Research Group, Department of Health and Human Performance, Universidad Politécnica de Madrid, 28040 Madrid Spain

FUNDING INSTITUTE(S):
ImFINE Research Group, Department of Health and Human Performance, Universidad Politécnica de Madrid, 28040 Madrid Spain
Universidad Politécnica de Madrid, 28040 Madrid Spain

Resolution of October 1, 2020 of the Universidad Politécnica de Madrid recruituing a predoctoral contract for research personnel in training to carry out the Doctorate program on the research topic "Relationship between adverse reactions to food (ARF), physical performance and health in a Mediterranean population" (Ref. CPGI/007/2020).

Regarding the reference CPGI/007/2020 for a Predoctoral Contract financed by research groups within the framework of R&D projects, for the completion of the doctorate program in their schools, faculties, R&D centers and institutes, and as established in article 9 of the Rectoral Resolution of October 4, 2012 on the bases for predoctoral contracts, the Resolution is published on October 28, 2020.

AWARDED TO:

Pantoja Arévalo, Lisset Shyrlenne
Trainee research staff
PhD student, Researcher

González Gross, María Marcela
Thesis director and principal investigator
Principal Researcher

Madrid, October 28, 2020
VICE-RECTORATE FOR RESEARCH, INNOVATION AND DOCTORATE

Madrid, 28 de octubre de 2020
VICERRECTORADO DE INVESTIGACIÓN,
INNOVACIÓN Y DOCTORADO

Vicerrectorado de Investigación, Innovación y Doctorado
UNIVERSIDAD POLITÉCNICA DE MADRID

7.2. Appendix B: Summarized Curriculum Vitae

LISSET SHYRLENNE PANTOJA ARÉVALO
CURRÍCULUM VITAE



I. EDUCATION

PhD Studies in Physical Activity and Sport Sciences *2018 – to present*
Faculty of Physical Activity and Sport Sciences (INEF)
Universidad Politécnica de Madrid
Madrid, Comunidad de Madrid, ES

Master's Thesis *2016 - 2017*
Food Science Research Institute of The Spanish National Research Council (CIAL-CSIC)
Universidad Politécnica de Madrid and Universidad Complutense de Madrid
Madrid, Comunidad de Madrid, ES

Master's degree in Food Engineering applied to Health *2015 - 2016*
Agricultural, Food and Biosystems Engineering Technical School (ETSIAAB))
Universidad Politécnica de Madrid
Madrid, Comunidad de Madrid, ES

Food Engineer *2007 - 2012*
College of Agriculture, Food and Nutrition (CAAN)
Universidad San Francisco de Quito
Quito, Pichincha, EC

International Baccalaureate® in Experimental Sciences *2006 - 2007*
The International Baccalaureate® Organization (IBO)
Colegio Politécnico (COPOL)
Guayaquil, Guayas, EC

II. PROFESSIONAL ASSOCIATIONS AND SOCIETIES

Collegiate professional number 20608-M *2023 to present*
Official College of Biologists of the Community of Madrid (COBCM)
Madrid, Comunidad de Madrid, ES

Member of the European Academy of Allergy and Clinical Immunology *2023 to present*
European Academy of Allergy and Clinical Immunology (EAACI)
Utrecht, The Netherlands, NL

Member of the Spanish Network for Research in Physical Exercise and Health *2021 to present*
Spanish Network for Research in Physical Exercise and Health (EXERNET)
Madrid, Comunidad de Madrid, ES

Associate Member of the Latin American Nutrition Society *2019 to present*
Latin American Nutrition Society (SLAN)
Asunción, PY

Member of the Spanish Society of Nutrition *2019 to present*
Spanish Society of Nutrition (SEÑ)
Madrid, Comunidad de Madrid, ES

III. AWARDS AND RECOGNITIONS

Erasmus+ Plus Research, Teaching and Training Programme Grant *2023*
International Mobility Office
Universidad Politécnica de Madrid
Madrid, Comunidad de Madrid, ES

Qualification and Expertise in Food Allergy, Food Intolerance and Gluten related Disorders *2023*
AESKU Kipp Institute
Wendelsheim, DE

Accessit prize for oral communication with the best scientific and nutritional content *2021*
Scientific Committee of the Association of Nutrition and Dietetic Nurses (AdENyD)
Zaragoza, Aragón, ES

Official Predoctoral Contract of the Universidad Politécnica de Madrid for research personnel in research training (reference: CPGI/007/2020) *2020*
Universidad Politécnica de Madrid
Madrid, Comunidad de Madrid, ES

Entrepreneurial Initiative Award *2020*
Federico González Suárez FGS Educational Unit
Santo Domingo de Los Colorados, EC

Qualification and Expertise of POC and diagnosis tests of Food Allergy and Food Intolerance *2019*
DST Diagnostische Systeme & Technologien GmbH
Schwerin, Paulsstadt, DE

IV. WORK EXPERIENCE

Predoctoral Fellow *2018 - 2024*
ImFINE Research Group
Health and Human Performance Department
Faculty of Physical Activity and Sport Sciences (INEF)
Universidad Politécnica de Madrid
Madrid, Comunidad de Madrid, ES

Researcher *2015 - 2017*
Research Group of Bioactivity and Allergenicity of Food Proteins and Peptides (BIOPEP CIAL-CSIC)
Food Science Research Institute of The Spanish National Research Council (CIAL-CSIC)
Universidad Politécnica de Madrid and Universidad Complutense de Madrid
Madrid, Comunidad de Madrid, ES

Technical consultant *2012 - 2015*
Bioscience Department
Merck KGaA
Quito, Pichincha, EC

Pool Operator & Lifeguard *2009 – 2010*
Continental Pools INC
Savage, Maryland, US

Laboratory technician *2007 - 2008*
Health Center I
Ministry of Public Health
Guayaquil, Guayas, EC

V. SCIENTIFIC PUBLICATIONS

FIRST AUTHOR PUBLICATIONS

1. Pantoja-Arévalo L, Gesteiro E, Matthias T, Urrialde R, González-Gross M. **Association between Food-Specific Immunoglobulin G4 Antibodies in Adults with Self-Reported Signs and Symptoms Attributed to Adverse Reactions to Foodstuffs**. *Biomedicines*. 2023 Dec 17;11(12):3335. doi: 10.3390/biomedicines11123335.

2. Pantoja-Arévalo, L., Gesteiro, E., Matthias, T., Urrialde, R., & González-Gross, M. **A dataset description for the preliminary evaluation of type 2 food allergy correlating symptomatology, body composition, physical activity and antibody titers of food-specific immunoglobulin G subclass 4 in Spanish adults**. *Materia.* 2023: 10.

3. Pantoja-Arévalo L, Gesteiro E, Calonge-Pascual S, Pérez-Ruiz M, Urrialde R, González-Gross M. **Design and validity of the Spanish version of two questionnaires related to adverse reactions to foodstuffs**. *Nutrición Hospitalaria*. 2023 Aug 28;40(4):800-810. English. doi: 10.20960/nh.04631.

4. Pantoja-Arévalo L, Gesteiro E, López-Seoane J, Nehari A, Matthias T, Urrialde R, González-Gross M. **Food Allergen-Specific Substitutive Diet as a Proposed Tool for Adverse Reactions to Foodstuffs Management: The ALASKA Study**. *Proceedings*. 2023; 91(1):313. doi: 10.3390/proceedings2023091313.

5. Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, López-Seoane J, Wusterhausen P, Matthias T, Urrialde R, González-Gross M. **The multifactorial approach and the food allergen-specific substitutive diet as a tool to manage and ameliorate adverse reactions to foodstuffs in adulthood: study protocol for a randomized controlled trial-the ALASKA study.** *Trials*. 2024 Jul 20;25(1):494. doi: 10.1186/s13063-024-08307-2.

CO-AUTHOR PUBLICATIONS

6. Rivera-Ochoa M, López-Gil JF, Brazo-Sayavera J, Pantoja-Arévalo L, González-Gross M, Vizmanos-Lamotte B, Guadalupe-Grau A. **Clustering Health Behaviors in Mexican Adolescents: The HELENA-MEX Study**. *Res Q Exerc Sport*. 2024 Mar;95(1):281-288. doi: 10.1080/02701367.2023.2195458.

ABSTRACT PUBLICATIONS

1. Pantoja-Arévalo, Lisset; Gesteiro, Eva; Pérez-Ruiz, Margarita; Chiesa, Ilaria; Navarro, Ana; Wusterhausen Patricia; Matthias Torsten; Urrialde, Rafael; González-Gross, Marcela. **Food-specific IgE antibody reactions and self-reported symptomatology in Spanish adults**. Flash talks (FT). *Allergy*. 2024 Oct 79: 165-357. doi: 10.1111/all.16299.

2. Pantoja-Arévalo, Lisset; Gesteiro, Eva; Tang, Songxin; Prieto, Lucas; Matthias, Torsten; Pérez-Ruiz, Margarita; Urrialde, Rafael; González-Gross, Marcela. **High food-specific IgE antibody reactions in older Spanish adults with good physical fitness.** *Journal of Strength and Conditioning Research*. 2024 Oct 38(10): e626-e637. doi: 10.1519/JSC.0000000000004910.

3. Pantoja-Arévalo, Lisset; Gesteiro, Eva; Pérez-Ruiz, Margarita; Tang, Songxin; Chiesa, Ilaria; Navarro, Ana; Urrialde, Rafael; González-Gross, Marcela. **Lactose and fructose intolerance, symptomatology and physical fitness in Spanish adults. The ALASKA study.** *Journal of Physiology and Biochemistry*. 2024 Jul. doi: 10.1007/s13105-024-01031-0.

4. Pantoja-Arévalo, Lisset; Gesteiro, Eva; Pérez-Ruiz, Margarita; Navarro, Ana; Chiesa, Ilaria; Wusterhausen, Patricia; Matthias, Torsten; Urrialde, Rafael; González-Gross, Marcela. **Food-specific serum IgG4 and IgE antibody reactions and physical fitness in Spanish adults. The ALASKA study.** *Journal of Physiology and Biochemistry*. 2024 Jul. doi: 10.1007/s13105-024-01031-0.

5. Pantoja-Arévalo, Lisset; Gesteiro, Eva; Piccardi, Annica; Aparicio-Ugarriza, Raquel; Pedrero Chamizo, Raquel; Ríos, Yolanda; González-Gross, Marcela. **Food literacy changes before and during confinement of the COVID-19 pandemic in Spain.** *Annals of Nutrition and Metabolism*. 2022 Sept. doi: 10.1159/000526374.

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8. Pantoja-Arévalo, Lisset; Gesteiro, Eva; Dangers, Marc; Urrialde, Rafael; González-Gross, Marcela. **Nivel alto de respuesta a IgG$_4$ en hombres y mujeres españoles con sintomatología asociada a alergias e intolerancias alimentarias.** *Archivos Latinoamericanos de Nutrición. 2021*. doi: 10.37527/2021.71.S1.

9. Pantoja-Arevalo, L., Gesteiro, E., Dangers, M., & Gonzalez-Gross, M. **Prevalence of IgG$_4$ antibodies against mediterranean food allergens in patients with symptoms associated with adverse reactions to food. In annals of nutrition and metabolism.** *Karger*. 2020 76 (4): 86-86.

ACADEMIC PUBLICATIONS

1. *Pantoja Arévalo Lisset Shyrlenne.* **Caracterización proteómica de digeridos gástricos simulados de proteínas de suero lácteo**. Master's degree Tesis. *Universidad Politécnica de Madrid Repository. 2016*.

2. *Pantoja Arévalo Lisset Shyrlenne.* **Caracterización del contenido de aceite de dos oleaginosas: sacha inchi (*Plukenetia volubilis L.*) y canola (*Brassica napus y Brassica rapa*).** First degree Tesis. *San Francisco de Quito University Repository. 2012.*

VI. SCIENTIFIC CONGRESSES

1. XXXIII Congress of the Spanish Nutrition Society (SEÑ). *2 Poster scientific communications*. 19-21 June 2024. Granada, Spain.

2. International Congress of the European Academy of Allergy and Clinical Immunology (EAACI). *Oral scientific communication*. 31 May – 3 June 2024. Valencia, Spain.

3. XV International Symposium in Strength Training. *Poster scientific communication*. 15-16 December 2023. Madrid, Spain.

4. 14th European Nutrition Conference FENS and Serbian Nutrition Society Congress. *Oral scientific communication*. 14-17 November 2023. Belgrade, Serbia.

5. XII International Congress of the Spanish Association of Sports Sciences. *Oral scientific communication*. 21-23 June 2023. Madrid, Spain.

6. XXXI Congress of the Spanish Nutrition Society (SEÑ). *Oral and 2 Poster scientific communications*. 15-17 September 2022. Cartagena, Spain.

7. XIX Latin American Congress of Nutrition (SLAN). *Poster scientific communication*. 31 October – 4 November 2021. Asunción, Paraguay.

8. VII Fitness and Health network (EXERNET) Symposium: Evidence-based exercise prescription. *Oral scientific communication*. 22-23 October 2021. Cuenca, Spain.

9. XVII Congress of the Latin American Federation of Nutritional Therapy, Clinical Nutrition and Metabolism (FELANPE). *Poster scientific communication*. 2-6 July 2021. Guayaquil, Ecuador.

10. Virtual Congress-Forum of the Association of Nutrition and Dietetic Nurses (AdENyD). *Oral scientific communication*. 3-4 June 2021. Zaragoza, Spain.

11. Congress of the Ibero American Nutrition Foundation (FINUT). *Poster scientific communication*. 26-28 April 2020. Mexico DF, Mexico.

12. XXVIII Congress of the Spanish Nutrition Society (SEÑ). *Poster scientific communication*. 20-22 June 2019. Soria, Spain.

VII. LANGUAGES

Spanish - C2 Native
English - C2 Bilingual