

UNIVERSIDAD POLITÉCNICA DE MADRID
Facultad de Ciencias de la Actividad Física y del Deporte



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

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Lisset Shyrlenne Pantoja Arévalo

Master of Food Engineering applied to Health

Madrid, 2024



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Dr. Eva Gesteiro Alejos

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That the Doctoral Thesis entitled *“Relation between adverse reactions to food, physical performance and health in a Mediterranean population. Relación entre reacciones adversas a alimentos, rendimiento físico y salud en una población Mediterránea”* presented by Mrs. **Lisset Shyr lenne Pantoja Arévalo** to the superior judge of the university Tribunal designated by the Universidad Politécnica de Madrid, has been carried out under my **supervision** over the years of **2019-2024**, being expression of the scientific technical and interpretative capacity of its author in such advantageous conditions that make **her** worthy of the **PhD Doctorate degree with International mention**, as long as the mentioned university Tribunal considers it.

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This Doctoral Thesis does not include content that has been previously used, either fully or partially, to earn another academic degree or diploma. Except where otherwise indicated, this Doctoral Thesis is **my own work**.

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Dedication

"To my very esteemed thesis director, Prof. Dr. Marcela González-Gross, thank you for being an inspiring role model and for your consistent guidance during this process",

"To my supportive family, your endless love, belief and encouragement fueled my determination to accomplish this goal"

"May this achievement signify the continuing of my academic and research career and the beginning of fresh new challenges".

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Abstract

Introduction: Even though athletes have higher probability to develop a compromised immune function, given their continuous exposure to environmental and contact allergens; only few is known about the relation between health, physical performance and immune and non-immune-mediated adverse reactions to foodstuffs (ARFS). Food-specific serum (s)IgE and sIgG₄ antibody reactions (AbR) have been used as a tool to explore immune-mediated ARFS, type 1 (T1FH) and type 2 food hypersensitivities (T2FH), respectively. Likewise, lactose (LCI) and fructose (FCI) intolerance have been considered relevant non-immune-mediated ARFS.

Objectives: The main goal of this Doctoral Thesis was to analyze the relationship between physical fitness and ARFS, and to assess the overall health and physical performance of ARFS individuals before and after a food allergen-specific substitutive diet (FASSD), as part of the ALASKA Study project.

The specific objectives were: (1) To determine the prevalence of ARFS in a preliminary study and analyze the associated body systems and variables that may impact this prevalence in ARFS individuals. (2) To design and validate questionnaires to investigate the diseases, signs, symptoms and the frequency of food and beverages consumption in ARFS adults. (3) To develop a robust protocol for the ARFS management in adults. (4) To evaluate the frequency of immune and non-immune-mediated ARFS in Spanish adults and to analyze its correlation with physical fitness. (5) To describe the effect of a FASSD on sIgE and sIgG₄ AbR, physical performance and on ARFS symptomatology of Spanish adults with regular physical activity.

Methods: This Doctoral Thesis was based on the cross-sectional and randomized controlled trial of the ALASKA Study with 254 Spanish adult volunteers (61% females). Food-specific sIgE and sIgG₄ AbR against 61 food allergens were determined through line immunoassay (AESKU.GROUP GmbH, Wendelsheim, Germany). LCI and FCI were determined by subject's exhaled air using the Cerascreen® hydrogen and methane breath test kits (Cerascreen GmbH, Schwerin, Germany). Physical performance was measured through cardiorespiratory fitness, handgrip strength, leg power, sit-to-stand speed, body balance and resting heart rate. Statistical analysis was performed using IBM-SPSS Statistics v.25.0 and GraphPad Prism 10 softwares. Statistical significance was set at 0.05.

Results: (1) The most frequent self-reported symptomatology attributed to ARFS in a preliminary study of Spanish adults belonged mainly to skin and subcutaneous tissue (43%), followed by gastrointestinal (GI) (41%), and those related to nervous system (NS) problems (33%); (2) A final construct of questions was designed and validated for the following ARFS tools: 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). 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), respectively. (3) The ALASKA Study protocol has been developed as a strategy to manage and ameliorate ARFS in adulthood. The strategy relied on a multifactorial-variable approach and a six-month treatment using a FASSD. The FASSD was designed with emphasis in the common lacking micronutrients in ARFS population: niacin, magnesium, potassium, phosphorus, calcium, zinc, vitamin B12, folate, iron and fiber.

(4) Legume, egg and nuts seemed to be the most prevalent food groups explaining more than 60% of the positive prevalence of sIgG₄ AbR. Similarly, legume, seeds and nuts appeared to be the most prevalent food groups explaining more than 40% of the positive prevalence of sIgE AbR of Spanish adults. Excellent cardiorespiratory fitness was related to the highest levels of prevalence of subjects with sIgG₄ and sIgE AbR- both positive (41%); in comparison with subjects with good (34%) and normal cardiorespiratory fitness (15%) (all $p<0.05$). When analyzing subjects with sIgE-AbR-only, no significant differences were observed between the cardiorespiratory fitness categories. The level of excellent cardiorespiratory fitness was related to the lowest levels of LCI and FCI, when both were positive (8%); compared to subjects with good cardiorespiratory fitness (14%) ($p<0.05$). When analyzing the subjects with LCI-only and FCI-only, no significant differences were observed between each of the cardiorespiratory fitness categories. (5) Waist circumference, body weight, body fat %, T1FH and T2FH, estimated VO₂max, body balance and PSIMP-ARFSQ-10 symptomatology significantly improved after the FASSD intervention (all $p<0.05$).

Conclusion: (1) The most frequent self-reported signs and symptoms in the studied Spanish sample of adults with suspicion of ARFS included skin and subcutaneous tissue, GI, and NS problems. Sex and age might be a potential variable of interest for further ARFS research. (2) Both designed and validated questionnaires could be used to evaluate food and beverages consumption; also, to investigate the link between some specific diseases, signs and symptoms in ARFS adult population. (3) 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 dietary intervention to avoid crucial micronutrient deficiencies that a current strict food-allergen avoidance or elimination diet may provoke.

(4) Excellent cardiorespiratory fitness is associated to higher prevalence of sIgG₄ and sIgE AbR- both positive compared to the good and normal cardiorespiratory fitness levels. FCI is associated with higher self-reported GI symptomatology than LCI. Excellent cardiorespiratory fitness level was associated with less LCI and FCI- both positive prevalence in Spanish adults with ARFS self-reported symptomatology. (5) The FASSD greatly influenced the PSIMP-ARFSQ-10 score in individuals with T1FH and T2FH. After the ALASKA Study intervention, immune-mediated reactions decreased along with body fat%; improvements were observed in physical performance variables, like cardiorespiratory fitness and body balance.

Key words: Allergens, Cardiorespiratory Fitness, Exercise Test, Physical Fitness, Food Hypersensitivity, Immunoglobulin E, Immunoglobulin G, Sports Medicine.

Resumen (español)

Introducción: A pesar de que los deportistas pueden ser más propensos a tener un sistema inmunológico debilitado debido a su constante exposición a alérgenos ambientales y de contacto, todavía hay mucho desconocimiento sobre cómo afecta la salud, el rendimiento físico y las reacciones adversas a los alimentos y bebidas (RAAB), inmuno-mediadas y no-inmuno mediadas. La medición de la respuesta de anticuerpos (AbR) IgE en suero (sIgE) y AbR sIgG₄ frente a alimentos y bebidas se ha empleado para investigar el desarrollo de las RAAB, en particular la hipersensibilidad alimentaria tipo 1 (T1FH) y tipo 2 (T2FH), respectivamente. Asimismo, la intolerancia a la lactosa (LCI) y a la fructosa (FCI) se han considerado RAAB no-inmunomediadas comunes y relevantes.

Objetivos: El objetivo principal de esta Tesis Doctoral fue evaluar la salud general de sujetos con RAAB según diferentes categorías de condición física antes y después de un tratamiento dietético sustitutivo específico de alérgenos alimentarios, como parte del Estudio ALASKA.

Los objetivos específicos fueron: (1) Evaluar la prevalencia de RAAB, como un ensayo inicial, y examinar la relación de los sistemas del organismo humano con la sintomatología autorreportada de sujetos con sospecha de RAAB. (2) Diseñar y validar cuestionarios para la investigación de la sintomatología y de la frecuencia de consumo de alimentos y bebidas en adultos con sospecha de RAAB. (3) Desarrollar un protocolo robusto para la gestión de RAAB en adultos. (4) Evaluar la prevalencia de RAAB inmuno-mediadas y no-inmuno-mediadas en adultos españoles y analizar su relación con la condición física. (5) Describir el efecto de una intervención dietética sustitutiva sobre las RAAB y la sintomatología entre todas las categorías de condición física establecidas en el Estudio ALASKA.

Métodos: Esta Tesis Doctoral se basa en un estudio transversal y un ensayo controlado aleatorio, el Estudio ALASKA, sobre 254 voluntarios adultos españoles (61% mujeres). Se determinaron AbR sIgE y sIgG₄ específicos de alimentos frente a 61 alérgenos alimentarios mediante inmunoensayo en línea (AESKU.GROUP GmbH, Wendelsheim, Alemania). Las intolerancias alimentarias, LCI y FCI, se determinaron mediante el aire exhalado de los sujetos voluntarios utilizando los kits de prueba de aliento de hidrógeno y metano de Cerascreen® (Cerascreen GmbH, Schwerin, Alemania). El rendimiento físico se midió considerando las siguientes variables: capacidad cardiorrespiratoria (CCR), fuerza de prensión manual, potencia de las piernas, velocidad de bipedestación, equilibrio corporal y frecuencia cardíaca en reposo. El análisis estadístico se realizó utilizando el IBM-SPSS Statistics v.25.0 y GraphPad Prism 10 softwares. La significación estadística se estableció en 0,05.

Resultados: (1) Las enfermedades, signos y síntomas (sintomatología) autorreportados más frecuentes atribuidos a las RAAB pertenecían principalmente a los siguientes sistemas: piel y tejido subcutáneo (43%), digestivo o gastrointestinal (GI) (41%), y nervioso (33%). El sexo y la edad se consideraron como posibles variables de interés que influirían en futuros estudios de investigación sobre las RAAB; (2) Se diseñó y validó un conjunto final de preguntas para las siguientes herramientas: Cuestionario de Frecuencia de Consumo de Alimentos y Bebidas para identificar Reacciones Adversas de origen Alimentario (CFCAB-RAA-18); y cuestionario de Patologías y Sintomatología asociadas a Reacciones Adversas a Alimentos (PSIMP-RAA-10). Los coeficientes de confianza 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), respectivamente. (3) El protocolo del estudio ALASKA se desarrolló como una estrategia para gestionar y mejorar las RAAB en la edad adulta. La intervención FASSD fue diseñada con especial énfasis en los micronutrientes comunes que suelen ser de riesgo en la población con RAAB: niacina, magnesio, potasio, fósforo, calcio, zinc, vitamina B12, folato, hierro y fibra.

(4) En el caso de AbR sIgG₄, las legumbres, los huevos y los frutos secos fueron los grupos de alimentos más prevalentes, y explicaron más del 60% de los casos positivos. Considerando las AbR sIgE, las legumbres, las semillas y los frutos secos fueron los grupos de alimentos más prevalentes y explicaron más del 40% de los casos positivos en los adultos españoles. Los sujetos con CCR excelente tuvieron una mayor prevalencia de reacciones positivas para los dos anticuerpos (sIgG₄ y sIgE) (41%) en comparación con aquellos con CCR buena (34%) y normal (15%) (todos $p < 0,05$). Al analizar sujetos con reacciones positivas sólo a sIgE, no se observaron diferencias significativas entre las categorías de CCR. Analizando las RAAB no-inmuno-mediadas, se encontraron diferencias significativas en la puntuación PSIMP-RAA-10 de sintomatología GI considerando los sujetos que sólo presentaban LCI ($4,2 \pm 2,8$) y los que sólo presentaban FCI ($5,8 \pm 2,9$) ($p < 0,05$). El nivel de CCR excelente se relacionó con los niveles más bajos de prevalencia positiva de sujetos con LCI y FCI- ambos positivos (8%); en comparación con sujetos con CCR buena (14%) ($p < 0,05$). Al analizar a los sujetos con LCI-solo e FCI-solo, no se observaron diferencias significativas entre cada una de las categorías de CCR. (5) La circunferencia de la cintura, el peso corporal, el porcentaje de grasa corporal, T1FH y T2FH, el VO₂ máx estimado, el equilibrio corporal y la sintomatología relacionada con ARFS mejoraron significativamente después de la intervención dietética sustitutiva (todos $p < 0,05$).

Conclusión: (1) Los signos y síntomas autorreportados más frecuentes en la muestra estudiada de adultos españoles con sospecha de RAAB incluyeron problemas de piel y tejido subcutáneo, GI y del sistema nervioso. (2) Ambos cuestionarios validados podrían usarse para analizar la asociación entre el consumo de ciertos alimentos y bebidas con las RAAB; además de investigar el vínculo entre algunas enfermedades, signos y síntomas específicos con las RAAB. (3) Abordar a las RAAB con el análisis de múltiples variables, como factores influyentes, conduce a una estrategia novedosa para la gestión robusta de las RAAB.

La intervención con el tratamiento dietético sustitutivo ha sido diseñada para evitar las deficiencias de micronutrientes cruciales que puede provocar una dieta estricta de eliminación de alérgenos alimentarios como las que se emplean en la actualidad. (4) Un nivel excelente de CCR se asocia con una mayor prevalencia de AbR IgE y sIgG₄- ambos positivos en comparación con los niveles buenos y normales de CCR. Un nivel excelente de CCR se asoció con menos LCI e FCI- ambas positivas en adultos españoles con sintomatología autorreportada de RAAB. (5) La dieta sustitutiva influyó en gran medida en la puntuación de sintomatología PSIMP-ARFSQ-10, mejorando todos los síntomas digestivos, cutáneos, nerviosos y respiratorios, las reacciones inmuno-mediadas y el porcentaje de grasa corporal. Algunas variables de rendimiento físico, como la CCR y el equilibrio corporal, también mostraron mejoras después del tratamiento dietético con la dieta sustitutiva.

Palabras clave: Alérgenos, Capacidad Aeróbica Máxima, Capacidad Cardiorrespiratoria, Condición Física, Hipersensibilidad Alimentaria, Inmunoglobulina E, Inmunoglobulina G, Medicina Deportiva.

Table of Contents

<i>Acknowledgement</i>	XV
<i>Abstract</i>	XVII
<i>Resumen (español)</i>	XXI
<i>List of Tables</i>	XXIX
<i>List of Figures</i>	XXXI
<i>Abbreviations and Acronyms</i>	XXXIII
1. Introduction.....	1
1.1. Statements	1
1.1.1. Grants and subventions.....	1
1.1.2. Funding statements.....	2
1.2. Structure of the Doctoral Thesis.....	3
1.2.1. Timeline of the Doctoral Thesis	3
1.2.2. State of the Art.....	7
1.2.3. List of publications.....	8
1.2.4. Contributions of this Doctoral Thesis	11
1.3. Research Background	14
1.3.1. Definition of adverse reactions to foodstuffs	14
1.3.2. Prevalence of adverse reactions to foodstuffs.....	15
1.3.3. Classification of adverse reactions to foodstuffs	16
1.3.4. Biomarkers and identification tools.....	18
1.3.5. Physical performance and adverse reactions to foodstuffs.....	19
1.3.6. Current treatments of adverse reactions to foodstuffs	21
1.3.7. Research question, hypothesis and objectives	22
1.4. Objectives	23
1.4.1. General objective	23
1.4.2. Specific objectives	23

1.4.2.1.	Objective 1.....	23
1.4.2.2.	Objective 2.....	24
1.4.2.3.	Objective 3.....	24
1.4.2.4.	Objective 4.....	24
1.4.2.5.	Objective 5.....	24
2.	Materials and Methods.....	25
2.1.	Study design	25
2.1.1.	Geographical location of the study.....	26
2.1.2.	Intervention.....	27
2.1.3.	M1 Cross-sectional study	28
2.1.4.	M5 Randomized Controlled Trial.....	29
2.2.	Study protocol	30
2.2.1.	Timeline of the ALASKA Study intervention.....	30
2.3.	Inclusion and exclusion criteria	32
2.4.	Recruitment and data storage	32
2.5.	Physical, clinical assessments and questionnaires	34
2.5.1.	Main assessments of the ALASKA Study.....	34
2.5.2.	Variables and instruments of the ALASKA Study.....	35
2.5.2.1.	Forms and questionnaires	38
2.5.2.2.	Clinical assessments	40
2.5.2.3.	Physical assessments	44
2.6.	Ethical considerations.....	45
2.7.	Statistical analysis	46
3.	Results	49
3.1.	Preliminary study	49
3.1.1.	Sample characteristics of the preliminary study	49
3.1.2.	Signs and symptoms.....	50
3.1.3.	Food allergen-specific immunoglobulin G ₄ antibody reactions	52
3.1.4.	Food allergen-specific immunoglobulin G ₄ and age	53
3.1.5.	Food allergen-specific immunoglobulin G ₄ and symptomatology	58

3.2.	Design and validity of the PSIMP-ARFSQ-10 y FBFC-ARFSQ-18 questionnaires.....	61
3.2.1.	Phase 1: Questionnaires development.....	61
3.2.2.	Phase 2: Pilot test and reliability.....	63
3.2.3.	Phase 3: Content validity.....	64
3.2.4.	Phase 4: Face validity.....	68
3.3.	Development of the study protocol.....	69
3.3.1.	Study protocol characteristics.....	69
3.4.	Cross-sectional study results.....	77
3.4.1.	Sample characteristics of the ALASKA Study at month 1.....	77
3.4.2.	Adverse reactions to foodstuffs and age.....	81
3.4.2.1.	Type 1 food hypersensitivity.....	81
3.4.2.2.	Type 2 food hypersensitivity.....	82
3.4.2.3.	Lactose and Fructose intolerance.....	85
3.4.3.	Physical performance.....	86
3.4.3.1.	Physical performance and sample characteristics.....	86
3.4.3.2.	Physical performance and adverse reactions to foodstuffs.....	88
3.4.4.	Physical performance as a risk factor of adverse reactions to foodstuffs.....	90
3.5.	Randomized controlled trial results.....	93
3.5.1.	Sample characteristics of the ALASKA Study at month 5.....	93
3.5.2.	Anthropometry and body composition.....	93
3.5.3.	Physical performance.....	94
3.5.4.	General Health.....	98
3.5.5.	Quality of life.....	100
3.5.6.	Relative risk ratios.....	100
4.	Discussion.....	101
4.1.	General discussion.....	101
4.1.1.	Pilot study and variables of influence.....	105
4.1.2.	Design and validity of questionnaires for the assessment of adverse reactions to foodstuffs in adults.....	107
4.1.3.	ALASKA Study protocol.....	109

4.1.4.	M1 – Cross-sectional study	110
4.1.5.	M5 – Randomized controlled trial	113
4.2.	Strengths and limitations	117
4.3.	Future research lines.....	120
4.4.	Conclusions.....	120
4.4.1.	Conclusion 1	120
4.4.2.	Conclusion 2.....	121
4.4.3.	Conclusion 3.....	121
4.4.4.	Conclusion 4.....	122
4.4.5.	Conclusion 5.....	123
4.4.6.	General conclusion	123
5.	References	125
6.	Annexes.....	149
6.1.	Annex A: Informed Consent Form of the ALASKA Study.....	149
6.2.	Annex B: Demographics form.....	157
6.3.	Annex C: Inclusion and exclusion criteria form.....	161
6.4.	Annex D: Blood bank registration sheet.....	169
6.5.	Annex E: Article 1	171
6.6.	Annex F: Article 2.....	189
6.7.	Annex G: Article 3.....	203
6.8.	Annex H: Article 4	223
6.9.	Annex I: Article 5	243
7.	Appendixes	277
7.1.	Appendix A: Funding Documentation.....	277
7.2.	Appendix B: Summarized Curriculum Vitae	279

List of Tables

Table 1.2.3.1 Articles included in this Doctoral Thesis.....	10
Table 1.2.4.1 Contributions of this Doctoral Thesis by articles	11
Table 2.5.2.1 Summary of the variables and instruments of the ALASKA Study	35
Table 3.1.1.1 Sample characteristics.....	49
Table 3.1.4.1 Food-specific IgG ₄ AbR levels in adults of different age range groups	54
Table 3.2.2.1 Descriptive data of the pilot sample for the adaptation of FBFC-ARFSQ-18 and PSIMP-ARFSQ-10	63
Table 3.2.3.1 Descriptive data of experts for the evaluation of FBFC-ARFSQ-18.....	64
Table 3.2.3.2 Descriptive data of experts for the evaluation of PSIMP-ARFSQ-10.....	65
Table 3.2.3.3 Likert-type scale values offered by the panel of experts in all FBFC-ARFSQ-18 items for validation	65
Table 3.2.3.4 Likert-type scale values offered by the panel of experts in all PSIMP-ARFSQ-10 items for validation	66
Table 3.2.3.5 Number of experts pointing to each Likert-type scale value, mean score and Aiken's V coefficient for all items evaluated by experts.....	67
Table 3.3.1.1 Individual checklist example of the ALASKA Study intervention	70
Table 3.3.1.2 Food allergen-specific panels of IgE and IgG ₄ antibody reactions for the detection of type I and type II food hypersensitivity in human serum samples	74
Table 3.3.1.3 Design of a healthy Food-allergen Specific Substitutive Diet.....	75
Table 3.4.1.1 Sample characteristics of the ALASKA Study at M1	78
Table 3.4.1.2 Symptomatology, physical activity and physical performance compared by sex and age	80
Table 3.4.2.1 Food-specific Immunoglobulin E antibody reactions compared by sex and age	83
Table 3.4.2.2 Food-specific Immunoglobulin G ₄ antibody reactions compared by sex and age	84
Table 3.5.2.1 Baseline characteristics of the ALASKA Study intervention.....	94
Table 3.5.3.1 ALASKA Study assessments before and after the FASSD intervention.....	95

List of Figures

Figure 1.2.1.1 PhD timeline	6
Figure 1.2.2.1 Doctoral Thesis Structure Diagram	8
Figure 1.3.3.1 Adverse reactions to foodstuffs classification	17
Figure 2.1.1.1 Geographical location of the Faculty of Physical Activity and Sport Sciences of the Universidad Politécnica de Madrid	27
Figure 2.1.3.1 Diagram of the M1 Cross-sectional study	28
Figure 2.1.4.1 Diagram of the M5 Randomized Controlled Trial	29
Figure 2.2.1.1 Timeline of the ALASKA Study intervention.....	30
Figure 2.5.1.1 Main assessments of the ALASKA Study protocol	34
Figure 2.5.2.1.1 Screen capture of the personalized schedule set up in the ALASKA Study RedCap profile for each study participant	39
Figure 3.1.3.1 Food-specific IgG ₄ AbR in Spanish men and women. (a) Prevalence of food-specific IgG ₄ AbR in men. (b) Prevalence of food-specific IgG ₄ AbR in women	53
Figure 3.1.5.1 Symptomatology profile of subjects with level 3 of food-specific IgG ₄ AbR. (a) Skin and subcutaneous tissue symptomatology profile. (b) Digestive or gastrointestinal symptomatology profile. (c) Nervous system symptomatology profile.	60
Figure 3.4.2.1 Prevalence of type I and type II hypersensitivity reactions according to age groups at M1.	81
Figure 3.4.2.3.1 Prevalence of lactose and fructose intolerance according to age and sex groups.	85
Figure 3.4.3.1.1 Physical Performance variables and sample characteristics.....	87
Figure 3.4.3.2.1 Physical performance according to the types of adverse reactions to foodstuffs	89
Figure 3.4.4.1 Odds ratio associations between physical performance and ARFS food groups	90
Figure 3.5.4.1 PSIMP-ARFSQ-10 symptomatology scores according to physical fitness level before and after the ALASKA Study intervention	99

Abbreviations and Acronyms

Ab	antibody.
AbR	antibody reactions.
<i>a.m.</i>	<i>ante meridiem</i> (in the morning).
ARFS	adverse reactions to foodstuffs.
BMI	Body mass index
CeD	coeliac disease.
EAACI	European Academy of Allergology and Clinical Immunology.
EFSA	European Food Safety Authority.
<i>e.g.</i>	<i>exempli gratia</i> , “for example”.
FASSD	food allergen-specific substitutive diet.
FBFC-ARFSQ-18	Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs.
FA	food allergy.
FDA	Food and Drug Administration.
FI	food intolerance.
<i>gold standard</i>	reference method.
HDLc	high-density lipoprotein cholesterol.
HRQoL	Health-related quality of life
<i>i.e.</i>	<i>id est</i> , “that is”
IgE	Immunoglobulin E.
IgG ₄	Immunoglobulin G subclass 4.
IPAQ	The International Physical Activity Questionnaire.
IPAQ-PA	physical activity according to The International Physical Activity Questionnaire.
LDLc	low-density lipoprotein cholesterol.
M1	month 1 timepoint of the ALASKA Study.
M5/6	between month 5 and month 6 timepoint of the ALASKA Study.
OIT	oral immunotherapy.
OR	Odds ratio.
PSIMP-ARFSQ-10	Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs.
QoL	quality of life.
RAPA	The Rapid Assessment of Physical Activity Questionnaire.
RAPA-PA	Physical activity according to The Rapid Assessment of Physical Activity Questionnaire
RCT	randomized controlled trial.
RedCap®	research electronic data capture platform.
sIgE	serum Immunoglobulin E.
sIgG ₄	serum Immunoglobulin G ₄ .

T1FH	type 1 food hypersensitivity.
T2FH	type 2 food hypersensitivity.
vs	versus. <i>Latin word meaning "against"</i> .
y	years of age.
24HDRI	24-hour dietary recall interview

1. Introduction

1.1. Statements

1.1.1. Grants and subventions

- Official Predoctoral Contract grant of the Universidad Politécnica de Madrid (reference: CPGI/007/2020). The author of this Doctoral Thesis, Lisset Shyrlenne Pantoja Arévalo, had been awarded with an official Predoctoral Contract of the Universidad Politécnica de Madrid for research personnel in research training (reference: CPGI/007/2020) for performing the PhD Thesis entitled “Relation between adverse reactions to food, physical performance and health in a Mediterranean population” from the academic year of 2020 to 2024.

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

- I+D+i R&D project of the Universidad Politécnica de Madrid: “Relation between adverse reactions to food, physical performance and health in a Mediterranean population” (project number: P2011600273) conferred on October 28th, 2020.
- Erasmus+ Plus mobility, research, teaching and training international grant (ID number: 67335) supported by the Vice-rectorate for Internationalization of the Universidad Politécnica de Madrid. The international mobility took place at the Institute of Translational Immunology of the Johannes Gutenberg University in Mainz- Germany from July 2nd, 2023, to October 6th, 2023.

1.1.2. Funding statements

- This research was partially funded by own funds of the ImFINE Research Group. Additional funding from the Instituto de Salud Carlos III through CIBEROBN (reference: CB12/03/30038), which is co-funded by the European Regional Development Fund.
- Lisset Shyrlenne Pantoja Arévalo was supported by Universidad Politécnica de Madrid by means of an official Predoctoral Contract of the Universidad Politécnica de Madrid for research personnel in research training (reference: CPGI/007/2020) from the academic year of 2020 to 2024.
- This Doctoral Thesis was linked to the I+D+i R&D project of the Universidad Politécnica de Madrid: “Relation between adverse reactions to food, physical performance and health in a Mediterranean population” (project number: P2011600273).
- This project was additionally funded by Aesku.Diagnostics GmbH through an agreement with the Universidad Politécnica de Madrid. AESKUBLOTS® kits (Aesku.Diagnostics GmbH, Wendelsheim, Germany) were manufactured and blind-processed by Aesku.Diagnostics GmbH. However, the design, management, statistical analysis, and reporting of the study have been performed entirely independent of Aesku.Diagnostics GmbH.
- This research work was conducted in the ImFINE Research Group facilities of the Faculty of Physical Activity and Sport Sciences 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 these results.

1.2. Structure of the Doctoral Thesis

1.2.1. Timeline of the Doctoral Thesis

Development of the Doctoral Thesis project

This Doctoral Thesis involved 5 years of research work (Figure 1.2.1.1), encompassing the project design, funding applications, scholarships, ethical considerations, and clinical trial registration as initial tasks. The Doctoral Thesis project was first written on the 10th of September 2018 for the application of several research scholarships while, as PhD student, I was attending to the Complementary Training classes of the Doctorate Program of Physical Activity and Sport Sciences of the Faculty of Physical Activity and Sport Sciences of the Universidad Politécnica de Madrid. The PhD research process started as funding applications to different calls for aids programs, where this PhD project was finalist in the prestigious “La Caixa” InPhinit doctoral scholarships, retaining in research centers and universities in Spain and Portugal of the 2019 call. Finally, the project was born by means of a contract collaboration between the private industry and the Universidad Politécnica de Madrid that resulted in a Predoctoral official academic contract.

Clinical research using blood and breath human samples was intended, so the application to the Ethics Committee of the Universidad Politécnica de Madrid started on the 17th of February 2020 and was approved on the 17th of July 2020. Consequently, the Clinical Trials registration was done at the end of the COVID-19 pandemic and was accepted on the 5th of December 2022.

COVID-19 pandemic

The 2019 COVID-19 pandemic, caused by the virus SARS-CoV-2, had been expanded from Wuhan, the capital city of Hubei Province in China, to a growing number of countries by

2020 [1]. Due to the gravity of circumstances, the “state of alarm” was declared in Spain on the 14th of March 2020 by the Boletín Oficial del Estado (BOE) of the Real Decreto 463/2020 [2]. Since then, Spain passed through continuous lockdown measures for a period of at least three consecutive months. These measures included leaving home only to buy essentials from supermarkets and pharmacies and going to work in those cases in which work in person was essential (pharmacies, healthcare, supermarkets, cleaning operatives, etc.) while maintaining social distance [3]. The mandatory use of facemasks started on the 21st of May 2020 and ended gradually from the 4th of July 2020. On this date, the Council of Ministers approved the agreement declaring the end of the health crisis caused by COVID-19. With the publication of this agreement in the BOE, the obligation to use masks also ends, although their use is still highly recommended in some cases. The COVID-19 pandemic led to lockdown, changed several daily habits, including social interaction and the ability to accomplish the minimum requirements of physical activity and a healthy diet. It was conceivable that from a situation with complete food availability and with a shift from eating out to mandatory in-house consumption, could have altered the dietary patterns of many Spanish individuals [4]. After the mandatory measurements were lifted, vaccination against COVID-19 in Spain, as a national vaccination strategy, began on December 27th 2020, within the framework of a global effort to combat the COVID-19 pandemic, and ended the 31st of January 2022 with the third vaccination doses.

Prior to the COVID-19 outbreak, a pilot study was conducted which yielded preliminary data leading to the publication of the first article of the Doctoral Thesis project. The pandemic then delayed the start of the fieldwork of this Doctoral Thesis work. However, the COVID-19 pandemic restrictions were seen as an opportunity and the creation of the online-adapted ALASKA Study project took place on the 6th of October 2020 using an academic online platform, The Research Electronic Data Capture (RedCap®), which is a secure web platform

that enables remote use, as necessary during the pandemic when social interaction was still limited. Consequently, a robust post-pandemic adapted ALASKA Study protocol, with standardized online forms and questionnaires, was constructed, enhanced, and implemented.

The ALASKA study field work

After the ALASKA Study protocol was developed and published [5], field work started on April 2022 with the participants' recruitment. The participants' intervention was performed from May 2023 until July 2024 focusing on the variables of interest that had been previously identified and studied in the pilot study and the ones that had been established during the development of the ALASKA Study protocol (multifactorial approach). After completing the first phase of the field work, an international experience at the Institute of Translational Immunology of the Johannes Gutenberg University in Mainz- Germany took place from the 2nd of July 2023 to 6th October 2023 to pursue personal, academic and professional skills in the field of adverse reactions to foodstuffs (ARFS), more specifically, type 1 and type 2 food hypersensitivity (T1FH and T2FH, respectively) in German human serum samples with different diseases related to ARFS and gluten-related disorders and sensitivities in murine serum samples. Cardiorespiratory fitness assessments, from human and murine organisms, were previously characterized by the cardiorespiratory research team of the Institute of Translational Immunology and results were stored and registered. After returning from this rewarding research international stay, the focus in this Doctoral Thesis program had been directed to the publishing of the ALASKA Study results of phase 1 (M1) and phase 2 (M5) and to complete the present final Doctoral Thesis manuscript for dissertation.

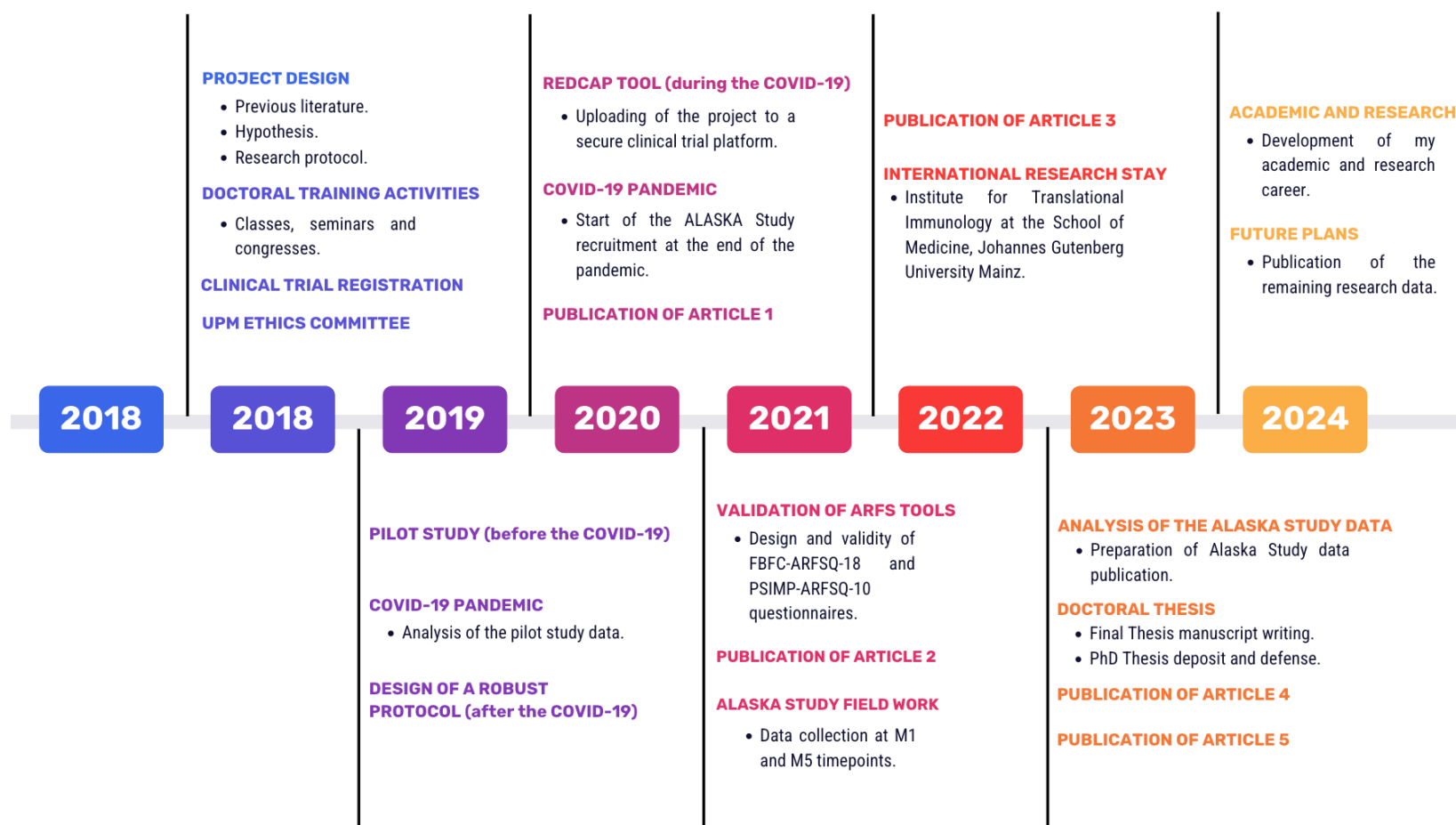


Figure 1.2.1.1 PhD timeline

ARFS, adverse reactions to foodstuffs; COVID-19, pandemic of the coronavirus SARS-CoV-2 disease; FBFC-ARFSQ-18, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; I/E, inclusion and exclusion criteria; M1, month 1 of the ALASKA Study; M5/6, month 5 or month 6 of the ALASKA Study; PSIMP-ARFSQ-10, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; Redcap®, research electronic data capture; UPM, Universidad Politécnica de Madrid. Own elaboration.

1.2.2. State of the Art

This Doctoral Thesis structure has been developed in accordance with the Universidad Politécnica de Madrid Doctoral Thesis template and formatting guidelines, which, in many respects, follow formatting standards common to many other national and international academic institutions. This document is divided into three main components (front matter, main matter and end matter), each of which has several subcomponents or sections, as shown in Figure 1.2.2.1. The front matter refers to the initial pages that appear at the start of the document and before all the sections of the Doctoral Thesis. The main matter is the main body of the PhD Thesis; and the end matter refers to the final pages that appear at the end of the present manuscript.

This Doctoral Thesis consists of the following structure of sections. Section 1 contains a general introduction of the whole Doctoral Thesis, a description of the structure of the Doctoral Thesis work and an explanation of the research question and general objectives. Section 2 explains the general materials and methods of the Doctoral Thesis including the study design, the study protocol, the inclusion and exclusion criteria, recruitment and data storage, physical and clinical assessments, questionnaires, ethical considerations and statistical analysis. Section 3 incorporates the Doctoral Thesis scientific publications, following the required format by the scientific journals (introduction, material and methods, results, discussion and conclusions). Within this section, each article published and/or submitted as part of this Doctoral Thesis is presented (Table 1.2.3.1) and organized chronologically following the Doctoral Thesis timeline (Figure 1.2.1.1). Section 4 contains a general discussion of the entire Doctoral Thesis and summarizes the main outcomes of the Doctoral Thesis findings. Lastly, this section also includes the general conclusions of the Doctoral Thesis. All the presented

articles are interconnected, follow a logical order and are part of the overall Doctoral Thesis work.

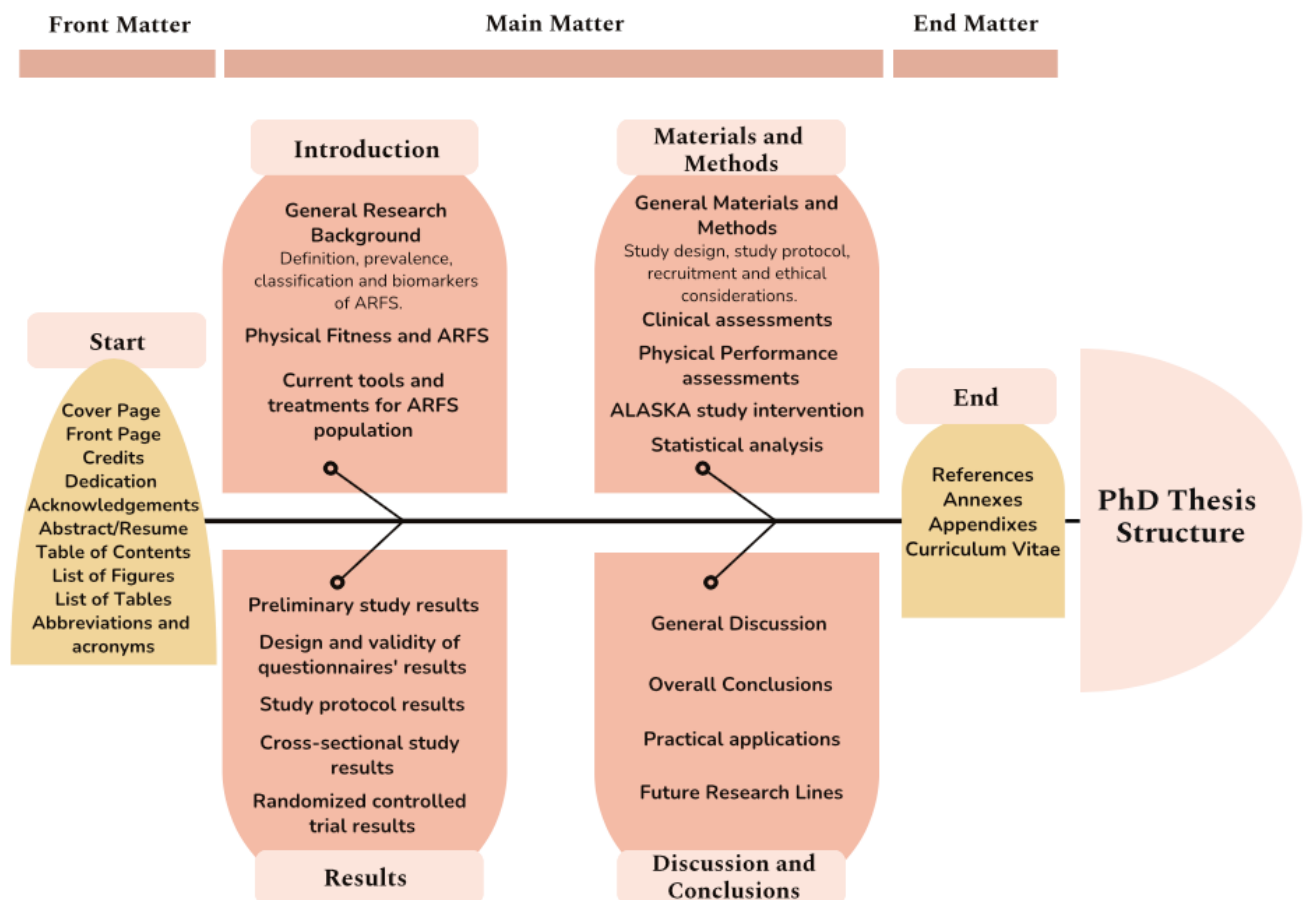


Figure 1.2.2.1 Doctoral Thesis Structure Diagram

ARFS, adverse reactions to foodstuffs; FBFC-ARFSQ-18, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; G4, Immunoglobulin G subclass 4; M1, month 1 of the ALASKA Study; M5, month 5 of the ALASKA Study; PSIMP-ARFSQ-10, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs.

1.2.3. List of publications

Published articles

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 Mar 17;11(12):3335. doi: 10.3390/biomedicines11123335.

2. 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.** *Nutr Hosp*. 2023 Aug 28;40(4):800-810. doi: 10.20960/nh.04631.
3. 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.

Submitted articles:

4. Pantoja-Arévalo L, Gesteiro E, Tang S, Pérez-Ruiz M, Urrialde R, González-Gross M. **Is there a relationship between physical performance factors and adverse reactions to foodstuffs? - The ALASKA Study.** *Sports*. 2024 Aug 20.
5. Pantoja-Arévalo L, Gesteiro E, Pérez-Ruiz M, Díaz-Martínez ÁE, Urrialde R, González-Gross M. **Effect of the food allergen-specific substitutive diet on health, quality of life and physical performance in adults with adverse reactions to foodstuffs: A randomized controlled trial - The ALASKA Study.** *Public Health*. 2024 Oct 15.

Table 1.2.3.1 Articles included in this Doctoral Thesis

Article	Title	Journal/Publisher	JCR/ Scopus/ Other • IF	Status
1	Association between food-specific Immunoglobulin G ₄ antibodies in adults with self-reported signs and symptoms attributed to adverse reactions to foodstuffs	Biomedicines/ MDPI	Q1 • 4.0	Published
2	Design and validity of the Spanish version of two questionnaires related to adverse reactions to foodstuffs	Nutrición Hospitalaria/ Grupo ARAN	Q3 • 1.2	Published
3	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/ Biomed Central BMC of Springer Nature	Q2 • 2.0	Published
4	Is there a relationship between physical performance factors and adverse reactions to foodstuffs? - The ALASKA Study	Sports/ MDPI	Q2 • 3.0	Submitted
5	Effect of the food allergen-specific substitutive diet on health, quality of life and physical performance in adults with adverse reactions to foodstuffs: A randomized controlled trial - The ALASKA Study	Public Health Nutrition/ Cambridge University Press	Q1 • 4.7	Submitted

ALASKA Study, Allergies and food intolerances in adults and athletes; BMC, BioMed Central; G₄: Immunoglobulin G subclass 4; IF, Impact Factor; JCR, Journal Citation Reports; MDPI, Multidisciplinary Digital Publishing Institute; Q1, top 25% of journals in a particular field based on Impact Factor; Q2, second quartile, comprising journals with Impact Factors higher than the median but lower than the top 25%; Q3, third quartile, encompassing journals with Impact Factors lower than the median but higher than the bottom 25%.

1.2.4. Contributions of this Doctoral Thesis

The contributions of this Doctoral Thesis are explained below. In-depth explanation of the input for each article is provided and organized in the following columns.

Table 1.2.4.1 Contributions of this Doctoral Thesis by articles

Article	What is already known on this topic?	What does this PhD Thesis add?
Article 1 Association between food-specific Immunoglobulin G4 antibodies in adults with self-reported signs and symptoms attributed to adverse reactions to foodstuffs	There is lack of current research data about the characterization of Spanish adults with suspicion of having ARFS. There is also an urgent need for the evaluation of ARFS subjects regarding their symptomatology, sex, PA, physical fitness and body composition, to identify the specific variables of influence in ARFS Spanish adult population. Only few is known about the associations of the IgG4 and these variables of possible influence. IgG4 biomarker, has been recently studied, as T2FH in patients with FI symptomatology and related diseases. Almost none is published about the relationship between body composition and the prevalence of food allergen-specific FA or FI.	Selected variables of interest, in the PA and sport sciences field, were determined as a preliminary overview in a specific sample of ARFS Spanish adults. The most frequent symptomatology has been identified among subjects with ARFS suspicion and IgG4, as T2FH biomarker, has been evaluated in Spanish adults suspecting to have FA or FI. A first clinical screening has been approached in this publication. It has been determined that most of Spanish adults self-reporting symptomatology related to ARFS did not accomplish the PA recommendations of the WHO.
Dataset of Article 1 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	Dataset publications analyzing ARFS, such as T1FH, T2FH, LCI and FCI, have not yet considered all variables of interest related to ARFS, such as general health, physical and clinical measurements. Previous published datasets focus on the evaluation of general health avoiding the assessment of variables related to PA, body composition and physical performance.	A specific dataset, including physical and clinical assessments, was created to evaluate the mentioned variables, their associations and have a clearer preliminary clinical picture and data about PA of Spanish adults with suspicion of ARFS.

(Table 1.2.4.1 continues)

Article	What is already known on this topic?	What does this PhD Thesis add?
Article 2 Design and validity of the Spanish version of two questionnaires related to adverse reactions to foodstuffs	There is a lack of validated tools, in Spanish language, to measure precisely the symptomatology and the food frequency consumption in individuals with ARFS considering their variety of possible diseases, signs and symptoms associated to ARFS, as well as their dietary consumption habits. Specific validated tools are needed for research and clinical purposes to relate physical assessments, such as physical performance, in a more accurate way and to clarify the pathogenesis of ARFS in the Mediterranean population.	Two specific questionnaires were designed and validated, in Spanish language, to measure the symptomatology and the food frequency consumption of Spanish adults with suspicion of having any ARFS, such as FA or FI. The published validated tools were: 1) FBFC-ARFSQ-18; and 2) PSIMP-ARFSQ-10.
Article 3 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	A robust protocol to investigate, beyond the diagnosis, the ARFS characteristics in Mediterranean population is needed to perform a complete characterization of Spanish adults with related symptomatology. Body composition, PA, physical fitness and QoL related variables are key for the analysis of this specific population. There are still no research studies analyzing the overall clinical and physical picture of this specific ARFS population.	The ALASKA Study protocol was developed and included all clinical and physical variables of interest considering preliminary analysis and publications of this Doctoral Thesis work. In this publication, the ALASKA Study was organized in a robust protocol considering 10 main assessments as part of the ARFS management and the dietary intervention, FASSD, was proposed as a treatment to ameliorate the symptomatology in Spanish ARFS population with different physical fitness levels.
Article 4 Is there a relationship between physical performance factors and adverse reactions to foodstuffs? - The ALASKA Study	The study of the main relationship between ARFS and physical performance is needed to further comprehend the clinical picture of ARFS in individuals with different physical condition to improve their ARFS treatment. It has been previously studied that the differences in the positivity of ARFS can be seen in individuals when they are exposed to the same type or amount of food allergen at different stages of life and with different symptomatology profiles. However, the differences in this positivity, considering their physical performance have not been investigated yet.	Article 4 explained the prevalence of ARFS, T1FH and T2FH, according to age groups. Symptomatology, PA and physical performance were evaluated according to sex and age groups. Finally, physical performance variables were evaluated according to the type of ARFS; and the probability of suffering any of the studied types of ARFS was calculated according to the measured physical performance levels in a sample of Spanish adults.

(Table 1.2.4.1 continues)

Article	What is already known on this topic?	What does this PhD Thesis add?
Article 5 Effect of the food allergen-specific substitutive diet on health, quality of life and physical performance in adults with adverse reactions to foodstuffs: A Randomized Controlled Trial - The ALASKA Study	Despite acceptable prognosis regarding dietary treatments for population suffering from ARFS, such as elimination diets, it may have negative long-term effects on the metabolic, well-being and physical health of affected individuals. Health problems such as relevant nutritional deficiencies, muscle weakness, weight gain and muscle atrophy are on the one hand significantly related issues to dietary treatment adherence, QoL and overall well-being of the adult population presenting ARFS.	Article 5 evaluated a dietary intervention, the FASSD, that avoided the need to eliminate essential nutrients, which are necessary for the optimal metabolic and physical well-being of population with ARFS and to prevent the risk of muscle dysfunction and weight gain. In this article, body composition variables such as weight and body fat, played a beneficial role in the intervention group. The risk of suffering T1FH and T2FH between the control group following a standard Spanish healthy diet and the intervention group following the FASSD was calculated.

ARFS, adverse reactions to foodstuffs; FA, food allergy; FBFC-ARFSQ-18, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; FI, food intolerance; IgG₄, Immunoglobulin G₄; PA, physical activity; PSIMP-ARFSQ-10, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs; T1FH, type 1 food hypersensitivity; T2FH, type 2 food hypersensitivity; QoL, quality of life; WHO, World Health Organization.

1.3. Research Background

1.3.1. Definition of adverse reactions to foodstuffs

ARFS have been officially defined since approximately 1995 and updated in 2024, by the European Academy of Allergology and Clinical Immunology (EAACI), as any abnormal, exaggerated or irregular reaction to exogenous stimuli which are commonly derived from components of food and beverages ingestion [6]. ARFS are currently considered a significant cause of morbidity worldwide and a considerable burden on current public health in both developed countries and emerging economies [7]. Immune-mediated and non-immune-mediated food and beverages allergen-specific reactions, such as food allergies (FA)/ food hypersensitivities and food intolerances (FI), can be indicative of ARFS [8].

Recently, there has been a clear alarming increasing tendency of chronic health conditions of the inactive and sedentary adult population in the past decade [9]. In fact, studies have shown that approximately one in every three adults in Europe are physically inactive [10].

Athletes seem to have higher probability to develop a compromised immune function, given their continuous exposure to environmental and contact allergens, strict demands of sports activities and recurrent stress [11]. However, only few is known about the relation between health, physical performance and immune and non-immune-mediated ARFS in Spanish adults. The current research thoroughly examined immune-mediated ARFS, such as T1FH and T2FH; likewise, non-immune-mediated ARFS, such as lactose intolerance (LCI) and fructose intolerance (FCI), were evaluated for their associations with physical performance and health in a Spanish sample within the framework of the ALASKA Study project. [5].

1.3.2. Prevalence of adverse reactions to foodstuffs

The prevalence of ARFS (T1FH, T2FH, LCI and FCI) is currently increasing worldwide and it represents a public health concern. Actually, it has been considered by the World Health Organization (WHO), since 2020, that allergy is the current fourth most common chronic disease category in the world [12]. The prevalence of ARFS has been specially increasing in the adult population. The scarcity of unified data, considering the same diagnostic procedure, makes it difficult to estimate a precise prevalence of T1FH, T2FH, LCI and FCI among the world population. It is important to mention that several types of improved diagnosis based on the molecular mechanisms of ARFS have also been on the rise, which could possibly explain the recent increasing prevalence of ARFS [13]. More than 150 million Europeans have been estimated to be suffering from chronic general allergic disease, including about 20 million suffering from specific FA pathogenesis, which is a type of FH, and more than 80 million from FI [14,15]. Some other studies have suggested around 2% of prevalence, raising up to more than 30%, respectively, for FA and FI with an adult female dominance of more than 60 % of prevalence in self-reported studies [16,17].

Typically, ARFS are often regarded as pediatric problems; thus, these reactions have not been in-depth studied in the adult population and no accurate current strategies focusing on the adult population have been established up to this present study. ARFS can begin at any age; in fact, the frequency of self-reported ARFS cases in adults is rising, with a reported prevalence of approximately one adult affected in every four children [18-20]. It has been estimated that by 2050, half of the world's population will be affected by at least one type of allergy. Moreover, by that time, one in every five people globally will be aged 60 years or over, and more than one in every three adults in Europe will be physically inactive [18]. Therefore, the general health, physical performance and quality of life (QoL) of the adult population emerge as an essential object of study for the coming years [21]. A novel strategy involving a protocol

with the assessment of multiple variables and a dietary treatment to manage and ameliorate ARFS was developed and proposed as part of this Doctoral Thesis [5].

1.3.3. Classification of adverse reactions to foodstuffs

According to the European Food Safety Authority (EFSA), EAACI, and some other authors [22-25], ARFS are classified as non-immune-mediated and immune-mediated reactions. However, this classification may vary among different institutions. Hence, it is crucial to outline and determine the specific clinical definition and classification to be used. Regarding the present Doctoral Thesis work, and to achieve the established research goals, individuals' specific responses to several food and beverages components were considered. Therefore, ARFS have been classified by pathogenic mechanism of action in immune-mediated reactions (T1FH and T2FH), and non-immune-mediated reactions (LCI and FCI). Thus, an adapted classification has been constructed and published specifically for the present study (Figure 1.1.3.1) [5].

This research has considered the assessment of Immunoglobulin E (IgE) as a biomarker of T1FH, commonly known as FA, because of its clinical and therapeutic significance. Research on IgE-mediated food reactions has been ongoing for a considerable period of time, dating back to approximately 1967 when Gunnar Johansson and Hans Bennich, of the Uppsala University, identified IgE as the mediator of allergic diseases [26]. Nowadays, IgE-mediated food reactions are utilized as a standard method for evaluating FA in medical facilities. IgE-mediated food reactions occur when the immune system is activated, leading to a lymphocyte T helper 2 response that causes IgE to bind to Fcε receptors on cells such as mast cells and basophils, resulting in the pathophysiology of the reaction. The release of histamine and other preformed mediators by these cells leads to rapid symptom onset, a well-known characteristic of T1FH [26].

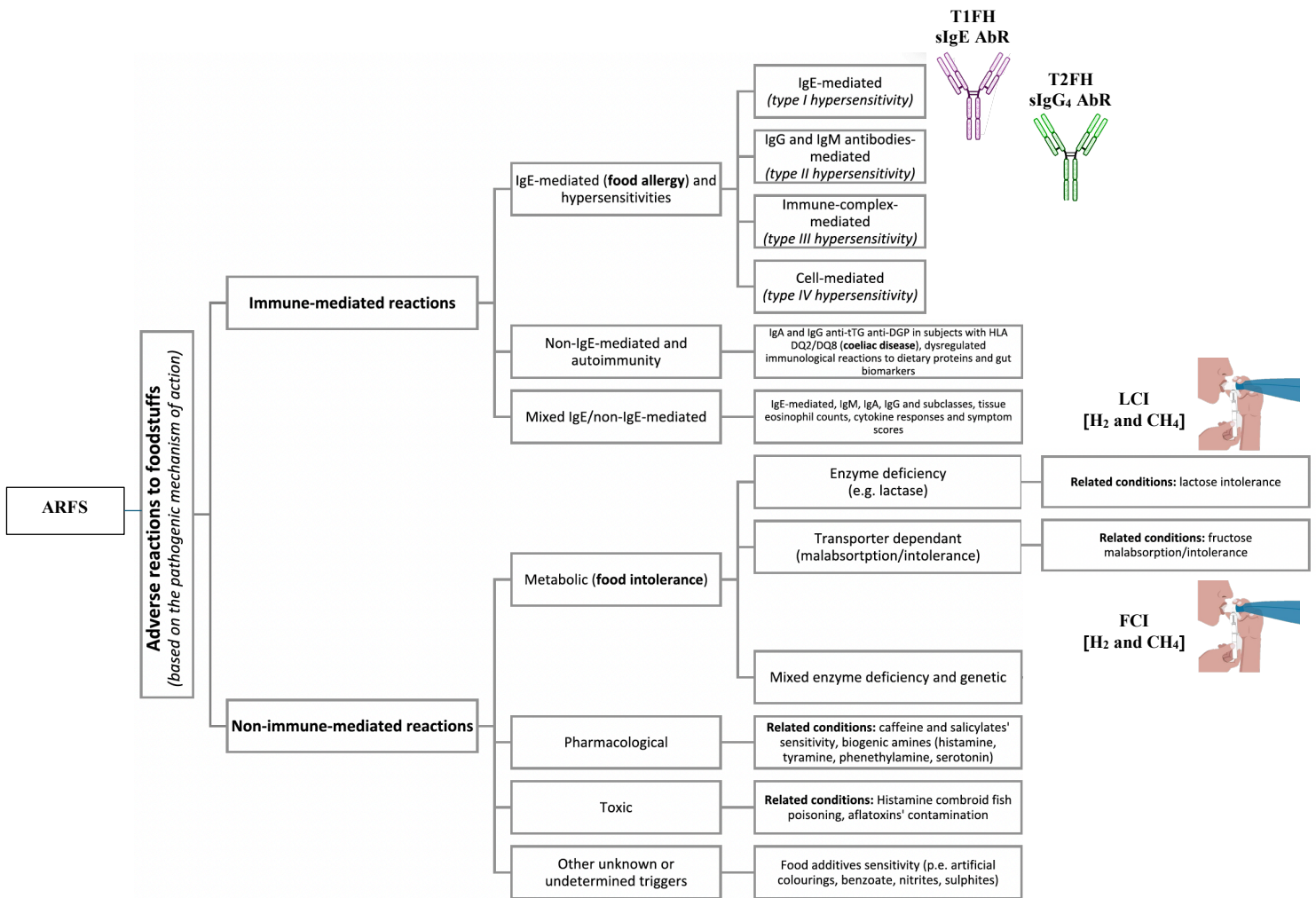


Figure 1.3.3.1 Adverse reactions to foodstuffs classification

AbR, antibody reactions; ARFS, adverse reactions to foodstuffs; CH₄, methane; FCI, fructose intolerance; H₂, hydrogen; LCI, lactose intolerance; sIgE AbR, serum Immunoglobulin E antibody reactions; sIgG₄ AbR, serum Immunoglobulin G₄ antibody reactions; T1FH, type 1 food hypersensitivity; T2FH, type 2 food hypersensitivity. Adapted from Article 3 [5].

In this study, the therapeutic significance of Immunoglobulin G₄ (IgG₄) biomarker in assessing T2FH, has been also considered. IgG₄ does not indicate immediate food reactions, but rather delayed onset in food hypersensitivity and specific symptoms in the digestive, skin, subcutaneous tissue, and nervous system [27]. Regarding its pathophysiological mechanism, mast cell degranulation can also be triggered by IgG₄. In addition to FcεRI, both humans and mice also have mast cells and basophils that possess Fcγ receptors which can attach to IgG

antibodies [28]. IgG antibodies have a complex structure and biology, with four subclasses known as IgG₁, IgG₂, IgG₃, and IgG₄ [29]. Food allergen-specific IgG₄ has been studied as a therapeutic tool to ameliorate FA-related symptoms [5,30]. Recent studies have interpreted IgG₄ as a novel biomarker because after the allergic cascade, it has the potential to form immune complexes, specially related to obesity, that may lead to inflammation, impact on physical performance, pain and weight gain [30-32].

1.3.4. Biomarkers and identification tools

Immune-mediated ARFS, such as food allergen-specific serum (s)IgE and sIgG₄ antibody reactions (AbR), have been recently used as a tool to explore T1FH and T2FH, respectively [27,33]. The sIgE and sIgG₄ AbR are typically measured in blood serum samples of individuals who exhibit specific diseases, signs, symptoms and dietary habits related to ARFS [34]. Likewise, non-immune-mediated ARFS, such as LCI and FCI, are typically determined by breath tests, using hydrogen and methane concentrations from individuals' exhaled air and following the double-blind placebo-controlled food challenges (DBPCFC) procedures. DBPCFC or oral challenges have been a widely accepted, and the *gold standard*, method for evaluating ARFS, both immune and non-immune-mediated reactions. [5]

Assessing and quantifying symptomatology and dietary habits is also crucial in diagnosing both immune and non-immune-mediated reactions [35]. sIgE and sIgG₄, as well as LCI and FCI, have been usually reported together with symptomatology and food and beverages consumption profiles. ARFS may affect different body systems (gastrointestinal (GI), skin, subcutaneous tissue and nervous system) and the consumption or avoidance of certain food groups may also impact the severity of the ARFS pathogenesis [27]. The present study evaluates the prevalence of immune-mediated and non-immune-mediated AbR of participants in contrast

with their ARFS-related symptomatology score and with their food/ beverage frequency of consumption incidence [34].

Several approaches of tools have been launched to identify food and beverages frequency consumption and symptomatology in the adult population with ARFS; however, they were initially created for evaluating just one disease or only one food and beverage allergen or component. For example, questionnaires focusing on coeliac disease (CeD), Gluten Food Frequency Questionnaire (G-FFQ); on asthma, the Global Allergy and Asthma European Network (GA2LEN) FFQ [36]. Similarly, various tools have been designed to screen general ARFS, such as the Food Allergy Questionnaire (FAQ) [37]; and the Allergy Questionnaire for Athletes (AQUA©) [38]. Thus, in order to rigorously assess ARFS population food and beverages intake and ARFS-related symptomatology, this study aimed 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*); 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, were part of the first steps to facilitate the identification of ARFS population under the umbrella of the subsequent ARFS analysis initiative through a follow-up or intervention study, the ALASKA Study.

1.3.5. Physical performance and adverse reactions to foodstuffs

There is increasing evidence of a higher prevalence of FA and digestive-related symptomatology in elite athletes. An athlete's food and beverages dietary consumption can be

influenced by intrinsic and extrinsic factors that can either ameliorate or worsen symptomatology associated to exercise-induced FA or FI. For athletes suffering from immune-mediated allergy to environmental allergens, such as pollen, exercise may exacerbate their physical and digestive condition due to increased ventilation during exercise [39]. For athletes without any related diagnosed ARFS disease, but presenting related symptomatology, exercise may worsen their physical and digestive performance due to increased metabolic inflammation, especially in the adipose tissue [40]. The time, place, and surroundings where athletes eat, as well as their well-being, on a daily basis have been identified as important factors [41]. Previous research has shown that variations in ARFS positivity can be observed in people exposed to the same food allergen at various life stages, with different food intake, exercise habits, body composition and with different symptomatology profiles [42].

ARFS encompasses a range of related aspects that can be affected by those intrinsic and extrinsic factors. Optimal cardiorespiratory fitness and other physical performance variables have been shown to have a potential positive impact on the health of adults with chronic diseases in developed countries. However, another perspective suggests that increasing exercise could potentially modify mechanisms of the immune system. These modifications may probably signify not only positive, but negative consequences in the long-term. In general, there is a lack of robust data regarding the direct relationship between ARFS and physical performance variables. The present study evaluated the prevalence of ARFS according to age, analyzed the physical performance variables according to the type of ARFS, and determined the probability of suffering any of the studied types of ARFS according to physical performance variables in a sample of Spanish adults [43].

1.3.6. **Current treatments of adverse reactions to foodstuffs**

Current oral immunotherapy (OIT) treatment options for ARFS are only United States Food and Drug Administration (FDA)-approved for certain T1FH or IgE-mediated food hypersensitivity, like peanut allergies, due to the extensive research and development of this condition [44]. However, ongoing clinical trials are investigating the use of multiple-allergen OIT as well as exploring different methods of administration such as sublingual, epicutaneous, and dietary approaches [45]. Studying new nutritional strategies and trends that ARFS population can use before and during regular exercise, training or competitions is crucial when dealing with symptoms related to ARFS. This will determine particular foods and beverages that athletes can eat to potentially reduce ARFS-related symptoms, enhance body composition and physical performance.

Present dietary treatments for T1FH or T2FH are not ideal in terms of both nutritional and physical condition aspects. The current recommended dietary treatment involves adhering to a strict elimination diet, free of allergens; otherwise, utilizing adrenaline, as well as epinephrine, for systemic reactions triggered by allergens [46,47]. Despite acceptable prognosis regarding current dietary treatments such as these elimination diets, it may have negative long-term effects on the metabolic, well-being and physical health of affected individuals. Health problems such as relevant nutritional deficiencies, muscle weakness and atrophy are on the one hand significantly related issues to treatment adherence, QoL and overall well-being of the ARFS adult population. The present study proposes a dietary intervention, the food allergen-substitutive diet (FASSD), that could prevent the need to eliminate essential nutrients, necessary for the optimal metabolic and physical well-being of ARFS population and prevent the risk of muscle dysfunction or weight gain [48].

1.3.7. Research question, hypothesis and objectives

Given recent athletes' reports on food hypersensitivity and its related symptomatology, there is a widespread inquiry about ARFS and how it may impact the physical performance and health of individuals exhibiting symptoms associated with ARFS [49]. This study seeks to investigate how ARFS, such as food hypersensitivity and FI, are related to various physical and clinical variables from a multidisciplinary perspective, what are the factors across the pathogenesis of ARFS that may influence the physical performance of ARFS population, how can they be effectively evaluated, and what are the current aspects, both affecting and improving, in ARFS Spanish population when correctly treated. Considering all the reported health benefits regarding physical exercise, while also considering the prevalent impact of immunological issues on athletes, the null hypothesis (H_0) would be: "those who have higher physical performance are more likely to experience any type of ARFS".

The main goal of this Doctoral Thesis was to evaluate ARFS population, using a multifactorial protocol and questionnaires related to the pathogenesis of T1FH, T2FH, LCI and FCI, and determine their relationship with physical performance. Additionally, to evaluate the overall health, body composition and physical performance variables at baseline and after a FASSD treatment, as part of the ALASKA Study project.

The secondary objectives were: (1) To determine the prevalence of ARFS in a preliminary study and analyze the main associated body systems and factors that may impact this prevalence in individuals with self-reported ARFS. (2) To design and validate questionnaires to investigate the diseases, signs, symptoms and the frequency of food and beverages consumption in Spanish adults with suspected ARFS. (3) To develop a robust protocol for the ARFS management in Spanish adults with different levels of physical fitness. (4) To evaluate the frequency of immune and non-immune-mediated ARFS in Spanish adults and to analyze its correlation with physical fitness. (5) To describe the effect of a FASSD

intervention on immune-mediated ARFS and on the symptomatology associated to ARFS of Spanish adults with different physical fitness levels.

1.4. Objectives

The overall perspective of the objectives of this Doctoral Thesis work encompassed all aspects of the established hypothesis. The research question was the phenomenon of ARFS pathogenesis at varying physical fitness levels; thus, the focus consisted of observing variables in a preliminary study, designing and validating specialized tools, developing a robust protocol for clinical and physical analysis, studying the ARFS and physical fitness relationship and evaluating dietary ARFS treatment effects on clinical and physical variables.

1.4.1. General objective

The main goal of this Doctoral Thesis was to evaluate ARFS population, using a multifactorial protocol and questionnaires related to the pathogenesis of T1FH, T2FH, LCI and FCI, and determine their relationship with physical performance. Additionally, to evaluate the overall health, body composition and physical performance variables at baseline and after a FASSD treatment, as part of the ALASKA Study project.

1.4.2. Specific objectives

The following specific secondary aims were considered:

1.4.2.1. Objective 1

(1) To determine the prevalence of IgG₄-mediated ARFS in a preliminary study and analyze its relationship with self-reported signs and symptoms and to identify factors that may impact this prevalence in individuals with self-reported ARFS.

1.4.2.2. Objective 2

(2) To design and validate two questionnaires to investigate the diseases, signs, symptoms and the frequency of food and beverages consumption in Spanish adults with suspected ARFS.

1.4.2.3. Objective 3

(3) To develop a robust protocol for the ARFS management in Spanish adults with different levels of physical fitness.

1.4.2.4. Objective 4

(4) To evaluate the frequency of immune and non-immune-mediated ARFS in Spanish adults and to analyze its correlation with physical fitness.

1.4.2.5. Objective 5

(5) To describe the effect of a FASSD intervention on IgE and IgG₄ AbR and on symptomatology of Spanish adults suspecting of ARFS with different physical fitness levels.

2. Materials and Methods

The general materials and methods of the overall Doctoral Thesis work is described in the following sections.

2.1. Study design

This Doctoral Thesis manuscript is based on the data obtained from the ALASKA Study. The ALASKA Study protocol was approved on the 17th of July 2020 by the Ethics Committee of the Universidad Politécnica de Madrid (reference number: 20200602); registered and accepted on the 5th of December 2022 on ClinicalTrials.gov, Clinical Trials (NCT05802017). The methodology of this study was carried out following the ALASKA Study standard protocol published on the 20th of July 2023 and described elsewhere, in the journal *Trials*, as part of this Doctoral Thesis work [5]. The main variables and instruments of this Doctoral Thesis have been described and summarized in Section 2.5.2.

This study consisted of a cross-sectional study on 254 Spanish adult volunteers (61% females) (see Figure 2.1.3.1); and a randomized controlled trial (RCT) on 101 Spanish adult volunteers (62% females) (see Figure 2.1.4.1), equal or older than 18 years of age. Subjects of study were classified in equal or under 45y ($n=135$, younger adults) and over 45y ($n=119$, older adults).

Participants of the cross-sectional study

A starting number of 295 Spanish adults, after the first month of the ALASKA Study measurements (M1 timepoint, Figure 2.2.1.1), were eligible to participate. 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 were evaluated and considered as a valid sample for the cross-sectional analysis (Figure 2.1.3.1) [43].

Participants of the randomized controlled trial

After two-hundred and fifty-four volunteers were successfully measured at the first month of the ALASKA Study, 62 subjects did not compromise to attend two more visits. Consequently, all these subjects were invited and included in the ALASKA Study intervention and were measured for five to six months (M5 or M5/6 timepoint, Figure 2.2.1.1). However, 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 timepoint. A final sample of 101 volunteers were eligible subjects for the ALASKA Study intervention analysis (Figure 2.1.4.1) [48].

Both the cross-sectional study and the RCT were carried out consecutively as part of the ALASKA Study and this current Doctoral Thesis project.

2.1.1. Geographical location of the study

The field work of this research was conducted from April 2022 to July 2024 in the facilities of the ImFINE Research Group of the Department of Health and Human Performance of the Faculty of Physical Activity and Sport Sciences (INEF) of the Universidad Politécnica de Madrid located in the Region of Madrid, Spain (Figure 2.1.1.1.).



Figure 2.1.1.1 Geographical location of the Faculty of Physical Activity and Sport Sciences of the Universidad Politécnica de Madrid

2.1.2. Intervention

The RCT of the ALASKA Study consisted of a dietary intervention, the FASSD, and the analysis of clinical and physical variables at the first month of the ALASKA Study and after five to six months of follow-up. This investigation had a matched pair trial design (established by demographics), and a 1:2 allocation ratio between the control (Group A) and the intervention group (Group B) respectively. Individuals assigned to the Group A were instructed to follow a general nutritional advice with dietary recommendations for a healthy lifestyle, while subjects assigned to the Group B were instructed to follow a personalized FASSD treatment based on each subject's food hypersensitivity results (T1FH and T2FH). In-depth information on the instructions of both dietary treatments can be found in the published ALASKA Study protocol (supplementary material of the published ALASKA Study protocol: Annexes I and II [5]). The duration of the ALASKA Study intervention for both groups, A and B, was between five to six months.

2.1.3. M1 Cross-sectional study

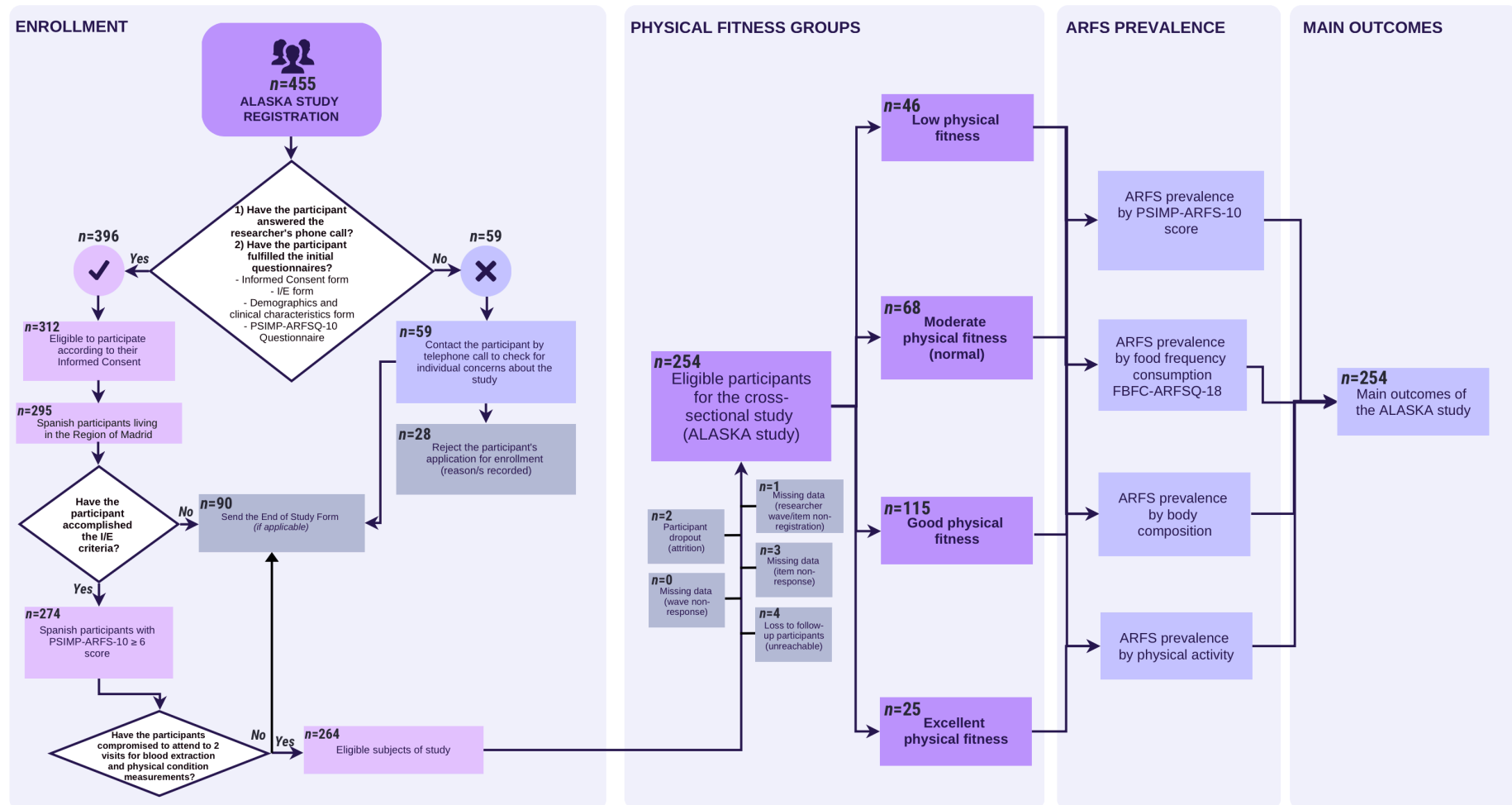


Figure 2.1.3.1 Diagram of the M1 Cross-sectional study

ARFS, adverse reactions to foodstuffs; FBFC-ARFSQ-18, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; I/E, inclusion and exclusion criteria; M1, month 1 of the ALASKA study; M5/6, month 5 or month 6 of the ALASKA study; PSIMP-ARFSQ-10, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs.

2.1.4. M5 Randomized Controlled Trial

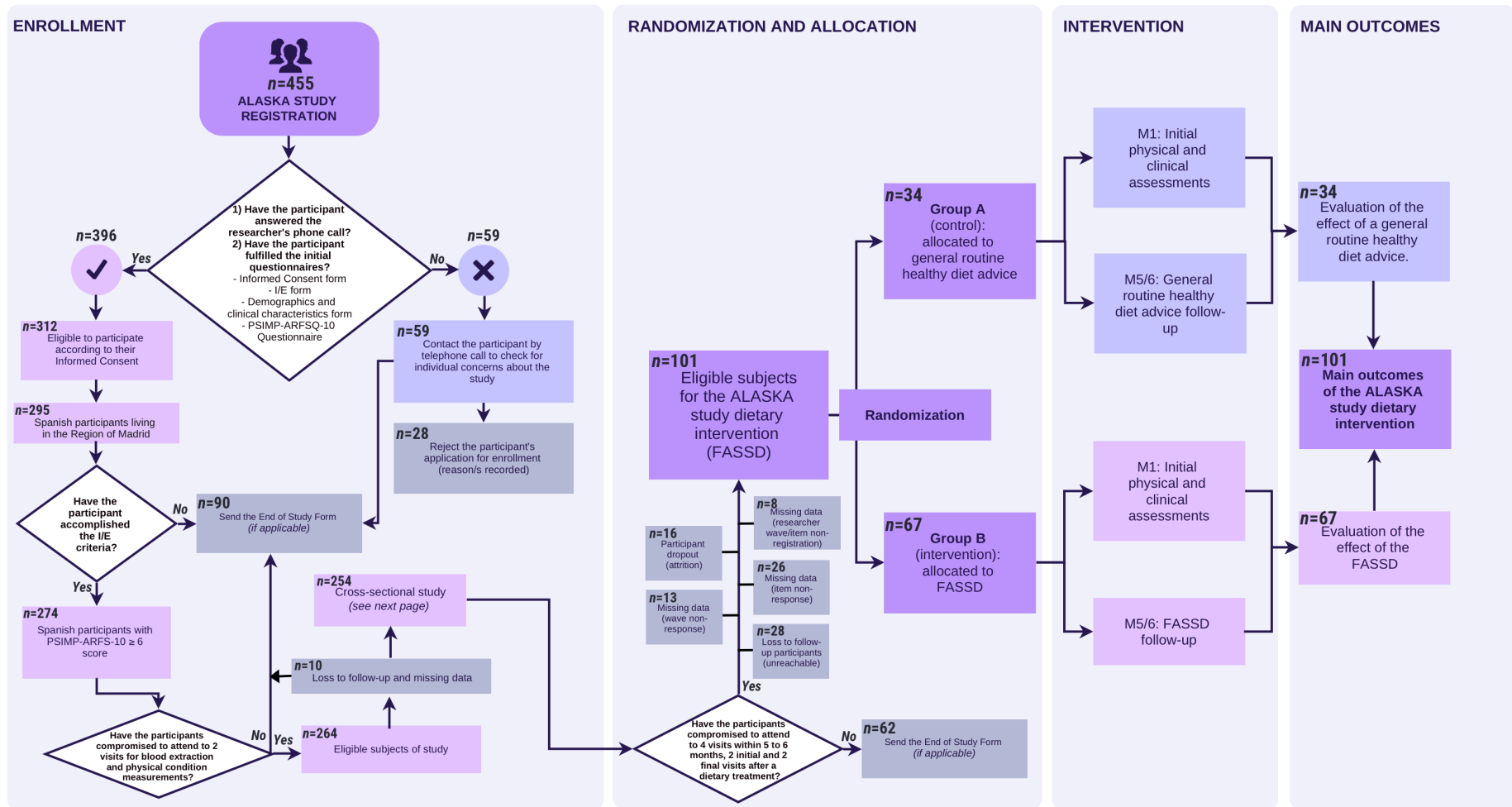


Figure 2.1.4.1 Diagram of the M5 Randomized Controlled Trial

ARFS, adverse reactions to foodstuffs; FBFC-ARFSQ-18, Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs; I/E, inclusion and exclusion criteria; M1, month 1 of the ALASKA study; M5/6, month 5 or month 6 of the ALASKA study; PSIMP-ARFSQ-10, Pathologies and Symptomatology Questionnaire associated with Adverse Reactions to Foodstuffs.

2.2. Study protocol

The long, detailed and complete version of the ALASKA Study protocol along with all forms and questionnaires, can be found in Article 3 [5]. A schematic representation of the timeframe of the ALASKA Study is outlined in Figure 2.2.1.1 to aid in understanding the overall study. "Mx" where x is a numeric value, stands for the month number in the study (e.g. M1: month 1 of the ALASKA Study). A and B were the two face-to-face meetings that were scheduled within the same month.

2.2.1. Timeline of the ALASKA Study intervention

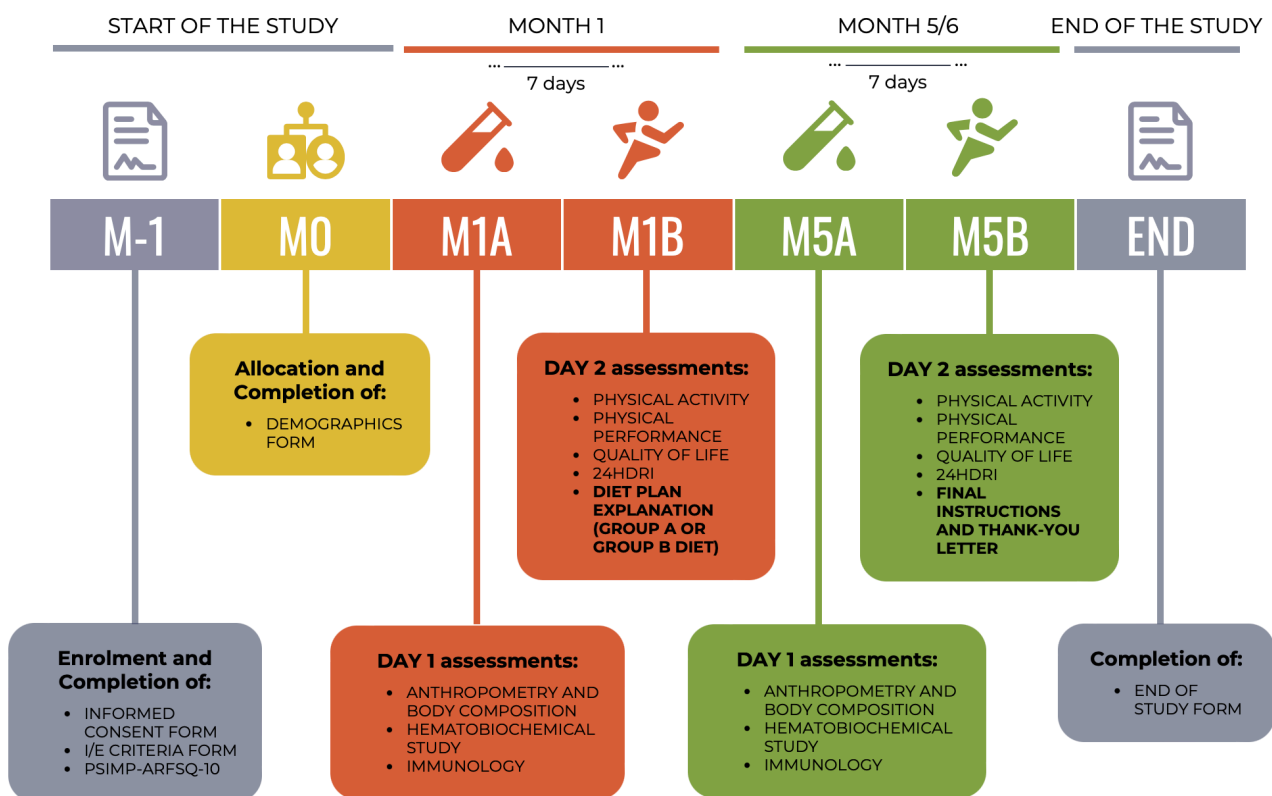


Figure 2.2.1.1 Timeline of the ALASKA Study intervention

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.

Individuals from the Madrid metropolitan area and nearby areas who agreed to take part in this research by signing the informed consent form and any other required paperwork, were asked to attend two in-person visits at the Biochemistry Laboratory of the Faculty of Physical Activity and Sport Sciences of the Universidad Politécnica de Madrid (M1 timepoint). Following approximately five to six months, participants were requested to once again attend two in-person visits of equal characteristics (M5 timepoint). Overall, participants needed to come to two face-to-face appointments in the first month and two more in-person visits at M5/6.

Preliminary online questionnaires, using RedCap® platform, were completed before the study commencement. All online questionnaires and forms were carefully explained and described in the published ALASKA Study protocol [5]. Informed consent, demographics, inclusion and exclusion criteria and symptomatology forms were completed during a preliminary phase (M-1 and M0) before the study commencement.

The first visit at M1 (M1A) consisted of checking blood pressure, fasting blood sample collection (hematobiochemical and immunology studies), taking anthropometric measurements, and assessing body composition. The collection of blood samples was carried out by a qualified and licensed nurse who was employed by a private subcontracted company (Synlab Group) specifically for this project. During the second visit at M1 (M1B), physical performance measurements were assessed (body balance, leg power, sit-to-stand speed, resting heart rate, handgrip strength and cardiorespiratory fitness). Physical measurements were conducted by trained personnel in the field of Physical Activity and Sport Sciences. Physical activity and QoL questionnaires were completed and the 24-hour dietary recall interview (24HDRI) was performed by a trained professional in the field of nutrition. All measurements were performed by specialized professionals in their respective

fields of expertise and all the characteristics of the physical and clinical measurements are detailed in Section 2.5 of this document.

2.3. Inclusion and exclusion criteria

Participants aged equal or over 18 years 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 (less than 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 or binge eating), were not currently adopting a special diet (for FA, FI, CeD, or any other food hypersensitivity), had no medical restrictions on 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 equal or over 6. The validated PSIMP-ARFSQ-10 tool was employed to assess diseases, signs, and symptoms associated with ARFS [34]. Additional information regarding exclusion and inclusion criteria can be found in the published ALASKA Study protocol [5].

2.4. Recruitment and data storage

Volunteers with signs, symptoms, and/or diagnosed diseases related to ARFS and equal or older than 18 years, including men and women, were recruited. The recruitment period was active for approximately 1 to 3 months and according to the enrollment response. Around 90 to 120 initial email requests were received each month to join the ALASKA Study, not including participants who were later excluded for different reasons (answering the initial researcher-participant phone call, completing the initial received questionnaires and informed consent and

accomplishing the required inclusion and exclusion criteria). Enrollment mass e-mail campaigns, social media, digital display screens in public areas of the Universidad Politécnica de Madrid, and the local online contact flyer/website (<https://short.upm.es/kwgpo>) of the ImFINE Research Group were used to recruit interested Spanish adults to participate in the ALASKA Study.

The first approach with possible participants was carried out with an initial one-to-one informative phone call conversation between the researcher and the potential participant to share a wider vision about the characteristics of the study. Engaged participants were registered in the study and an anonymous alphanumeric unique participant code was assigned to these participants (the format of the alphanumeric participant code was ALWXYZ, where the two static initial letters were pointing to the study name, ALASKA Study, and the next four random-digits was a unique numeric code). Complete records of each participant were stored, using the unique mentioned participant alphanumeric code, in the Research Electronic Data Capture (RedCap®) platform locally hosted in the Supercomputing and Visualizing Center of Madrid (CESVIMA), belonging to the Universidad Politécnica de Madrid. Digital databases of each assessment and instrument were created promoting the data quality and avoiding the need of duplicating questionnaire measurements. Databases were locked using a secure password and with access only to the coordinators and principal investigator (PI) of the ALASKA Study (Doctoral Thesis Director and PI of the study, Prof. Dr. Marcela González-Gross, Doctoral Thesis Co-director and coordinator of the study, Dr. Eva Gesteiro Alejos and Doctoral Thesis researcher and coordinator of the study, Lisset Pantoja Arévalo, MSc). Additional researchers, after the authorization of the PI, had also limited-blind access to the RedCap® profile of the study (Doctoral Thesis support researcher in statistical analysis, Dr. Carlos Quesada, and Doctoral Thesis Laboratory Technician support, LT. Ana Navarro Sánchez, BSc).

2.5. Physical, clinical assessments and questionnaires

During the ALASKA Study, ten main assessments outlined in Figure 2.5.1.1 were conducted. Demographics, symptomatology scores, hematobiochemical study, immunology, enzymatic activity, anthropometry, body composition, physical performance, QoL and the FASSD treatment, as the ALASKA Study intervention, were carried out during this research.

2.5.1. Main assessments of the ALASKA Study

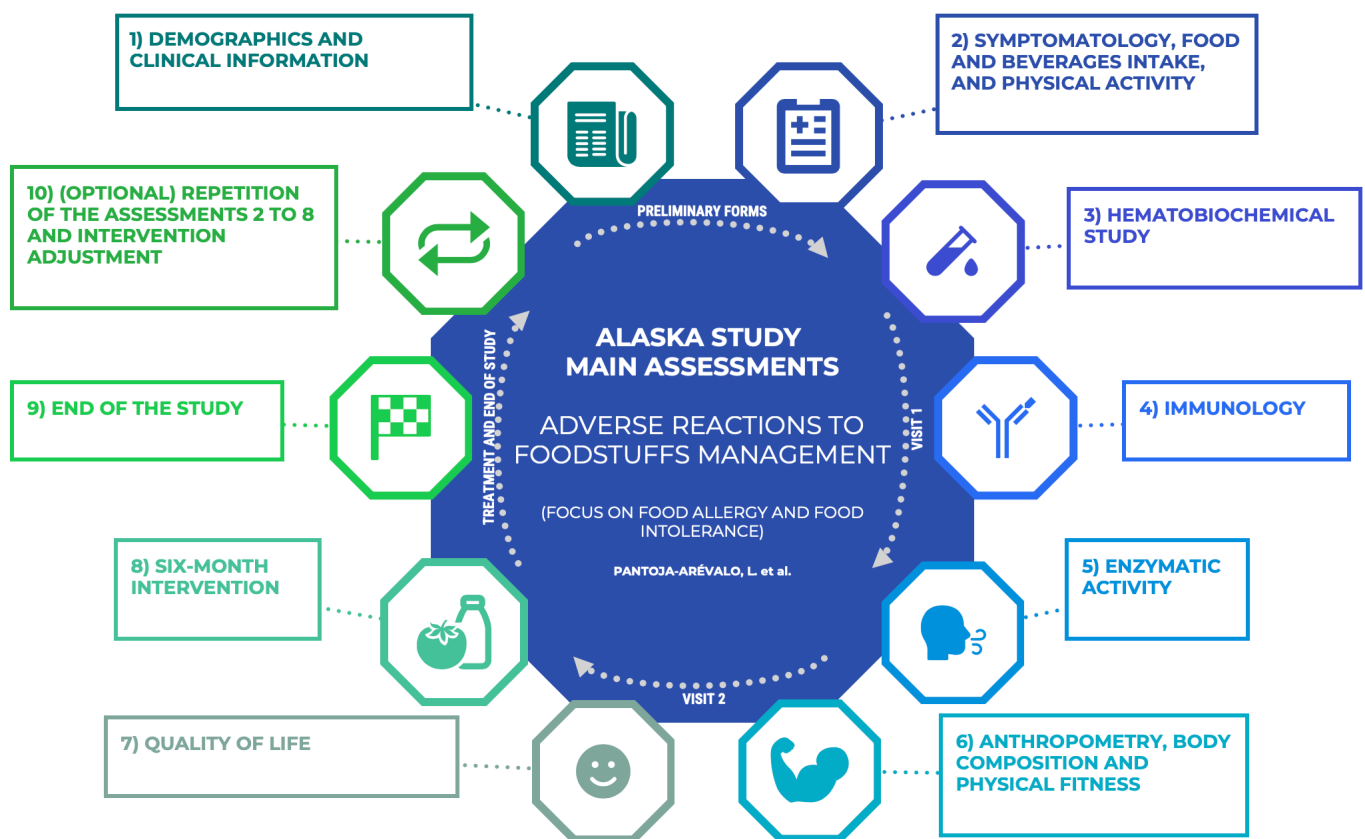


Figure 2.5.1.1 Main assessments of the ALASKA Study protocol

Sourced from Article 3 [5].

2.5.2. Variables and instruments of the ALASKA Study

A detailed description of the ALASKA Study's main variables and instruments can be found in the published ALASKA Study protocol of Article 3 [5]. However, a summarized description of the measured variables in this Doctoral Thesis field work is presented in the following Table 2.5.2.1.

Table 2.5.2.1 Summary of the variables and instruments of the ALASKA Study

	Timepoint when assessed	Variables/ Assessments	Questionnaires/ Instruments	References
Demographics	M0	Date of birth, age, sex, level of education, employment situation, occupation, living situation (number of people living together) and home city	Demographics form	See Annex B
Anthropometry and Body Composition	M1A, M5A	Height (cm)	Stadiometer	(Seca GmbH & Co. KG., model 213 1721009, Hamburg, Germany)
	M1B, M5B	Waist-to-hip ratio (ISAK criteria)	Anthropometric tape	(Cescorf Ltd., 2-meter Innovare measuring tape, Porto Alegre City, Brazil)
	M1A, M5A	Weight (kg) and BIA with segmental multifrequency analysis	BIA bascule	(Tanita, model MC-780MA-N, Tokyo, Japan)
	M1A, M5A	Body mass index (kg/m ²)	Height and weight instruments	[50,51]

	Timepoint when assessed	Variables/ Assessments	Questionnaires/ Instruments	References
Physical performance	M1A, M5A	Resting heart rate	Tensiometer	(Omron Healthcare Co. Ltd., model M3 Comfort HEM-7155-E(C), Kyoto, Japan)
	M1B, M5B	Handgrip strength (hand/ upper extremity strength)	Dynamometer	(Takei, model T.K.K. 5401 Grip-D, Tokyo, Japan)
	M1B, M5B	Power strength (leg/ lower extremity strength)	Force platform	(Tanita, model BM-220 7341(0), Tokyo, Japan)
		Sit-to-stand speed		
		Body balance		
	M1B, M5B	Estimated VO ₂ max	Åstrand-Ryhming Step Test	[52]
		Pulse rate	Pulse oximeter	(GE Healthcare, Ohmeda Tuffsat, Boston, Massachusetts, USA)
		Pace/cycles	Metronome	(KORG Inc., model MA-1, Tokyo, Japan)
Physical Activity	M1B, M5B	Physical activity habits and WHO accomplishment	IPAQ-LF	[53,54]
			RAPA	[55]
Health	M-1, M5A	Diseases, signs and symptoms (PSIMP-ARFSQ-10 score)	PSIMP-ARFSQ-10	[5]
	M1A, M5A	Hematobiochemical study	Complete blood count	(Beckman Coulter, Inc., UniCel DxH 800 Coulter Cellular Analysis System, Brea, CA, USA)
			Biochemical composition	(Beckman Coulter Diagnostics Inc., Beckman Coulter® AU5800, Brea, California, USA)
			Vitamin profile	(Roche Diagnostics Ltd., Cobas e411, Rotkreuz, Switzerland)

	Timepoint when assessed	Variables/ Assessments	Questionnaires/ Instruments	References	Timepoint when assessed
Nutrition	M1A, M5A	Immunology	T1FH, food allergen-specific sIgE AbR T2FH, food allergen-specific sIgG ₄ AbR	In-line immunoassay analyzer	(Aesku.Diagnostics GmbH, Helia® Helmed, Wendelsheim, Germany)
	M1A	Enzymatic activity and malabsorption	Lactose intolerance Fructose intolerance	Lactose/ fructose breath test kits	(Cerascreen GmbH, Schwerin, Germany)
	M1B	Dietary intervention	Group A diet Group B diet	General nutritional advice healthy diet FASSD	[5]
	M1B, M5B	Food and beverages record		24-h Dietary Recall Interview (24HDRI)	
	M2, M3, M4	Dietary adherence		Dietary Adherence Questionnaire (DAQ)	
Quality of Life (QoL)	M1B, M5B	Food and beverages frequency consumption		FBFC-ARFSQ-18	[34]
	M1B, M5B	Health-related QoL		12-Item Short Form Health Survey (SF-12)	[56]
	M1B, M5B	FA-related QoL		Food Allergy Quality of Life Questionnaire-Adult Form (S-FAQLQ-AF)	[57]

AbR, antibody reactions; DAQ, Dietary adherence questionnaire; BIA, bioelectrical impedance analysis; FA, Food allergy; FASSD, Food Allergen-specific substitutive diet; FBFC-ARFSQ-18, food and beverages frequency consumption questionnaire to identify adverse reactions to foodstuffs; IPAQ-LF, The International Physical Activity Questionnaire Long Form; ISAK, International Society for the Advancement of Kinanthropometry; 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; 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; VO₂ max, maximal oxygen consumption; WHO, World Health Organization; 24HDRI, 24-h Dietary Recall Interview.

2.5.2.1. Forms and questionnaires

All subjects of the ALASKA Study completed the forms and questionnaires listed in Table 2.5.2.1. using an online format through RedCap® platform. The ALASKA Study RedCap® profile was created after uploading all the project's characteristics, such as the Ethics Committee information and the Clinical Trials registration number by the PI of this project. The questionnaires and forms for the performance of the ALASKA Study protocol were also added to the ALASKA Study RedCap® online profile. All questionnaires and forms used for this study were either validated beforehand or validated specifically for this Doctoral Thesis project. Two specific questionnaires were validated for the purposes of this Doctoral Thesis, before the ALASKA Study data collection, and as a part of this Doctoral Thesis project (Article 2) [34]: the food and beverages frequency consumption questionnaire to identify adverse reactions to foodstuffs (FBFC-ARFSQ-18); and the pathologies and symptomatology questionnaire associated with adverse reactions to foodstuffs (PSIMP-ARFSQ-10). Both questionnaires were essential for assessing the ARFS population, particularly for examining their food and beverages consumption and evaluating their symptoms.

Before the beginning of the ALASKA Study (M(-1)), informed consent form, inclusion/exclusion criteria and the PSIMP-ARFSQ-10 were requested to all the registered subjects. At the beginning of the ALASKA Study (M0) (Figure 2.2.1.1), demographic information was gathered from all participants who met the specified criteria for inclusion and exclusion and all who have signed and agreed to the informed consent form (see Annexes A, B and C of this manuscript, for the informed consent form, demographics form and inclusion/exclusion criteria forms). Demographics form included, sex, age, education level, job and living situation. The PSIMP-ARFSQ-10 questionnaire was employed to determine diseases, signs, and symptoms associated with ARFS and to calculate, using a minimum PSIMP-ARFSQ-10

score of 6, if a participant was eligible to be included in the ALASKA Study [34]. The self-perceived health-related QoL (HRQoL) of the subjects of study were assessed using the validated questionnaires: The Short-Form Health Survey 12 (SF-12) [58] and the Food Allergy Quality of Life Questionnaire - Adult Form (FAQLQ-AF) [57].

Customized schedules were arranged in the ALASKA Study RedCap profile to ensure timely delivery of corresponding adequate questionnaires to each participant based on their enrollment date (Figure 2.5.2.1.1).

Data Collection Instrument	Mes -1 Fase preliminar (M_-1) (1)	Mes 1 Fase inicial (M_1) (2)	Mes 2 (M_2) (3)	Mes 3 Fase trimestre (M_3) (4)	Mes 4 (M_4) (5)	Mes 5 Fase semestre (M_5) (6)	Realizado por el investigador (7)
Consentimiento Informado (CI) (survey)	✓						
Datos Personales (DP) e Historia Clínica (HC) (survey)	✓						
Criterios de Inclusión/ Exclusión (I/E) (survey)	✓						
Cuestionario de Patologías y Sintomatología asociadas a Reacciones Adversas a Alimentos, PSIMP-RAA-10 (survey)		✓				✓	
Cuestionario de Toma de Muestra Sanguínea (BSQ) (survey)		✓				✓	
Cuestionario de Frecuencia de Consumo de Alimentos y Bebidas para identificar Reacciones Adversas de origen Alimentario, CFCAB-RAA-18 (survey)		✓				✓	
Cuestionario Internacional De Actividad Física (IPAQ) (survey)		✓				✓	
Valoración del Nivel y la Intensidad de la Actividad Física (RAPA) (survey)		✓				✓	
Calidad de vida (SF-12) (survey)		✓				✓	
Calidad de Vida en Alergia y/o Intolerancia a Alimentos - Versión para adultos (FAQLQ-AF) (survey)		✓				✓	
Cuestionario de adherencia a la dieta sustitutiva (CADS) (survey)			✓	✓	✓	✓	
Cuestionario De Hidratación (CH) - version ALASKA study (survey)						✓	
Formulario de Eventos Adversos (survey)							✓
Formulario de Fin de Estudio (survey)							✓

Figure 2.5.2.1.1 Screen capture of the personalized schedule set up in the ALASKA Study RedCap profile for each study participant
Presented in the original language of the ALASKA Study RedCap® profile (Spanish).

2.5.2.2. Clinical assessments

Blood collection procedure

Blood collection was performed by a nursing health professional in M1A and M5A timepoints. All participants were asked to attend in fasting conditions for both the M1A and M5A visits. Before the blood sampling, subjects answered the BSQ questionnaire to assess their pre-analytical conditions. Three 8.5-ml serum (Vacutainer® BD serum-separating tube SST™ II Advance, REF368968) (BD Becton, Dickinson and Company NYSE: BDX, Sunnyvale, CA, USA), two 4-ml lithium heparin LH Vacutainer® BD tube, REF368884) (BDX, Sunnyvale, CA, USA); and two 3-ml dipotassium ethylene diamine tetraacetic acid, K2-EDTA-containing blood sample tubes (Vacutainer® BD K2-EDTA tube, REF367838) (BD Becton, Dickinson and Company NYSE: BDX, Sunnyvale, CA, USA) were extracted from the median cubital and cephalic veins for biochemistry, immunology and hematology analysis, respectively. Thirty minutes before the blood collection, all the clinical analysis equipment was calibrated beforehand.

After the blood collection, one serum tube and both heparin lithium-containing tubes from each participant were centrifuged at 3000 RPM for 15 minutes (Eppendorf AG, 5702R Centrifuge, Hamburg, Germany). Besides both K2-EDTA-containing blood sample tubes were stirred in a blood tube mixer (Boule AG, Boule Mixer 820, Spånga, Sweden). The two remaining tubes with serum and whole blood, respectively, were anonymized and sent to Synlab Group for blinded hemogram and biochemical studies. Similarly, one aliquot sample of 1ml of serum was sent to the Biochemical Laboratory of the Spanish Agency for Health Protection in Sports (AEPSAD); while one aliquot sample of 0.5ml of serum and one aliquot sample of 0.5ml of lithium heparin were both sent to AESKU Group facilities for blinded immunological analysis.

Samples storage

Two 1-ml aliquots of total blood samples with K2-EDTA, four 0.5-ml aliquots of serum samples and two 0.5-ml aliquots of plasma containing lithium heparin anticoagulant were saved, from participants who gave their consent to use their samples for further analysis, and storage at -80°C (ultrafreezing conditions) (Thermo Fisher Scientific, Ultrafreezer Model 906, CA, USA) for further analysis. Colored 2-ml microtubes were used for the ultrafreezing storage of 0.5ml aliquots of serum (yellow, REF05-408–140), 0.5ml aliquots of plasma (green, REF05-408–142) and 1ml aliquots of whole blood (red, REF05-408–139) (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

Local blood bank registry book was constructed and written and the following characteristics of the aliquots were saved: project name, investigator(s), principal investigator, location, freezer temperature, number or name of freezer, location of the cryogenic box inside of the freezer, aliquot code, number of cryogenic box, milliliters of the aliquots and date of storage. A blood bank registry sheet was developed for the purposes of this research (Annex D).

Hematobiochemical study

Complete blood cell count (CBC) was measured in all the study subjects, as a technique to measure the general health status of individuals. Hemogram testing was performed to obtain the CBC of all subjects of this study at the first and the fifth month of the ALASKA Study (M1A and M5A timepoints). Each subject's hemogram was obtained using the Beckman Coulter® UniCel DxH 800 Hematology analyzer (Beckman Coulter® Cellular Analysis System, Brea, CA, USA). This cell counter combined hardware technology, computer algorithms, impedance, flow cell volume, conductivity, and five light scatter measurements to analyze platelets (PLTs), white blood cells (WBCs), and nucleated red blood cells (NRBCs).

The repeatability of all measured biomarkers was good with coefficient of variation of 0.91 to 1.23% from the mean of standard values within the acceptable normal range [59].

The biochemical analysis was carried out, as well at M1A and M5A timepoints, through the spectrophotometric, enzymatic and ion-selective electrodes (ISE) techniques, using a Beckman Coulter® AU5800 (Beckman Coulter® Diagnostics Inc., Brea, California, USA). Parameters measured by spectrophotometry were glucose, cholesterol (total, LDLc and HDLc), albumin, urate, creatinine, urea, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase, creatine kinase (CK), iron, calcium; triglycerides were determined by the enzymatic technique; and sodium, potassium and chloride by ISE. All measured biomarkers showed good repeatability, with a coefficient of variation ranging from 0.60 to 2.60% from the mean of standard values within the acceptable normal range [60].

The Roche Cobas e411 analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland) was used to measure lactate dehydrogenase, magnesium, amylase, lipase, vitamin B12, folic acid and vitamin D by electrochemiluminescence (ECLIA) technique. The Cobas e411 analyzer showed good repeatability with values around 1.79% for the coefficient of variation from the mean of standard values within the acceptable normal range [61].

Immunology

Immune-mediated reactions, specifically T1FH and T2FH were measured in all participants at both timepoints of the ALASKA Study intervention, M1A and M5A. Quantitative food allergen-specific sIgE, for the determination of T1FH, and sIgG4 AbR, for the identification of T2FH, were determined and reported in kUA/l. Ready-to-use AESKUBLOTS® kits were performed using the immunoblot multiplex technology of the Helia® Helmed Line Immunoassay analyzer (Aesku.Diagnostics GmbH, Wendelsheim,

Germany). Positive Ab reactions were considered as ≥ 3.5 kUA/l. sIgE and sIgG₄ AbR measured biomarkers showed good repeatability coefficient of variation ranging from 0.81 to 1.10% from the mean of each participants' own values.

Food allergen-specific reactions were determined against a panel of 61 food antigens (almond, amaranth, anacard, apple, baker's yeast, banana, barley, black pepper, broccoli, buckwheat, cabbage, carrot, celery, chicken, cocoa, cod, coffee, corn, cow, cow's milk, egg white, egg yolk, garlic, ginger, goat's milk, grape, green beans, green pea, hazelnut, kiwi, lamb, leek, lemon, mango, mustard, nut, oat, octopus, onion, orange, peach, peanut, pineapple, pork, potato, quinoa, rice, rye, salmon, sesame, sheep's milk, shrimp, soybean, spelt, squid, strawberry, tomato, trout, tuna, turkey, wheat) and organized in 12 main food groups (egg, cereal, fish, fruit, gluten-free cereal, legume, meat, milk, nuts, seeds, shellfish and mollusks, and vegetables).

Enzymatic activity and malabsorption

Non-immune-mediated reactions, particularly LCI and FCI, were assessed in subjects of the ALASKA Study at M1 timepoint. Breath tests involving the determination of hydrogen and methane concentrations were measured in participant's exhaled air for the assessment of LCI and FCI. Ready-to-use Cerascreen® H₂/ CH₄ breath test kits were used for the collection of the exhaled air samples (Cerascreen® GmbH, Schwerin, Germany) and hydrogen and methane concentrations in these breath samples were quantified using the BreathTraker® gas breath analyzer (QuinTron Instrument Company Inc., Milwaukee, WI, USA).

Two breath test Cerascreen® kits, one with lactose substrate and another with fructose substrate were delivered to selected participants at M1A timepoint. Face-to-face explanations were carried out to facilitate participants' comprehension on the performance of the kits. An explanatory video was also published for the purposes of this research [62] (see "Additional

File 1 – Annex VI: Breath test instructions” of the ALASKA Study protocol for further detail on the breath test instructions). Participants were observed during this M1A first visit of the ALASKA Study and were carefully selected to take the breath test according to their commitment and PSIMP-ARFSQ-10 score. This selection was made because of the price and stock limitations (after the COVID-19 pandemic, there were several glass and breathing-related material manufacture companies with discontinued references; QuinTron Instrument Company Inc. had the glass-made breath collecting tubes discontinued by that time).

2.5.2.3. Physical assessments

Standard and normative methods were followed for all the described physical evaluations of Table 2.5.2.1. Standardized procedures of the International Society for the Advancement of Kinanthropometry (ISAK) were followed for quantifying the described anthropometric assessments (height, waist-to-hip ratio, weight, and body mass index). Body composition was performed in fasting conditions using multi-frequency segmental bioelectrical impedance analysis (BIA) using the MC-780MA-N Tanita device (Tanita, Tokyo, Japan) 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 [5,63]. BMI was evaluated according to the classification used by the United States National Institute of Health (NIH) and the WHO [51]; where BMI was described like a health indicator as underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), class I obesity (30.0-34.9) and class II obesity (35.0-39.9). Physical activity was arranged in accordance with the WHO 2020 guidelines on physical activity and sedentary behavior [64].

The physical performance-related variables considered in this research were resting heart rate, hand grip strength, leg power, sit-to-stand speed, body balance and cardiorespiratory fitness. The Tanita BM-220 force platform together with the Zaritz software (Tanita, Tokyo,

Japan) were used for the assessment of body balance, leg power, and sit-to-stand speed. Hand grip strength was measured to evaluate muscle strength, and upper extremity function using a digital 5401 Grip-D dynamometer (Takei Scientific Instruments Co. Ltd, Tokyo, Japan). Before the performance of the test, the dynamometer regulation was set at 3.5 and 4 centimeters between the phalanges according to the participant's hand size. Handgrip strength was performed in both hands, right and left, while standing (anthropometric neutral position). Subjects were instructed to rest for 30 seconds between each hand measurement, and the highest values were recorded after duplicate testing. Cardiorespiratory fitness was determined by estimating the maximal oxygen consumption ($\text{VO}_{2\text{max}}$) using the Åstrand-Ryhming Step Test (stÅ-R) [65,66]. $\text{VO}_{2\text{max}}$ values were estimated according to Å-R's nomograms by the determination of submaximal heart rate values using a professional oximeter device (General Electric, GE, Ohmeda Tuffsat) (GE Healthcare, Boston, Massachusetts, USA). Submaximal heart rate was recorded every minute during a total test time of 5 minutes. Final submaximal heart rate was recorded as the maximal value during the last 30 seconds of the submaximal fifth minute. For the final reading of the estimated $\text{VO}_{2\text{max}}$, values were adjusted according to the Å-R's nomograms, the participant's final submaximal heart rate, sex, age and weight [65]. $\text{VO}_{2\text{max}}$ values were predicted by age and sex using the international guidelines published by the American Heart Association for the evaluation of cardiorespiratory fitness in clinical practice of the adult population [67,68].

2.6. Ethical considerations

This research was performed in accordance with the Ethical Guidelines of the Declaration of Helsinki of 1964, revised in Fortaleza (2013) (current in force Declaration of Helsinki at the time of the ALASKA Study project duration) [69] and following the Spanish and European regulations on data protection [70]. The study protocol was approved by the

Ethics Committee of the Universidad Politécnica de Madrid (REF.20200602); and registered on ClinicalTrials.gov, Clinical Trials (NCT05802017). A signed informed consent model was obtained from all subjects of study using the ALASKA Study Informed Consent form uploaded and available to be digitally signed in RedCap® 14.0.27 platform.

AESKUBLOTS® kits were manufactured and blind-processed by Aesku.Diagnostics GmbH (Aesku.Diagnostics GmbH, Wendelsheim, Germany). However, the design, management, statistical analysis, and reporting of the study have been performed entirely independent of Aesku.Diagnostics GmbH.

This research work was conducted in the ImFINE Research Group facilities and the Biochemistry Laboratory of the Faculty of Physical Activity and Sport Sciences of the Universidad Politécnica de Madrid. Industries and/or private institutions had no role in the design, execution, data analyses, interpretation of the data, or decision to submit these results.

2.7. Statistical analysis

Statistical analysis was performed using IBM-SPSS® Statistics software v.25.0. Descriptive statistics were assessed as mean $\pm$ standard deviation (SD) for continuous variables, and as frequency with percentage for categorical variables. Subjects were described and evaluated by sex (male / female or men / woman, according to each journal article guidelines) and age (young adults, ≤ 45 y) and older adults (older adults, >45 y) groups. Cardiorespiratory fitness was classified into four groups according to the 95% of the good age-sex-predicted VO₂max value below which there is risk of developing major health problems (mainly cardiovascular diseases, diabetes and/or hypertension) [71]. The following physical fitness categories were considered: low cardiorespiratory fitness (under 85% of the predicted good VO₂max), normal cardiorespiratory fitness (85-95% of the predicted good VO₂max), good cardiorespiratory fitness (96-119% of the predicted good VO₂max), and excellent

cardiorespiratory fitness (equal or over 120% of the predicted good VO₂max) [71]. Age-sex adjustments were applied to all physical performance variables. Median cut off values of maximal hand grip strength and cardiorespiratory fitness were determined to classify the sample into low and high physical performance groups and to create the dichotomic datasets of physical performance 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 (η^2) was used as an indicator of effect size and η^2 was categorized as small effect sizes (≥ 0.01 to < 0.06), medium effect sizes (≥ 0.06 to < 0.14), and large effect sizes (≥ 0.14) [72].

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 population value, in terms of SD). The mean scores for PCS-12 and MCS-12 were both rated at 50 points. The SD was 10 points on average. Each deviation of 10 points above or below 50 corresponded to one SD 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 dichotomic datasets of physical performance variables. Odds ratios (OR) were used as measurement of association and probability model. Relative risk, also called relative risk ratios, were calculated using the dichotomic datasets of T1FH and T2FH to identify the relative risk factors in developing T1FH and T2FH under the FASSD intervention group against the control group. Relative risk ratios were computed as a measurement of association and as a random-effects risk model. Statistical significance was set at 0.05.

3. Results

3.1. Preliminary study

3.1.1. Sample characteristics of the preliminary study

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 were analyzed, 143 women and 62 men with a mean ($\pm$ SD) age of 45.5 years old ($\pm$ 14.9y, from 20 to 79y).

Descriptive data from the sample are shown in Table 3.1.1.1. More than half of the participants were over normal weight (56.59%) and did not meet the WHO recommendations for physical activity (52.7%).

Table 3.1.1.1 Sample characteristics

Assessment	Mean $\pm$ SD or %			
	Total (n=205)	Men (n=62)	Women (n=143)	Min-Max
Age	45.46 $\pm$ 14.91	47.45 $\pm$ 13.27	44.59 $\pm$ 15.53	20-79
Body composition				
Height (m)	1.67 $\pm$ 0.09	1.76 $\pm$ 0.06	1.63 $\pm$ 0.07	1.50-1.92
Weight (kg)	73.39 $\pm$ 16.12	82.92 $\pm$ 15.63	69.26 $\pm$ 14.54	42.30-130.60
Fat free mass (kg)	47.72 $\pm$ 10.12	52.93 $\pm$ 10.65	45.46 $\pm$ 9.03	30.71-74.75
Muscular mass (%)	59.28 $\pm$ 12.20	56.96 $\pm$ 13.29	60.28 $\pm$ 11.60	26.91-97.12
Bone mass (kg)	2.77 $\pm$ 0.76	3.08 $\pm$ 1.23	2.63 $\pm$ 0.34	1.90-12.11
Fat mass (%)	33.17 $\pm$ 9.07	32.46 $\pm$ 9.43	33.47 $\pm$ 8.92	10.80-60.30
Body mass index	26.32 $\pm$ 4.99	28.05 $\pm$ 4.93	25.56 $\pm$ 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	--

(Table 3.1.1.1 continues)		Mean $\pm$ SD or %		
Assessment	Total (n=205)	Men (n=62)	Women (n=143)	Min-Max
Physical activity (IPAQ-SF)				
Low (%)	87.30	95.20	83.90	--
Moderate (%)	12.70	4.80	16.10	--
High (%)	0	0	0	--
Sitting time (min/week)	2577.37 $\pm$ 943.82	2499.68 $\pm$ 978.15	2611.05 $\pm$ 930.03	1260-5040
Sitting time (h/week)	42.96 $\pm$ 15.73	41.66 $\pm$ 16.30	43.52 $\pm$ 15.50	21-84
Symptomatology				
Dermatological	43.27	37.40	45.50	--
Digestive or GI	40.74	47.90	38.60	--
NS	33.33	52.40	34.50	--

IPAQ-SF, The International Physical Activity Questionnaire-Short Form; GI, Gastrointestinal; NS, Nervous System.

3.1.2. 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%), GI (40.74%) and those related to NS (33.33%) problems. When comparing these signs and symptoms by sex (Table 3.1.1.1); 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); and after that, 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 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%) ($\chi^2 (4) = 41.11$; $p < 0.001$; $\eta^2 = 0.93$). 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 significantly higher frequency of subjects who reported signs and symptoms of the subcategory of abdominal bloating and/or abdominal pain (18%) in overweight than in subjects of normal weight (15.4%) ($\chi^2 (4) = 14$; $p = 0.007$; $\eta^2 = 0.58$). Furthermore, there was 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%) ($\chi^2 (5) = 24.49$; $p < 0.001$; $\eta^2 = 0.72$).

Regarding the different reported ‘number of signs and symptoms’ in men and women, there was 22% of women who reported a mean of four self-reported signs and symptoms versus 14.5% of men. There was 21% of men who 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 ($\chi^2 (5) = 17.40$; $p = 0.004$; $\eta^2 = 0.17$).

3.1.3. Food allergen-specific immunoglobulin G₄ antibody reactions

The most prevalent food-specific IgG₄ AbR is shown in Figure 3.1.3.1. The positive population response ($\geq 50\%$, equal or greater than 50% of levels 2 + 3 IgG₄ AbR) 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' 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 versus six foodstuffs.

When comparing sex along high level 3 of IgG₄ AbR, 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 moderate level 2 of IgG₄ AbR, goat's milk IgG₄ AbR was also significantly higher for women than men ($Z=2.18$; $p=0.029$; $\eta^2=0.023$) (Figure 3.1.3.1).

There were some foodstuffs, which directly correlated with each other. As expected, gluten allergen correlated with the level of IgG₄ AbR against wheat, rye, barley (all $p < 0.001$), and against oats ($p=0.037$). The variable 'number of signs and symptoms' had different correlations with food-specific IgG₄ AbR: 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 (MCT) were performed and revealed that the level of IgG₄ AbR against pineapple was higher (levels 2 and 3) when subjects presented from 3 to 5 self-

reported ‘number of 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 ($p>0.05$).

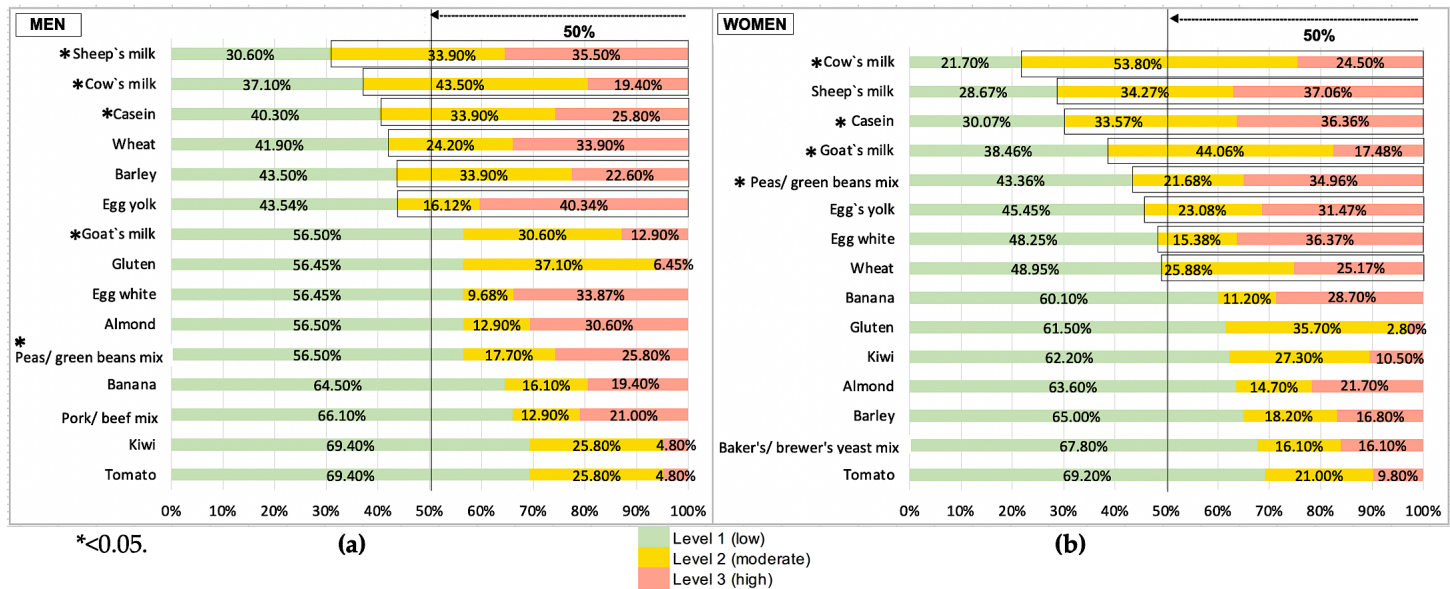


Figure 3.1.3.1 Food-specific IgG₄ AbR in Spanish men and women. (a) Prevalence of food-specific IgG₄ AbR in men. (b) Prevalence of food-specific IgG₄ AbR in women

3.1.4. Food allergen-specific immunoglobulin G₄ and age

Food-specific IgG₄ AbR between the four studied age range groups are shown in Table

3.1.4.1. Seventeen most prevalent foodstuffs IgG₄ AbR were analyzed. When evaluating level 3 positive responses, subjects belonging to the 20-34-year-old group showed higher prevalence of casein IgG₄ AbR 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 ($\chi^2(3)=12.99$; $p=0.005$; $\eta^2=0.06$). Similarly, subjects from the 35-49-year-old group had higher prevalence of egg white IgG₄ AbR 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 ($\chi^2(3)=8.32$; $p=0.040$; $\eta^2=0.04$).

Table 3.1.4.1 Food-specific IgG₄ AbR levels in adults of different age range groups

Age range	(20-34)									
	Total				Men			Women		
	n=57				n=13			n=44		
Level of IgG ₄ AbR	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%

Values bearing different letters were significantly different (a > b > c).

(Table 3.1.4.1 continues)

Age range

Level of IgG ₄ AbR	(35-49)									
	Total				Men			Women		
	n=67				n=22			n=45		
	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%
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%

Values bearing different letters were significantly different (a > b > c).

(Table 3.1.4.1 continues)

Age range	(50-64)									
	Total				Men			Women		
	n=59				n=20			n=39		
Level of IgG ₄ AbR	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%

Values bearing different letters were significantly different (a > b > c).

(Table 3.1.4.1 continues)

Age range	(65-79)									
	Total				Men			Women		
	n=22				n=7			n=15		
Level of IgG ₄ AbR	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 > c).

3.1.5. Food allergen-specific immunoglobulin G₄ and symptomatology

Figure 3.1.5.1 shows the frequency of self-reported signs and symptoms of subjects when presenting positive level 3 of food-specific IgG₄ AbR 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 IgG₄ AbR against tomato (75%), almond (56%), egg white (55%) and egg yolk (54.10%). Likewise, all GI 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 IgG₄ AbR 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 IgG₄ AbR 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 IgG₄ AbR against legume mix (peas/ green beans mix) (38.20%).

Positive (level 3) food-specific IgG₄ AbR 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 IgG₄ AbR 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 IgG₄ AbR reaction count. However, when age range variable was adjusted as covariate, both symptomatology variables explained 30% of the variance of total IgG₄ AbR reaction count. Significance improved from 0.239 to 0.149 but no statistical differences were found between symptomatology and the total IgG₄ AbR reaction count.

(a)

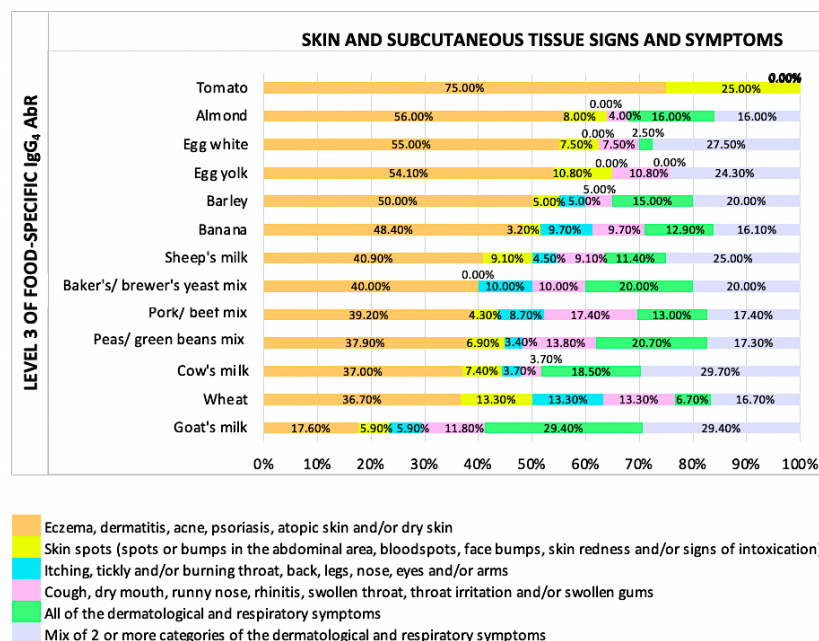
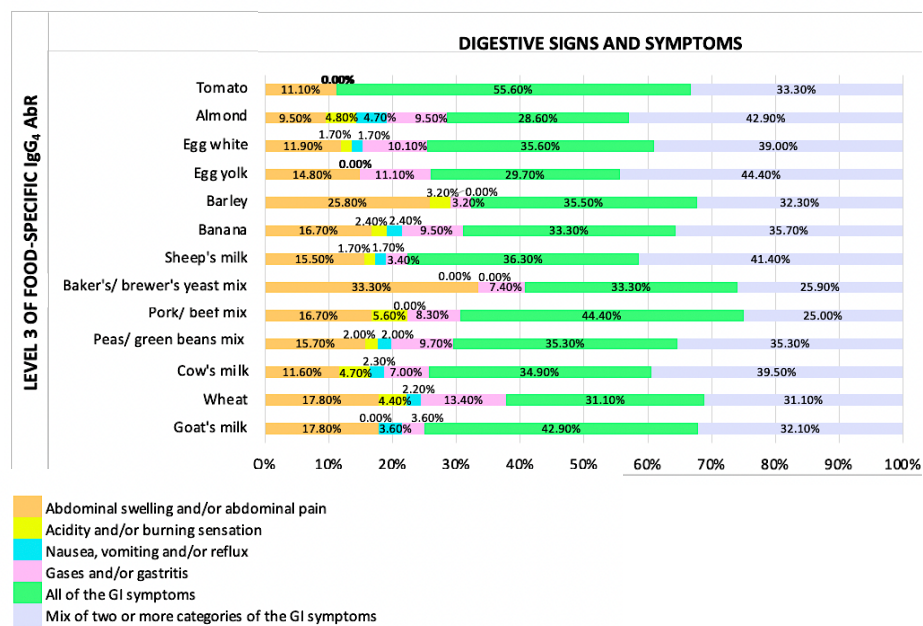


Figure 3.1.5.1 Symptomatology profile of subjects with level 3 of food-specific IgG₄ AbR. (a) Skin and subcutaneous tissue symptomatology profile. (b) Digestive or gastrointestinal symptomatology profile. (c) Nervous system symptomatology profile.

(Figure 3.1.5.1 continues)

(b)



(c)

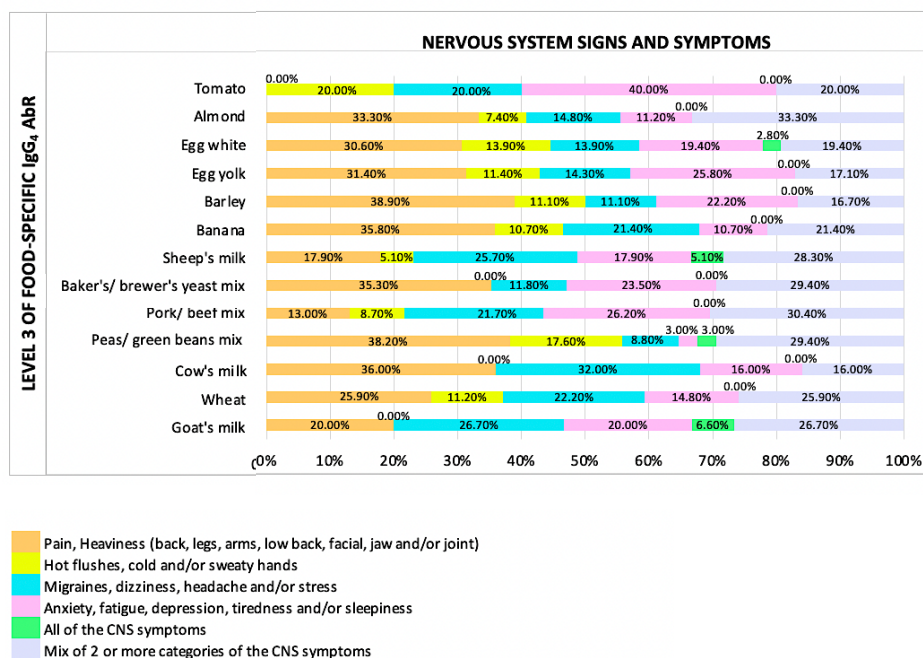


Figure 3.1.5.1 Symptomatology profile of subjects with level 3 of food-specific IgG₄ AbR. (a) Skin and subcutaneous tissue symptomatology profile. (b) Digestive or gastrointestinal symptomatology profile. (c) Nervous system symptomatology profile.

3.2. Design and validity of the PSIMP-ARFSQ-10 y FBFC-ARFSQ-18 questionnaires.

The FBFC-ARFSQ-18 and the PSIMP-ARFSQ-10 questionnaires were designed and validated using the expert judgement method over the following phases: 1) questionnaires development, 2) pilot test and reliability, 3) content validity, and 4) face validity.

Results obtained over each of these phases are explained below.

3.2.1. 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 [[73-76](#)].

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 [[76](#)]. Items 3 and 4 describe the most reported aspects of concern when it comes to ARFS: type of diet (vegan, gluten-free, fermentable oligosaccharides, 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 [[77-79](#)]. 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 [80,81]. Items 5 to 15 corresponded to 11 food groups designed to be answered based on the consumption of a participant or patient in the last 6 months with a scale describing the monthly, weekly or daily intake of each foodstuff. There is plenty of information on pediatric 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 3 to 6 months [82,83]. Trying to fill in the gaps, the present study proposes the FBFC-ARFSQ-18, describing the food and beverages intake during the last 6 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 4 body categories of diseases and symptomatology (digestive, skin and subcutaneous tissue, nervous system, respiratory system) and 1 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 diagnostics. 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 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 [84]. A final construct of questions was designed for both tools, ensuring no overlap (Supplementary Material, Annex I “Description and development of the final questionnaires FBFC-ARFSQ-18 and PSIMP-ARFSQ-10”, of Article 2 [34]).

3.2.2. Phase 2: Pilot test and reliability

The pilot test was completed by sixteen administrative and service personnel evaluators of the Faculty of Physical activity and Sport Sciences of the Universidad Politécnica de Madrid, aged 24 to 66 years (50% women, 48.38 ± 13.96 y), during December 2021 (Table 3.2.2.1). 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 completion 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 completion time for both questionnaires, reported by the administrative and service personnel evaluators, 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.

Table 3.2.2.1 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

(Table 3.2.2.1 continues)

Evaluators	Age (years)	M/F	Country of birth	Residence	Qualification	Number of diseases, symptoms and signs related to ARFS
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.

3.2.3. Phase 3: Content validity

A total of 20 Spanish experts accomplished the inclusion criteria, 5 experts of the FBFC-ARFSQ-18 and 3 experts of the PSIMP-ARFSQ-10 were excluded. Two experts did not accomplish one of the inclusion criteria. The other six, for various reasons, as not answering the evaluation questionnaire, or sending an incomplete evaluation. All experts were different individuals and none of the experts evaluated both questionnaires (10 food science and/or nutrition professionals aged mean $\pm$ SD 55.6 $\pm$ 11.37y evaluated the FBFC-ARFSQ-18; and 10 medicine and/or nursing professionals aged mean $\pm$ SD 61.5 $\pm$ 5.38y evaluated the PSIMP-ARFSQ-10) (Tables 3.2.3.1 and 3.2.3.2).

Table 3.2.3.1 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; PhD, Doctor of Philosophy.

Table 3.2.3.2 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; PhD, Doctor of Philosophy.

Resulting values of the Likert-type scale by the 10 selected experts for both questionnaires are shown in Tables 3.2.3.3 and 3.2.3.4. 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 (3.2.3.5).

Table 3.2.3.3 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

(Table 3.2.3.3 continues)

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 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 9 experts.

Table 3.2.3.4 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.

3.2.4. Phase 4: Face validity

Food groups of the FBFC-ARFSQ-18 were organized in 10 food groups in Section 2 of the FBFC-ARFSQ-18 (Supplementary Material: Annex I Description and development of the final questionnaires FBFC-ARFSQ-18 and PSIMP-ARFSQ-10). 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 did not represent a relevant or possible causative of ARFS were grouped where possible and listed into each food group (e.g., currant, passion fruit, or any other less common food in the Mediterranean Spanish area), these foodstuffs could be also 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, 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 10 experts commented at least one section of the FBFC-ARFSQ-18 and the PSIMP-ARFSQ-10 tool (except for expert number 9 and 5, correspondingly of each questionnaire). The average of completion 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. Supplementary Material, Annexes II and III “Spanish version of the FBFC-ARFSQ-18 and PSIMP-ARFSQ-10 questionnaires”, of Article 2 [\[34\]](#).

3.3. Development of the study protocol

3.3.1. Study protocol characteristics

The ALASKA Study protocol was developed according to The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [85]. Table 3.3.1.1 displays a checklist example of a participant's timeline of the ALASKA Study.

The ALASKA study protocol was proposed as a multifactorial strategy to manage ARFS in the adult population, and was organized in the following 10 main factors: 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) six-month intervention, 9) end of study and adverse events, and 10) (optional step) repetition of the assessments 2 to 8 and intervention adjustment.

Description of the immunological profiles and the substitutive diet

Food allergen-specific sIgE and sIgG₄ AbR panels were defined as participant's immunoglobulin profiles (Table 3.3.1.2).

The FASSD was designed considering seven principal characteristics explained in Table 3.3.1.3. Substitutive foodstuffs of the FASSD were 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.

Table 3.3.1.1 Individual checklist example of the ALASKA Study intervention

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	RedCap® randomization automated tool (RRAT)	--	✓	--	--	--	--	--	--	--	--
INTERVENTIONS		--									
GROUP A: General routine healthy diet advice (CONTROL)		--									
GROUP B: Food-allergen specific substitutive diet (FASSD)		--	--	--	--	--	--	--	--	--	--
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 (IMFINE-HYD)	--	--	--	--	--	--	--	--	✓	--
Food and beverages record	24-h Dietary Recall Interview (24HDRI)	--	--	--	✓	--	--	--	--	✓	--

ASSESSMENT (Table 3.3.1.1 continues)	INSTRUMENTS	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
Physical activity	The International Physical Activity Questionnaire Long Form (IPAQ-LF) (RedCap® platform)	--	--	--	✓	--	--	--	--	✓	--
	The Rapid Assessment of Physical Activity (RAPA) questionnaire (RedCap® platform)	--	--	--	✓	--	--	--	--	✓	--

3. HEMATO-BIOCHEMICAL 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, Helia® Helmed, Wendelsheim, Germany)	--	--	✓	--	--	--	--	✓	--	--
--	--	----	----	---	----	----	----	----	---	----	----

ASSESSMENT (Table 3.3.1.1 continues)	INSTRUMENTS	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
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 dynamometer (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, Brazil)	--	--	--	✓	--	--	--	--	✓	--

ASSESSMENT (Table 3.3.1.1 continues)		INSTRUMENTS	M-1	M0	M1A	M1B	M2	M3	M4	M5A	M5B	M6
Aerobic capacity	Estimated VO _{2max}	Åstrand-Ryhming Step Test (stÅ-R) method	--	--	--	✓	--	--	--	--	✓	--
	Pulse rate	Pulse oximeter (GE Healthcare, Ohmeda Tuffsat, 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; I/E, inclusion and exclusion; 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; H₂, hydrogen; CH₄, 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.

Table 3.3.1.2 Food allergen-specific panels of IgE and IgG₄ antibody reactions for the detection of type I and type II food hypersensitivity in human serum samples

ARFS	biomarker	AESKUBLOTS® kit (reference/name)	Food allergen-specific panel
T1FH	IgE	421001/ 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)
		421011/ 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)
		431401/ 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)
T2FH	IgG ₄	431402/ 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)
		431403/ 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; IgG₄, immunoglobulin G₄; T1FH, type I food hypersensitivity; T2FH, type II food hypersensitivity.

Table 3.3.1.3 Design of a healthy Food-allergen Specific Substitutive Diet

Characteristic	Source	FASSD
1) essential food groups for a balanced diet	SENC Spanish nutritional pyramid [86]	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 [87,88]	B2, B3, Mg, K, P, Ca, Zn, B12, B9, Fe and fiber
3) foodstuffs with equal or greater micronutrients than the replaced foodstuff	BEDCA [89], USDA[90] and NCCDB [91] 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 ≥ 20 , CH ₄ ≥ 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; FASSD, Food-allergen specific substitutive diet; MD, Mediterranean Diet.

The FASSD was constructed according to the following references: SENC Spanish nutritional pyramid [86], micronutrients deficiency in ARFS population [87,88] and national and international food composition databases [89-91].

3.4. Cross-sectional study results

3.4.1. Sample characteristics of the ALASKA Study at month 1

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 3.4.1.1. Men were significantly older than women (Table 3.4.1.2); 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 3.4.1.1 showed statistically significant anthropometric differences between men and women's BMI, height, weight, waist circumference, waist-to-hip ratio and body composition variables (all $p < 0.05$).

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 3.4.1.2). Estimated VO₂max, maximal HGS and leg power distribution values were significantly higher in men than women (all $p < 0.001$). 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 3.4.1.2).

Mean ages for young adults (≤ 45 y) and older adults (> 45 y) were 33.77 ± 7.55 y and 54.99 ± 5.90 y, respectively. Both mean GI and nervous system symptomatology PSIMP-ARFSQ-10 scores were higher in young adults than older adults ($p < 0.05$). GI PSIMP-ARFSQ-10 scores were still higher for the group of young adults vs older adults in women ($p < 0.05$). Self-reported PA in men was worse in older adults than in young adults, with twice as many older adults not meeting WHO recommendations compared to young adults (20% vs 10%). Regarding maximal HGS, there were no significant differences by age groups in men, while

young adult women showed higher maximal HGS than older adults ones ($p<0.05$). Leg power and STS speed were significantly higher in young adults than older adults, in both, men and women (both $p<0.001$). Estimated VO_2max was significantly higher in young adults, maintaining its significance when analyzing the specific CRF levels between young adults and older adults. The proportion of subjects showing good CRF was 70% of young adults and 50% of older adults (Table 3.4.1.2).

Table 3.4.1.1 Sample characteristics of the ALASKA Study at M1

Mean ±SD or n (%)					
Assessment	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)	--	NS 0.275
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)	--	
University education (college graduate, engineer, high degree)	105 (41.3)	41 (41.4)	64 (41.3)	--	
Master's degree	60 (23.7)	23 (23.2)	37 (23.9)	--	
PhD/doctorate's degree	15 (5.9)	7 (7.1)	8 (5.2)	--	
Job situation					
Employed or freelance	209 (82.3)	86 (86.9)	123 (79.4)	--	NS 0.140
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)	--	
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)	--	NS 0.220
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)	--	
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)	--	

(Table 3.4.1.1 continues)		Mean $\pm$ SD or <i>n</i> (%)			
Assessment	Total (<i>n</i> =254)	Men (<i>n</i> =99)	Women (<i>n</i> =155)	Min-Max	<i>p</i> -values _{sex}
Body composition					
Fat mass (%)	25.08 $\pm$ 8.63	20.48 $\pm$ 6.62	28.02 $\pm$ 8.50	5.60-51.60	<0.001
Fat mass (kg)	17.93 $\pm$ 9.07	16.74 $\pm$ 8.07	18.68 $\pm$ 9.61	3.90-51.00	<0.05
Fat free mass (kg)	51.66 $\pm$ 10.12	61.91 $\pm$ 6.97	45.12 $\pm$ 5.18	31.20-79.30	<0.001
Muscle mass (kg)	49.06 $\pm$ 9.64	58.82 $\pm$ 6.64	42.82 $\pm$ 4.92	29.60-75.40	<0.001
Bone mass (kg)	2.60 $\pm$ 0.48	3.09 $\pm$ 0.33	2.29 $\pm$ 0.26	1.60-3.90	<0.001
Body water (%)	51.89 $\pm$ 5.95	56.27 $\pm$ 4.13	49.10 $\pm$ 5.21	35.90-66.60	<0.001
Body water (kg)	35.85 $\pm$ 7.38	43.80 $\pm$ 4.56	30.77 $\pm$ 3.10	24.30-56.90	<0.001
Visceral fat rating (VFR ¹)	5.96 $\pm$ 4.18	8.27 $\pm$ 4.43	4.48 $\pm$ 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.

3.4.2. Adverse reactions to foodstuffs and age

Figure 3.4.2.1 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 young adults were against legume (53%) and seeds (45%), while against legume (46%) and nuts (42%) in older adults. Analogously, the most prevalent T2FH in young adults were against egg (66%) and nuts (63%), while against legume (68%) and egg (60%) in older adults.

Table 3.4.1.2 Symptomatology, physical activity and physical performance compared by sex and age

Mean $\pm$ SD or n (%)															
		Total (n=254)	Men (n=99)	Women (n=155)	Min-Max	p-values _{sex}	$\leq 45y$ (n=135)			$>45y$ (n=119)			p-values _{age}	p-values _{age-men}	p-values _{age-women}
							Total $\leq 45y$	Men (n=43)	Women (n=92)	Total $>45y$	Men (n=56)	Women (n=63)			
Symptomatology	Age (years)	43.71 $\pm$ 12.61	46.23 $\pm$ 13.04	42.10 $\pm$ 12.10	18.00-77.00	<0.05	33.77 $\pm$ 7.55	33.91 $\pm$ 7.94	33.71 $\pm$ 7.40	54.99 $\pm$ 5.90	55.70 $\pm$ 6.68	54.37 $\pm$ 5.08	<0.001	<0.001	<0.001
	(PSIMP-ARFSQ-10 score) ¹ [34]														
	GI system	4.87 $\pm$ 2.77	4.77 $\pm$ 2.71	4.94 $\pm$ 2.81	0.00-15.00	NS 0.319	5.29 $\pm$ 2.56	4.98 $\pm$ 2.56	5.43 $\pm$ 2.56	4.40 $\pm$ 2.93	4.61 $\pm$ 2.83	4.21 $\pm$ 3.02	<0.01	NS 0.252	<0.05
	Skin and subcutaneous tissue	2.92 $\pm$ 2.65	2.64 $\pm$ 2.54	3.10 $\pm$ 2.71	0.00-14.00	NS 0.089	2.84 $\pm$ 2.48	2.70 $\pm$ 2.19	2.91 $\pm$ 2.62	3.00 $\pm$ 2.84	2.59 $\pm$ 2.79	3.37 $\pm$ 2.85	NS 0.321	NS 0.417	NS 0.155
	Nervous system	3.04 $\pm$ 2.75	2.07 $\pm$ 2.34	3.66 $\pm$ 2.83	0.00-16.00	<0.001	3.36 $\pm$ 2.76	2.44 $\pm$ 2.35	3.79 $\pm$ 2.85	2.67 $\pm$ 2.71	1.79 $\pm$ 2.31	3.46 $\pm$ 2.82	<0.05	NS 0.084	NS 0.236
	Respiratory system	1.56 $\pm$ 1.86	1.70 $\pm$ 1.84	1.47 $\pm$ 1.87	0.00-11.00	NS 0.173	1.47 $\pm$ 1.69	1.77 $\pm$ 1.48	1.33 $\pm$ 1.77	1.66 $\pm$ 2.04	1.64 $\pm$ 2.08	1.68 $\pm$ 2.01	NS 0.200	NS 0.370	NS 0.123
Physical activity	Other pathologies/ diseases	2.49 $\pm$ 2.34	2.14 $\pm$ 2.13	2.72 $\pm$ 2.45	0.00-10.00	<0.05	1.81 $\pm$ 2.00	1.42 $\pm$ 1.79	1.99 $\pm$ 2.07	3.27 $\pm$ 2.47	2.70 $\pm$ 2.22	3.78 $\pm$ 2.58	<0.001	=0.001	<0.001
	Total PSIMP-ARFSQ-10 score	14.88 $\pm$ 7.97	13.31 $\pm$ 7.33	15.88 $\pm$ 8.21	6.00-44.00	<0.05	14.77 $\pm$ 7.46	13.30 $\pm$ 6.67	15.46 $\pm$ 7.74	15.00 $\pm$ 8.53	13.32 $\pm$ 7.86	16.49 $\pm$ 8.88	NS 0.410	NS 0.495	NS 0.221
	(IPAQ-SF)														
	Accomplish WHO recommendations	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)			
	Handgrip strength (HGS)														
Physical performance	Total maximal HGS (kg)	34.46 $\pm$ 9.91	44.41 $\pm$ 6.30	28.11 $\pm$ 5.66	17.30-66.00	<0.001	34.18 $\pm$ 9.96	45.36 $\pm$ 6.39	28.95 $\pm$ 6.36	34.78 $\pm$ 9.90	43.68 $\pm$ 6.18	26.88 $\pm$ 4.20	NS 0.314	NS 0.095	<0.05
	Power, speed and balance														
	Leg power (f/w)	1.55 $\pm$ 0.24	1.61 $\pm$ 0.26	1.51 $\pm$ 0.23	1.10-2.39	<0.001	1.65 $\pm$ 0.25	1.78 $\pm$ 0.22	1.58 $\pm$ 0.23	1.44 $\pm$ 1.19	1.47 $\pm$ 0.20	1.41 $\pm$ 0.17	<0.001	<0.001	<0.001
	Sit-to-stand speed (RFD/w)	12.61 $\pm$ 3.75	12.79 $\pm$ 4.10	12.49 $\pm$ 3.51	0.01-23.60	NS 0.271	13.85 $\pm$ 3.58	15.27 $\pm$ 3.23	13.18 $\pm$ 3.55	11.20 $\pm$ 3.43	10.88 $\pm$ 3.66	11.49 $\pm$ 3.23	<0.001	<0.001	=0.001
	Body balance (BP T-score)	50.12 $\pm$ 9.42	49.43 $\pm$ 10.46	50.55 $\pm$ 8.69	1.00-63.00	NS 0.178	50.43 $\pm$ 8.61	50.33 $\pm$ 8.33	50.48 $\pm$ 8.77	49.76 $\pm$ 10.28	48.75 $\pm$ 11.86	50.67 $\pm$ 8.64	NS 0.575	NS 0.230	NS 0.448
	Cardiorespiratory fitness (CRF)														
	Estimated VO ₂ max (ml/kg*min)	39.25 $\pm$ 9.92	42.79 $\pm$ 10.06	36.98 $\pm$ 9.17	21.45-68.96	<0.001	43.28 $\pm$ 9.39	48.80 $\pm$ 8.98	40.70 $\pm$ 8.44	34.67 $\pm$ 8.45	38.18 $\pm$ 8.29	31.55 $\pm$ 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. 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.

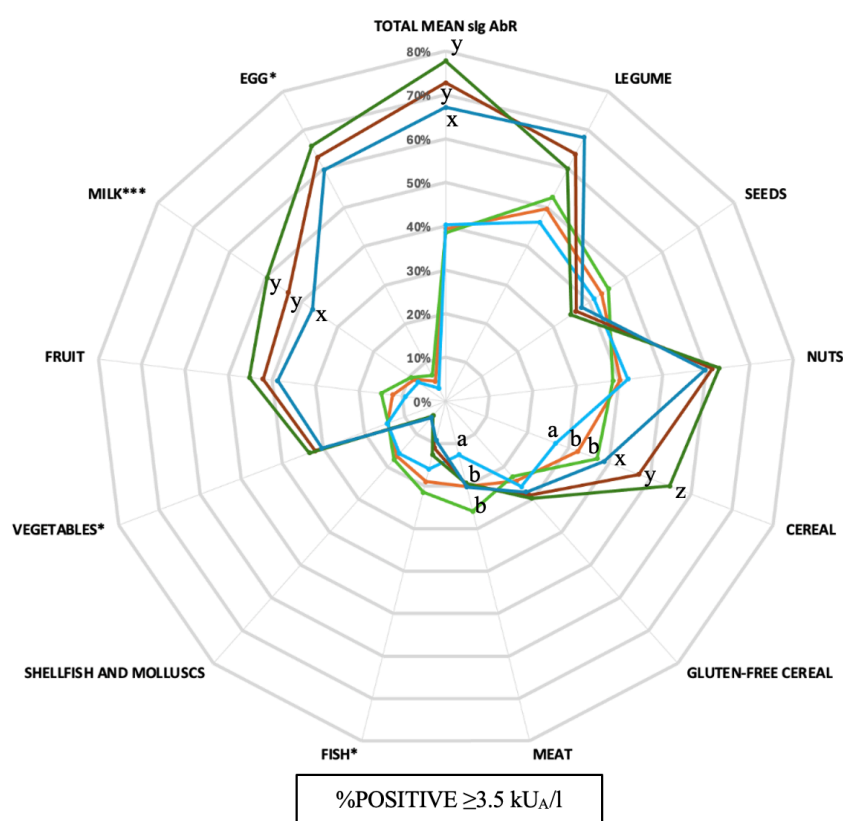


Figure 3.4.2.1 Prevalence of type I and type II hypersensitivity reactions according to age groups at M1.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. 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 ($a \neq b \neq c$; $x \neq y \neq z$).

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.

3.4.2.1. Type 1 food hypersensitivity

Mean total sIgE AbR was 4.22 ± 4.9 kUA/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 3.4.2.1). 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 young adults vs older adults were 4.6 ± 5.6 and 3.8 ± 3.9 kUA/l, respectively

($p>0.05$). However, cereal and meat food groups showed statistically higher prevalence of positive sIgE AbR in young adults compared to the older adults' group (both $p<0.05$).

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 $p<0.05$).

3.4.2.2. Type 2 food hypersensitivity

Mean total sIgG₄ AbR was 13.65 ± 13.93 kUA/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 3.4.2.1). 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 young adults vs older adults were 14.37 ± 14.12 and 12.84 ± 13.71 kUA/l, respectively ($p>0.05$). However, higher prevalence of positive sIgG₄ AbR against cereal and milk food groups were found in young adults compared to the older adults' age group (both $p<0.05$).

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 older adults compared to young adults. Further information regarding T1FH and T2FH and their corresponding associations between sex and age groups are referred in Table 3.4.2.1.

Table 3.4.2.1 Food-specific Immunoglobulin E antibody reactions compared by sex and age

	Mean $\pm$ SD or <i>n</i> (%)													
	Total (<i>n</i> =254)	Men (<i>n</i> =99)	Women (<i>n</i> =155)	Min-Max	<i>p</i> -values _{sex}	$\leq 45y$ (<i>n</i> =135)			$>45y$ (<i>n</i> =119)			<i>p</i> -values _{age}	<i>p</i> -values _{age-men}	<i>p</i> -values _{age-women}
						Total $\leq 45y$	Men (<i>n</i> =43)	Women (<i>n</i> =92)	Total $>45y$	Men (<i>n</i> =56)	Women (<i>n</i> =63)			
Age (years)	43.71 $\pm$ 12.61	46.23 $\pm$ 13.04	42.10 $\pm$ 12.10	18.00-77.00	SIG <0.05	33.77 $\pm$ 7.55	33.91 $\pm$ 7.94	33.71 $\pm$ 7.40	54.99 $\pm$ 5.90	55.70 $\pm$ 6.68	54.37 $\pm$ 5.08	SIG <0.001	SIG <0.001	SIG <0.001
sIgE AbR														
Total sIgE _{CALCULATED} ^a AbR (kUA/l)	4.22 $\pm$ 4.87	4.11 $\pm$ 4.41	4.28 $\pm$ 5.15	0.12-20.65	NS 0.394	4.56 $\pm$ 4.87	4.84 $\pm$ 5.21	4.43 $\pm$ 5.78	3.83 $\pm$ 3.87	3.56 $\pm$ 3.64	4.08 $\pm$ 4.09	NS 0.118	NS 0.076	NS 0.339
+ (%) ^b	100 (39.4)	41 (41.4)	59 (38.1)	--	NS 0.298	52 (38.5)	20 (46.5)	32 (34.8)	48 (40.3)	21 (37.5)	48 (40.3)	NS 0.384	NS 0.186	NS 0.156
Legume IgE AbR (kUA/l)	11.51 $\pm$ 16.66	11.98 $\pm$ 17.17	11.20 $\pm$ 16.38	0.16-54.98	NS 0.359	12.87 $\pm$ 17.25	15.23 $\pm$ 18.84	11.77 $\pm$ 16.44	9.96 $\pm$ 15.90	9.49 $\pm$ 15.49	10.37 $\pm$ 16.38	NS 0.082	NS 0.050	NS 0.302
+ (%)	126 (49.6)	49 (49.5)	77 (49.7)	--	NS 0.489	71 (52.6)	24 (55.8)	47 (51.1)	55 (46.2)	25 (44.6)	55 (46.2)	NS 0.156	NS 0.138	NS 0.337
Seeds IgE AbR (kUA/l)	7.28 $\pm$ 11.75	7.57 $\pm$ 11.97	7.09 $\pm$ 11.64	0.10-81.39	NS 0.374	8.40 $\pm$ 12.73	11.20 $\pm$ 15.39	7.10 $\pm$ 11.13	6.00 $\pm$ 10.43	4.79 $\pm$ 7.51	7.07 $\pm$ 12.43	NS 0.052	SIG <0.01	NS 0.495
+ (%)	110 (43.3)	45 (45.5)	65 (41.9)	--	NS 0.291	61 (45.2)	24 (55.8)	37 (40.2)	49 (41.2)	21 (37.5)	49 (41.2)	NS 0.261	SIG <0.05	NS 0.302
Cereal IgE AbR (kUA/l)	5.84 $\pm$ 11.44	6.46 $\pm$ 11.11	5.44 $\pm$ 11.67	0.11-86.76	NS 0.244	7.14 $\pm$ 14.10	7.59 $\pm$ 13.59	6.93 $\pm$ 14.40	4.37 $\pm$ 7.13	5.60 $\pm$ 8.77	3.27 $\pm$ 5.09	SIG <0.05	NS 0.190	SIG <0.05
+ (%)	82 (32.3)	28 (28.3)	54 (34.8)	--	NS 0.139	50 (37.0)	15 (34.9)	35 (38.0)	32 (26.9)	13 (23.2)	32 (26.9)	SIG <0.05	NS 0.103	NS 0.157
Nuts IgE AbR (kUA/l)	5.60 $\pm$ 7.57	4.85 $\pm$ 6.23	6.08 $\pm$ 8.29	0.13-37.11	NS 0.104	5.97 $\pm$ 8.36	5.95 $\pm$ 7.56	5.98 $\pm$ 8.74	5.19 $\pm$ 6.57	4.02 $\pm$ 4.89	6.23 $\pm$ 7.65	NS 0.207	NS 0.064	NS 0.426
+ (%)	102 (40.2)	39 (39.4)	63 (40.6)	--	NS 0.422	52 (38.5)	19 (44.2)	33 (35.9)	50 (42.0)	20 (35.7)	50 (42.0)	NS 0.286	NS 0.199	NS 0.073
Gluten-free cereal IgE AbR (kUA/l)	4.54 $\pm$ 11.20	3.70 $\pm$ 8.94	5.07 $\pm$ 12.42	0.09-100.00	NS 0.172	5.27 $\pm$ 13.60	3.74 $\pm$ 9.33	5.98 $\pm$ 15.19	3.71 $\pm$ 7.57	3.67 $\pm$ 8.71	3.75 $\pm$ 6.47	NS 0.135	NS 0.484	NS 0.137
+ (%)	62 (24.4)	20 (20.2)	42 (27.1)	--	NS 0.107	31 (23.0)	7 (16.3)	24 (26.1)	31 (26.1)	13 (23.2)	31 (26.1)	NS 0.285	NS 0.200	NS 0.367
Fish IgE AbR (kUA/l)	3.57 $\pm$ 9.14	2.94 $\pm$ 6.62	3.97 $\pm$ 10.44	0.12-92.41	NS 0.192	3.54 $\pm$ 7.41	3.53 $\pm$ 6.66	3.54 $\pm$ 7.77	3.60 $\pm$ 10.81	2.49 $\pm$ 6.61	4.58 $\pm$ 13.48	NS 0.479	NS 0.221	NS 0.272
+ (%)	48 (18.9)	18 (18.2)	30 (19.4)	--	NS 0.408	29 (21.5)	11 (25.6)	18 (19.6)	19 (16.0)	7 (12.5)	19 (16.0)	NS 0.132	SIG <0.05	NS 0.468
Meat IgE AbR (kUA/l)	3.68 $\pm$ 9.18	3.07 $\pm$ 7.26	4.07 $\pm$ 10.23	0.07-66.73	NS 0.199	2.75 $\pm$ 5.34	2.52 $\pm$ 4.40	2.85 $\pm$ 5.75	4.75 $\pm$ 12.10	3.50 $\pm$ 8.88	5.86 $\pm$ 14.35	SIG <0.05	NS 0.255	SIG <0.05
+ (%)	50 (19.7)	17 (17.2)	33 (21.3)	--	NS 0.211	35 (25.9)	10 (23.3)	25 (27.2)	15 (12.6)	7 (12.5)	15 (12.6)	SIG <0.01	SIG <0.01	SIG <0.05
Vegetables IgE AbR (kUA/l)	2.23 $\pm$ 5.78	1.93 $\pm$ 4.46	2.43 $\pm$ 6.50	0.10-55.92	NS 0.252	2.22 $\pm$ 5.39	1.84 $\pm$ 5.25	2.39 $\pm$ 5.47	2.25 $\pm$ 6.23	2.00 $\pm$ 3.79	2.48 $\pm$ 7.80	NS 0.481	NS 0.433	NS 0.468
+ (%)	36 (14.2)	13 (13.1)	23 (14.8)	--	NS 0.352	19 (14.1)	4 (9.3)	15 (16.3)	17 (14.3)	9 (16.1)	17 (14.3)	NS 0.481	NS 0.081	NS 0.269
Shellfish/ mollusks IgE AbR (kUA/l)	2.22 $\pm$ 4.62	2.14 $\pm$ 4.25	2.28 $\pm$ 4.85	0.09-47.90	NS 0.409	2.75 $\pm$ 5.73	2.72 $\pm$ 5.90	2.76 $\pm$ 5.69	1.63 $\pm$ 2.79	1.70 $\pm$ 2.30	1.57 $\pm$ 3.19	SIG <0.05	NS 0.119	NS 0.066
+ (%)	43 (16.9)	11 (11.1)	32 (20.6)	--	SIG <0.05	24 (17.8)	4 (9.3)	20 (21.7)	19 (16.0)	7 (12.5)	19 (16.0)	NS 0.351	NS 0.164	NS 0.343
Milk IgE AbR (kUA/l)	1.74 $\pm$ 6.17	1.90 $\pm$ 6.56	1.64 $\pm$ 5.93	0.00-46.60	NS 0.373	1.57 $\pm$ 5.46	1.04 $\pm$ 2.44	1.81 $\pm$ 6.40	1.94 $\pm$ 6.91	2.56 $\pm$ 8.43	1.39 $\pm$ 5.22	NS 0.316	NS 0.127	NS 0.331
+ (%)	22 (8.7)	8 (8.1)	14 (9.0)	--	NS 0.397	13 (9.6)	4 (9.3)	9 (9.8)	9 (7.6)	4 (7.1)	9 (7.6)	NS 0.280	NS 0.310	NS 0.348
Fruits IgE AbR (kUA/l)	1.51 $\pm$ 2.97	1.68 $\pm$ 3.39	1.40 $\pm$ 2.67	0.10-23.94	NS 0.230	1.66 $\pm$ 3.02	1.97 $\pm$ 3.64	1.52 $\pm$ 2.69	1.33 $\pm$ 2.91	1.46 $\pm$ 3.19	1.22 $\pm$ 2.65	NS 0.189	NS 0.233	NS 0.245
+ (%)	31 (12.2)	14 (14.1)	17 (11.0)	--	NS 0.227	20 (14.8)	7 (16.3)	13 (14.1)	11 (9.2)	7 (12.5)	11 (9.2)	NS 0.089	NS 0.350	NS 0.065
Egg IgE AbR (kUA/l)	0.90 $\pm$ 2.96	1.14 $\pm$ 3.34	0.75 $\pm$ 2.70	0.10-29.98	NS 0.148	0.59 $\pm$ 1.22	0.80 $\pm$ 1.69	0.48 $\pm$ 0.91	1.26 $\pm$ 4.11	1.41 $\pm$ 4.18	1.13 $\pm$ 4.08	SIG <0.05	NS 0.187	NS 0.073
+ (%)	13 (5.1)	5 (5.1)	8 (5.2)	--	NS 0.484	9 (6.7)	2 (4.7)	7 (7.6)	4 (3.4)	3 (5.4)	4 (3.4)	NS 0.117	NS 0.299	SIG <0.05

^a sIgE_{CALCULATED}: mean serum IgE AbR. ^b (+) Percentage of positive food-specific serum (sIgE) antibody reactions (≥ 3.5 kUA/l).

AbR: antibody reactions.

Table 3.4.2.2 Food-specific Immunoglobulin G₄ antibody reactions compared by sex and age

Mean $\pm$ SD or n (%)														
	Total (n=254)	Men (n=99)	Women (n=155)	Min-Max	p-values _{sex}	$\leq 45y$ (n=135)			$>45y$ (n=119)			p-values _{age}	p-values _{age-men}	p-values _{age-women}
						Total $\leq 45y$	Men (n=43)	Women (n=92)	Total $>45y$	Men (n=56)	Women (n=63)			
sIgG₄ AbR														
Total sIgG ₄ CALCULATED ^a AbR (kUA/l)	13.65 $\pm$ 13.93	16.24 $\pm$ 16.18	12.00 $\pm$ 12.04	0.04-77.80	SIG <0.01	14.37 $\pm$ 14.12	17.14 $\pm$ 15.58	13.07 $\pm$ 13.28	12.84 $\pm$ 13.71	15.54 $\pm$ 16.73	10.43 $\pm$ 9.86	NS 0.191	NS 0.314	NS 0.090
+ (%) ^b	185 (72.8)	68 (68.7)	117 (75.5)	--	NS 0.118	105 (77.8)	33 (76.7)	72 (78.3)	80 (67.2)	35 (62.5)	45 (71.4)	SIG <0.05	NS 0.066	NS 0.167
Legume IgG ₄ AbR (kUA/l)	17.60 $\pm$ 20.46	18.07 $\pm$ 24.18	17.30 $\pm$ 17.76	0.06-100.00	NS 0.385	16.46 $\pm$ 19.38	16.86 $\pm$ 22.35	16.27 $\pm$ 17.95	18.90 $\pm$ 21.63	19.01 $\pm$ 25.65	18.81 $\pm$ 17.52	NS 0.172	NS 0.332	NS 0.192
+ (%)	162 (63.8)	56 (56.6)	106 (68.4)	--	SIG <0.05	81 (60.0)	24 (55.8)	57 (62.0)	81 (68.1)	32 (57.1)	49 (77.8)	NS 0.092	NS 0.448	SIG <0.05
Seeds IgG ₄ AbR (kUA/l)	10.04 $\pm$ 16.97	13.54 $\pm$ 19.56	7.80 $\pm$ 14.72	0.01-83.21	SIG <0.01	9.87 $\pm$ 17.46	12.45 $\pm$ 18.88	8.66 $\pm$ 16.72	10.23 $\pm$ 16.47	14.39 $\pm$ 20.19	6.54 $\pm$ 11.18	NS 0.432	NS 0.314	NS 0.190
+ (%)	92 (36.2)	45 (45.5)	47 (30.3)	--	SIG <0.01	47 (34.8)	20 (46.5)	27 (29.3)	45 (37.8)	25 (44.6)	20 (31.7)	NS 0.311	NS 0.427	NS 0.376
Cereal IgG ₄ AbR (kUA/l)	20.83 $\pm$ 30.11	25.85 $\pm$ 34.37	17.62 $\pm$ 26.66	0.00-100.00	SIG <0.05	23.07 $\pm$ 29.68	29.38 $\pm$ 33.20	20.12 $\pm$ 27.59	18.29 $\pm$ 30.51	23.14 $\pm$ 35.30	13.98 $\pm$ 25.02	NS 0.104	NS 0.187	NS 0.080
+ (%)	120 (47.2)	50 (50.5)	70 (45.2)	--	NS 0.204	74 (54.8)	27 (62.8)	47 (51.1)	46 (38.7)	23 (41.1)	23 (36.5)	SIG <0.01	SIG <0.05	SIG <0.05
Nuts IgG ₄ AbR (kUA/l)	17.23 $\pm$ 20.70	22.77 $\pm$ 24.40	13.69 $\pm$ 17.10	0.02-85.98	SIG <0.001	17.00 $\pm$ 20.19	22.22 $\pm$ 23.40	14.56 $\pm$ 18.13	17.49 $\pm$ 21.34	23.20 $\pm$ 25.35	12.43 $\pm$ 15.54	NS 0.424	NS 0.422	NS 0.224
+ (%)	156 (61.4)	66 (66.7)	90 (58.1)	--	NS 0.085	85 (63.0)	28 (65.1)	57 (62.0)	71 (59.7)	38 (67.9)	33 (52.4)	NS 0.296	NS 0.389	NS 0.119
Gluten-free cereal IgG ₄ AbR (kUA/l)	8.29 $\pm$ 16.23	12.23 $\pm$ 19.42	5.78 $\pm$ 13.28	0.02-100.00	SIG <0.001	8.38 $\pm$ 16.91	14.42 $\pm$ 22.15	5.56 $\pm$ 13.02	8.18 $\pm$ 15.48	10.54 $\pm$ 17.04	6.09 $\pm$ 13.75	NS 0.461	NS 0.164	NS 0.405
+ (%)	73 (28.7)	39 (39.4)	34 (21.9)	--	SIG =0.001	40 (29.6)	20 (46.5)	20 (21.7)	33 (27.7)	19 (33.9)	14 (22.2)	NS 0.370	NS 0.104	NS 0.472
Fish IgG ₄ AbR (kUA/l)	5.53 $\pm$ 18.71	10.09 $\pm$ 24.69	2.62 $\pm$ 12.86	0.01-100.00	SIG <0.001	6.88 $\pm$ 21.40	12.95 $\pm$ 28.71	4.04 $\pm$ 16.38	4.00 $\pm$ 15.05	7.89 $\pm$ 21.11	0.54 $\pm$ 3.13	NS 0.111	NS 0.157	SIG <0.05
+ (%)	28 (11.0)	20 (20.2)	8 (5.2)	--	SIG <0.001	17 (12.6)	10 (23.3)	7 (7.6)	11 (9.2)	10 (17.9)	1 (1.6)	NS 0.199	NS 0.256	SIG <0.05
Meat IgG ₄ AbR (kUA/l)	5.41 $\pm$ 13.21	7.84 $\pm$ 15.82	3.86 $\pm$ 11.02	0.04-88.95	SIG <0.01	4.87 $\pm$ 12.69	5.84 $\pm$ 12.62	4.41 $\pm$ 12.76	6.03 $\pm$ 13.81	9.38 $\pm$ 17.85	3.05 $\pm$ 7.82	NS 0.243	NS 0.136	NS 0.225
+ (%)	50 (19.7)	26 (26.3)	24 (15.5)	--	SIG <0.05	26 (19.3)	10 (23.3)	16 (17.4)	24 (20.2)	16 (28.6)	8 (12.7)	NS 0.428	NS 0.278	NS 0.215
Vegetables IgG ₄ AbR (kUA/l)	5.74 $\pm$ 12.41	7.18 $\pm$ 14.28	4.82 $\pm$ 11.00	0.01-95.51	NS 0.070	6.35 $\pm$ 14.11	7.54 $\pm$ 16.25	5.79 $\pm$ 13.05	5.06 $\pm$ 10.17	6.90 $\pm$ 12.71	3.41 $\pm$ 6.90	NS 0.205	NS 0.413	NS 0.094
+ (%)	81 (31.9)	37 (37.4)	44 (28.4)	--	NS 0.067	45 (33.3)	15 (34.9)	30 (32.6)	36 (30.3)	22 (39.3)	14 (22.2)	NS 0.300	NS 0.329	NS 0.080
Shellfish/ mollusks IgG ₄ AbR (kUA/l)	0.95 $\pm$ 4.06	1.06 $\pm$ 3.75	0.89 $\pm$ 4.25	0.04-34.51	NS 0.370	1.01 $\pm$ 4.36	1.46 $\pm$ 5.17	0.80 $\pm$ 3.94	0.89 $\pm$ 3.69	0.75 $\pm$ 2.11	1.00 $\pm$ 4.69	NS 0.402	NS 0.178	NS 0.387
+ (%)	12 (4.7)	7 (7.1)	5 (3.2)	--	NS 0.080	6 (4.4)	3 (7.0)	3 (3.3)	6 (5.0)	4 (7.1)	2 (3.2)	NS 0.412	NS 0.487	NS 0.488
Milk IgG ₄ AbR (kUA/l)	24.95 $\pm$ 36.38	27.00 $\pm$ 37.01	23.63 $\pm$ 36.03	0.00-100.00	NS 0.236	27.18 $\pm$ 36.41	29.77 $\pm$ 35.79	25.97 $\pm$ 36.82	22.41 $\pm$ 36.34	24.88 $\pm$ 38.10	20.22 $\pm$ 34.85	NS 0.149	NS 0.259	NS 0.165
+ (%)	111 (43.7)	45 (45.5)	66 (42.6)	--	NS 0.327	67 (49.6)	23 (53.5)	44 (47.8)	44 (37.0)	22 (39.3)	22 (34.9)	SIG <0.05	NS 0.081	NS 0.056
Fruits IgG ₄ AbR (kUA/l)	7.30 $\pm$ 11.53	8.02 $\pm$ 13.13	6.84 $\pm$ 10.40	0.10-23.94	NS 0.213	8.44 $\pm$ 12.41	8.66 $\pm$ 12.95	8.34 $\pm$ 12.22	6.00 $\pm$ 10.34	7.54 $\pm$ 13.37	4.64 $\pm$ 6.41	NS 0.092	NS 0.338	SIG <0.05
+ (%)	107 (42.1)	43 (43.4)	64 (41.3)	--	NS 0.368	61 (45.2)	21 (48.8)	40 (43.5)	46 (38.7)	22 (39.3)	24 (38.1)	NS 0.147	NS 0.173	NS 0.253
Egg IgG ₄ AbR (kUA/l)	39.93 $\pm$ 39.64	41.21 $\pm$ 41.82	39.12 $\pm$ 38.29	0.00-64.44	NS 0.342	42.91 $\pm$ 40.14	44.18 $\pm$ 42.31	42.32 $\pm$ 39.31	36.55 $\pm$ 38.95	38.92 $\pm$ 41.69	34.45 $\pm$ 36.55	NS 0.101	NS 0.269	NS 0.105
+ (%)	160 (63.0)	61 (61.6)	99 (63.9)	--	NS 0.359	89 (65.9)	28 (65.1)	61 (66.3)	71 (59.7)	33 (58.9)	38 (60.3)	NS 0.152	NS 0.268	NS 0.225

^a sIgG₄CALCULATED: mean serum IgG₄ AbR. ^b (+) Percentage of positive food-specific serum (sIgG₄) antibody reactions (≥ 3.5 kUA/l).

AbR: antibody reactions.

3.4.2.3. 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 3.4.2.3.1).

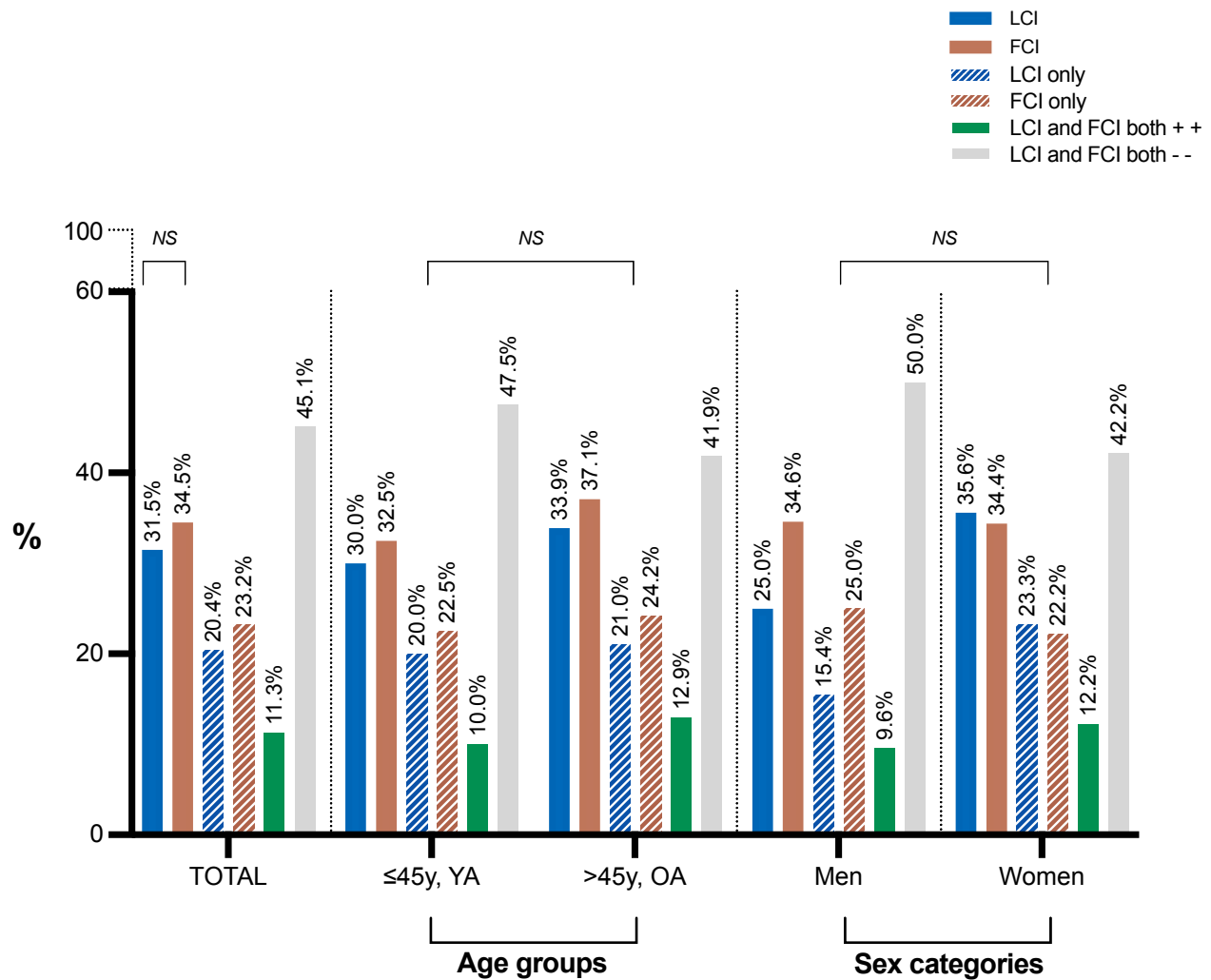


Figure 3.4.2.3.1 Prevalence of lactose and fructose intolerance according to age and sex groups. OA, older adults; YA, younger adults; LCI, lactose intolerance; FCI, fructose intolerance; NS, statistically non-significant; y, years of age.

3.4.3. Physical performance

3.4.3.1. Physical performance and sample characteristics

Significant associations were found between the physical performance variables (cardiorespiratory fitness as estimated $\text{VO}_{2\text{max}}$, maximal handgrip strength, leg power, sit-to-stand speed, body balance and resting heart rate distributions) and the studied categories of sex, age, BMI and symptomatology (Figure 3.4.3.1.1). There were no significant differences between physical performance variables and self-reported physical activity; or between physical performance variables and the subjects' living situation (number of people living with) (data not shown). As expected, significant associations, endorsing men, were observed between estimated $\text{VO}_{2\text{max}}$, maximal handgrip strength, and leg power, with sex categories (all $p < 0.001$). Besides, women exhibited significantly higher resting heart rate values. There were no significant associations between sit-to-stand speed and body balance, with sex categories. Young adults significantly outperformed older adults in cardiorespiratory fitness, leg power, and sit-to-stand speed (all $p < 0.001$). Cardiorespiratory fitness and leg power were significantly associated with BMI and it was observed that greater distribution values of estimated $\text{VO}_{2\text{max}}$ and leg power were significantly related to BMI values of < 25 ($p < 0.01$). Furthermore, greater distribution values of maximal handgrip strength, leg power, and resting heart rate 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 $p < 0.05$).

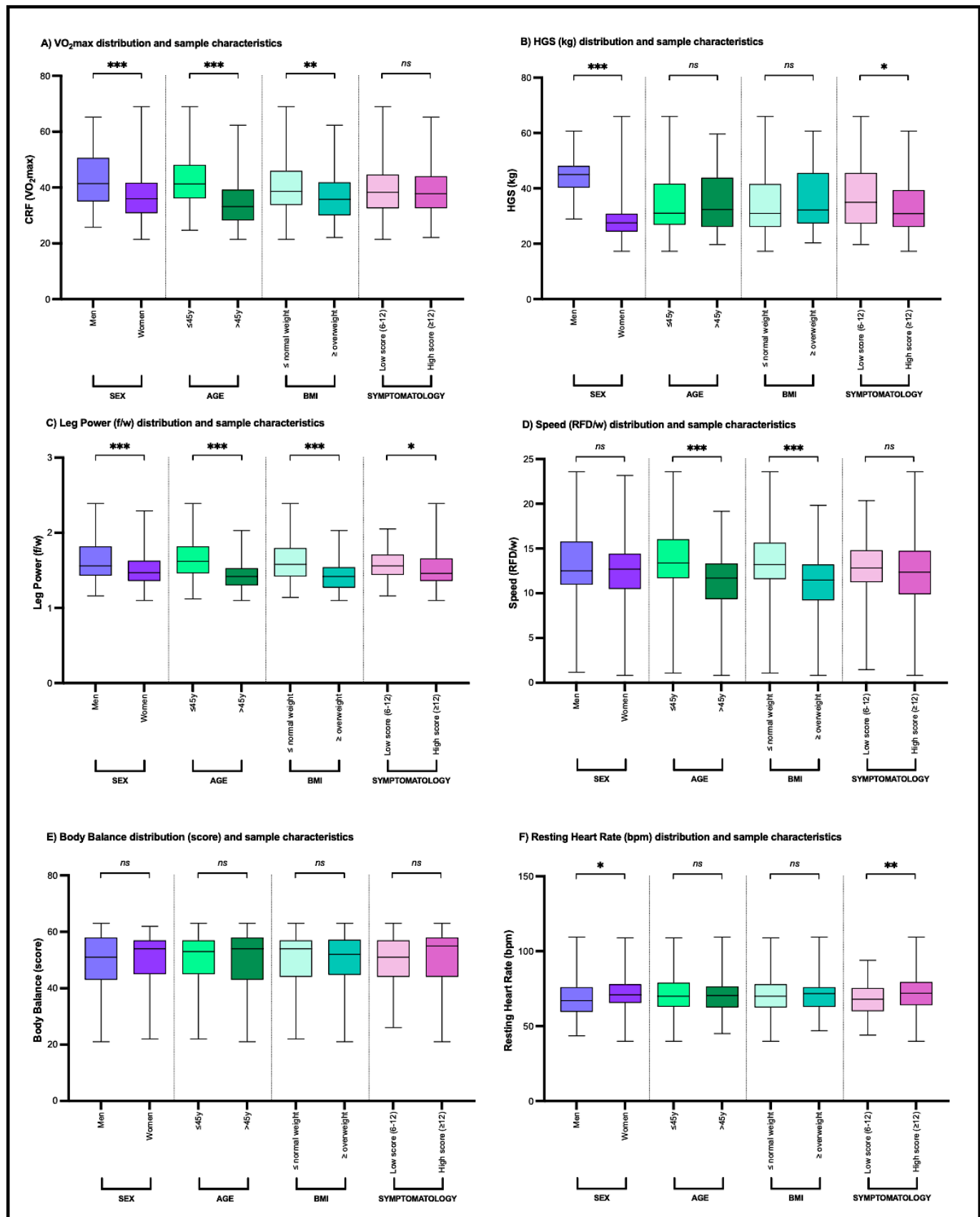


Figure 3.4.3.1.1 Physical Performance variables and sample characteristics

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. 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.

3.4.3.2. Physical performance 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 physical performance. Meanwhile, positive LCI subjects were significantly associated with low physical performance compared to high physical performance (Supplementary material of Article 4 [\[43\]](#)). Maximal handgrip strength showed a significant decrease with positive LCI compared to negative LCI ($p<0.05$). Nonetheless, there were no significant variations in cardiorespiratory fitness between the established positive groups of FA and FI. Nevertheless, some associations have been observed while studying the 12 food groups and physical performance variables (Table 3.4.2.2).

Positive food-specific sIgG₄ AbR were associated to several physical performance variables. Food-specific sIgG₄ AbR were positively related to higher cardiorespiratory fitness values for the cereal, fish, nuts and shellfish food groups; higher maximal handgrip strength values for milk and meat food groups; higher sit-to-stand speed values for legume food group; and higher balance values for fish food group. In contrast, food-specific sIgG₄ AbR were related to lower sit-to-stand speed values for cereal food group, and resting heart rate values for egg food group (all $p<0.05$). No significant differences in higher leg power were found, or in higher or lower physical performance values for food-specific sIgE AbR (Supplementary material of Article 4 [\[43\]](#)).

Older adult subjects had more significant positive food-specific sIgE and sIgG₄ AbR against food groups when higher levels of the analyzed physical performance variables were observed. Older adult subjects showed significant higher values of cardiorespiratory fitness when positive cereal, fish and seeds sIgG₄ AbR, or when positive egg sIgE AbR; besides, higher maximal handgrip strength when positive fish sIgG₄ AbR, or when positive meat sIgE AbR; moreover,

higher leg power when positive vegetables sIgE AbR; furthermore, higher sit-to-stand 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.

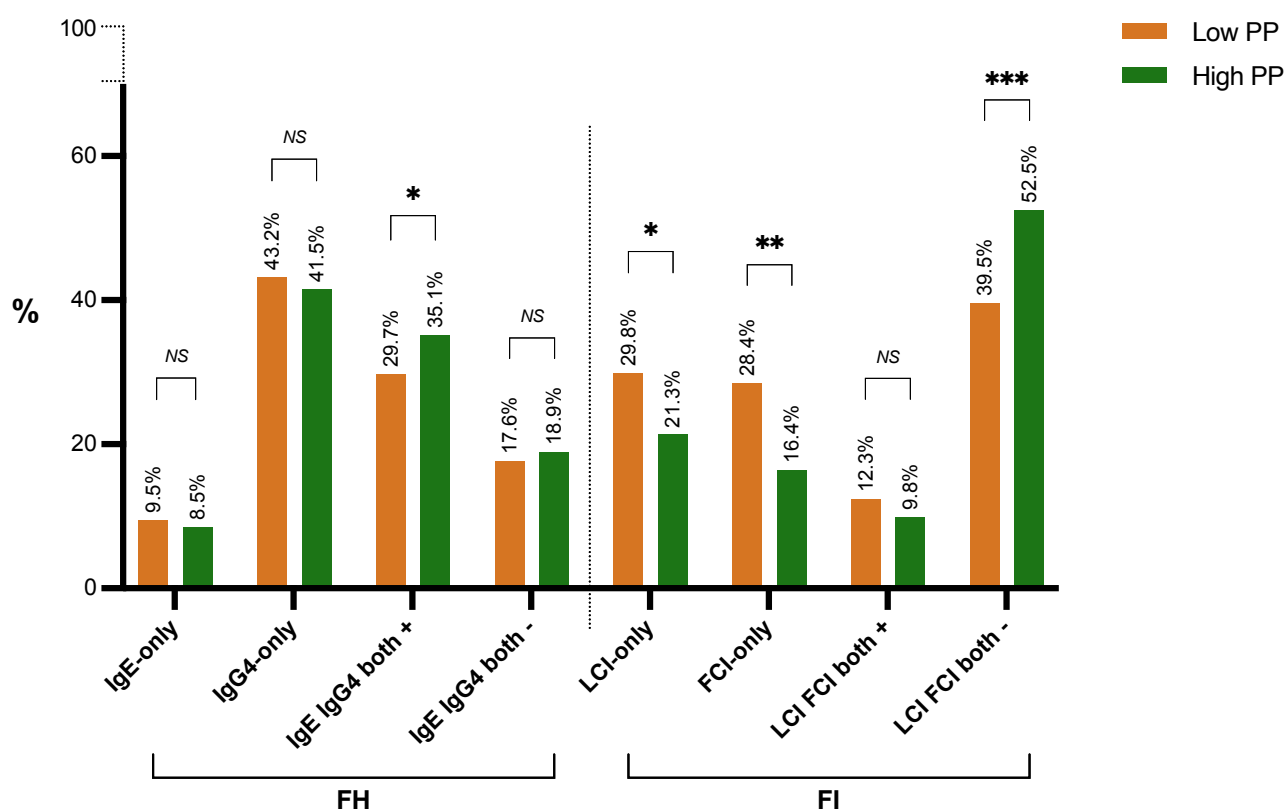


Figure 3.4.3.2.1 Physical performance according to the types of adverse reactions to foodstuffs

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

3.4.4. Physical performance as a risk factor of adverse reactions to foodstuffs

In order to comprehend the connection between physical performance 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 physical performance was associated with having any of the analyzed categories of ARFS (Figure 3.4.4.1).

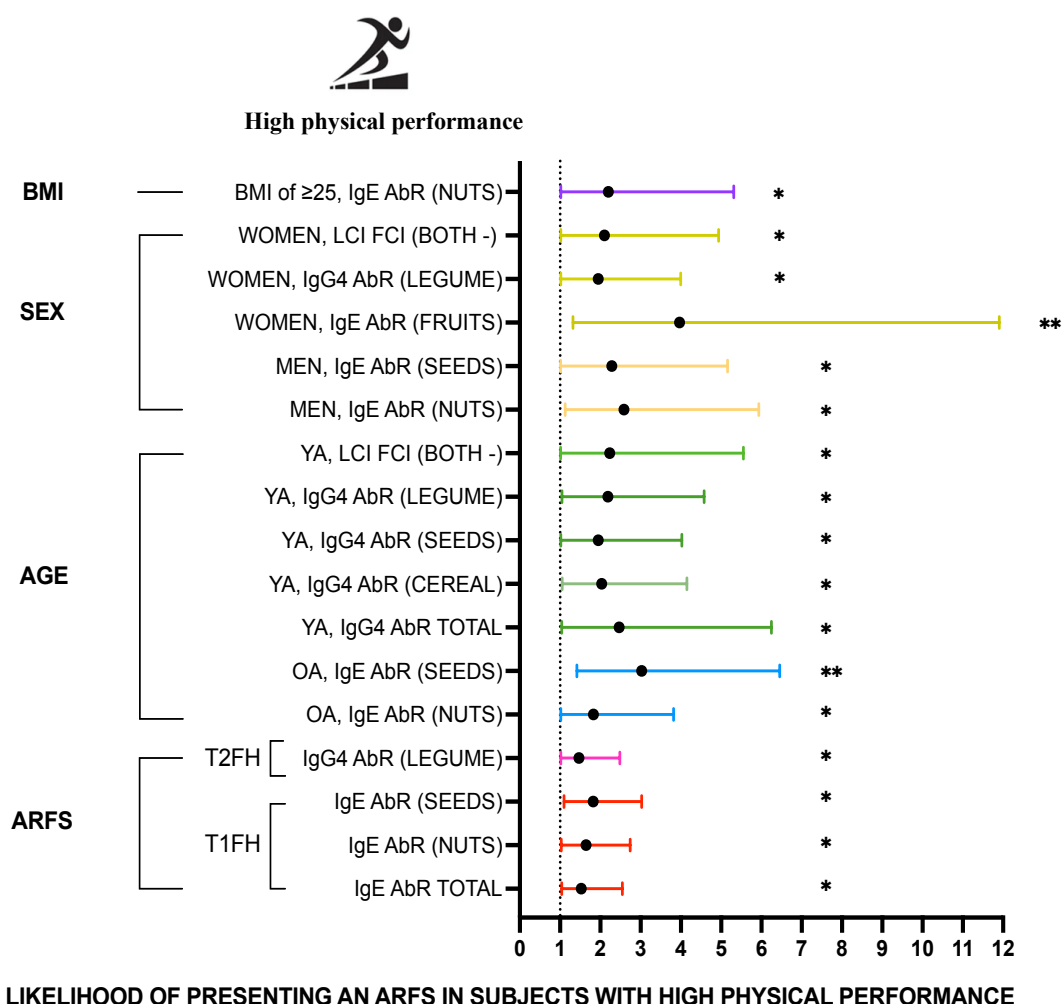


Figure 3.4.4.1 Odds ratio associations between physical performance and ARFS food groups
LCI, lactose intolerance; FCI, fructose intolerance; OA, older adults ($>45y$); OW, overweight; T1FH, type 1 food hypersensitivity; T2FH, type 2 food hypersensitivity; YA, young adults ($\leq 45y$); Both -, both negative.

Subjects with high physical performance were over 1.5 times as likely to have experienced a T1FH compared to low physical performance 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 physical performance compared to low physical performance 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 physical performance subjects compared to those with low physical performance who were women (76.6% vs 62.6%, respectively; OR = 1.95, 95% CI (1.03, 3.99); $p < 0.05$); and subjects aged ≤ 45 y (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 IgG₄-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 young adults with high physical performance 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); $p < 0.05$).

3.5. Randomized controlled trial results

3.5.1. Sample characteristics of the ALASKA Study at month 5

A total sample of 101 Spanish adults participated in the ALASKA Study intervention (62% women) with a mean age of 45.35 ± 11.76 y. Data regarding demographics and baseline characteristics of the ALASKA Study are presented in Table 3.5.2.1. There were no significant differences of socio-demographic characteristics (education level, job and living situation) or BMI categories between control group (Group A) and intervention group (Group B) in the baseline timeline point. Around 60% of the studied sample presented normal weight. Expected significant differences were observed between men and women's anthropometric and body composition features (data published elsewhere [43], data not shown). No further statistical differences were observed between the results presented in Table 3.5.2.1 (all $p > 0.05$).

Table 3.5.3.1 presents the main variables measured before and after the ALASKA Study intervention (anthropometry, body composition, physical performance, PA, symptomatology, immunology, hematobiochemical study and QoL) and their statistical significance for the control and intervention FASSD groups (Group A and Group B, respectively). No physical activity or QoL significant effects were self-reported by subjects of the ALASKA Study intervention.

3.5.2. 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 ($F_{1,66}=13.61$; $p < 0.001$; $\eta^2=0.17$), body fat mass ($F_{1,66}=9.27$; $p=0.003$; $\eta^2=0.12$) body fat mass percentage ($F_{1,66}=8.59$; $p=0.005$; $\eta^2=0.12$) and waist circumference ($F_{1,66}=42.04$; $p < 0.001$; $\eta^2=0.39$) 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 intervention with the healthy diet ($p > 0.05$).

Table 3.5.2.1 Baseline characteristics of the ALASKA Study intervention

		Mean $\pm$ SD or <i>n</i> (%)			Min-Max	<i>p</i> -values control-intervention
		Total (<i>n</i> =101)	Control Group A (<i>n</i> =34)	Intervention Group B (<i>n</i> =67)		
Age (years)		45.35 $\pm$ 11.76	46.59 $\pm$ 11.59	44.72 $\pm$ 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 $\pm$ 8.34	167.59 $\pm$ 9.78	168.93 $\pm$ 7.55	150.00 - 186.00	NS 0.449
Weight (kg)		68.61 $\pm$ 14.01	68.64 $\pm$ 15.61	68.60 $\pm$ 13.24	41.80 - 124.80	NS 0.989
BMI (kg/m ²)		24.17 $\pm$ 4.67	24.44 $\pm$ 5.13	24.04 $\pm$ 4.45	17.10 - 38.50	NS 0.686
BMI (WHO categories)						
<18.5 underweight (%)		3 (3.0)	2 (5.9)	1 (1.5)	--	NS 0.475
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)	--	
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)	--	NS 0.723
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)	--	
Master's 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)	--	
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.

3.5.3. Physical performance

No significant change in hand grip strength was observed over time whereas the rest of physical performance tests changed over time (leg power strength, 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 hand grip strength variable ($p > 0.05$). There were significant group $\times$ time interactions in cardiorespiratory fitness ($F_{1,66}=2.13$; $p < 0.034$; $\eta^2=0.039$) and body balance ($F_{1,66}=15.50$; $p < 0.001$; $\eta^2=0.190$) for subjects belonging to the Group B.

Table 3.5.3.1 ALASKA Study assessments before and after the FASSD intervention

	Mean $\pm$ SD or <i>n</i> (%)						<i>p</i> -values		
	M1 (<i>n</i> =101)			M5/6 (<i>n</i> =101)			M1 vs M5/6		
	Total Baseline	Control Group A (<i>n</i> =34)	FASSD Intervention Group B (<i>n</i> =67)	Total Intervention	Control Group A (<i>n</i> =34)	FASSD Intervention Group B (<i>n</i> =67)	<i>p</i> -values BASELINE-INTERVENTION	<i>p</i> -values CONTROL	<i>p</i> -values FASSD
ANTHROPOMETRY									
BMI (kg/m ²)	24.17 $\pm$ 4.67	24.44 $\pm$ 5.13	24.04 $\pm$ 4.45	24.09 $\pm$ 4.78	24.45 $\pm$ 5.25	23.90 $\pm$ 4.56	NS 0.601	NS 0.979	NS 0.458
Height (cm)	168.48 $\pm$ 8.34	167.59 $\pm$ 9.78	168.93 $\pm$ 7.55	168.10 $\pm$ 8.33	167.38 $\pm$ 9.64	168.46 $\pm$ 7.64	NS 0.366	NS 0.421	NS 0.452
Weight (kg)	68.61 $\pm$ 14.01	68.64 $\pm$ 15.61	68.60 $\pm$ 13.25	66.80 $\pm$ 14.66	68.63 $\pm$ 16.42	65.87 $\pm$ 13.72	<0.01	NS 0.991	<0.001
Waist circumference (cm)	79.40 $\pm$ 12.77	80.25 $\pm$ 12.86	78.97 $\pm$ 12.80	76.02 $\pm$ 12.45	80.20 $\pm$ 13.62	73.89 $\pm$ 11.33	<0.001	NS 0.933	<0.001
Hip circumference (cm)	94.94 $\pm$ 10.92	95.70 $\pm$ 10.67	94.56 $\pm$ 11.10	95.37 $\pm$ 8.17	100.33 $\pm$ 9.96	92.85 $\pm$ 5.69	NS 0.634	<0.001	NS 0.151
Waist-to-hip ratio	0.84 $\pm$ 0.11	0.84 $\pm$ 0.09	0.84 $\pm$ 0.12	0.80 $\pm$ 0.10	0.80 $\pm$ 0.08	0.80 $\pm$ 0.11	<0.001	<0.001	<0.01
BODY COMPOSITION									
Fat mass percentage (%)	25.04 $\pm$ 8.55	24.12 $\pm$ 8.64	25.56 $\pm$ 8.59	23.35 $\pm$ 8.71	27.44 $\pm$ 7.87	21.27 $\pm$ 8.42	NS 0.143	NS 0.053	<0.01
Fat mass (kg)	17.96 $\pm$ 9.09	16.83 $\pm$ 9.03	18.59 $\pm$ 9.19	15.14 $\pm$ 9.02	17.36 $\pm$ 9.37	14.02 $\pm$ 8.69	<0.05	NS 0.774	<0.01
Fat free mass (kg)	50.85 $\pm$ 9.18	49.80 $\pm$ 9.91	51.39 $\pm$ 8.82	50.98 $\pm$ 9.48	49.27 $\pm$ 10.44	51.86 $\pm$ 8.91	NS 0.757	NS 0.150	NS 0.447
Muscle mass (kg)	48.29 $\pm$ 8.74	47.29 $\pm$ 9.44	48.80 $\pm$ 8.39	48.44 $\pm$ 9.03	46.77 $\pm$ 9.94	49.29 $\pm$ 8.49	NS 0.711	NS 0.139	NS 0.403
Bone mass (kg)	2.56 $\pm$ 0.44	2.51 $\pm$ 0.47	2.59 $\pm$ 0.42	2.55 $\pm$ 0.45	2.50 $\pm$ 0.50	2.57 $\pm$ 0.43	NS 0.357	NS 0.524	NS 0.448
Body water (%)	52.04 $\pm$ 6.25	50.70 $\pm$ 6.23	52.72 $\pm$ 6.20	51.88 $\pm$ 6.11	50.70 $\pm$ 5.75	52.48 $\pm$ 6.24	NS 0.473	NS 0.987	NS 0.394
Body water (kg)	35.39 $\pm$ 6.88	34.44 $\pm$ 7.31	35.88 $\pm$ 6.65	35.01 $\pm$ 7.01	34.44 $\pm$ 7.64	35.30 $\pm$ 6.71	NS 0.225	NS 0.984	NS 0.202
Visceral fat ratio	6.00 $\pm$ 3.98	6.15 $\pm$ 4.17	5.93 $\pm$ 3.91	6.00 $\pm$ 3.96	6.41 $\pm$ 4.23	5.79 $\pm$ 3.84	NS 0.998	NS 0.239	NS 0.462
PHYSICAL PERFORMANCE									
Handgrip strength (HGS)									
Total maximal HGS (kg)	33.72 $\pm$ 8.76	32.60 $\pm$ 9.56	34.28 $\pm$ 8.34	33.83 $\pm$ 9.07	32.54 $\pm$ 9.20	34.49 $\pm$ 9.00	NS 0.734	NS 0.882	NS 0.661
Power, speed and balance									
Leg power (f/w)	1.59 $\pm$ 0.24	1.56 $\pm$ 0.22	1.60 $\pm$ 0.25	1.50 $\pm$ 0.18	1.55 $\pm$ 0.22	1.47 $\pm$ 0.15	<0.001	NS 0.076	<0.001
Sit-to-stand speed (RFD/w)	13.62 $\pm$ 2.89	13.36 $\pm$ 2.61	13.75 $\pm$ 3.03	12.53 $\pm$ 2.63	13.35 $\pm$ 2.61	12.12 $\pm$ 2.55	<0.001	NS 0.543	<0.001
Body balance (BP T-score)	49.08 $\pm$ 10.05	49.35 $\pm$ 9.17	48.94 $\pm$ 10.53	54.91 $\pm$ 7.87	54.03 $\pm$ 6.66	55.36 $\pm$ 8.43	<0.001	<0.01	<0.001
Cardiorespiratory fitness (CRF)									
Estimated VO ₂ max (ml/kg*min)	38.59 $\pm$ 9.85	36.51 $\pm$ 9.21	39.65 $\pm$ 10.06	39.56 $\pm$ 11.63	35.38 $\pm$ 9.55	41.67 $\pm$ 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)	NS 0.705	NS 0.432	NS 0.289
Normal CRF (%)	27 (26.7)	11 (32.4)	16 (23.9)	25 (24.8)	12 (35.3)	13 (19.4)			
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)			

(Table 3.5.3.1 continues)

(Table 3.5.3.1 continues)	Mean ±SD or <i>n</i> (%)						<i>p</i> -values		
	M1 (<i>n</i> =101)			M5/6 (<i>n</i> =101)			M1 vs M5/6		
	Total Baseline	Control Group A (<i>n</i> =34)	FASSD Intervention Group B (<i>n</i> =67)	Total Intervention	Control Group A (<i>n</i> =34)	FASSD Intervention Group B (<i>n</i> =67)	<i>p</i> -values BASELINE- INTERVENTION	<i>p</i> -values CONTROL	<i>p</i> -values FASSD
PHYSICAL ACTIVITY (IPAQ-SF)									
Accomplish WHO recommendations	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 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 sIgG4 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
Low-density lipoprotein LDLc (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
High-density lipoprotein HDLc (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

(Table 3.5.3.1 continues)

	Mean $\pm$ SD or <i>n</i> (%)						<i>p</i> -values		
	M1 (<i>n</i> =101)			M5/6 (<i>n</i> =101)			M1 vs M5/6		
	Total Baseline	Control Group A (<i>n</i> =34)	FASSD Intervention Group B (<i>n</i> =67)	Total Intervention	Control Group A (<i>n</i> =34)	FASSD Intervention Group B (<i>n</i> =67)	<i>p</i> -values BASELINE- INTERVENTION	<i>p</i> -values CONTROL	<i>p</i> -values FASSD
AST/GOT (U/L)	22.97 $\pm$ 7.00	22.50 $\pm$ 8.08	23.21 $\pm$ 6.44	23.06 $\pm$ 9.86	21.59 $\pm$ 7.11	23.81 $\pm$ 10.97	NS 0.923	NS 0.371	NS 0.647
GGT (U/L)	21.50 $\pm$ 12.77	23.21 $\pm$ 17.94	20.63 $\pm$ 9.15	20.98 $\pm$ 12.27	23.00 $\pm$ 16.66	19.96 $\pm$ 9.27	NS 0.316	NS 0.791	NS 0.317
Albumin (g/dL)	44.48 $\pm$ 2.44	43.62 $\pm$ 2.12	44.91 $\pm$ 2.48	43.25 $\pm$ 2.37	42.41 $\pm$ 2.35	43.67 $\pm$ 2.29	<0.001	<0.01	<0.001
Creatinine (mg/dL)	0.80 $\pm$ 0.19	0.75 $\pm$ 0.18	0.83 $\pm$ 0.18	0.80 $\pm$ 0.17	0.78 $\pm$ 0.17	0.82 $\pm$ 0.17	NS 0.675	<0.05	NS 0.312
Creatine kinase (CK)	141.47 $\pm$ 170.96	144.41 $\pm$ 178.58	139.97 $\pm$ 168.31	145.55 $\pm$ 242.98	120.68 $\pm$ 80.92	158.18 $\pm$ 292.74	NS 0.880	NS 0.366	NS 0.639
Amilase (U/L)	76.42 $\pm$ 28.56	73.90 $\pm$ 24.36	77.70 $\pm$ 30.56	73.42 $\pm$ 32.10	68.19 $\pm$ 21.18	76.07 $\pm$ 36.28	NS 0.145	<0.01	NS 0.576
Lipase (U/L)	30.12 $\pm$ 15.17	26.67 $\pm$ 12.76	31.87 $\pm$ 16.07	33.27 $\pm$ 41.87	26.66 $\pm$ 12.28	36.63 $\pm$ 50.46	NS 0.376	NS 0.995	NS 0.372
LDH (U/L)	179.31 $\pm$ 28.77	180.38 $\pm$ 31.51	178.76 $\pm$ 27.50	162.84 $\pm$ 22.08	165.17 $\pm$ 23.22	161.65 $\pm$ 21.56	<0.001	<0.001	<0.001
Iron (μ g/dL)	100.21 $\pm$ 38.57	88.59 $\pm$ 32.22	106.10 $\pm$ 40.37	99.85 $\pm$ 32.32	88.35 $\pm$ 29.10	105.69 $\pm$ 32.51	NS 0.925	NS 0.967	NS 0.933
Calcium (mg/dL)	9.73 $\pm$ 0.33	9.63 $\pm$ 0.32	9.78 $\pm$ 0.33	9.67 $\pm$ 0.34	9.56 $\pm$ 0.34	9.73 $\pm$ 0.34	<0.05	NS 0.113	NS 0.169
Sodium (mEq/L)	141.18 $\pm$ 2.00	141.21 $\pm$ 1.39	141.16 $\pm$ 2.25	141.75 $\pm$ 1.77	141.74 $\pm$ 1.42	141.76 $\pm$ 1.94	<0.05	NS 0.098	NS 0.061
Potassium (mEq/L)	4.47 $\pm$ 0.38	4.49 $\pm$ 0.38	4.46 $\pm$ 0.38	4.53 $\pm$ 0.35	4.50 $\pm$ 0.32	4.55 $\pm$ 0.36	NS 0.180	NS 0.825	NS 0.161
Chloride (mEq/L)	105.31 $\pm$ 2.05	105.91 $\pm$ 1.60	105.00 $\pm$ 2.19	105.52 $\pm$ 2.11	105.97 $\pm$ 1.95	105.30 $\pm$ 2.17	NS 0.300	NS 0.873	NS 0.248
Magnesium (mg/dL)	2.11 $\pm$ 0.13	2.11 $\pm$ 0.13	2.11 $\pm$ 0.13	2.06 $\pm$ 0.14	2.07 $\pm$ 0.14	2.07 $\pm$ 0.15	<0.01	NS 0.051	<0.05
Vitamin B12 (pg/mL)	540.17 $\pm$ 210.59	510.52 $\pm$ 187.00	555.21 $\pm$ 221.42	540.05 $\pm$ 211.02	536.48 $\pm$ 192.73	541.86 $\pm$ 221.10	NS 0.991	NS 0.126	NS 0.353
Folic acid (ng/mL)	9.53 $\pm$ 4.38	9.03 $\pm$ 3.65	9.78 $\pm$ 4.70	9.95 $\pm$ 5.52	9.15 $\pm$ 4.52	10.36 $\pm$ 5.95	NS 0.421	NS 0.847	NS 0.431
Vitamin D (ng/mL)	33.19 $\pm$ 11.56	32.18 $\pm$ 8.68	33.70 $\pm$ 12.81	36.45 $\pm$ 11.27	37.39 $\pm$ 13.36	35.98 $\pm$ 10.13	<0.01	<0.05	NS 0.063
QUALITY OF LIFE									
SF-12									
MCS	74.36 $\pm$ 20.66	76.18 $\pm$ 20.89	73.43 $\pm$ 20.64	75.15 $\pm$ 22.92	75.00 $\pm$ 21.50	75.22 $\pm$ 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 $\pm$ 18.66	84.71 $\pm$ 21.07	89.10 $\pm$ 17.30	88.91 $\pm$ 20.29	87.35 $\pm$ 26.66	89.70 $\pm$ 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 $\pm$ 16.19	80.44 $\pm$ 18.23	81.27 $\pm$ 15.18	82.03 $\pm$ 17.85	81.18 $\pm$ 21.50	82.46 $\pm$ 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 $\pm$ 1.16	2.52 $\pm$ 1.13	2.65 $\pm$ 1.18	2.75 $\pm$ 1.16	2.61 $\pm$ 1.16	2.82 $\pm$ 1.16	NS 0.284	NS 0.712	NS 0.301

^a Number of pathologies/diseases, signs and symptoms related to ARFS [34]. AbR, antibody reactions; BMI, body mass index; BP, body power; f, force; HGS, hand grip strength; HRQoL, health-related quality of life; IPAQ-SF, The International Physical Activity Questionnaire – short form; MCS, mental component score; PAL, physical activity level; PCS, physical component score; PSIMP-ARFSQ-10, Pathologies and symptomatology questionnaire associated with adverse reactions to foodstuffs; RAPA, Rapid Assessment of Physical Activity; RFD, Rate of Force Development in N/s; S-FAQLQ-AF, Food Allergy Quality of Life Questionnaire-Adult Form; SF-12, 12-Item Short Form Health Survey; 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.

3.5.4. General Health

Symptomatology

The PSIMP-ARFSQ-10 symptomatology score was significantly reduced in Group B after the FASSD dietary intervention. All body systems' PSIMP-ARFSQ-10 score significantly decreased in Group B after the FASSD intervention: GI ($F_{1,66}=36.37$; $p<0.001$; $\eta^2=0.036$), skin and subcutaneous tissue ($F_{1,66}=8.59$; $p<0.005$; $\eta^2=0.115$), nervous system ($F_{1,66}=11.02$; $p<0.001$; $\eta^2=0.143$) and respiratory system ($F_{1,66}=7.03$; $p<0.010$; $\eta^2=0.096$), while Group A did not show any statistically significant changes in PSIMP-ARFSQ-10 scores before and after the intervention with the healthy diet.

The amelioration of the symptomatology in subjects with food hypersensitivity showed positive results across all types of symptomatology PSIMP-ARFSQ-10 scores: GI, skin and subcutaneous tissue, nervous system, and respiratory system. In addition, subjects of different physical fitness levels in Group B showed positive symptomatology trends after the FASSD intervention (Figure 3.5.4.1).

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 Group B 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 Group A before and after the ALASKA Study intervention.

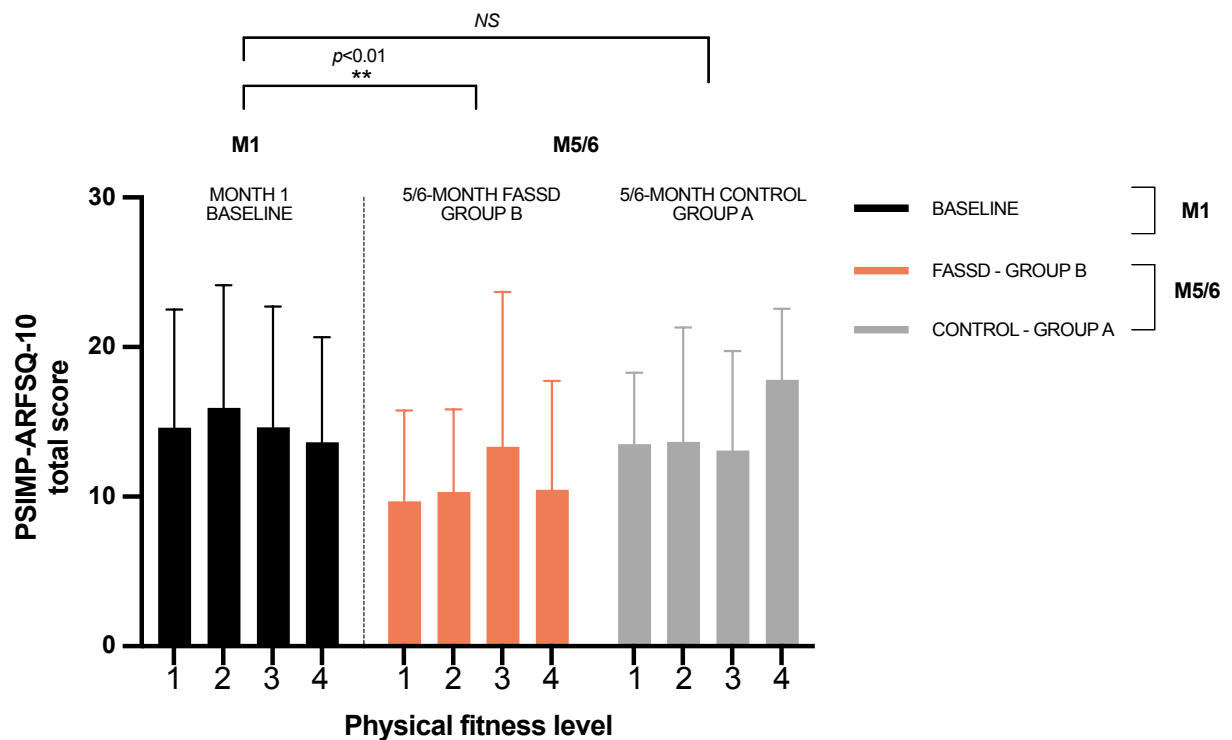


Figure 3.5.4.1 PSIMP-ARFSQ-10 symptomatology scores according to physical fitness level before and after the ALASKA Study intervention

FASSD, food allergen-specific substitutive diet; M1, month 1; M5/6, between month 5 and 6; M5B, visit 2 at month 5/6; NS, not statistically significant; PSIMP-ARFSQ-10, Pathologies and symptomatology questionnaire associated with adverse reactions to foodstuffs.

Hematobiochemical study

Despite all blood biomarkers were inside the normal and expected ranges at the M1 timepoint of the ALASKA Study, there were slight significant improvements in Group B for leucocytes, neutrophils and monocytes biomarkers post-intervention. These parameters had a significant decrease ($p < 0.01$), yet this reduction occurred within the normal value range of the adult population. However, the overall hematobiochemical profile of the subjects in the ALASKA Study following the FASSD intervention could not be considered physiologically different or better, as the values fall within the normal range.

3.5.5. Quality of life

Among the participants belonging to Group B, no significant differences in mental, physical or food hypersensitivity-related QoL were observed before and after the ALASKA Study intervention ($p>0.05$).

3.5.6. Relative risk ratios

In order to further comprehend the risk of developing or ameliorating a positive food hypersensitivity, relative risk ratios were calculated between Group A and Group B of the ALASKA Study intervention, logistic regression models were constructed, adjusting the data using Groups A and B at M1 and M5/6 timepoints. Relative risk ratios were determined to measure how the probability of having positive food hypersensitivity was associated with being exposed to either the FASSD or the standard healthy control diet. Group A had a significantly higher risk of developing both T1FH or T2FH (relative risk=2.55, 95% CI: 1.70-3.84, and relative risk=3.12, 95% CI: 2.16-4.51, respectively), $p<0.001$; while Group B, had significantly lower risk of T1FH or T2FH (relative risk =0.11, 95% CI: 0.04-0.28 and relative risk =0.13, 95% CI: 0.03-0.45, respectively), $p<0.01$).

4. Discussion

4.1. General discussion

This Doctoral Thesis aimed to analyze the phenomenon of ARFS pathogenesis and relate it with physical performance and general health variables. In order to achieve this overall aim, specific goals were established, which were to perform a preliminary study, to design and validate specialized questionnaires, to establish a reliable multifactorial protocol for assessing individuals with ARFS (T1FH, T2FH, LCI and FCI), to analyze the relationship between physical performance and ARFS, and to evaluate the overall health, body composition and physical performance variables of individuals with ARFS before and after a FASSD intervention, as part of the ALASKA Study project.

A comprehensive understanding of the clinical picture of ARFS, a concerning growing issue in global health, is essential for its accurate diagnosis and management. Cataloguing which symptoms can be attributed to food hypersensitivity and FI, as well as identifying which foods are causing the symptoms, is a complicated clinical task because while foods can act as triggers, there are also other factors such as irritants, infections, environmental allergens, and physical condition that can contribute to the severity of diseases, signs and symptoms [27].

In order to effectively evaluate the ARFS population, a first insight into the characteristics of this population was necessary (Article 1 [27]). Characterizing this population was also essential for conducting this Doctoral Thesis to a significant contribution in the area of ARFS and physical fitness. At the time when this study began, there were almost no scientific publications analyzing the ARFS and their relationship with some physical assessment such as body composition, physical fitness or physical performance. Most of the studies were focused

on the relation between these physical assessments and asthma, osteoporosis and mental health [92-94].

Both clinical and self-reporting questionnaires (Article 2 [34]) were necessary for the accurate evaluation of ARFS population. Clinical techniques and molecular methods for the identification of ARFS were available, especially a huge variety of tools for the identification of T1FH which is the IgE-mediated food hypersensitivity or commonly known as FA [24,95]. However, self-reporting questionnaires, which could identify ARFS-related symptomatology and food frequency consumption, by the time that the ALASKA Study began, were limited to pediatric applications, non-Spanish language questionnaires, gluten-related hypersensitivity [96], GI disorders [97] and general food frequency questionnaires [98].

Consequently, at the time when the ALASKA Study commenced, the ARFS management was limited to the diagnosis and clinical assessments regarding its identification, rarely accompanied by physical assessments such as body composition, physical performance or QoL measurements in the same study (Article 3 [5]). This Doctoral Thesis unified general health variables of interest to construct a multifactorial approach for the ARFS management. This multi-variable approach contributed to the investigation regarding ARFS pathogenesis, offering the deserved importance of human physical condition when evaluating ARFS chronic diseases, such as T1FH, T2FH, LCI and FCI.

Once these significant preliminary actions were completed, the ALASKA Study was conducted. The link between ARFS and physical condition was assessed to meet the needs of the lacking information on Spanish ARFS population and their physical condition. A cross-sectional study was conducted after the first month of measurements of the ALASKA Study intervention (Article 4 [43]).

The most prevalent sIgE and sIgG4-mediated ARFS were determined, being legume and egg the most reactive food groups for sIgE and sIgG4-mediated ARFS, respectively.

However, there were significant differences between total mean sIgG₄ AbR of young adults ≤ 45 years of age and those over 45 years of age, being young adults the group with the highest total mean sIgG₄ AbR. Cereal IgE and sIgG₄ AbR were significantly higher in the young adults group. Previous literature has explored the allergenicity of grains and legume, finding that they have increased in prevalence to almost 14% in adults following a plant-based diet, and to nearly 49% of prevalence in the general adult population [99,100].

High physical performance was related to positive immune-mediated reactions, both positive IgE and IgG₄ AbR, and to negative non-immune-mediated reactions, LCI and FCI both negative [43]. Lactose in sports nutrition is known for maximizing the availability of readily oxidizable carbohydrates that could optimize physical performance. Lactose could also play a role in a post-exercise recovery setting, as a vehicle for the delivery of glucose and galactose, for the optimization of muscle and liver glycogen [101]. Similarly, endurance athletes consume high amounts of fructose during physical activity. It has been studied that fructose is a useful energy source for muscles during exercise and that the impact of high fructose diets on metabolism depends on physical activity levels. Research studies have also shown that exercise training can prevent the stimulation of lipogenic genes in the liver caused by a high fructose, FCI or sucrose diet in rats, as well as the development of insulin resistance, high triglyceride levels, and liver steatosis [102].

After conducting the cross-sectional study, the ALASKA Study intervention was carried out from M1 to M5/6 timepoints of the study using a RCT study model. The effect of a FASSD intervention over ARFS, body composition, QoL and physical performance variables was assessed and relative risk-related to the pathogenesis were identified (Article 5 [48]). The main achieved outcome of the ALASKA Study intervention was the improvement of T1FH and T2FH prevalence (sIgE and sIgG₄ AbR) and the PSIMP-ARFSQ-10 symptomatology score in participants of Group B. These progressions were supported by improvements in body

composition and anthropometric measures related to weight, body fat percentage, and waist circumference. Cardiorespiratory fitness and body balance showed improvements even though participants consistently maintained the same physical activity habits, as reported by themselves, throughout the entire five to six-month period of the ALASKA Study intervention [48].

In addition to OIT treatments, which have shown good efficacy towards the amelioration of ARFS, especially for T1FH (sIgE-mediated food hypersensitivity), dietary treatments for the overall ARFS pathogenesis are still being studied. Dietary treatments have been commonly used for T1FH and T2FH, and more frequently for T2FH, where epinephrine is unnecessary (delayed reactions or T2FH), when there is not an immediate adverse reaction, and a special dietary approach can be considered as treatment [16]. Existing dietary intervention studies in both adults and children, have not yet reported proper randomization and they have been recommended to perform direct comparisons of various dietary approaches and increased statistical power [103]. In contrast, the FASSD, studied in Article 5 of this Doctoral Thesis, compared the proposed substitutive diet with a standard Spanish healthy diet [48]. Current dietary approaches have been reported as elemental diets, empiric diets and test-directed elimination diets. Limitations based on costly elemental formulas, adherence and dental problems (due to reduced saliva production) have been reported in elemental diet studies [104]. Similarly, empiric diets have shown scientific limitations when not correctly recording the specific foodstuff (food or beverage) being avoided [105]. Lastly, test-directed elimination diets, which are widely used, have been reported that they may lead to nutritional deficiency issues in either children and adults [88]. On the contrary, the FASSD, developed on this Doctoral Thesis, was designed to include palatable food and beverages, to be personalized controlling each subjects' avoiding foodstuffs, and to meet the minimum macro and micronutrient requirements that may not be met while following an elimination diet [48].

This Doctoral Thesis introduced a new methodological approach to manage ARFS, considering multiple health relevant factors, such as symptomatology, dietary habits, blood parameters, immune response, enzyme concentrations, physical performance variables (cardiorespiratory fitness, hand grip strength, leg power, resting heart rate, body balance and sit-to-stand speed), BMI, anthropometry, body composition and QoL. This research also introduced the initial RCT examining how a substitutive diet, the FASSD intervention, may impact the ARFS pathogenesis in terms of the same mentioned factors of ARFS population. The results of this research offered novel evidence related to the developed ALASKA Study protocol.

4.1.1. Pilot study and variables of influence

ARFS currently represent a growing global health concern [7]. Thus, their attributable signs and symptoms, as well as possible related clinical or physical factors, are of crucial interest for the development of research and evaluation of ARFS. Regarding the preliminary study (Article 1), it was determined that some influencing variables in ARFS population might be age, sex, and the specific body systems involved in the self-reported symptomatology (GI, nervous system, skin and subcutaneous tissue). In a previous study, the evaluation of food and beverages allergen-specific reactions have been previously proposed as an obesity and weight loss therapy [106]. This present study was aligned with the previous findings, revealing that nearly 40% of the population with suspected ARFS with food allergen-specific IgG4 AbR had a normal weight, while over 50% were above the normal weight levels, including overweight and obese individuals. Obesity type I and type II were frequent among the studied participants. However, due to just a few studies regarding this effect [107], this study was required and recommended to clarify, in future research studies, if there were other influencing factors when food and beverages allergen-specific reactions were present [27].

In this first article of this Doctoral Thesis, it was determined that there was a frequent number of symptoms related to ARFS in a group of Spanish adults aged 20 to 79 residing in the Region of Madrid. The self-reported pathogenesis of the sample being studied centered on diseases, signs and symptoms related to skin, GI, and central nervous system, all related to ARFS suspicion. Besides the symptomatology profile, T2FH biomarker, sIgG₄ AbR, was measured. The most common IgG₄-mediated food hypersensitivity in men and women were primarily linked to dairy, wheat, barley, and egg yolk. Differences by sex were found being barley the most prevalent in men and peas and green beans in women. The majority of common allergens align with those identified in earlier research; however, peas and green beans emerged as novel findings in this study involving Spanish adults with the mentioned symptomatology and BMI characteristics. In fact, legume, peas and green beans have been recently reported to be associated with severe reactions and have been described as new important allergen sources [100,108]. As a scientific hypothesis, a case report was published suggesting that there may be a risk of developing this type of legume-based allergy due to new trends, particularly vegan diets rich in substitutive foodstuffs of animal protein [109]. Besides, one of the most prevalent alternatives to gluten-containing cereals in the modern industry is legume-derived food products [110]. Consequently, CeD patients and gluten sensitive individuals might be also at risk of developing this specific legume food hypersensitivity. Actually, it has been reported that deamidation of distinct glutamines in HLA-DQ2 CeD patients, can be mediated by tissue transglutaminase (tTG) and tTG activity has been detected in green peas [111]. As in this Doctoral Thesis work have been found that peas are prevalent allergens in women, further studies are needed to analyze if there is a consistent relationship between this sensitivity and CeD. A noteworthy result of this initial study was that peas and green beans were not part of the current mandatory European allergen declaration list. Currently, there are 14 major food allergens as listed ingredients identified in Annex II of Regulation (EU) No 1169/2011 causing

FA or FI that are mandatory to declare and apply to food business operators at all stages of the food chain, resulting as: cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk, nuts, celery, mustard, sesame, sulphur dioxide and sulphites, lupine, mollusks and all their derivatives [112].

4.1.2. Design and validity of questionnaires for the assessment of adverse reactions to foodstuffs in adults

Following the preliminary study and the observed previous scenario with the first scientific publication of this Doctoral Thesis [27], it had been determined that the self-reported clinical picture played a significant role regarding individuals suspecting of ARFS, as well as their dietary habits. It was evident that two particular questionnaires were necessary to assess Spanish adults with suspicion of having ARFS. The design and validity of the PSIMP-ARFSQ-10 questionnaire involved a score value per each item. The score of this questionnaire currently covers not only one disease and its symptoms but also gathers information on a wide range of current specific diseases, signs, and symptoms associated with ARFS. The determination of each set of diseases, signs, and symptoms of each body system (GI, nervous system, skin and subcutaneous tissue) was based on recent literature [73] and the expert input from Spanish professionals in the field. Diseases, signs and symptoms indicative of FA or FI should be recorded along with the identification of their corresponding clinical biomarkers in individuals with ARFS suspicion. Certain symptoms may occur simultaneously, while certain diseases may also manifest together, either as a result or effect of one another. Hence, it is essential to thoroughly examine the clinical picture, identifying diseases, signs and symptoms when ARFS is suspected for a proper evaluation of the overall pathogenesis [113]. Due to the findings in the second article of the Doctoral Thesis [34], individuals with ARFS suspicion can self-report

diseases, signs, and symptoms using the published PSIMP-ARFSQ-10 symptomatology questionnaire.

Furthermore, the design and validity of the FBFC-ARFSQ-18 was carried out and it was used to identify, not only one self-reported food and beverage source per food group, but rather including a variety of animal or plant sources according to the self-reported food group (e.g. for the “Milk and dairy derivatives food group”, individuals were able to report, not just cow’s milk, but also goat’s milk, sheep’s milk and mare’s or other animal’s milk) [34]. Food frequency questionnaires have been commonly and widely used to evaluate nutrient and type of food consumption in different populations for epidemiological studies and to determine the correlation with diseases like chronic and non-communicable diseases, such as T1FH, T2FH, LCI and FCI [114]. Besides, distinguishing the frequency of food and beverages consumption related to ARFS in individuals with positive T1FH, T2FH, LCI or FCI has been crucial in determining the specific risk factors for these conditions [115]. This food frequency questionnaire has been also used as a tool for discriminating or establishing inclusion/exclusion criteria in the ALASKA Study. In this Doctoral Thesis, it was used to identify the type of allergen source or foodstuffs being consumed by the subjects of study, to exclude participants following vegan or non-Mediterranean dietary habits, and to investigate the correlation between the self-reported food and beverages consumption and the ARFS prevalence.

The findings of this study allowed to establish, in Article 2 [34], that both questionnaires, designed to assess the food consumption, diseases and symptomatology in a Spanish population with suspected ARFS, could be used for research and clinical applications to analyze the association between certain food and beverages consumption with ARFS, such as, T1FH, T2FH, LCI and FCI; also, to investigate the link between some specific diseases, signs and symptoms with ARFS due to their optimal validity values. The FBFC-ARFSQ-18 and the

PSIMP-ARFSQ-10 questionnaires should facilitate research projects and be used together (recommended) with the clinical diagnostic tools of T1FH, T2FH, LCI and FCI.

4.1.3. **ALASKA Study protocol**

The ALASKA Study protocol was developed to address ARFS (specifically T1FH, T2FH, LCI and FCI) in Spanish adults equal or over 18 years old. Performing multiple assessments to evaluate ARFS resulted in a novel multifactorial approach for managing ARFS [5]. These multiple assessments were selected after reviewing the available literature about the key aspects when analyzing ARFS [34], physical performance and the current dietary treatments regarding T1FH, T2FH, LCI and FCI. The final assessments included in this published study protocol were symptomatology, dietary habits, blood parameters, immune response, enzyme concentrations, physical performance variables (cardiorespiratory fitness, hand grip strength, leg power, resting heart rate, body balance and sit-to-stand speed), BMI, anthropometry, body composition, QoL and the FASSD intervention. As a proposed dietary treatment, the FASSD was created as a customized instrument to prevent relevant micronutrient deficiencies that may be triggered by following strict elimination diets [88]. As a proposed dietary treatment, the FASSD was created as a customized instrument to prevent relevant micronutrient deficiencies that may be triggered by following strict elimination diets [88]. The FASSD emphasized important food groups for a healthy diet and focused specifically on vitamins B2, B3, B9, B12, magnesium, potassium, phosphorus, calcium, zinc, iron, and fiber. The ALASKA Study protocol can be utilized in both research and clinical settings to examine and assess general ARFS and explore its connection to associated clinical (symptomatology, dietary habits, blood parameters, immune and non-immune responses) and physical assessments (physical performance variables, BMI, anthropometry, body composition and QoL). In the research

setting, this multifactorial approach can be also used to investigate the association of current positive ARFS to new potential biomarkers linked to food [5].

A high-quality methodology is required to achieve effectiveness in academic and clinical research centers [116]. Especially, protocols describing clinical trials play a crucial role in establishing the safety, appropriate dosages or settings, and effectiveness of new treatments for the studied chronic diseases. A clinical-trial protocol serves as a comprehensive document outlining the objectives, design, methodology, statistical considerations, and overall organization of a study [117]. Research protocols must follow official reference guidelines. Reference guidelines include the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement [85], International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [118], Guideline for Good Clinical Practice (GCP) [119], and reporting guidelines, like the Consolidated Standards of Reporting Trials (CONSORT) [120]. In the European Union, clinical research must be conducted according to a study protocol which includes official guidelines. The ALASKA Study protocol was developed following the SPIRIT recommendations [85].

Once the study protocol was released and published [5], the multifactorial ARFS management and its proposed strategy were incorporated into the ALASKA Study field work. Consequently, a multivariable model was performed to assess ARFS more comprehensively, incorporating key predictor variables and commonly reported influencing aspects in T1FH, T2FH, LCI and FCI, as well as the novel FASSD dietary intervention.

4.1.4. M1 – Cross-sectional study

At M1 timepoint, the analyzed sample of 254 Spanish adults showed demographic homogeneous characteristics, serving as a uniform convenient starting point. Sex and age played a powerful role on determining ARFS and the physical performance of Spanish adults.

When it comes to understanding population effects as well as subpopulation differences, homogeneous convenience samples (i.e., samples that are intentionally limited to specific sociodemographic subgroups and therefore homogeneous on one or more sociodemographic factors) should provide more accurate accounts of population effects and subpopulation differences [121].

In the present study, novel associations were identified among various physical fitness and physical performance levels, age groups, T1FH, T2FH, LCI and FCI biomarker values. Generally, the cardiorespiratory fitness had been only measured together with asthma allergic incidence in previous studies, but data regarding the direct relationship to food hypersensitivity was still lacking until Article 4 of this Doctoral Thesis [43] [122]. The in-dept analysis of the direct and novel relationship between physical fitness tests and the prevalence of certain positive food hypersensitivity reactions is still generally unexplored in the nutrition and sport science fields. In the present study, it was determined that the role that any ARFS may have over physical performance is clearly different depending on the type of ARFS, meaning T1FH, T2FH, LCI or FCI, and the food group involved in the disease. 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 [123]. 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 [124].

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 [125]. In this current research, adults with positive LCI showed significantly reduced hand grip strength values. These results are aligned

with previous findings about the strong correlation between hand grip strength and GI symptoms, enzymatic FI like LCI, and other chronic diseases [126] [127] [128].

This study investigated the relationship between physical performance ARFS in adult population, focusing on associations with specific biomarkers, such as sIgE and sIgG₄ AbR and LCI and FCI. Prevalence rates of ARFS were identified, revealing novel links between physical performance metrics and food hypersensitivity. Results demonstrated that sIgG₄ AbR, primarily linked to T2FH, was more prevalent in the analyzed sample compared to T1FH. Specific food allergens displayed distinct prevalence patterns influenced by age, gender, and physical performance. According to previous studies [99] [129], our findings revealed that adults under 45 years of age exhibited a higher prevalence of sIgE AbR against legume and seeds, while adults over 45 years of age showed broader associations between cardiorespiratory fitness and higher prevalence of sIgE AbR against legume and nuts [130]. Regarding sex differences, sIgE-mediated food hypersensitivity against shellfish and mollusks was higher among women; in the case of sIgG₄-mediated food hypersensitivity this sex differences were positive in 60% of the studied food groups [131]. Physical fitness measures revealed diverse relationships with food hypersensitivity biomarkers across age groups and food categories. Cardiorespiratory fitness emerged as a significant variable in adults over 45 years of age regarding sIgE AbR as differences were found between normal and good physical fitness groups in nearly half of the studied food groups, suggesting its importance in inflammation and overall health assessment. Adults with higher physical performance level showed less FI (LCI and FCI) and more food hypersensitivities (T1FH and T2FH); while those with lower physical performance showed a reverse pattern (more FI and less food hypersensitivities) [132]. Despite these findings, the role of sIgG₄ AbR in the allergic response remains unclear, necessitating further research to clarify its relationship to disease progression and severity. The study also highlighted LCI as a factor negatively impacting handgrip strength, aligning with prior research on the interplay between

FI, GI symptoms and physical performance [133]. Collectively, the findings underscore the intricate connections between food hypersensitivity, physical performance, and allergen exposure, emphasizing the need for comprehensive studies to unravel the mechanisms underlying these relationships.

4.1.5. M5 – Randomized controlled trial

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 [5], on various variables including anthropometric measurements, body composition, physical performance, symptomatology, immunology, hematobiochemical study results, and QoL assessments.

Regarding the ALASKA Study intervention using the FASSD, self-reported symptoms have been also ameliorated in previous research with elimination diets of only the allergen causing the symptoms to the individuals [88]. The gluten-free diet was suggested as an appropriate dietary approach for patients with self-reported gluten/wheat-dependent symptoms [134]. In the present study, all subjects with well-characterized ARFS-related symptomatology were carefully selected before the ALASKA Study intervention commencement. The FASSD dietary intervention was effectively personalized for each participant to substitute their own positive food and beverages allergens, leading to improvements in self-reported symptoms, body composition, body balance and cardiorespiratory fitness due to improvements in ARFS prevalence and adipose tissue [40].

During the past decade, researchers have started to investigate the link between physical performance and ARFS. Diets based on IgG AbR diets have been used by collegiate athletes (19 years old), leading to improvements in run time, concentration, and memory [135];

however, in the present study improvements in body balance and VO₂max were observed. During the past decade, researchers have started to investigate the link between physical performance and ARFS. Diets based on IgG AbR diets have been used by collegiate athletes (19 years old), leading to improvements in run time, concentration, and memory [135]; however, in the present study improvements in body balance and VO₂max were observed.

Regarding anthropometry and body composition evaluation, statistically significant group x time interactions were observed in body weight, body fat mass, percentage of body fat mass, and waist circumference before and after the FASSD intervention. However, there were no significant changes in anthropometric and body composition variables before and after intervention in the Group A. Murine models have shown that individuals with a positive food hypersensitivity generally have higher body weight and epididymal adipose tissue mass [40]. Since T1FH and T2FH levels have dropped in Group B following the FASSD intervention, it is expected that body weight, body fat mass, and waist circumference will also decrease. Likewise, alterations in muscle mass are usually anticipated with high protein diets and/or exercise programs; thus, as predicted, there was no significant change in muscle mass throughout the duration of the ALASKA Study intervention.

Food hypersensitivity and other types of ARFS commonly manifest with GI symptoms in approximately half of diagnosed patients [27,136]. [27,136]. 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 [137]; however, healthy blood biomarkers can be affected over time and nutritional deficiencies can affect the overall health of individuals with food hypersensitivity. It has been observed that the symptomatology related to ARFS can be improved after dietary exclusions [137]; however, healthy blood biomarkers can be affected

over time and nutritional deficiencies can affect the overall health of individuals with food hypersensitivity [47]. During long-term exclusion or substitutive diets lasting months, regular fluctuations in blood biomarkers such as glucose and LDL-cholesterol may occur periodically [138]. During long-term exclusion or substitutive diets lasting months, regular fluctuations in blood biomarkers such as glucose and LDL-cholesterol may occur periodically [138]. While exclusion diets have been linked to nutritional deficiencies and negative nutrient blood markers [88], it was noted as a positive outcome that none of the measured blood markers worsened following the FASSD dietary intervention [48].

The main international societies in the field of food hypersensitivity management have released evidence-based guidelines covering mostly T1FH drug treatments and immunotherapies [139]. 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 [140]. [140]. However, negative effects of elimination diets have been reported, particularly those related to nutrient deficiencies [88]. In the present study, where a substitutive dietary intervention was applied, subjects decreased their sIgE AbR and sIgG₄ AbR, which are T1FH and T2FH biomarkers, respectively.

Although SF-12 scores are typically expected to fluctuate over time, the ALASKA Study Group B did not show significant enhancements before and after the FASSD intervention. QoL improvement was also expected, given that a specific food hypersensitivity tool was used to evaluate food hypersensitivity-related QoL. Dietary interventions are mostly expected to influence QoL of individuals when weight loss intention is involved [141]. 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 or worsened after the FASSD intervention. Their starting

point might not be clearly different from their end point regarding emotions, mental or physical perceptions towards food hypersensitivity. Previous review studies suggest that physical therapy treatments could have a significant impact on QoL, except when certain pathologies are present [142,143]. Further research is needed to identify the influencing factors that could have a significant effect on the QoL of individuals with food hypersensitivity.

The relative risk ratio of developing any of the studied food hypersensitivity was significantly different between Group A (following a standard healthy diet) and Group B (following the FASSD), being lower in the latter [48].

This RCT evaluated the impact of the FASSD, as outlined in the ALASKA Study protocol, on adults with suspected ARFS. The intervention aimed to personalize dietary adjustments by replacing allergens with suitable alternatives, resulting in improvements in body composition, body balance, cardiorespiratory fitness, symptomatology and food hypersensitivity prevalence. Significant reductions in body weight, body fat mass, and waist circumference were observed in Group B, attributed to decreases in T1FH and T2FH biomarkers; while muscle mass remained stable, aligning with predictions for substitutive diets [144], the FASSD intervention successfully reduced food allergen-specific antibody responses (sIgE for T1FH and sIgG4 for T2FH), suggesting the FASSD diet's efficacy in mitigating ARFS symptoms without adverse effects on body composition. In contrast, Group A did not show any body composition improvements.

Symptomatology related to ARFS, including GI, skin, respiratory, and nervous system symptoms, improved following the FASSD intervention. Unlike elimination diets often associated with nutrient deficiencies [88] [21], the FASSD maintained stable blood biomarkers, supporting its long-term viability.

QoL measures, such as SF-12 scores, did not show any significant changes, likely due to the participants' pre-existing health conditions. This suggests that while dietary intervention effectively addressed physiological factors, emotional and mental health perceptions were not substantially influenced.

The relative risk of developing food hypersensitivity was significantly lower in Group B compared to Group A, highlighting the potential of tailored dietary approaches like FASSD in managing ARFS while minimizing negative health impacts.

4.2. Strengths and limitations

Strengths

Notable differences in physical fitness and exercise levels have been observed among adults depending on their race and income [145,146]. The current study focuses on a well-defined group of Caucasian Spanish adults, the majority of whom are employed (>80%) and have attained a university degree or higher (>70%). 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 designed and validated specific questionnaires for the accurate analysis of ARFS population before the recruitment of volunteers.

Data collection and clinical analysis of the pilot study were performed before the COVID-19 pandemic. This condition benefited the uniformity of the sample and has allowed analysis of statistical differences between self-reported symptoms and self-reported physical activity before and after the pandemic. 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 SARS-CoV-2 infection may still have

persistent signs and symptoms, radiological abnormalities, and compromised respiratory function [147].

This study proposes a novel methodological protocol for the evaluation and management of ARFS considering a variety of influencing factors such as the symptomatology, food and beverages intake, hematobiochemical, immunological (T1FH and T2FH) and non-immunological makers (LCI and FCI), anthropometry, body composition, physical performance and QoL.

This study proposes the first RCT analyzing the effect of a dietary intervention consisting of a substitutive diet (FASSD) in adults with suspected ARFS.

The findings of this Doctoral Thesis work provided evidence on novel explored relationships involving ARFS and physical performance.

Limitations

One of the limitations of the preliminary study of this overall work was that it started as a standard clinical consultation, in which patients were first enrolled without considering inclusion/ exclusion criteria as individuals suspecting of having ARFS. Following the clinical consultation that revealed intriguing findings, it was subsequently modified to fit the scientific method. In this preliminary study, data analysis was only performed to individuals accomplishing the established inclusion/ exclusion criteria in the ALASKA Study protocol. While this represents a limitation of the study, it also offers an advantage since patients were meticulously monitored and precisely assessed, aiming for data suitable for publication.

Regarding the design and validity of both questionnaires, PSIMP-ARFSQ-10 and FBFC-ARFSQ-18, the main limitation was that they were validated only for adults aged 18 to 77, for Spanish-speaking application and for the dietary preferences and types of food and

beverages consumed mostly in the Mediterranean area. However, it works also as an advantage to have such a specific tool for the Mediterranean adult population with suspected ARFS.

The ARFS biomarkers analyzed in this Doctoral Thesis work were focused on food hypersensitivity (T1FH and T2FH) and FI (LCI and FCI). There might be other interesting biomarkers associated to ARFS. Thus, when analyzing other types of ARFS, apart from food hypersensitivity 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. However, this limitation might also remain as an advantage because all the proposed assessments in the published multifactorial study protocol can be measured together with other ARFS biomarkers of interest.

Participants in the ALASKA Study mostly accomplished the WHO current physical activity recommendations. It would be interesting to perform the same analysis in population who self-report that they do not accomplish the WHO current physical activity recommendations.

Finally, almost half of initial subjects' collected data couldn't be included in the final data analysis for multiple reasons (participant dropout, wave non-response, item non-registration by the researcher, item non-response by the participant and loss to follow-up participants). However, in the published ALASKA Study protocol it was anticipated that the missing rate would be elevated. Therefore, it is recommended that future studies consider a greater initial recruitment of participants or, alternatively, design an accurate strategy, apart from a monthly adherence questionnaire, to enhance adherence.

4.3. Future research lines

Results indicate intriguing patterns of food-specific IgE and IgG₄ antibody responses related to body composition, physical performance, sex, age, and symptomatology. Future research of the same kind will be considered and the possible measurement of more health-related biomarkers such as microbiota determination, genetic analysis, other immunological biomarkers such as TG2, DGP, gliadins, mTG, and mTG neo epitopes and other ARFS-related diseases such as CeD and non-coeliac gluten sensitivity (NCGS). Further research is needed to investigate the precise role of sIgE and sIgG₄ AbRs in ARFS and physical fitness. Since food hypersensitivities are common in adults, there is a need to further explore the potential benefits of dietary interventions like the FASSD on the analyzed physiological variables. Dietary patterns and the type of exercise or sport are also important aspects to be considered for future research studies regarding ARFS.

Future research on ARFS based on the ALASKA Study protocol could be performed considering other age groups, such as children, adolescents, elderly; geographical areas, or dietary patterns.

4.4. Conclusions

4.4.1. Conclusion 1

The most frequent ARFS self-reported signs and symptoms, in Article 1, were related to skin and subcutaneous tissue, GI, and nervous system. Age and sex were influencing variables of contrast when analyzing ARFS.

4.4.2. Conclusion 2

The PSIMP-ARFSQ-10 (Pathologies and symptomatology questionnaire associated with adverse reactions to foodstuffs) and the FBFC-ARFSQ-18 (Food and Beverages Frequency Consumption Questionnaire to Identify Adverse Reactions to Foodstuffs) questionnaires were designed and validated for ARFS pathogenesis (diseases, signs and symptoms), in Spanish language, for adults and for the Mediterranean population dietary characteristics. Both questionnaires are current tools that can be used when evaluating and/ or investigating FA, FI, CeD, NCGS or any other ARFS on Spanish adults.

4.4.3. Conclusion 3

The ALASKA study protocol has been created as a global approach to assess and manage ARFS, especially T1FH, T2FH, LCI and FCI, in adults over 18 years old. Utilizing various assessments for ARFS, including factors like symptomatology, food and beverages consumption, hematobiochemical and immunological evaluations, enzymatic activity, physical fitness, physical performance, anthropometry, body composition and QoL signifies a comprehensive new approach to ARFS management. The FASSD has been developed as a personalized tool for ARFS adult Spanish population to prevent significant micronutrient deficiencies that a strict avoiding diet or eliminating food and beverages products could cause. The FASSD identifies key food groups necessary for a balanced diet and emphasizes the importance of some micronutrients, such as vitamin B2, vitamin B3, vitamin B9, vitamin B12, magnesium, potassium, phosphorus, calcium, zinc, iron, and fiber. The ALASKA study protocol can be currently utilized in both research and clinical settings to explore and assess general ARFS, as well as to analyze its association with related diseases, symptoms, dietary

intake, physical fitness, physical performance, anthropometry, body composition, QoL and other innovative potential food-specific biomarkers.

4.4.4. Conclusion 4

Age group (equal or under 45 vs over 45 years) is a determining influencing factor between adults scoring at least 6 points in the PSIMP-ARFSQ-10 associated to food hypersensitivity (T1FH and T2FH). Younger adults equal or under 45 years of age presented a higher prevalence of food-specific sIgE AbR positivity compared to adults over 45 years. The most self-reported pathologies/diseases, signs and symptoms in both age groups were the ones belonging to the GI system. FCI was associated with higher ARFS GI symptoms than LCI. Excellent cardiorespiratory fitness level was associated with less of both positive LCI and FCI prevalence in Spanish adults with ARFS symptomatology.

Adults up to 45 years had the most prevalent sIgE-mediated or T1FH against legume. Analogously, adults older than 45 years of age presented the most prevalent sIgG₄-mediated or T2FH against legume. Total positive sIgG₄ AbR was associated with higher values of physical performance, being significantly related with higher values of BODY BALANCE, compared to negative sIgG₄ AbR. Non-immune-mediated reactions or FI were linked to lower values of physical performance; specifically, LCI was inversely associated with maximal hand grip strength. The probability of developing total positive sIgE immune-mediated reactions or T1FH 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 odds of developing ARFS in high physical performance adults.

4.4.5. Conclusion 5

The ALASKA Study intervention had more positive effects in body weight, body fat mass, waist circumference, cardiorespiratory fitness and body balance of subjects following the FASSD than those under the healthy control diet. The FASSD greatly influenced the PSIMP-ARFSQ-10 symptomatology score, improving all GI, skin, nervous, and respiratory symptoms in individuals with T1FH and T2FH. The FASSD also had a direct positive effect on the immunological biomarkers of IgE and IgG₄, leading to improvements in T1FH and T2FH in participants of the intervention following the FASSD. The ALASKA Study intervention did not play a significant role in HR-QoL or FH-related QoL aspects of individuals with FH. There is a significant higher risk of developing either positive T1FH or T2FH in subjects following the control diet than those following the FASSD. Further research studies are still needed to clarify this phenomenon, the relationship between physical performance related variables and ARFS.

4.4.6. General conclusion

This Doctoral Thesis comprehensively addresses the prevalence, evaluation, management, and intervention strategies in adults with suspected ARFS. The main findings highlight the importance of age, sex, cardiorespiratory fitness and physical performance in understanding the pathophysiology and prevalence of immune-mediated (T1FH and T2FH) and non-immune-mediated (LCI and FCI) ARFS. The study successfully developed and validated tools such as the PSIMP-ARFSQ-10 and FBFC-ARFSQ-18 questionnaires, tailored to assess ARFS pathogenesis in the context of the Mediterranean dietary pattern.

A multi-factorial management protocol, the ALASKA Study, was established, incorporating clinical assessments, physical fitness measures and a substitutive diet proposal,

the FASSD. This protocol, once evaluating the effects of the FASSD, demonstrated significant benefits in improving ARFS symptoms (skin, GI, respiratory and nervous system-related health), body composition, and immunological markers, particularly IgE- and IgG₄-mediated responses. The FASSD intervention neither reduced nor increased the prevalence of micronutrient deficiencies often associated with restrictive diets compared to a standard Spanish healthy diet.

The study found that younger adults exhibited a higher prevalence of IgE-mediated reactions, whereas older adults were more prone to IgG₄-mediated responses, with legumes being the most commonly implicated food group.

Physical performance played a crucial role, with higher levels being linked to increased immune-mediated ARFS. After following the FASSD during five to six months, subjects with higher physical performance levels showed a better improvement of symptoms.

Overall, the ALASKA Study protocol and FASSD intervention represent significant advancements in ARFS management, offering a structured approach to address food and beverages substitutions and enhance overall health. Future research is necessary to explore the interplay between ARFS, physical performance, and QoL, and to refine these tools for broader applications in clinical and research settings.

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