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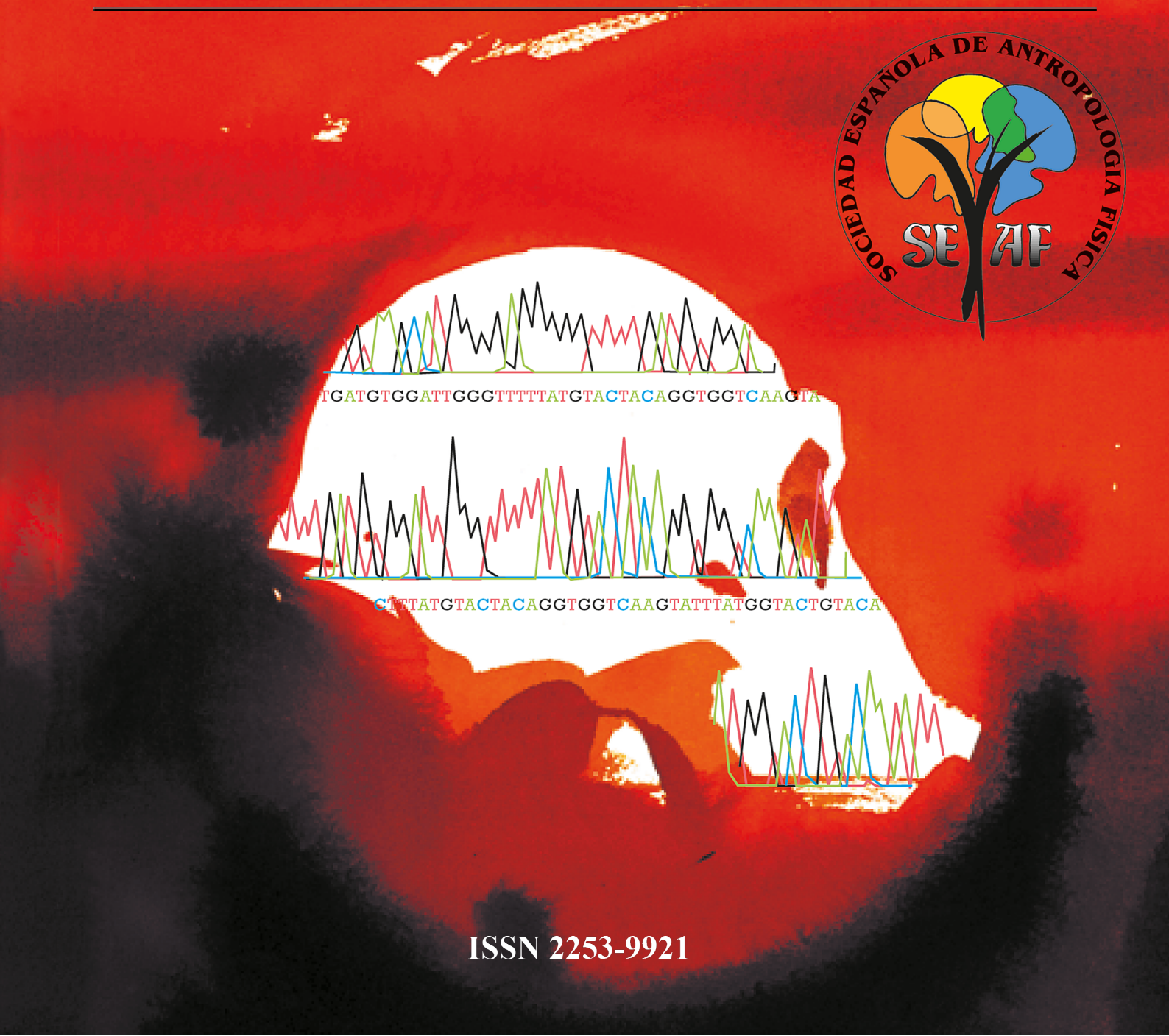

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Dr. Luis Alberto Vargas. De viajes, música y placeres. Un recorrido por los divertimentos antropológicos

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Luis Alberto Vargas Guadarrama nació en México D.F. en enero de 1941. Fue médico cirujano por la UNAM, antropólogo físico por la Escuela Nacional de Antropología e Historia, y doctor en Biología, especializado en Antropología por la Universidad de París. Investigador titular C en el Instituto de Investigaciones Antropológicas de la Universidad Nacional Autónoma de México, donde fue secretario académico durante once años y director durante cuatro.

Durante 23 años impartió los contenidos de Antropología de la Salud de la materia Historia y Filosofía de la Medicina en la Facultad de Medicina de la UNAM, así como Teoría de la Antropología Física y Antropología del proceso biocultural alimentación-nutrición en la Maestría en Antropología de la UNAM. En esa misma Maestría ofreció materias donde relacionaba la Antropología con la alimentación y con la salud. También formó parte del grupo de profesores de la *Università di Scienze Gastronomiche en Pollenzo y Colorno*, Italia. Durante 10 años enseñó de Antropología en la Maestría de Arquitectura para la Facultad del Hábitat de la Universidad Autónoma de San Luis Potosí (México).

Miembro del Sistema Nacional de Investigadores con el nivel III, de la Academia Mexicana de Ciencias y de la Academia Nacional de Medicina, fue durante cinco años presidente de la Unión Internacional de Ciencias Antropológicas y Etnológicas y del *XII International Congress of Auxology*. Participó activamente en 17 sociedades científicas. Entre las tesis que dirigió contó con 13 de licenciatura, dos de especialidad, 18 de maestría y más de 20 de doctorado, donde sus tutorados abordaron temas como: la medicina indígena, la alimentación, primatología, antropología forense, etc., realizando investigaciones sobre identificación humana, ergonomía y antropología del trabajo y estudios sobre la vejez, por mencionar algunos.

Fue investigador visitante en *Michigan State University* y profesor en la *State University of New York* en Albany, e impartió seminarios, cursos y talleres en la universidad Autónoma de Madrid, de Granada, de Córdoba, y de Zaragoza, en España, así como las italianas de Perugia, Bolonia, y la boliviana de Potos, de ahí el título de este texto "***de viajes***". Fue Asesor en Nutrición de la Organización Panamericana de la Salud, con sede en Washington D.C., trabajó en Latinoamérica entre 1985 – 1987, y fue responsable de la Unidad de Educación Médica Continua de la Facultad de Medicina de la UNAM de 1991 a 1993.

Sus áreas de trabajo fueron diversas: en la antropología física realizó estudios sobre crecimiento, desarrollo humano y osteología; en la antropología aplicada estudió el proceso de alimentación-nutrición, en los campos de evaluación antropométrica analizó el estado de nutrición y el papel de la cultura en la alimentación; en la antropología del trabajo se ocupó en particular del campo de la ergonomía; también abordó la antropología del proceso salud-enfermedad y el estudio antropológico del proceso de envejecimiento. En el campo de la divulgación científica intervino en diversas actividades destinadas al público general, entre las que destacan sus participaciones en radio y televisión, tanto en México como en el extranjero, iniciadas en 1975, presentando los avances de las disciplinas en las que colaboraba. Fue miembro de los comités editoriales de Cuadernos de Nutrición, ¿Cómo ves?, Estudios de Antropología Biológica, Antropología Física latinoamericana, Etnobiología, Anales de Antropología, y Gaceta Médica de México, por mencionar algunas.

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Su vida, estuvo llena de anécdotas interesante, profundas y científicamente conmovedoras. Sus primeros atisbos de la Antropología Física se debieron a la amistad de su padre y el resto de su familia con el Dr. Eusebio Dávalos Hurtado, el primer antropólogo físico titulado en México, quien además era médico homeópata y fue director del Museo Nacional de Antropología, y durante varios sexenios director del INAH.

A ello, el Dr. Vargas me comentó:

> …Cuando el Dr. Dávalos era director del Museo Nacional de Antropología en Moneda lo visitó mi padre para pedirle piezas arqueológicas prestadas para montar una exposición para un congreso de radiología que se llevaría a cabo en el aún no inaugurado Conservatorio Nacional de Música. Dávalos explicó que esto no era posible, pero se interesó en los conocimientos de mi padre y, desde entonces, se hicieron grandes amigos y colaboradores, estudiando restos humanos con lesiones. De ahí surgió la relación con el mundo antropológico que me tocó a mí por continuidad. Poco después se inició la amistad de mi madre con Conchita, la esposa de Dávalos y con sus seis hijas, que eran menores que mis hermanos y yo, lo que duró hasta la muerte de mi madre…
> …Los Dávalos iban con frecuencia a nuestra casa y en ocasiones los acompañaban Jorge Gurría, Gonzalo Obregón y otra serie de personajes y se armaban grandes tertulias a las que acudíamos los de menor edad. Entre ellos estaba un huraño muchacho cuya madre pidió a la mía que lo invitara a nuestra casa para que tuviera trato social. Era y es Eduardo Matos, quien pasó la transición de niño a adolescente y joven en nuestra casa. Ha afirmado en varias ocasiones que su vocación de arqueólogo surgió por el préstamo por mi padre del libro "Dioses, tumbas y sabios". Por influencia de Dávalos se hizo arqueólogo, mientras yo estudiaba medicina, pero nos acompañábamos a nuestras respectivas escuelas con frecuencia. Recuerdo el momento en que un sábado llamó Dávalos a la casa buscando a Eduardo y le otorgó el nombramiento de Subdirector de Monumentos Prehispánicos bajo el Arqueólogo D. Ignacio Marquina, a quien veíamos como un hombre muy anciano. El Dr. Dávalos fue mi profesor en la ENAH y me ofreció la beca para hacer el doctorado en París. Daba la clase en la Dirección del INAH a los muy escasos alumnos avanzados de Antropología Física …
> …Un recuerdo valioso fue cuando acompañamos a Dávalos para preparar el gran proyecto en Teotihuacán. Fuimos con Piña Chán y recuerdo su gesto de ponerse el dedo doblado sobre la nariz y decirle a Dávalos donde ubicar el espacio inicial de bienvenida, museo y cafetería, al estar seguro de que no tenía construcciones valiosas. Al final le dijo: "*Eusebio, ya que me haces dueño de la pelota, me reservo la Ciudadela para explorarla yo*…"
> …Una historia adicional, fue cuando mi padre y su amigo Manuel Monroy buscaron el apoyo del Dr. Eusebio Dávalos y se dedicaron a organizar conciertos en Tepotzotlán (antes de que hubiera supercarretera a Querétaro), la Catedral, el Castillo de Chapultepec con obras como El Mesías, la Misa en Si de Bach o la de Beethoven. Cuando falleció el Dr. Dávalos, como homenaje, se interpretó el Réquiem de Mozart…

Durante su estancia en Europa falleció Eusebio Dávalos, lo que truncó lo que hubiera sido una fructífera colaboración.

Los inicios…

En el actualmente llamado Colegio Ciudad de México la trayectoria del Dr. Vargas fue larguísima. En 1947 inició la primaria en el *Mexico City School*. Cuando terminó, sus padres decidieron que la escuela requería una secundaria y se reunieron para hacerla y fue de su primera generación; al año siguiente inauguraron el espacio de la nueva en Campos Elíseos 139, donde está ahora, frente a una glorieta con una fuente.

Al respecto el Dr. Vargas me platicó…

> …Mis hermanos también estuvieron ahí, así como mis hijos y ahora los nietos. Fui profesor de Anatomía unos 20 años, lo que explica a muchos de mis conocidos y amigos. Al mismo tiempo fui

> miembro del Consejo Académico de la escuela. Cuando llegué a la Dirección del IIA me retiré de eso, pero ahora nuestra hija Lety es una especie de eminencia gris ahí, ya que es la persona de total confianza de la directora y deshace entuertos, apoya a la administración y organiza todo lo que se te ocurra, aunque formalmente su cargo es organizar las reuniones de exalumnos suspendidas por la pandemia…

Desde muy pequeño fue melómano y asistía con sus padres a Bellas Artes y a los conciertos de la UNAM en el Teatro Metropolitan.

El Dr. Vargas continúa…

> …Tengo las notas de mi madre sobre los impresionantes solistas y directores que escuché entonces, sin estar muy consciente de ello. Ya en Medicina, durante el tercer año (1960) y estando en el Hospital General nos organizábamos todos los viernes para salir corriendo de clase con el Dr. Ruy Pérez Tamayo para ir al tercer piso a los conciertos. Para entonces, ya me acompañaba Lety. Durante el intermedio bajábamos al primer piso para conversar con Ruy y con mi padre sobre lo escuchado. Con frecuencia mi padre nos llevaba a merendar al Hotel del Prado. Pero lo mejor era después, ya que, por estar en el Hospital ABC como radiólogo, mi padre recibía como pacientes a músicos nacionales y de fuera. Un hecho inolvidable fue haber conocido a Pina Carmirelli y meses después, ser guiados por ella en su auto para conocer Roma. En esos tiempos entró como gerente de la orquesta Enrique F. Gual, gran amigo de la familia, quien después fue director del Museo de San Carlos y había sido en España amigo de Picasso, Dalí, García Lorca, Buñuel y otros. Las visitas a Bellas Artes se enriquecían con la prima de unos amigos cercanos, quien estudiaba chelo. Con ella conocimos y tratamos a varios integrantes de la orquesta…

Durante el primer año de sus estudios en la UNAM fue alumno del llamado Grupo Piloto, en el que se impartió una enseñanza muy selecta, donde se incluyó un curso de Antropología Física impartido por Juan Comas y Santiago Genovés.

Durante esta época, el Dr. Vargas me comentó…

> …Estuve cercano al grupo de fisiólogos por un gran maestro: Alberto Guevara Rojas, gran amigo de mi padre, quien antes de entrar a la carrera me envió a su laboratorio de electrolitos en el Hospital General. Esas determinaciones se hacían a mano y tardaban horas. Uno de los pasos era agregar gotitas de ácido a la muestra para que se notara un leve cambio de color. Yo me equivocaba de todas todas, sin entonces saber que era daltónico. Años después se lo comenté a D. Alberto y se puso furioso. Comentó que era un mal fisiólogo al no haber considerado esa posibilidad y pensar que yo era simplemente descuidado…

Durante su formación académica inicial estudió Anatomía en el libro inglés de Hamilton, donde colaboraron y le otorgaron un enfoque a la materia, distinguidos antropólogos físicos británicos. En esos años su padre y Dávalos habían colaborado haciendo estudios de restos humanos depositados en el INAH, con el propósito de identificar alteraciones patológicas. Colaboró con ellos Arturo Romano y el Dr. Vargas padre fomentó en la Dirección de Antropología Física el uso de rayos X y al final de su vida fue brevemente profesor de Paleopatología en la ENAH. Más tarde tuvo la ventaja de ingresar como estudiante al Servicio de Medicina Interna del Hospital General de México, dirigido por Fernando Martínez Cortés, quien había sido secretario de la Facultad de Medicina y por cuya iniciativa se enseñó antropología física en el Grupo Piloto, y era además amigo de Juan Comas y vecino de Johanna Faulhaber, con quien se turnaba para llevar a sus hijos por las mañanas al Colegio Alemán.

El Servicio de Medicina Interna se caracterizaba por enseñar una medicina con enfoque humanístico y se trataba a los pacientes como personas y no como objetos. A Luis Alberto le inquietó el que, a pesar del tratamiento afectuoso y efectivo para los pacientes, muchos volvían a ingresar al Hospital en peores condiciones. Esto era frecuente entre quienes padecían de cirrosis hepática causada por su mala alimentación y el consumo excesivo de alcohol. Su tesis profesional fue un manual educativo para los pacientes. La mayoría de ellos expresaba ideas

relativas a la salud y la enfermedad que eran muy diferentes a las que mostraban los libros médicos. Inquieto por esta situación, al final de su formación médica y antes de graduarse, ingresó en 1965 a la Escuela Nacional de Antropología para cursar la carrera de Antropología Física. Al mismo tiempo daba clases de Anatomía en una Preparatoria. En la Antropología buscaba conocimientos y recursos para comprender mejor a sus pacientes y ello le llevó a estudiar el contexto de la alimentación de los mexicanos, así como la manera como atienden sus problemas de salud, lo que ha constituido dos de los ejes de su trabajo académico.

En la ENAH tuvo como maestros a Johanna Faulhaber, Arturo Romano, Javier Romero, Felipe Montemayor, María Teresa Jaén y a Eusebio Dávalos, quienes le ofrecieron una visión amplia de la disciplina y en particular Romero, Faulhaber, Montemayor y Dávalos le interesaron en dedicarse a los estudios de personas vivas, aunque por la influencia de Romano y Jaén incursionó en la osteología. Uno de sus primeros trabajos fue el estudio del esqueleto de una persona quien tenía la pierna dentro de grilletes, lo que le llevó a entender el contexto biológico, social y cultural de dicha práctica. Durante esta etapa obtuvo el título de médico.

Por propuesta de Eusebio Dávalos, obtuvo una beca para estudiar el Doctorado en Antropología Física en Francia, antes de obtener la Maestría en la ENAH. En París se incorporó al laboratorio de Georges Olivier y tuvo cercanía con quien sería el director de su tesis, Jean Hiernaux y recibió conocimientos prácticos de Nicole Petit Marie y Françoise Demoulin. Al principio de su estancia se entrevistó con el decano de los profesores franceses, Henri Victor Vallois quien había sido el profesor de Dávalos y, aunque lo trató poco, tuvo admiración por Eugène Schreider, quien había realizado trabajos de fisiología antropológica entre los otomíes. Visitó el laboratorio de ergonomía de Alain Wisner que le causó gran impresión. Inició los estudios de doctorado en la Facultad de Medicina, pero poco después el laboratorio cambió su sede a la Facultad de Ciencias en la recién inaugurada Torre *Jussieu*.

Tuvo la oportunidad de cursar un Diploma de Estudios con Profundidad en el Laboratorio de Seres Organizados de la Universidad de París, donde tuvo como maestro al célebre paleontólogo Jean Piveteau, y realizó trabajo de campo con el fin de constatar cambios de la composición de las poblaciones en una pequeña zona cercana a París y conocer a destacados biólogos evolucionistas. Su tesis fue sobre los cambios en el color de la piel de la cara interna del brazo, la frente, el pezón y la línea entre el pubis y el ombligo en un grupo de mujeres jóvenes, donde demostró cambios de coloración solamente perceptibles por un reflectómetro, pero que permitieron identificar, por su brusco cambio, el momento de la ovulación.

De esta época el Dr. Vargas recordó…

> …La historia de lo de la pigmentación es curiosa. En París tomaba clase con un *maestrazo*, Jean Hiernaux, quien había medido color de piel en África. Nos comentó que la curva de la población era curiosa, unimodal en los hombres y bimodal en las mujeres. Leticia estaba embarazada y yo tenía un ejemplo vivo de los cambios de coloración durante el embarazo y sugerí que ello podía deberse a diferencias en la producción de melanina o del flujo de sangre en la piel durante el ciclo menstrual. De ahí surgió mi interés. Quien realmente registró los datos fue Lety, ya que tenían que ser mujeres y ella las tenía a la mano en la escuela de Pediatría Social donde estudiaba. Desde luego mis amigos mexicanos se ofrecían de ayudantes para sostener los pechos mientras medíamos la coloración del pezón. Recuerda que en esos tiempos no había computadoras y nuestra gran envidia era la calculadora eléctrica del Prof. Georges Olivier ¡donde se podía calcular la raíz cuadrada! Nunca publiqué los hallazgos al no estar seguro del análisis estadístico. Compramos un reflectómetro en México y las lecturas eran aberrantes. Lo llevé al entonces Centro de Instrumentos y encontraron que la falla se debía a la inestabilidad del voltaje de la red en México. Entonces no había reguladores de voltaje. Sin embargo, eso me abrió amistades muy valiosas…

La tesis fue aprobada y obtuvo el Doctorado en Biología en el campo de Antropología, y al final de su estancia en Europa fue becado para realizar una estancia de un mes en Londres. Su interés en el estudio de personas vivas

le llevaron a entrevistarse con destacados antropólogos británicos, entre ellos Don Brothwell, Joseph S. Weiner y, sobre todo, Otto Edholm quien había publicado un libro sobre la Biología humana y el trabajo.

Buscando aspectos novedosos para llevar a cabo su tesis para la ENAH buscó a Caroline Berry del Museo Británico de Historia Natural de Londres, quien había hecho interesantes estudios sobre los caracteres discontinuos en el cráneo de Londres, quien había hecho interesantes estudios sobre los caracteres discontinuos en el cráneo de ratones que podrían aplicarse a los humanos.

De regreso a México, continuó colaborando por un año en la Sección de Antropología Física del Museo Nacional de Antropología, actividad que hacía por las tardes, ya que por las mañanas colaboraba con el Departamento de Anatomía de la Facultad de Medicina para poco después (1971), y por intervención de Juan Comas, ser contratado en la Sección de Antropología del Instituto de Investigaciones Históricas cuyo director era Miguel León Portilla y Alfredo López Austin el secretario académico.

Al no poder ocupar varios cargos simultáneamente, fue sustituido por su esposa, la Dra. Leticia Esthela Casillas en el Departamento de Anatomía, con lo que iniciaron una intensa actividad académica conjunta. En ese tiempo publicó con Eduardo Matos dos trabajos de corte etnohistórico, uno sobre las representaciones de las anomalías de los pies y otro sobre el embarazo y el parto en Mesoamérica. Por consejo de Juan Comas hizo su tesis de Maestría en Antropología Física en la ENAH sobre los caracteres discontinuos en cráneos de Tlatilco. El maestro buscaba que fuera plenamente reconocido como colega en México y no como un médico interesado en la Antropología. En ese tiempo fue invitado a colaborar con la Procuraduría General de Justicia del Distrito Federal en tareas forenses, de lo que resultó un artículo sobre superposición radiológica del esqueleto del cráneo con fotografías de las supuestas víctimas. Este campo no le interesó y recomendó que lo asumiera Arturo Romano, quien ahí realizó una valiosa línea de trabajo. En el Departamento de Anatomía estudió el dimorfismo sexual en fémures resguardados allí. Pero se dio cuenta lo valioso que era contar con los estudiantes universitarios para hacer estudios sobre crecimiento y nutriología, y sobre aplicaciones ergonómicas. Surgieron así varios trabajos con Leticia E. Casillas y otros colaboradores sobre aspectos antropométricos de los estudiantes, pruebas fisiológicas, datos para el diseño de mobiliario escolar desde el jardín de infancia hasta la universidad, y técnicas de campo para valorar el crecimiento y estado de nutrición de menores. La contribución más importante de esta etapa fue la publicación de las tablas de peso para jóvenes de distintas estaturas, ya que se sabe que esta edad es la de referencia para el resto de la vida. Las tablas fueron reproducidas por el IMSS y un resumen era publicado cada año en el Diccionario de Especialidades Farmacéuticas, que sirvió como referencia nacional hasta la adopción del índice de la masa corporal (IMC) del que publicó tal vez el primer artículo mexicano señalando su utilidad y limitaciones.

Tiempo después, Leticia pasó a ser funcionaria en la Dirección General de Servicios Médicos de la UNAM y tuvieron acceso a un contingente mayor de estudiantes universitario; posteriormente ella se hizo cargo de la dirección de los centros de desarrollo infantil de la misma universidad, quienes atendían a un gran grupo de hijos de los trabajadores administrativos. Sobre los indicadores antropométricos del estado de nutrición se hicieron varias publicaciones señalando las limitaciones del índice de la masa corporal y destacando el valor y referencias de la anchura del codo para evaluar la robustez del esqueleto; también se publicó un capítulo sobre este aspecto aplicado a la evaluación de los ancianos. Asimismo, el Dr. Vargas realizó estudios sobre la distribución de la grasa corporal y la obesidad en general.

En la década de 1980 siguió su colaboración con Fernando Martínez Cortés quien era el Director Médico del Grupo Resistol y con Rubén Vasconcelos quien había sido su profesor en la Facultad de Medicina; junto con el diseñador Javier Castellanos hicieron varios estudios con fines ergonómicos y para prevenir el daño y los accidentes entre los trabajadores de la mencionada empresa. El libro *Salud en el trabajo* y varios artículos y tesis contienen los resultados del trabajo del grupo. Estas últimas actividades lo llevaron a ser profesor de Ergonomía en la Universidad Iberoamericana y después en la Maestría de Diseño Industrial de la UNAM, con cuyo posgrado colaboró en la dirección de varias tesis.

Llevó a cabo una estancia de dos años como asesor en Nutrición en la Organización Panamericana de la Salud que le permitió viajar por varios países de América y además le abrió el interés en la salud de los ancianos que se continuaron con su colaboración con el grupo de Víctor Manuel Mendoza en la FES Zaragoza, y además se integró al Seminario Universitario Interdisciplinario sobre Vejez y Envejecimiento coordinado por Verónica Montes de Oca, lo que desembocó en varias publicaciones.

Desde la década de 1970 y hasta su repentina muerte el 13 de septiembre de 2023 en Villagarcía de Arosa (Galicia, España), estuvo interesado en los procesos bioculturales, publicando trabajos y asesorando tesis sobre la lactancia materna, los prejuicios sobre los ancianos, la sed y otros temas. Sus contribuciones al estudio de la Nutriología y a la antropología aplicada a la prevención y solución de los problemas de salud, que no es parte de este escrito, siempre incluyeron una perspectiva Antropofísica.

A un año de su partida, aquellos que tuvimos la oportunidad de convivir con él, lo recordamos como un profesor generoso, melómano, atento y culto y es ahora que me hace mucho sentido aquel epígrafe del escritor Bernardo Esquinca cuando menciona que "*solo necesitamos un lugar para enterrar a los extraños, pues a quienes amamos, los llevamos enterrados dentro de nosotros*".

ELSEVIER

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Biodiversity of wild edible-medicinal and cosmetic plants with traditional uses in in the Moroccan province of Taounat in the Rif mountain

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RESUMEN

Los estudios etnobotánicos han revelado la importancia central de las plantas silvestres comestibles-medicinales y cosméticas en varios tipos de usos por parte de la población rural marroquí. El conocimiento de la biodiversidad y de los usos tradicionales de estas plantas debe salvaguardarse y transmitirse a las generaciones más jóvenes, para evitar el peligro de su desaparición. El objetivo de este estudio fue inventariar y caracterizar los usos tradicionales de las plantas comestibles espontáneas entre la población de la región de Taounat, situada al sur de la montaña del Rif. Se realizó una encuesta etnobotánica mediante entrevistas a la población del Rif, utilizando un cuestionario para recoger información sobre el órgano comestible, el modo de consumo, la frecuencia y el tipo de uso y las enfermedades tratadas. Se analizan los datos sobre el valor de uso, la frecuencia relativa de citas, el nivel de fidelidad y el factor de consenso de los informantes. Se identificaron 103 especies vegetales pertenecientes a 46 familias y se citaron 48 recetas. La mayoría eran de uso medicinal (35,29 %) y principalmente en el tratamiento de trastornos digestivos. Las partes más consumidas fueron las hojas. Los índices más elevados de Valor de Uso y la frecuencia relativa de citas corresponden a las plantas citadas por todos los informantes, a saber, Lavandula stoechas L. (Halhal) para uso medicinal, Juglans L. (Ljawz) para usos alimentarios y medicinales, mientras que los valores más elevados del Factor de Consenso de los Informantes se registraron para los problemas gástricos y la diabetes. Este estudio reveló la contribución de las plantas comestibles espontáneas a la riqueza de la biodiversidad vegetal y su riqueza en fitoterapia, etno-culinaria y otros usos en la región de estudio.

Keywords:

Wild edible-medicinal and cosmetic plant
Ethnobotanical survey
Taounat province
Morocco.

ABSTRACT

Ethnobotanical studies have revealed the central importance of wild edible-medicinal and cosmetic plants in several types of uses by the rural Moroccan population. Knowledge of these plants biodiversity and uses would make it possible safeguarding and transmission these traditional uses to younger generations and avoid their disappearance. The aim of this study is to inventory and characterize the traditional uses of spontaneous edible plants among the population of Taounat region, located in the south of the Rif Mountain. An ethnobotanical survey was carried out by interviews with the population, using a questionnaire to collect information on the edible organ, the mode of consumption (recipes), the frequency and the type of use and diseases treated. Use value, relative citation frequency, fidelity level and informant consensus factor data are assessed. One hundred and three plant species belonging to 46 families were identified and 48 recipes cited. The majority were of medicinal use (35.29 %) and mainly in the treatment of digestive disorders. The most consumed parts were the leaves. The highest rates of Use Value and relative citation frequency belong to the plants cited by all the informants, namely *Lavandula stoechas L. (Halhal)* for medicinal use, *Juglans L. (Ljawz)* for food and medicinal uses, while the highest informant consensus factor values were recorded for gastric problems and diabetes. This study revealed the contribution of spontaneous edible plants to the richness of plant biodiversity

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Introduction

Wild edible-medicinal and cosmetic plants (WEMCPs) are natural resources that were an integral part of the livelihoods of rural populations (Al Hatmi & Lupton, 2021), (Sundriyal & Eklabya, 2004). However, there is a need of more scientific research on the valorization of traditional knowledge of their uses in relation to their biodiversity in the literature. Previous studies have reported different uses of these plants as medicines, traditional handicrafts and as foods with significant nutritional value etc., (Tardio et al., 2006; Ullah et al., 2020; Nadiroğlu et Behçet, 2018; Bhatia, 2018; Ugulu, 2012. The WEMCs were also a food source in famine and crises periods (Reyes-García, 2015) and can currently contribute to diversifying the diet (Tbatou et al., 2016). In the current context, these WEMCPs, which respect seasonality and protect the environment, would in addition, contribute to biodiversity and the sustainability of food (Serra-Majem, 2020). However, neglect and underuse of these plants is observed today, due to globalization and food abundance, the development of profitable crops and monocultures, constituting a threat to the disappearance of the traditional knowledge and biodiversity that these plants hold. Morocco, characterized by a variable geography, climate and ecology, is known for its great plant biodiversity. Among this, a large number of WEMCs species and subspecies was identified with variability of their uses depending on the populations and sites studied (Nassif et Tanji, 2013; Aboukhalaf et al., 2022; Naciri et al., 2022; Fenane et al., 1999; Fennane & Ibn Tattou, 2005; Fennane & Ibn Tattou, 1998).

As part of the strategic orientations and public policies in Morocco (ADA, 2008) this study constitutes a contribution to the safeguarding of local cultural traditions around WEMCs for the proper exploitation, protection and conservation of these natural resources. The objective was therefore to discover the biodiversity and the methods of use and marketing of this WEMCPs plants used by the population of the Taounat region, located south of the Rif Mountain in Morocco.

Materials and Methods

The survey setting

The study took place in the Taounat province located in the Fez – Meknes Great Region. This predominantly rural province, is located in the southern Rif and is part of the Jbala country. The province borders are Chefchaouen province to the north, Taza province to the east, Ouezzane province to the northwest, Sidi Kacem province to the west, and the prefecture of Fez to the south (HCP, 2009).

Four localities, the commune of Ansar, the commune of Zrizer, the commune of Ghafssay and the commune of Khemis Zrizer, located in the study province (Figure 1), are selected to conduct the survey (Table 1).

Table 1. Geographical coordinates and ecological conditions of the Study Province.

Province of Taounate		
Ecological conditions (DRAFM 2018)	Climate	semi-arid with temperate winter and hot dry summer
	Temperature	Max 37°C, Min 10°C
	Altitude	521 m Douar Al Onsar and 467m of Khemis Zrizer
	Average annual rainfall	Declining from north (1,800 mm/year) to south (500 mm/year)
Geographic location (DRAFM 2018)		North: Chefchaouen et Al Hoceima South: Fez East: Taza West: Sidi Kacem
Geographical coordinates (DRAFM 2018)		Latitude: 34° 32′ 09″ North, Longitude 4° 38′ 24″ West

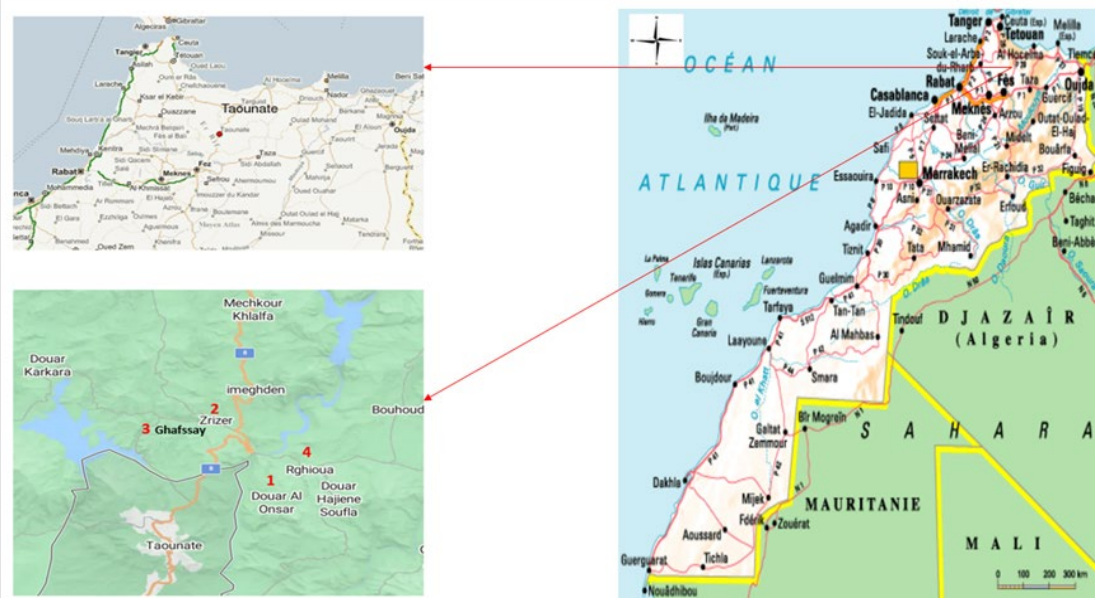

Figure 1. Map of the study area Taounate province (DRAFM, 2018)

Study region climate

The Taounate province, as a whole is characterized by a semi-arid climate with temperate winter and hot, dry summer, with rainfall declining from the north (1800 mm/year) to the south (500 mm/year).

The Taounate province is divided into two distinct parts:

• The northern part with mountainous terrain, which covers approximately 40% of the total area of the province, with altitudes varying up to 1800 m. It is crossed by six large rivers constituting the main tributaries of Wadi Ouergha.

• The southern part with hilly relief covers an area of approximately 3,300 km^2, and altitudes varying from 1000 m at Jbel Zeddour to 150 m along Wadi Inaouen (HCP, 2009) (Table 2).

Table 2. Distribution of the informant sample by localities.

Zone Name	Localities	Locality name	Participants number
Taounat	Locality 1	Douar El Ansar	30
	Locality 2	Zrizer	35
	Locality 3	Ghafssay	25
	Locality 4	Rghioua	10
Sample size			100

Survey Data Collection

The survey was conducted, using a questionnaire, in the local Arabic dialect language, during several visits to the study areas and over a period of 6 months from March to September 2022. The sample involved 100 people, aged 14 to 90 years, of both sexes and with different levels of education. It included people from rural areas and wild food plant sellers in local markets in four localities, distributed as shown in Table 3.

The information collected by the questionnaire used included a first part for the identification of the respondents sociodemographic and socioeconomic characteristics of each respondent, including age, gender, household size, occupation, education level, marital status, and place of residence (Table 3).

The second part concerned a participatory ethnobotanical survey (Betti et al., 2016). This part of the questionnaire is completed using individual direct interviews or in “focus groups”, and semi-structured group interviews carried out according to the method described by (Wentholt et al., 2001) consisting of interviewing women and men separately. In each interview, data for each plant cited included the local common name, information on local people's knowledge of this plants, their traditional uses (as food, medicinal, cosmetic, etc.), and when they use them (in

the past or in the present), as well as the parts of the plant used, the method of preparation, their uses as food in combination or not with other foods (recipes). For medicinal plants, information on the method of administration and the diseases treated were also recorded. The time spent on each interview was approximately one hour.

Plant samples were also collected, photographed and packaged in labeled plastic bags and brought back to the laboratory for scientific identification. Some plant samples were purchased from rural women participating in the survey.

Table 3. Socio-demographic and economic characteristi participant.

	N (%)
Total respondents	100(100)
Gender	
Females	77(77)
Males	23(23)
Type of survey	
Individual	35 (35)
Focus group	65 (65)
Area of residence	
Rural	100 (100)
Distribution by profession	
Housewife	48 (48)
Farmer	40 (40)
Others	12 (12)
Age (years)	
[14-30]	15 (15)
[31- 40]	24 (25)
[41- 90]	61(61)
Education level	
Middle and high school	12(12)
Primary	28(28)
Koranic	20(20)
Illiterate	40(40)
Marital status	
Married	43 (43)
Widowed	30 (30)
Single	27 (27)

Consent of participants

Participants, before being included in the study sample, were informed about the purpose of the study, voluntary participation and the possibility of ending their participation at any time, if they wish it. The confidentiality of their data is also respected.

Taxonomic identification of plants

The determination of the scientific nomenclature was carried out by comparing our samples with those kept in the herbarium of our laboratory (biotechnology, biochemistry, and nutrition laboratory of Chouaib Doukkali University) and out using a digital herbarium. Certain species were identified with the help of the National Herbarium (Rabat, Morocco), Agronomic and Veterinary Institute, and a botanist from the Faculty of Sciences in El-Jadida (UCD, Morocco) as well as using literature documents (Nègre, 1961; Quezel & Santa, 1962; Sijelmassi, 1993; Fennane & Ibn Tattou, 1998; Fennane et al., 1999; Valdés et al., 2002; Fennane & Ibn Tattou, 2005; Fennane et al., 2007; Dobignard & Chatelain, 2011, Tbatou et al., 2016).

Data analysis

The survey data concerning the number of citations and recipes of WEMCPs plants are entered using an Excel software database (Betti et al., 2001, 2002). Statistical analyzes are carried out using version 21 of SPSS software for Windows (Statistical Package for the Social Sciences).

The relative frequency of citations index (RFC) was used by Betti (2001, 2002), Tardio et al. (2006), Aboukhalaf et al (2022), Ghanimi et al (2022), Essaih et al. (2023) and calculated using the following formula:

$$\mathbf{RFC = FC / N\ (0 \leq RFC \leq 1)}$$

where:
FC = Number of informants mentioning the uses of the species
N = Total number of informants in the study

Informant consensus factor

The informant consensus factor (ICF) is used to indicate the degree of homogeneity of informants' knowledge for the use of the cited plants, and is calculated by the formula:

$$ICF = (Nur - Nt) / (Nur - 1)$$

where (Nur) denotes the number of usage reports and (Nt) refers to the number of taxa used. The value of the ICF is between 0 and 1. When this value tends towards 1, it indicates a high rate of consensus among informants (Ghanimi et al., 2022; Essaih et al., 2023).

Use value

The use value (UV) index assesses the relative importance of each plant species known locally to be used as herbal remedy. The use value is calculated according to the following formula:

$$UV = U/N$$

where U refers to the number of citations per species; and N is the number of informants who reported on the plant species (Salih & Ali, 2014).

Results and discussion

Sociodemographic and socioeconomic profile of respondents

A percentage of 65% of the data is collected using questionnaires completed in group surveys, namely during exchanges and debates with participants in focus groups (Table 2), (Figure 2), while individual surveys accounted for 35% of the data. In all the locations studied, the participants were aged 14 to 90 years, the most representative age group was 41 to 80 years old, followed by the age group 31 to 40 years old and finally the youngest group ages 14 to 30.

The majority (100%) of respondents were from rural areas with 77% female and 23% male. Socioeconomic status included farmers, housewives, herders, students, unemployed, traders, teachers, and fkih (religious teachers). The socio-economic categories most represented in rural areas are housewives (48%) and farmers (40%), followed by traders and teachers representing the socio-professional status of the population who travel to work in rural areas (12%). The Table 3 also shows that according to their distribution by level of education, the majority of

Figure 2. Photos of focus group (1), (2), (3), the individual (4), (5), and surveys (6). Plants collected by the housewife (Seloua ESSAIH)

respondents (40%) were illiterate, 28% were with primary level, 12% included both those with secondary and tertiary level and 20% attended Koranic school. According to their marital status, the majority of survey participants were married (43%), followed by widowers (30%) and singles (27%).

The data reported on the plant species cited in this work are obtained in the ethnobotanical study carried out among women and men aged 14 to 90 years in the mountainious province of Taounat, through individual surveys and focus group. This region is represented by the municipalities of Douar El ansar, Khemis Zrizer, Ghafsay and Rghioua. The results of this work revealed the identification of 103 plant species belonging to 46 families. The study also revealed a higher use of these plants in women (77%) than in men (23%). These results corroborate with the results of other work carried out in other provinces in the country, such as Tarfaya region (Idm'hand, Masnada and Cherifi, 2020) and in Casablanca Settat region (Essaih et al., 2023).

Floristic analysis and botanical families of the species mentioned

As presented in the Table 4, the plants collected and identified in this study are classified according to the alphabetical order of their botanical families, their local vernacular name, the type of use, the part used and for food category plants the form of their consumption and processing, for the medicinal and cosmetic plants, their mode and form of administration, the method of preparation used, uses in traditional medicine by the local community and ethnobotanical indices (use value (UV), relative citation frequency (RFC), fidelity level (FL) and informant consensus factor (ICF)).

The total of 103 species of WEMCPs , divided into 46 families were identified in the present study, being used in the past or are still used currently as declared by the population in the study areas. This number of the wild species cited by the surveyed population, is to our knowledge, higher than that found in the numerous studies carried out in the Mediterranean region. Indeed, this number exceeds those reported in the literature by other authors, namely 69 WEMCPs documented in the Guzman region of Spain (Tardio, Pardo-de-Santayana and Morales, 2006) and 66 WEMCPs in Bologna, Italy (Sansanelli and Tassoni, 2014) Similarly, 64 WEMCPs were recorded in the regions of Sidi Bennour (Aboukhalaf et al., 2023) and 78 WEMCPs in the region of Casablanca - Settat (Essaih et al., 2023) in Morocco.

Figure 3 shows the distribution of WEMCPs species cited by their families, and table 4 presents the number and percentages of citations of these species in alphabetical order as well as the type of their uses. The results show that botanically, the most represented families were herbaceous plants with a significant representation of Asteraceae in 16 different species (34.78%). These results are consistent with those of previous work carried out in other regions of Morocco by Ghanimi et al. (2022), Tbatou et al. (2016), Briguiche and Zidane (2019). These studies also reported that this family is considered to be at the top of the list of vascular flora naturally available and widely used by the Moroccan population (Essaih et al., 2023).

Other families grouped represented simultaneously (65,1%); these are: The Amaryllidaceae, Arecaceae, Berberidaceae, Ericaceae, Cannabaceae, Cupressaceae Cortinariaceae, Euphorbiaceae, Ericaceae, Fagaceae, Iridaceae, Illicaceae, Juncaceae, Juglandaceae, Lythraceae, Molliginaceae, Malvaceae, Moraceae, Oleaceae, Oxalidaceae, Palmaceae, Portulacaceae, Papaveraceae, Rubiaceae, Salicaceae, Tuberaceae, Thymelaeaceae, Urticaceae, Vervenaceae and the Zingibelaceae simultaneously.

In second place, among the families cited come the Lamiaceaes family with 15 citations (32.6%), the Apiaceas with 7 citations (15.21%), the Fabaceae with 5 citations (10,86%), the Brassicaceaes with 4 citations, the Brassicaceae (8.69%), Myrtaceae (6.52%), Poaceae and Solanaceaes with 3 citations (6.52%) simultaneously, the Anacardiaceae (4.34%), Apocynaceae(4.34%), Capparaceae (4.34%), Caryophyllaceae (4.34%), Chenopodiaceae (4.34%), Cactaceae (4.34%), Rosaceae (4.34%), and Verbenaceae (4.34%), with 2 citations simultaneously. The other families grouped represented simultaneously (65.1%) ; these are: The Amaryllidaceae, Arecaceae, Berberidaceae, Ericaceae, Cannabaceae, Cupressaceae Cortinariaceae, Euphorbiaceae, Ericaceae, Fagaceae, Iridaceae, Illicaceae, Juncaceae, Juglandaceae,

Table 4. List of the plants recorded in the survey localities and their ethnobotanical characteristics

Scientific Names / Vernacular Names (Voucher speciname)	***Families***	***RFC***	***UV***	***Type of use***	***Form of consumption and processing (Food category)***	***P P Part used U***	***Preparation Med. Cos* / Administration***	***Traditional medicine uses***
Allium roseum L/ ***Baslat dib*** *(BD 18)*	***Amaryllidaceae***	*0,85*	*0,86*	*Food*	*Fruit used as vegetables, cooked foods and to flavored salads (**Vegetables**) Also used dried as a condiment (**Spices**).*	*Fruit*		
Pistacia atlantica Desf / ***Lbtem*** *(BT33)*	***Anacardiaceae***	*0,75*	*0,75*	*Medicinal*	*Leaves prepared as infusion for Rheumatism.*	*Leaves*	***Infusion / Oral***	***Rheumatism***
Pisticia lentiscus L / ***Drou*** *(DR49)*	***Anacardiaceae***	*0,79*	*0,80*	*Food Medicinal*	*Extraction of the (**oil**) for ' donut preparation. Also used as nose drip and to cure asthma and angina.* *Use of the oil on the umbilical cord trace 'Belly' for menstrual pain.* *Infusion of leaves for gastric problems.*	*Seed* *Leaves*	***Oil / Externally*** ***Infusion / Oral*** ***Poultice / Externally*** ***Nose drip / inspiration***	***Gastric problems*** ***Menstrual pain*** ***Asthma*** ***Angina***
Ammi majus L. / ***Begueramel trilal*** *(TR2)*	***Apiaceae***	*0,65*	*0,70*	*Food Medicinal*	*The roots are used as a condiment with traditional Moroccan spices in infusion "Msakhen", to prepare balboula soup to activate the reproductive glands. Also used to flavor chicken (**Spices**)*	*Root*	***Infusion / Oral***	***Reproductive glands***
Foeniculum vulgare P.Mill./ ***Besbas lbouri*** *(BS22)*	***Apiaceae***	*1*	*1*	*Food*	*Aerial part used as (vegetables) to prepared tajines or dried to flavor the traditional recipes like barbecue.* *Stem used with the milk to prepare a different couscous from the mountains of Morocco (**Vegetable**).*	*Aerial part* *Stem*		
Daucus crinitus Desf./ ***Bû- zfûr*** *(BZ 15)*	***Apiaceae***	*0,54*	*0,54*	*Medicinal*	*Root Raw or cooked Oral for Gastric pain.*	*Root*	***Snack/ Oral***	***Gastric pain***
Ammodaucus leucotrichus Coss& Dur / ***Al camoun soufi*** *(CS10)*	***Apiaceae***	*1*	*1*	*Medicinal*	*Graine Cru Oral Tube digested for respiratory system infections.*	*Seed*	***Infusion/ Oral***	***Digestif pains***

Carum carvi / ***Karwiya*** *(KW 57)*	***Apiaceae***	*0,89*	*0,89*	*Medicinal*	*Fruit used as snack for respiratory system infections.*	*Fruit*	***Snack / Oral***	***Respiratory system infections***
Ammi visnaga L. Lam. / ***Maticht dib – Boukhnina*** *(BK 13)*	***Apiaceae***	*0,65*	*0,65*	*Food*	*Fruit used as (**Vegetables**).*	*Fruit*		
Ammi visnaga L. Lam. / ***Bouchnikh*** *(BC 10)*	***Apiaceae***	*0,89*	*0,90*	*Other Food*	*Seeds used fresh or dried to perfume tea (**Drinks**), garnish cake and bread (**Food decoration**).* *Stems are used as toothpicks.*	*Seed Stem*		
Laurier rose / ***Defla*** *(DF 19)*	***Apocynaceae***	*0,12*	*0,12*	*Medicinal*	*Aerial part boiled in water and used after as a foot bath for diabetics.*	*Aerial part*	***Foot bath / Externally***	***Diabetes***
Laurier rose / ***Defla roumya*** *(DR 28)*	***Apocynaceae***	*0,74*	*0,74*	*Medicinal*	*Leaves used for foot bath for diabetic person.*	*Leaves*	***Foot bath/ Externally***	***Diabetes***
Chamaerops humilis L./ ***Jemmakh doum*** *(JM72)*	***Arecaceae***	*0,80*	*0,90*	*Food Medicinal Others*	*Fruit consomed as snack for calming the digestif pains (**Snack**)* *Root-Aerial parts used to prepare home decorations such as small rugs and other traditional objects.*	*Fruit Root-Aerial part*	***Snack/ Oral***	***Digestif pains***
*Centaurea chamaerhaponticum Ball /**Tafgha*** *(TG 84)*	***Asteraceae***	*0,41*	*0,41*	*Medicinal*	*Dried leaves transformed into powder used with honey against fever.*	*Dried leaves*	***Compote / Oral***	***Fever***
Chamaemelum fuscatum (Brot.) Vasc. / ***Babounj*** *(BJ12)*	***Asteraceae***	*0,76*	*0,80*	*Food Medicinal*	*Flowers used fresh or dried as an infusion for gastric pains and as a natural sedative (**Drink**). As well as for decorating cakes. (**Garnish**).* *Tender stems consumed raw by shepherds and children (**Snacks**). Basals leaves, mixed with Lavatera cretica L. to make "Beqoula" dish (**Vegetables**).*	*Flowers Tender stems Basal leaves*	***Infusion / Oral***	***Gastric pains / sedative***
Dittrichia viscosa (L.) Greuter / ***Tarehla*** *(TH90)*	***Asteracea***	*0,80*	*0,80*	*Medicinal*	*Dried and powdered roots to heal wounds. Also used as an infusion for skin problems.*	*Root*	***Powdered / Oral***	***Heal wounds Skin problems***
Chrysanthemum coronarium L Rjel djaja/ ***Kraa djaja*** *(JD 12)*	***Asteraceae***	*0,47*	*0,47*	*Food*	*Tender stems, consumed raw by shepherds and children (**Snacks**).* *Basal leaves, mixed with Lavatera cretica L. to make "Beqoula" dish (**Vegetables**).*	*Tender stem Basal leaves*		

CompositaeArtemisia arborescens L. / ***Chiba*** *(CB5)*	***Asteraceae***	*1*	*1*	*Medicinal Food*	*Aerial part used as infusion for gastric pains also to flavor (**infusion**).*	*Aerial part*	***Infusion / Oral***	***Gastric pains***
Cynara humilis L / ***Timta*** *(TM 97)*	***Asteraceae***	*0,42*	*0,50*	*Food Medicinal Other*	*Flowers, net and stamens used for milk curdling. Receptacle cooked as a substitute for (**vegetables**). Roots dried and used as spices (**Seasoning**). Peeled or crushed and cooked as a garnish in traditional recipes (**Food decoration**). Dried and powdered roots to healwounds.*	*Flower Root dried*	***Powder / Oral***	***Heal wounds.***
Carlina gummifera (L.) Less / ***Dad lamlas*** *(DM45)*	***Asteraceae***	*0,12*	*0,13*	*Food Other*	*Fresh leaves used for milk curdling. Seed chewed like a (**chewing-gum**) Fruit consumed as (**Vegetable**).*	*Leaves Seed Fruit*		
Echinops spinosus L / ***Tasekra*** *(SK 13)*	***Asteraceae***	*0,42*	*0,42*	*Others*				
Leontodon saxatilis Lam / ***El-harricha*** *(EH 13)*	***Asteraceae***	*0,41*	*0,41*	*Food*	*Flowers used fresh or dried with tea dried (**Drinks**) and consumed as spices (**Seasoning**).*	*Flower*		
Scolymus hispanicus L / ***El-gernina*** *(GR18)*	***Asteraceae***	*0,44*	*0,44*	*Food*	*Basal leaves, mixed with Lavatera cretica L. to make "Beqoula" dish or cooked as vegetable on traditional recipes including tajines after being peeled from spines (**Vegetables**). Tender stems, consumed raw by shepherds and children (Snacks). Roots, peeled, cut or crushed and cooked as a garnish of traditional recipes (**Cooked roots**).*	*Basal leaves Tender stem Root*		
Chrysanthemum macrocarpum(Sch. Bip) Batt / ***Gahwan*** *(GH 29)*	***Asteraceae***	*0,32*	*0,32*	*Food*	*Stem eaten in the raw state by children and shepherds (**snack**).*	*Stem*		
Cirsium eriophorum (L.)Scop / ***Aalk*** *(Al 18)*	***Asteraceae***	*0,38*	*0,38*	*Food*	*Seeds consumed in the raw state as **chewing gum**,*	*Seed*		
Vertus de l'armoise blanche / ***Chih*** *(CH30)*	***Asteraceae***	*0,12*	*0,12*	*Medicinal*	*Aerial part boiled in water and used after as a foot bath for diabetics.*	*Aerial part*	***Foot bath / Externally***	***Diabet***

Pallenis spinosa (L.) Cass/ ***Ttafs*** *(TF68)*	***Asteraceae***	*0,34*	*0,34*	*Medicinal*	*Leaves prepared as infusion for digestif pains.*	*Leaves*	***Infusion / Oral***	***Digestif pains***
Artemisia campestris L / ***Chih lakhrissi*** *(CL 21)*	***Asteraceae***	*0,47*	*0,47*	*Cosmetic*	*Decoction leaves as infusion, drops used as face masks to treat "kleff" pregnancy marks or certain eye diseases.*	*Leaves*	***Infusion / Externally***	***Face care / Eye diseases***
Cynara scolymus / ***Lkherchouf lbeldi*** *(KB48)*	***Asteraceae***	*0,56*	*0,57*	*Food Medicinal*	*Stems Raw or coocked in Tagine (**Snack or vegetables**). Root decoction as infusion recommended in the treatment liver disease*	*Stem Root*	***Infusion / Oral***	***Liver disease***
Berbiris / ***Berztem*** *(BE 19)*	***Berberidaceae***	*0,04*	*0,04*	*Medicinal*	*Powder of seeds mixed with honey to prepare balls taken each morning to cure cancer.*	*Seed*	***Compote / Oral***	***Cancer***
Raphanus raphanistrum L / ***Bouhammou*** *(BH12)*	***Brassicaceae***	*0,42*	*0,42*	*Food*	*Young leaves, mixed with Lavatera cretica L. to make "Beqoula" dish (**Vegetables**) Flowers, cooked as a garnish for traditional bread (**Food decoration**).*	*Young leaves Flower*		
Lepiduim sativum / ***Hab rechad*** *(HD 94)*	***Brassicaceae***	*0,78*	*0,78*	*Medicinal*	*Fruit used as infusion for respiratory system infections and with milk and*	*Fruit*	***Infusion / Oral***	***Respiratory system infections***
Diplotaxis catholica (L) / ***El-harra/ El-kelkaza*** *(KK 50)*	***Brassicaceae***	*0,79*	*0,80*	*Food Medicinal*	*Young leaves, mixed with Lavatera cretica L. to make "Beqoula" dish (**Vegetables**) Flowers, cooked as a garnish for traditional bread (**Food decoration**). Drops recovered after infusion concentrated flowers to Oriel pain.*	*Young leaves Flower*	***Infusion / Oral Infusion / Externally***	***Oriel pain***
Telephium imperati L. / ***Khayata*** *(HY 62)*	***Brassicaceae***	*0,54*	*0,54*	*Medicinal*	*Leaves prepared as poultice for dried and powdered Leaves to heal wounds.*	*Leaves*	***Poultice / Externally***	***Heal wounds***
Calluna vulgaris / ***Khlanj*** *(KJ 14)*	***Ericaceae***	*0,30*	*0,31*	*Food Medicinal*	*Leaves prepared as (infusion) for digestif pains. Powdered seeds mixed with flour and corn to make traditional local bread.*	*Leaves Seed*	***Infusion / Oral***	***digestif pains***
Puntiaficus-indica (L.) Mill. / Lwarnaxi ***(karmous byad)*** *(KY)*	***Cactaceae***	*0,85*	*0,86*	*Food Medicinal*	*Fruit eaten fresh (**Snack**)or dried, mixed with olive oil and garlic in a paste to treat coughs.*	*Fruit*	***Snack / Oral***	***Coughs***

Opuntiaficus-indica (L.) Mill./ ***Sebbar, aknari, kermous ennssara, lkermous lhendi*** *(AK88)*	***Cactaceae***	*1*	*1*	*Food Medicinal Cosmetic*	*Fruit eaten as a snack and as a treatment to stop diarrhea (**Snack**) The gel from the inner surface of the leaves is used as a poultice for hair and face care.*	*Fruit leaves*	***Snack / Oral Poultice / Externally***	***Diarrhea / hair and face care***
Chanvre (Cannabis saliva L.)/ ***Kif*** *(KF91)*	***Cannabaceae***	*0,52*	*0,54*	*Medicinal Cosmetic Psycho actif use*	*Oil seed extraction to stop hair loss Decoction of Pistil, stigma and Bracté part for preparation the psycho-actif tobacco. The rest of the plant used for other purposes.*	*Seed Pistil Stigmat Bract Calyx*	***Poultice / Externally Steam / Externally Tabacco / Inhalation***	***Hair loss / Psychological state***
Capparis spinose / ***Kbar*** *(KR81)*	***Capparaceae***	*0,34*	*0,34*	*Medicinal*	*Fruit dried used for respiratory system infections*	*Fruit*	***Snack / Oral***	***Respiratory system infections***
Capparis spinosa L / ***Kebbar Afsas*** *(KS20)*	***Capparaceae***	*0,56*	*0,56*	*Medicinal*	*Fruit seed consumed as snack or as infusion for os pains.*	*Fruit*	***Infusion / Oral***	***Rheumatology***
Tetraclinis articulate (Vahl) Masters / ***Learaar*** *(AR 38)*	***Cupressaceae***	*1*	*1*	*Food Medicinal* *Others*	*Seeds used in decoction (**Seasoning Arome**), Leaves used in infusion combined with rosemary, with lavender and pennyroyal.* *Leaves Perfuming the environment.*	*Seed Leaves*	***Infusion / Oral***	***Hypoglycaemic - Diuretic against bronchitis***
Herniaria cinerea DC / ***Harras lehjar*** *(HL63)*	***Caryophyllaceae***	*0,38*	*0,39*	*Food Medicinal*	*Roots dried and consumed as spices (**Seasoning**). Aerial parts, used with tea (**Drinks**) Stems, used fresh or dried boiled to fight against diseases of the kidneys and gallbladder.*	*Root dried*	***Infusion / Oral***	***Diseases of the kidneys and gallbladder***
Silene vulgaris (Moench) Garcke / ***Tirecht*** *(TC39)*	***Caryophyllaceae***	*0,57*	*0,57*	*Others*	*Flowers used as detergent for washing clothes and white carpets it has a brightening effect.*	*Flower*		
Chenopodium murale L. / ***Berremram*** *(BR5)*	***Chenopodiaceae***	*0,28*	*0,29*	*Food Medicinal*	*Aerial parts dried and consumed in powder to fight against gastric pains. Roots boiled and consumed in Drinks to fight against kidney diseases. (**Drinks**) Aerial parts, mixed with L.cretica to prepare the dish beqoula (**Vegetable**). Aerial parts. Dried and consumed as*	*Aerial part Root*	***Powder / Oral***	***Gastric pains / kidney diseases***

					*spices (**Seasoning**).*			
*Chenopodium ambrosioides L / **Mkhinza** (MZ5)*	***Chenopodiaceae***	*0,88*	*0,89*	*Food Medicinal*	*Aerial parts crushed and mixed with orange juice to fight fever. Aerial parts, cooked as a garnish in some traditional recipes of soups (**Food decoration**).*	*Aerial part*	***Drink / Oral***	***Fever***
*Cortinarius / **Champignon** (CM 40)*	***Cortinariaceae***	*0,59*	*0,59*	*Food*	*Same use today as the aerial part used with eggs or to prepare other Moroccan dishes such as pastille (**Vegetable**).*	*Aerial part*		
*Mercurialis annua L / **Horiga lmalssa** (HM 12)*	***Euphorbiaceae***	*0,85*	*0,86*	*Medicinal Cosmetic*	*An infusion of the powder prepared from the leaves is recommended as a rinse for pruritus itchy skin of the scalp, skin and poultice applied for hair straightening.*	*Leaves*	***Infusion / Externally Poultice / Externally***	***Pruritus / Hair care***
*Arbutus unedo L./ **Sasnou, boukhennou** (SS12)*	***Ericaceae***	*1*	*1*	*Food*	*Fruit consomed as (**Snacks**).*	*Fruit*		
*Oak / **Balout** (AT21)*	***Fagaceae***	*1*	*1*	*Food*	*Seeds consumed raw (**Snacks**).*	*Seed*		
*Astragalus boeticus L./ **Krinbouch** (KN40)*	***Fabaceae***	*0,99*	*0,99*	*Food*	*Seeds, consumed raw by shepherds and children (**Snacks**).*	*Seed*		
*Lathyrus clymenum L./ **Rik el-hench** (RN40)*	***Fabaceae***	*0,22*	*0,22*	*Food*	*Seeds, consumed raw by shepherds and children (**Snacks**).*	*Seed*		
*Vicia sativa L / **Bouzghiba** (BG78)*	***Fabaceae***	*0,17*	*0,17*	*Food*	*Seeds eaten raw by children and shepherds (**Snacks**)*	*Seed*		
*Lotus corniculatus / **Karn laghzal** (KW37)*	***Fabaceae***	*0,17*	*0,17*	*Others*	*Aerial parts for animal feed.*			
*Ceratonia celua Kharoub / **Slaghwa** (SG27)*	***Fabaceae***	*0,87*	*0,88*	*Food Medicinal*	*Fruit consumed as (**snack**), Dreid fruit with honey or water for estomac pain.*	*Fruit*	***Drinks / Oral***	***Estomac pain***

Iris sisyrinchium L. / ***Lmessiw*** *(SW10)*	***Iridaceae***	*0,18*	*0,18*	*Food*	*Rhizome, consumed raw or roasted (**Snack**).*	*Rhizomes*		
Illicium verum / ***Lyansoun*** *(YS8)*	***Illiciaceae***	*0,93*	*0,93*	*Medicinal*	*Leaves dried prepared as an (**infusion**) to calm the nerves.*	*Leaves dried*	***infusion/ Oral***	***Nerves***
Juncus acutus L./ ***Essmar lgelmi*** *(SE60)*	***Juncaceae***	*0,62*	*0,62*	*Others*	*To prepare carpets, traditional bags and brooms.*			
Juglans L./ ***Ljawz*** *(JW80)*	***Juglandaceae***	*1*	*1*	*Medicinal Food*	*Their nuit consomed as dreid fruit (**Snack**).* *The leaves prepared as an infusion to calm the nerves.*	*Dried fruit Leaves*	***Infusion/ Oral***	***Nerves***
Rosmarinus officinalis / ***Azir lhour*** *(AZ90)*	***Lamiaceae***	*1*	*1*	*Medicinal Food*	*Aerial part used in infusion to cure gastric problems. Leaves, used as condiment to perfume tajines of chicken and fish (**Seasoning**).*	*Aerial part Leaves*	***Infusion / Oral***	***Gastric problems***
Calamanta officinalis / ***Manta*** *(MT4)*	***Lamiaceae***	*0,99*	*1*	*Food Medicinal*	*Aerial part used for seasoning olives using the traditional Moroccan Meslala method. (**Seasoning**)* *Also to flavor tea and milk and to attack the flu (infusion).*	*Aerial part*	***Infusion / Oral***	***Flu***
Origanum majorana / ***Merd douch*** *(MD10)*	***Lamiaceae***	*0,89*	*0,89*	*Medicinal*	*Leaves used in infusion to treat anxiety. Aerial part used to flavor tea and milk (**Drink**), prepared as (**infusion**) to attack the flu.*	*Leaves*	***Infusion / Oral***	***Anxiety, flu***
Lavandula officinalis / ***Khezama*** *(KZ 53)*	***Lamiaceae***	*0,96*	*0,96*	*Medicinal Other*	*Aerial part the steam from boiling the aerial part is indicated for genital problems delayed menstruation. The water from this boiling is indicated as a hygiene wash for the genital tract.* *Aerial part used with Peganum harmala other beliefs, which are ancient traditions "Bkhour" as conveying positive energy to clean the environment and leave a good scent.*	*Aerial part*	***Infusion / Externally***	***Delayed menstruation and genital tract***

Lavandula stoechas L. / ***Halhal*** *(HA 29)*	***Lamiaceae***	*1*	*1*	*Medicinal*	*Aerial part crushed used externally for treatment of hair loss, leaves used for footbath for diabetic person.*	*Aerial part Leaves*	***Poultice Applied/ Externally Foot bath/ Externally***	***Hair loss / Diabetes***
Thymus serpyllum / ***Ziitra*** *(ZT 15)*	***Lamiaceae***	*1*	*1*	*Food Medicinal*	*Leaves used in infusion to cure gastric problems, used as condiment to perfume tajines of chicken and fish (**Seasoning**), Used to perfume tea and milk (**Drink**).*	*Leaves*	***Infusion / Oral***	***Gastric problems***
Mentha suaveolens Ehrh / ***Mersita*** *(MS3)*	***Lamiaceae***	*1*	*1*	*Food Other (insecticide)*	*Aerial part used to flavour tea, powder of leaves for (**seasoning**) the pancakes, used also as an insecticide after crushing the leaves.*	*Aerial part Leaves*		
Mellissa officinalis / ***Hbak tranj*** *(HT50)*	***Lamiaceae***	*0,94*	*0,94*	*Food*	*Aerial part, used with tea or milk (**Drink**).*	*Aerial part*		
Romarinus officinalis / ***Azir Eklil ljabal*** *(EB39)*	***Lamiaceae***	*0,17*	*0,18*	*Food Medicinal*	*Aerial part are consumed fresh or dried to flavor tea or as an infusion to minimize gastric and head pains (**Drink**).* *Leaves, crashed and used as a garnish in traditional bread recipes (**Garnish**).*	*Leaves Aerial part*		***Gastric pain***
Salvia officinlis / ***Mrimeya*** ***(****MM 87)*	***Lamiaceae***	*0,70*	*0,70*	*Medicinal*	*Aerial part boiled in water and used after as a foot bath for diabetics.*	*Aerial part*	***Foot bath / Externally***	***Diabetics***
Thymus broussonetii Bois. / ***Zaatar*** *(ZH30)*	***Lamiacées***	*1*	*1*	*Food Medicinal*	*Aerial parts, used as seasoning of traditional recipes (**Seasoning**).* *Aerial parts, used as infusion or to perfume tea or milk (**Drinks**) and for gastric pain.*	*Aerial parts*	***Drink / Oral***	***Gastric pain***
Marrubium vulgare / ***Mreout*** *(MR87)*	***Lamiaceae***	*0,78*	*0,78*	*Medicinal*	*Aerial part used as infusion for respiratory system infections*	*Aerial part*	***Infusion / Oral***	***Respiratory system infections***

Mentha pulegium L. / ***Fleuo*** *(FU 15)*	***Lamiaceae***	*1*	*1*	*Medicinal*	*leaves consumed as an infusion with tea or to flavor milk.(* ***Drink****) for flu.*	*Leaves Aerial part*		
Ajuga iva (L.) Schreb / ***Chendgoura*** *(CG5)*	***Lamiaceae***	*0,98*	*0,98*	*Food*	*Aerial parts, cooked as a garnish in traditional breads, soups and couscous (****Garnish****).* *Aerial parts dried and consumed as spices (****Seasoning****).*	*Aerial parts*		
Salvia officinalis / ***Salmia*** *(SM22)*	***Lamiaceae***	*0,78*	*0,78*	*Medicinal*	*Leaves consumed as infusion to uterus pains and for diabetes. Aerial part are consumed fresh or dried to flavor tea.(****Drinks****).*	*Leaves Aerial part*	***infusion/ Oral***	***Uterus pains diabetes***
Lawsowia inermis / ***Henna*** *(HN 52)*	***Lythraceae***	*0,84*	*0,85*	*Cosmetic Medicinal*	*Dried leaves are used as a poultice with water for the skin and hair during Moroccan baths. So for the art of Elhenna Moroccan Nakch tattoo on women's hands and feet at traditional Moroccan festivals. Dried leaves mixed in powder with Chenopodium ambrosioides L (****Mkhinza****) leaves to lower temperature immediately. Dried leaves mixed in powder with the leaves of Chenopodium ambrosioides L - Mkhinza as a head poultice to stop fever immediately.*	*Leaves*	***Poultice / Externally*** ***Poultice / Externally***	***Hair and face care*** ***Fever***
Corrigiola telephiifolia Pourr. / ***Serghina*** *(SH28)*	***Molluginaceae***	*0,65*	*0,66*	*Food Medicinal Other*	*Aerial parts used as an infusion and mixed with natural yogurt for gastric problems, used to eliminate certain undigested non-food compounds in the case of Toukal in rural culture. Dried roots consumed as spices (****Seasoning****). The steam from burning the aerial parts of the plant is used as a disinfectant for the clothes of newborns.*	*Aerial parts, Dried roots*	***Infusion – steam Oral / Externally***	***Gastric problems/ disinfectant***
					Incense disinfecting the environment and for other beliefs which are "ancient traditions : "Bkhour" as conveying positive energy.			

Syzygium aromaticum / **Krounfoul** *(KF80)*	**Myrtaceae**	*0,75*	*0,76*	*Medicinal Food*	*Flower bud used to season, decorate traditional cakes (***Garnish***) and used for oral hygiene. Flower bud used as infusion to treat anxiety.*	*Flower bud*	**Infusion / Oral Machining / Oral**	**Anxiety Oral hygiene**
Eucalyptus globulus Labill. / **Lkalitos,Caliptus** *(KS82)*	**Myrtaceae**	*0,92*	*0,93*	*Medicinal Others*	*Leaves used as an infusion to fight fever. The stem produced by burning the plant's leaves is used as a household disinfectant.*	*Leaves Steams*	**Infusion / Oral Steam / Externally**	**Fever / Disinfectant**
Myrtus / **Rihan** *(RH 48)*	**Myrtaceae**	*0,68*	*0,68*	*Medicinal*	*Leaves used in infusion to cure gastric problems, flavored tea and poultice applied for hair straightening.*	*leaves*	**Infusion / Oral Poultice Applied / Externally.**	**Gastric problems / Hair care**
Lavatera cretica L. / **El-khobbiza / El-bakkoula** *(KZ1)*	**Malvaceae**	*1*	*1*	*Food*	*Moroccan dish Beqoula obtained from the aerial part of Lavatera cretica. (***Vegetables***)*	*Aerial parts*		
Ficus carica L / **Lghodan, karmouss, El karma, Chreha.** *(GK18)*	**Moraceae**	*1*	*1*	*Medicinal*	*Fruit consumed fresh or dried in powder form as an antioxidant and anticancer agent.*	*Fruit*	**Powder / Oral**	**Antioxidant and anticancer**
Olea europaea / **Zitoun lbari** *(TB 10)*	**Oleaceae**	*1*	*1*	*Medicinal*	*Olive For the diet for diabetics.*	*Fruit, Leaves*	**Oil / Oral**	**Diabetics**
Oxalis pes-caprae L. / **El-hommaida essafra** *(MD10)*	**Oxalidaceae**	*0,81*	*0,81*	*Food*	*Stems, chewed raw by shepherds and children (***Snacks***).*	*Stems*		
Phragmites communis Trin./ **Lakssiba** *(AB 12)*	**Poaceae**	*0,41*	*0,41*	*Medicinal*	*An infusion of the powder prepared from the rhizome is recommended as a rinse for pruritus of the scalp and skin.*	*Rhizome*	**Infusion / Externally**	**Pruritus**
Phragmites communis Trin / **Leksseb** *(EB39)*	**Poaceae**	*0,49*	*0,49*	*Medicinal*	*Raw root for face skin*	*Root*	**Poultice / Externally**	**Face skin**
Cynodon dactylon (L.) Pers /	**Poaceae**	*0,67*	*0,68*	*Food Medicinal*	*Rhizomes dried and consumed as spices (***Seasoning***). Roots used as an infusion*	*Rhizomes*	**Infusion / Oral**	**Flu**

Ennejem *(EN35)*					*to attack the condition of the flu.*			
Chamaerops humilis L. / ***Eddoum*** *(DD53)*	***Palmaceae***	*0,87*	*0,88*	*Food* *Others*	*Heart of the root consumed in the raw state by the shepherds and the children (**Snacks**).*	*Root*		
Portulaca oleracea L ./ ***Errejla*** *(ER5)*	***Portulacaceae***	*0,91*	*0,91*	*Food*	*Tender leaves and stems, cooked as vegetables in traditional recipes (**Vegetable**s).*	*leaves*		
Rumex pulcher L./ ***Homaida*** *(HI15)*	***Polygonaceae***	*0,79*	*0,79*	*Food*	*Young leaves, mixed with Lavatera cretica L. to make "Beqoula" dish. (**Vegetables**).* *Flowers, cooked as a garnish for traditional bread (**Garnish**).*	*leaves, Flowers*		
Papaver rhoeas L./ ***Belaaman*** *(LA44)*	***Papaveraceae***	*0,97*	*0,99*	*Food* *Cosmetic*	*Flowers dried and consumed as spices. (**Seasoning**) or mixed with flower water for traditional make-up called "Matleya akar Fassi".*	*Flowers*	***Powder / Externally***	***Facial treatment***
Cyperus rotundus L./ ***Oligue*** *(OG14)*	***Rosaceae***	*0,44*	*0,46*	*Food* *Medicinal* *Others*	*Fruit consumed fresh (**Snack**).* *Dried leaves are used in herbal teas as detersive gargles, to treat angina and used as infusion for gastric problems.*	*Fruit* *Leaves*	***Drink / Oral***	***Angina / Gastric problems***
Prunus amygdalus Stokes var amara DC./ ***Louz lhar*** *(LH 45)*	***Rosaceae***	*0,59*	*0,59*	*Medicinal*	*Dried fruit mixed in powder with honey to treat allergies.*	*Dried fruit*	***Compote / Oral***	***Allergies***
Rubia peregrina L./ ***El-fouwa*** *(FW29)*	***Rubiaceae***	*0,37*	*0,38*	*Food* *Medicinal*	*He stems are dried and prepared as an infusion (**Drinks**). The roots are dried, powdered and used as an ingredient in the Moroccan soup "Hssoua" for people suffering from anemia or for young brides.*	*Stems, roots*	***Powder / Oral***	***Anemia***
Lycium intricatum Boiss/ ***Seder*** *(ER 5)*	***Solanaceae***	*0,67*	*0,68*	*Food* *Medicinal*	*Fruits eaten raw (**Snaks**) or prepared as an infusion (**Drink**) for people suffering from kidney diseases. Liquid from the leaves in a fresh state used to fight against skin disease. The steam from the burning stems is also used to disinfect the environment and to have positive*	*Fruits* *Leaves* *stems*	***Snack / Oral*** ***Infusion / Oral*** ***Poultice / Externally***	***Skin disease / positive energy / disinfectant*** ***Hare care***

					energy - rural tradition. *The leaves are dried and powdered with Henna poultice for hare care.*			
Mandragora autumnalis Bertolt / ***Bayd elghoul*** *(AH22)*	***Solanaceae***	*0,87*	*0,87*	*Medicinal*	*Rhizome powder mixed with olive oil is used to prepare suppositories for rectal use against hemorrhoids and vaginal use against uterine problems.*	*Rhizome*	***Suppositories/ Rectal Suppositories/ Vaginal***	***Hemorrhoids Uterine problems***
Lycium intricatum Boiss / ***Sedra lmalssa*** *(SM 22)*	***Solanaceae***	*0,66*	*0,67*	*Others* *Cosmetic*	*The aerial part is used as a protective seat around houses against snakes.* *leaves dried and powdered, used as a cataplasm to leave hair soft.*	*Aerial part*	***Poultice / Externally***	
Populus alba L. / ***Sefsaf*** *(SF23)*	***Salicaceae***	*0,47*	*0,47*	*Medicinal*	*Leaves decocted for Gastric pain.*	*Leaves*	***Snack / Oral***	***Gastric pain***
Tuber oligospermum / ***Terfas*** *(TF91)*	***Tuberaceae***	*0,95*	*0,95*	*Food*	*Tuber used as a (**Vegetable**).*	*Tuber*		
Daphné garou / ***Lazzaz*** *LZ29)*	***Thymelaeceae***	*0,72*	*0,72*	*Cosmetic*	*Green leaves dried and powdered, used as a cataplasm to leave hair soft.*	*Leaves*	***Poultice / Externally***	***Hair care***
Urtica urens L. / ***Horriga lharcha/ Horriga lharra*** *(HU12)*	***Urticaceae***	*0,76*	*0,76*	*Medicinal*	*Aerial parts used as a foot bath to fight against rheumatic diseases.*	*Aerial parts*	***Foot bath / Externally***	***Rheumatic***
Verveine officinale L./ ***Baymût*** *(BY15)*	***Verbenaceae***	*0,47*	*0,47*	*Medicinal*	*Leaves used as infusion as a painkiller*	*Leaves*	***Infusion / Oral***	***Nerves***
Aloysia citriodora Palau / ***Lwiza*** *(LW12)*	***Verbenaceae***	*1*	*1*	*Medicinal*	*Leaves Decoction*	*Leaves*	***Decoction / Oral***	***Sedative, hypertension***
Peganum harmala / ***Harmal*** *(HR49)*	***Vervenaceae***	*0,92*	*0,92*	*Other*	*Incense disinfecting the environment and for other beliefs, which are "ancient traditions: "Bkhour" as conveying positive energy.*			
Alpinia officinarum Hance/ Khudenjal (DJ60)	***Zingiberaceae***	*0,48*	*0,48*	*Medicinal*	*Decoction root for emmenogogue*	***Root***	***Infusion / Oral***	***Emmenog -ogue***

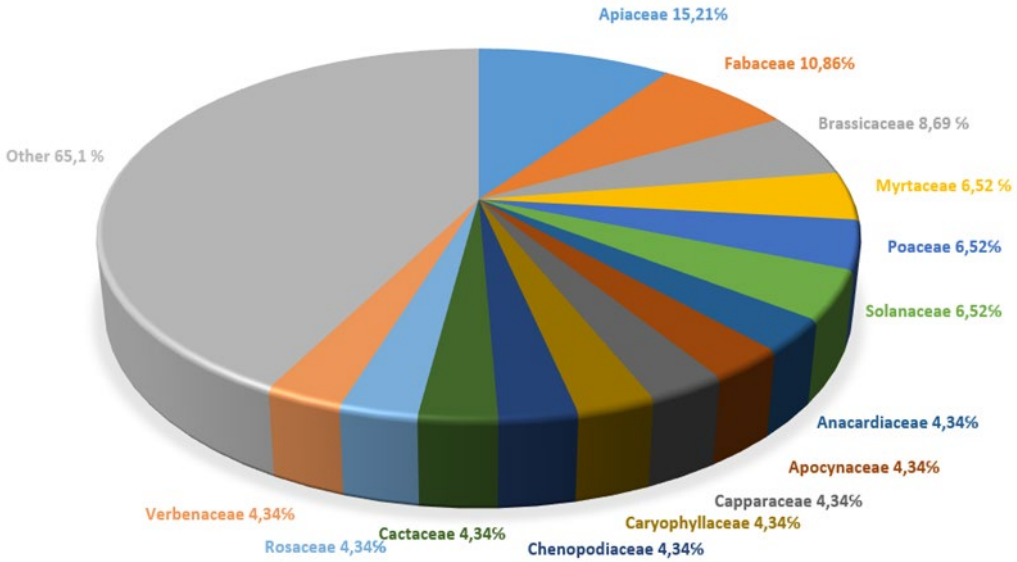

Figure 3. Distribution of the identified species by botanical families of the cited WEMCPs

Lythraceae, Molliginaceae, Malvaceae, Moraceae, Oleaceae, Oxalidaceae, Palmaceae, Portulacaceae, Papaveraceae, Rubiaceae, Salicaceae, Tuberaceae, Thymelaeaceae, Urticaceae, Vervenaceae and the Zingibelaceae simultaneously. The other families grouped represented simultaneously (65.1%) ; these are: The Amaryllidaceae, Arecaceae, Berberidaceae, Ericaceae, Cannabaceae, Cupressaceae Cortinariaceae, Euphorbiaceae, Ericaceae, Fagaceae, Iridaceae, Illicaceae, Juncaceae, Juglandaceae, Lythraceae, Molliginaceae, Malvaceae, Moraceae, Oleaceae, Oxalidaceae, Palmaceae, Portulacaceae, Papaveraceae, Rubiaceae, Salicaceae, Tuberaceae, Thymelaeaceae, Urticaceae, Vervenaceae and the Zingibelaceae simultaneously.

The predominance of Asteraceae is consistent with ethnobotanical studies conducted in North Africa such as Morocco (Ghanimi et al., 2022; Essaih et al., 2023; Aboukhalaf et al., 2022; Khabbach et al., 2012; Briguiche and Zidane, 2019), and Algeria (Sarri et al., 2014).

The different use types of the species mentioned

The results show that among all the uses of WEMCPs mentioned by the respondents, medicinal use is the majority in this region (35.29%), followed by food use (22.54%), then as both food and medicinal use (20.58%), other uses (5.88%), followed by both food - medicinal and other use (4.9%), both food and other use (3.92%), cosmetic use (1.96%), both cosmetic and medicinal use (1.96%), both cosmetic - medicinal and psychoactive use (0.98%) which is a use revealed for the first time in this study and which marks a difference in the use of WEMCPs with other regions of the country (Essaih et al., 2023; Aboukhalaf et al., 2022; Ghanimi et al., 2022; Khabbach et al., 2012; Briguiche and Zidane, 2016) and countries in the region such as Algeria (Sarri et al., 2014), Finally, both food - medicinal and cosmetic use and medicinal and other use simultaneously represent a 0.98%.

The identification of all of these uses shows that most of the WEMCPs are used by the population of the study area for various purposes in addition to being primary sources of food for the population in the study region and other regions such as CasaBlanca Settat province (Essaih et al., 2023), Al Haouz Region (Ghanimi et al., 2022), Sidi Bennour province (Aboukhalaf et al., 2022). These plants contribute to the therapy of people in the study region in the same way as in other national regions, in the Rif (Chaachouay et al., 2019), in the province of Tarfaya (Idm'hand et al., 2020), and in other international regions including Ethiopia (Hankiso et al., 2023).

This widespread use of medicinal plants has been explained by the lack of access to health care and

the insufficiency of health infrastructure and medicines as well as low income of the populations by (Liu et al., 2023; Guedje et al., 2010).

The culinary uses

In addition to their medicinal, cosmetic and psychoactive uses, the WEMCPs cited could also be classified into seven food categories, based on their culinary uses and consumption patterns of the population (Figure 4). The majority (37%) of these WEMCPs cited was used as a mainly warming drink in winter to combat the cold in this mountainous study region, 17% is consumed raw as a snack by shepherds and young people in the fields, due to their abundance and ease of access and the abundance of several fruit-producing plant species such as *Juglans L./ Ljawz* and *Oak / Balout*. A proportion of 16 % of WEMCPs is used as vegetables and spices simultaneously and 12 % for food decoration to enhance dishes. Other wild plants were used simultaneously in the form of gum and oil (1%).

The results of this study revealed a very great diversity of culinary uses where the difference in plant biodiversity determines the difference in frequency of use between the different culinary categories determined in comparison with other regions. This is in agreement with the difference in culinary uses revealed in this work with other regions of the country where the

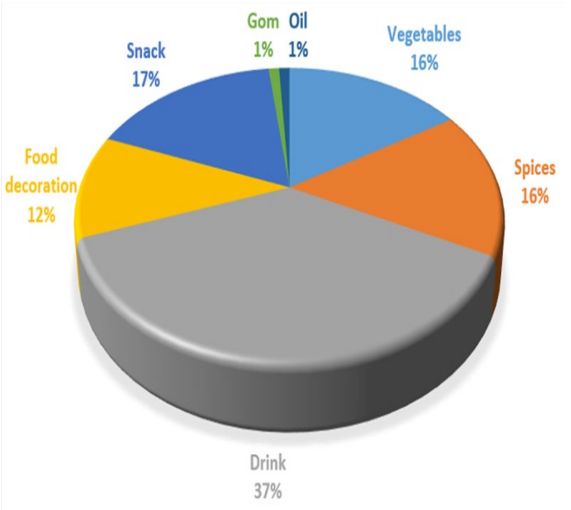

Figure 4. The consumption mode of the cited wild plants

majority of plants are consumed as vegetables e.g. in the populations of the regions of Chaouias and Doukalla (Essaih et al., 2023). This also confirms that culinary cultural traditions correspond to the conditions of the living environment to which populations adapt by exploiting local biodiversity. Thus, the appearance of a very high number 103 species compared to other research studies (Aboukhalaf et al., 2022) reflected this diversity of culinary uses with the appearance of new modes of consumption such as *Pisticia oil lentiscus L* which is extracted and was used in the past for the preparation of fritters.

Use value (UV) and relative frequency of citation (RFC)

The most popular plants are those with the highest RFC and UV index. The RFC frequency was calculated on the basis of the number of respondents having mentioned wild plants consumed in the municipality (RFC) and the UV index was calculated on the basis of the number of citations per species and the number of informants having declared the plant species. The highest RFC and UV value (RFC = 1, UV = 1) belongs to the plants cited by all informants. According to the data from this survey in the study region, these are *Lavandula stoechas L./ Halhal* which is for medicinal use*, Juglans L./ Ljawz* for food and medicinal use (UV = 1; RFC = 1) , *Lavatera cretica L. / El-khobbiza / El-bakkoula* (UV = 1; RFC = 1) for food use, *Thymus serpyllum / Ziitra* (UV = 1; RFC = 1) for food and medicinal use, *Olea europaea / Zitoun lbari* (UV = 1; RFC = 1) for food and medicinal use, *Mentha pulegium L. / Fleuo* (UV = 1; RFC = 1), *Foeniculum vulgare P.Mill / Besbas lbouri* (UV = 1; RFC = 1) for use food, *Ammodaucus leucotrichus Coss & Dur /Al camoun soufi* (UV = 1; RFC = 1) for medicinal use, *Ammi visnaga L. Lam. / Bouchnikh* (UV = 0.90; RFC = 0.89) for food and other use, Compositae *Artemisia arborescens L. / Chiba* (UV = 1; RFC = 1) for food and medicinal use, *Opuntiaficus-indica (L.) Mill ./ Sebbar, aknari* (UV = 1; RFC = 1) for food-medical and cosmetic use, *Arbutus unedo L./ Sasnou, boukhennou* (UV = 1; RFC = 1) for food use, *Oak/ Balout* (UV = 1; RFC = 1) for food use, *Rosmarinus officinalis / Azir lhour* (UV = 1; RFC = 1) for medicinal and food use, *Mentha suaveolens Ehrh / Mersita* (UV = 1; RFC = 1)

for food and other use, *Ficus carica L/ Lghodan, karmouss, El karma, Chreha* (UV=1; RFC=1) for food and medicinal use, *Thymus broussonetii Bois. / Zaatar* (UV = 1; RFC = 1) for food and medicinal use, *Aloysia citriodora Palau / Lwiza* (UV = 1; RFC = 1) for food and medicinal use.

In the following positions come the plants which were cited by more than 90% of the informants. These are *Astragalus boeticus L. / Krinbouch* for food use (UV = 0.99; RFC = 0.99), *Papaver rhoeas L. / Belaaman* as food and cosmetic (UV = 0.99; RFC = 0.97) *Calamanta officinalis / Manta* (UV = 0.99; RFC = 0.97) as food and medicinal, *Ajuga iva (L.) Schreb. / Chendgoura* (UV = 0.98; RFC = 0.98) for food use, *Lavandula officinalis / Khezama* for medicinal use (UV = 0.96; RFC = 0.96), *Tuber oligospermum / Terfas* for food use (UV = 0.95; RFC = 0.95), *Mellissa officinalis / Hbak tranj* for food use (UV = 0.94; RFC = 0.94), *Illicium verum / Lyansoun* for medicinal use (UV = 0.93; RFC = 0 .93), *Eucalyptus globulus Labill / Lkalitos*, Caliptus for medicinal and other uses (UV = 0.92; RFC = 0.93), *Peganum harmala/ Harmal* for other uses (UV = 0.92; RFC = 0.92) , *Portulaca oleracea L/ Errejla* for food use (UV = 0.91; RFC = 0.91). (Table 4).

All of these species have the highest UV index and RFC, as they were mentioned by a large number of informants in the survey population. Medicinal and food plant species with a high UV index must be subject to quantitative, qualitative, phytochemical and pharmaceutical analysis in order to identify and quantify their active components. These species must also be prioritized for conservation because of their preferred uses to avoid overexploitation that can threaten their populations. This observation has also been the subject of subsequent studies and is shared by other authors in the field at the national and international level (Sarri et al., 2014).

The used parts

The analysis of the information collected showed that the leaves are the most used part with a percentage of 42% (Figure 5), followed by the aerial part (28%), then the fruits (18%), the roots (15%), seeds (13%), flowers (11%), stems (11%), then rhizomes (4%), and finally, tubers, pistil, stigma, bract and calyx (1%) simultaneously.

These results corroborate those of other previous ethnobotanical research which reported that the leaves were the most commonly used part for the treatment of various diseases and in food recipes (Essaih et al., 2023; Hankiso et al., 2023). The wide use of these parts can be attributed to the fact that they are easy to access and can be collected quickly. Additionally, leaves serve as the primary site of photosynthesis in plants and in some cases, as a storage site for organic compounds responsible for the plant's biological characteristics. All of these factors contribute to the widespread use of this organ in various recipes, applications and remedies (Djamel et al., 2019; Eichert & Fernandez, 2023; Tamang et al., 2023).

Method of preparation and administration

Among the various methods of preparing WEMCPs used to facilitate administration, infusion constitutes the majority method of preparation declared by the population in this study region (41.25%), followed by poultice (13.75%), as snack (8.75%), foot bath and powder (7.5%) simultaneously, drink (6.25%), steam (5%), compote (3.75%), oil (2.5%) and finally smoking, this use appears for the first time in the research studies carried out by our team, and especially characterizes the region followed by the use as nose drops, chewing and suppositories (1.25 %) (Figure 6).

The decoction makes it possible to collect the most active ingredients and to attenuate or cancel the toxic effect of certain recipes (Wentholt et al., 2001). These figures confirm the results obtained by various authors at the national level in the Moroccan province of Tarfaya (Idm'hand, Msanda & Cherifi, 2020) and internationally in Nigeria (Ekpo et al., 2008), in Burkina Faso (Nadembega et al., 2011) and in Rawalpindi - Pakistan (Ahmad et al., 2017) where the preparations were made with water as a solvent. The decoction and infusion are highly valued and often preferred by local healers in Africa (Benarba, 2016).

The local population mainly uses external application (49%), especially in the treatment of skin diseases and hair care. Other methods of administration (rectal, vaginal, inhalation) are represented by a percentage of 9% (Figure 7, Table 4). The

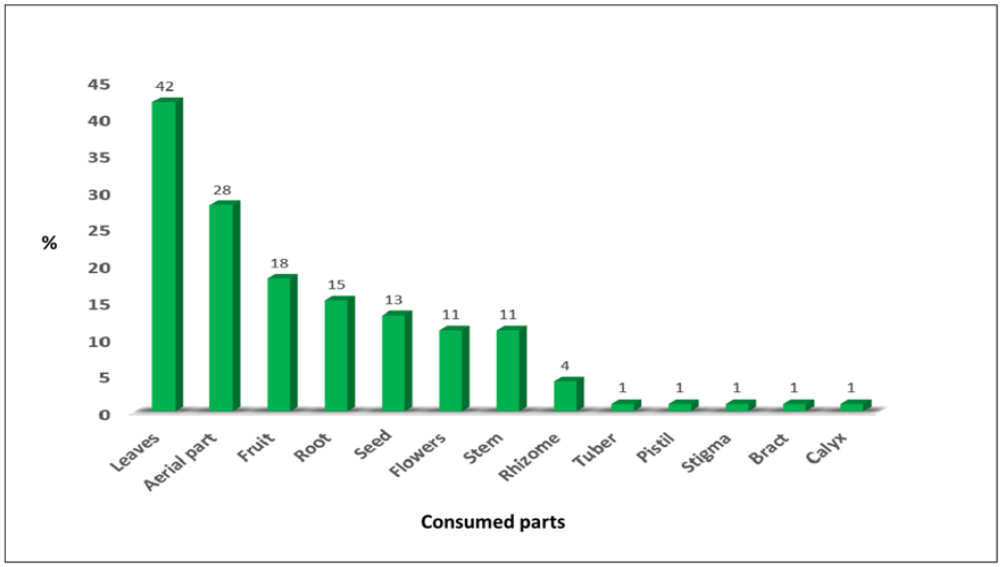

Figure 5. Distribution of the cited wild plants according to their consumed parts

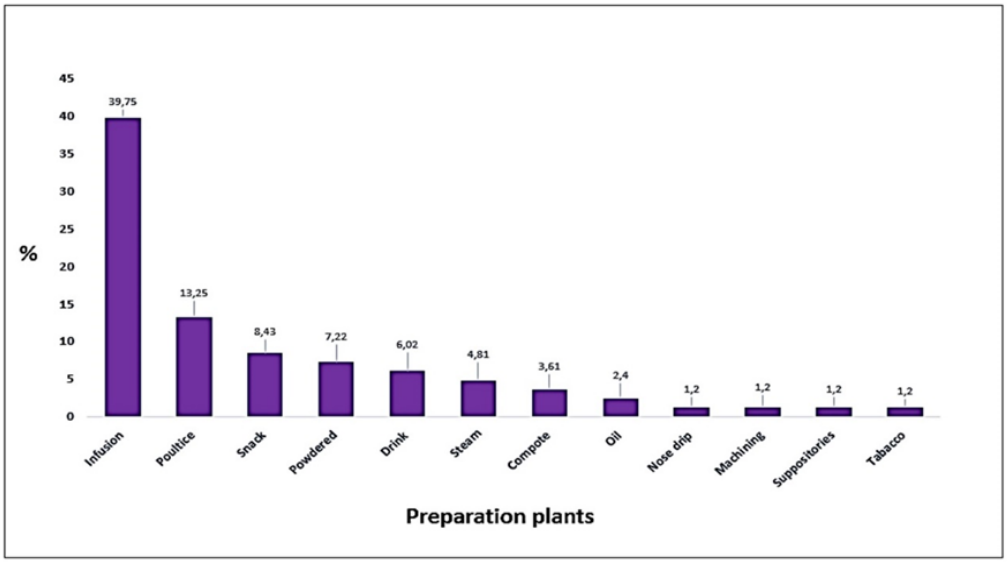

Figure 6. Methods of preparation of plants (%)

predominance of the external route makes the uses original in the study region (north of the country) and their difference well documented in the ethnobotanical studies carried out in the south of Morocco in Tarfaya (Idm'hand et al., 2020) and in Tata (Abouri et al., 2012) as well as in countries in the region such as Algeria, where the main route used and statistically in the majority is the oral route (Hadjadj et al. 2015; Madani et al., 2017).

In the present work, oral application is also used and constitutes the majority of preparation methods (42%) such as snacks, compote, oil, decoction, powder, infusion.

Informant consensus factor (ICF)

The very high value (0.999) of the study informants consensus factor for WEMCP was cited for gastric problems and digestif pain, diabetes, Heal wonds and skin problems, Face care, Cancer, Respiratory system infection, face care, respiratory system infection, hair care, fever, nerves, flu,

rheumatism, and others category (Table 5). This very high value confirms the robustness of the information. These results corroborate with those reported before other regions of the country such as in Messiwa region (Ghanimi et al., 2022) and in Casablanca- Settat region (Essaih et al., 2023).

Table 5. Informant consensus factor (ICF)

Category	Number of used reports	% of used reports	Number of taxa	ICF
Gastric problems and digestif pain	20	20,4	20	0,999
Diabetes	7	7,14	7	0,999
Heal wonds and skin problems	3	3,06	3	0,999
Face care	5	5,10	5	0,999
Cancer	3	3,06	3	0,999
Respiratory system infection	3	3,06	3	0,999
Hair care	9	9,18	9	0,998
Fever	3	3,06	3	0,999
Nerves	3	3,06	3	0,999
Flu	3	3,06	3	0,999
Rheumatism	3	3,06	3	0,999
Other	36	36,73	36	0,994

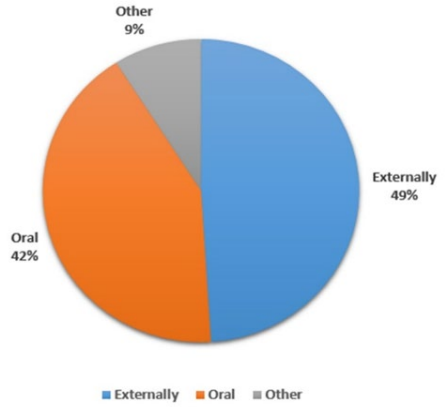

Figure 7. Routes of administration for herbal remedies (%)

Medicinal species used

The data from the present study shows that in this region, the medicinal plant species collected are consumed to treat a wide range of symptoms (Figure 8). Among these, gastric problems constituted the majority of pathologies (20.4%) treated with spontaneous plants in the study region. The same findings were recorded for medicinal plants listed in the town of El Jadida (Morocco) recommended to treat gastrointestinal disorders (Briguiche and Zidane, 2016) and in the region of Taounate (Northern Morocco) where these

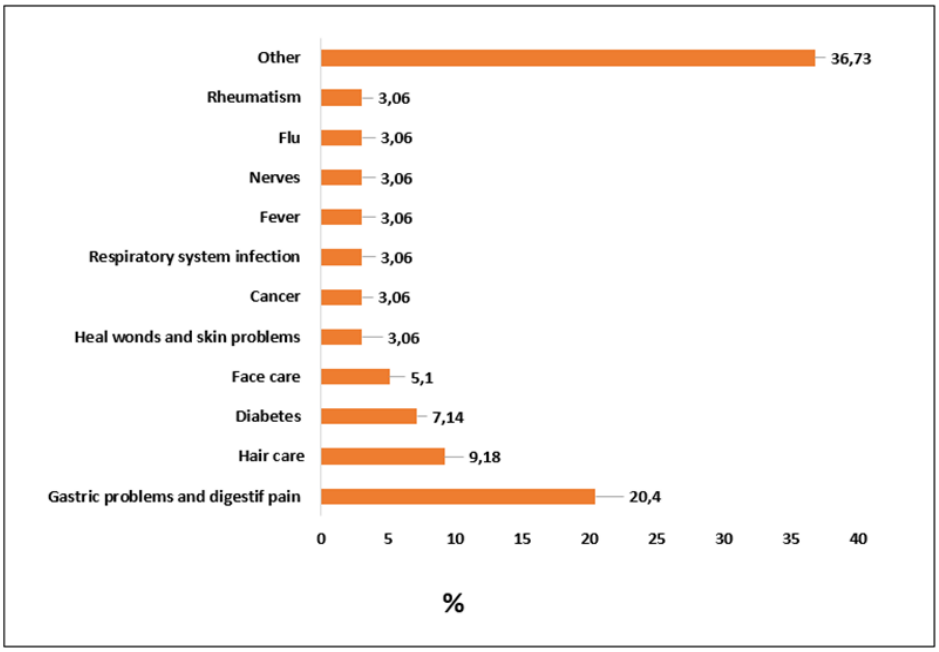

Figure 8. Pathologies groups

plants are reported to be the most used in the treatment of digestive problems (Sijelmassi, 1993).

The study respondents also reported using medicinal plants in hair care (9.18%), diabetes (7.14%), facial care (5.1%), wound healing and in skin problems, in cancer, respiratory system infection, fever, nerves, influenza, rheumatism (3.06%) simultaneously (Figure 7). Category of other disorders including menstrual pain, asthma, angina, reproductive glands, fever, eye diseases, live diseases, oriel pain, cough, psychological condition, rheumatology, hypoglycemic diuretic against bronchitis, kidney and gallbladder diseases, kidney diseases, pruritus, uterine pain, liver diseases, psychological state, anxiety, oral hygiene, disinfectant, allergies, anemia, skin diseases , hemorrhoids, emmenagogue, as a sedative, hypertension, positive energy represented in total with (36.73%) distributed over the different pathologies. This diversity of pathologies treated and medicinal uses of these plants, confirms the close relationship between the population studied and their good exploitation of their local floral biodiversity. Other work carried out in the Mediterranean region has confirmed the same results in Spain (Tardio et al., 2006), Algeria (Madani et al., 2017) and Italy (Sansanelli &Tassoni, 2014).

The insufficiency of health infrastructure and essential medicines, the low income of the population surveyed as well as the lack of access to health care also explain this important herbal medicine (Guedje et al., 2010; Idm'hand et al., 2020).

Socio-demographic and socio-economic characteristics

Figure 9 presents the distribution of knowledge of wild plants cited according to the sociodemographic characteristics of the population of the study region.

- *Level of education*

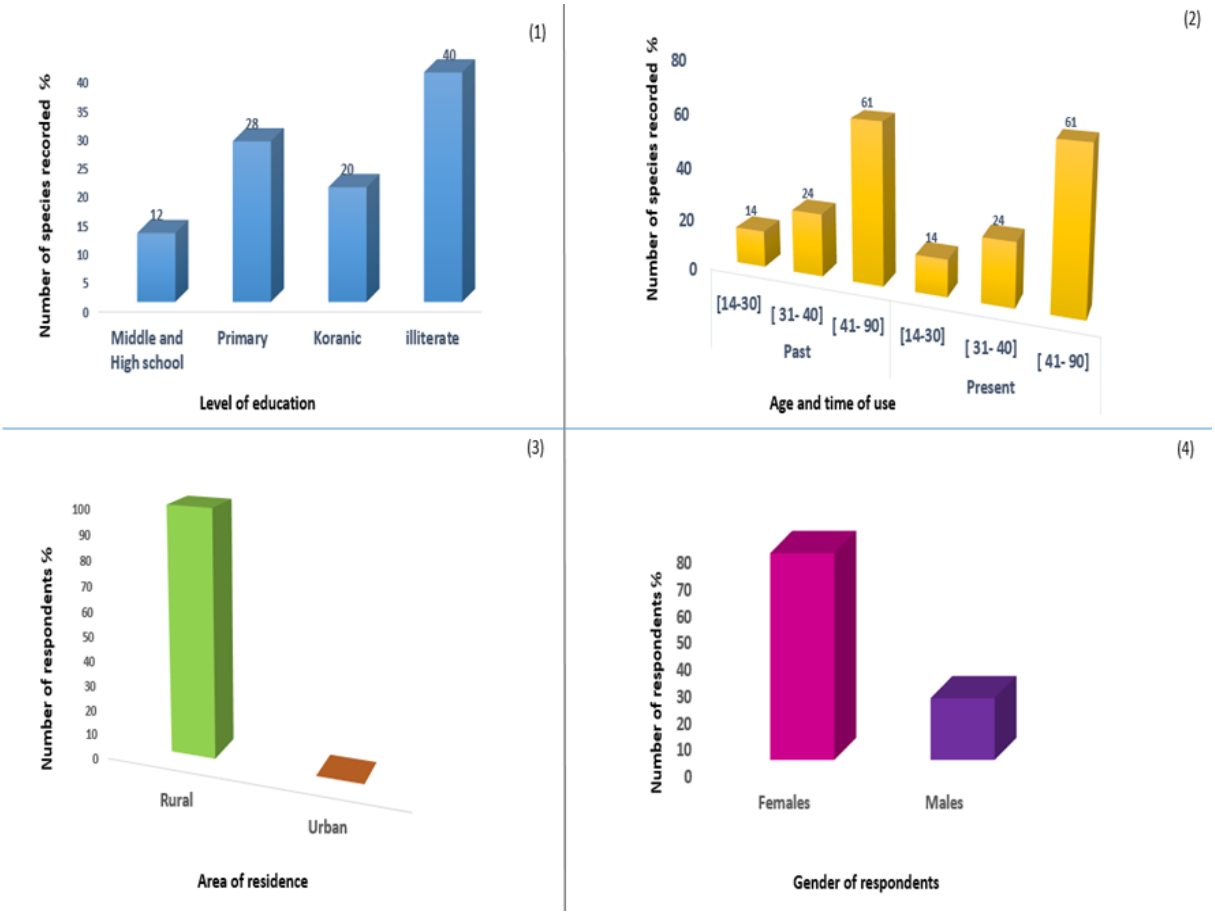

Figure 9. Number of recorded species/respondents according to education (1), age (2), area of residence (3), gender (4)

Knowledge of the plants consumed, assessed by the average number of citations, shows that the number of species cited by respondents decreases with the increase in the level of education. Figure (9-1) shows in fact that the greatest number of species of WEMCPs was cited by the illiterate (40%), followed by those at the Koranic school level (28%) then people of primary level (20%) and the smallest number was cited by those at secondary and high education levels simultaneously (12%). These results corroborate other ethnobotanical research on the same type of plants carried out in different regions of the country such as Messiwa (Ghanimi et al., 2022), and Casablanca- Settat (Essaih et al., 2023) of Morocco regions.

- *Depending on age and period of use*

Pleasantly surprisingly, for all age groups of the respondents, the number of WEMCPs species cited does not vary in the past and present (Figure 9-2). Indeed, the majority of people interviewed confirmed the story of their close relationship with nature explained by the ordinary uses cited linked to the consumption of these plants in the study region as well as the transmission of traditional knowledge of these uses from one generation to the next, and especially with the importance given to the region's plants on a national and international scale; the importance given to these plants is notably demonstrated by the creation of the National Agency for Medicinal and Aromatic Plants of Morocco.

- *Residence area*

As shown in Figure (9-3), no difference of knowledge level was revealed according to area of residence. This result is explained by the fact that all the survey respondents are (100%) rural and they carry out their professional activities at the local level. Indeed, the study area is a mountainous region with reliefs not allowing to combine work in a nearby urban environment while residing in a rural environment. Unlike in the Casa Blanca-Settat region where previous study data showing that (Essaih et al., 2023) respondents from the rural municipalities benefited from access to work in the cities while they reside in their rural environment.

- *According to gender*

Figure 9-4 shows that in this study, the use of wild plants was greater among women (77%) than among men (23%). The data collected shows that women were better informed than men about ethnobotany. Similar results are obtained in other research carried out in the province of Moulay Yaakoub, in Morocco (Mahraz et al., 2023), in western Mexico (Flores et al., 2023) and in the North. Waziristan in Pakistan (Ali Shah et al., 2023). One of the examples to cite of observations made during a visit to the study region, is the observation of this crucial role of rural women making extensive use of their time in the traditional cannabis crop in the Rif which, a family farming, with a complex work organization in complementarity with men teaching women how to undertake and carry out certain cultivation stages. Nevertheless, equity to create income rewarding this considerable effort of women in this work is needed to benefit from a better social environment. The same observation was also made previously during work carried out in the Moroccan Rif (Afsahi, 2015).

Conclusion

Data from the present study of Human Ecology about ethnobotanical knowledge conducted in the Taounat province of Rif mountains in Morocco revealed the importance of the use of WEMCPs in human populations for different purposes including food, traditional herbal medicine, cosmetics, psychoactive and other uses. This study identified a considerable number of 103 species of plants belonging to 46 botanical groups, used in various food recipes, in the treatment of a variety of conditions without forgetting their intervention in the well-being of women in the region.

The results of this study showed that the best knowledge of herbal medicine and traditional cuisine are held by the elderly women and that the majority of these various uses of plants are inventoried by the respondents with a low level of education or those that are illiterate. The study data also indicates that the most

used parts are the leaves made into decoctions and infusions and are taken orally. Digestive diseases are the conditions most frequently mentioned in these traditional remedies. The results also indicate that the most common plants in this area are those used as snacks and vegetables.

All these results constitute a valuable database for future scientific research for an in-depth evaluation of the composition and scientific validation through qualitative and quantitative studies in order to protect the population against the harmful effects of the consumption of certain plants containing toxic groups, while preserving this important cultural heritage. The latter is a contribution of this work to the knowledge and protection of biodiversity in the Rif Mountains in Morocco, and precisely in the studied province of Taounate, which has an environmental value of considerable socio-economic importance for populations, which must be preserved from hazardous exploitation.

List of abbreviations: TR= Taounat region/province, WEMCPs = wild edible medicinal and cosmetic plants, RFC= Relative Citation Frequency Index, UV= Use value Index, FL= Fidelity level Index, ICF= Informant consensus factor Index.

Ethics approval and consent to participate in the survey: The participants were informed about the survey objectives and a formal consent was given from all of them before any interviews were conducted.

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Perception and knowledge of organic food among the Moroccan population

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RESUMEN

Los recientes cambios en las pautas de producción y consumo de alimentos tienen importantes repercusiones en los sistemas alimentarios, con implicaciones para la salud humana y medioambiental. La agricultura ecológica se perfila como una palanca prometedora para fomentar el consumo sostenible de alimentos. El objetivo de este estudio es evaluar el nivel de consumo y la percepción de los marroquíes sobre los alimentos procedentes de la agricultura ecológica. Se realizó una encuesta a 423 participantes, principalmente de la provincia agrícola de El-Jadida en Marruecos. Se utilizó un cuestionario en línea creado con la herramienta Google Forms para recoger información sobre las características de los participantes, sus conocimientos y los factores determinantes y obstáculos que influyen en el consumo de alimentos ecológicos, así como las categorías consumidas de estos alimentos. Los datos de la encuesta muestran que el 81.7% de los encuestados consumían alimentos ecológicos por razones relacionadas principalmente con los beneficios para la salud humana y la reducción del impacto medioambiental, mientras que el 85.5% de los participantes declararon que el elevado coste de estos alimentos era la principal razón para no consumirlos. Además, el estudio reveló que una gran parte de los participantes, el 66.3%, tiende a confundir los productos ecológicos con los productos locales también llamados «Beldi». Los datos de este estudio confirman el creciente reconocimiento de la agricultura ecológica por parte de los marroquíes y ponen de relieve la necesidad de emprender acciones de sensibilización y desarrollo para seguir promoviendo este nuevo modo de producción agrícola sostenible.

Palabras claves:

Alimentos ecológicos
Alimentos locales
Consumo
Percepción
Población de Marruecos

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ABSTRACT

Recent changes in the patterns of food production and consumption have a significant impact on food systems with implications for human and environmental health. Organic farming is emerging as a promising lever for promoting sustainable food consumption. The aim of this study is to assess the consumption level and the perception of Moroccans regarding foods from organic farming. A survey was carried out on 423 participants, mainly from the agricultural province of El-Jadida in Morocco. An online questionnaire created with the Google Forms tool was used to collect information on the participants characteristics, their knowledge and the determining factors and obstacles, that influence the consumption of organic foods as well as the consumed categories of these foods. Survey data shows that 81.7% of respondents consumed organic foods for reasons mainly related to the benefits for human health and reduced environmental impacts, while 85.5% of the participants declared the high cost of these foods as the main reason for not eating them. In addition, the study revealed that a large part of the participants 66.3% tends to confuse organic products with local products also called "Beldi". The data from this study confirm the growing recognition of organic agriculture by Moroccans and highlight the need to undertake awareness and development actions to further promote this new mode of sustainable agricultural production.

Keywords:

Organic food
Local food
Consumption
Perception
Morocco Population

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Introduction

Sustainable food manifests itself as a key lever to overcome current challenges in order to protect both health and the environment (FAO & WHO, 2019). Indeed, the adoption of a lifestyle and traditional and local agricultural practices in the past have always made it possible to live in harmony with nature without harming the environment. However, the concern to feed the growing world population, estimated by food and agriculture organization (FAO) to increase by (60%) or 2.3 billion people by 2050 compared to 2009 (Alexandratos & Bruinsma, 2012) , as well as the industrial revolution, have led to changes of these practices toward increase of the production, an overexploitation and waste of natural resources leading to their depletion to the detriment of human health and that of the planet (Capone et al., 2012). The adoption of efficient production and food consumption behaviors limiting waste and losses would make it possible to modify or reverse this trend towards sustainable production and consumption (CIHEAM, FAO, Mediterra, 2016).

Global transition

Populations have moved from a society characterized by high birth and death rates to one with significantly lower mortality rates. This transition associated with the efforts of health systems is explained by the increase in life expectancy and the reduction in mortality rates often linked to communicable diseases thanks to medical progress, accessibility to health care and improvement of sanitary conditions (Antoine et al., 2010).

This transition generated population growth and a transition of the food system towards an increase in agricultural production. Furthermore, an economic transition due to industrial development and improving incomes has also changed eating habits. At the same time, societies have evolved from a dietary model based on seasonality and the consumption of local products towards a model focused on processed products, always available in abundance and rich in calories (Ronto et al., 2024). While this evolution has led to sufficient global production to feed the entire world population, malnutrition, hunger, food and nutritional insecurity however persist, affecting a significant part of our world, and continuing to increase despite adopted efforts (Sundaram et al., 2015). Paradoxically, while overnutrition is increasing and affecting a portion of the world's population, health problems linked to undernutrition still persist. The nutritional transition associated with these changes has led to health effects generating epidemiological profiles characterized by an increasing emergence of non-communicable diseases such as obesity, cardiovascular diseases, cancer and diabetes and has led to the double burden of malnutrition (Belahsen, 2014).

Effect on the environment and sustainable food

The intensive growth of global food production also has negative consequences on the environment, including the depletion of natural resources such as water scarcity, soil depletion, overfishing, deforestation, loss of biodiversity, pollution, biodiversity loss, air pollution and climate change. These are all major challenges that require the adoption of sustainable practices to overcome this situation and preserve the health of both humans and the planet (Dubey et al., 2021).

According to the Brundtland Report commissioned by the United Nations (UN) in 1987, sustainable development must meet the needs of the present without compromising the ability of future generations to meet their own needs (Brundtland, 1987). Indeed, development must precisely be economically efficient, socially equitable and ecologically sustainable (UN, 1992). Likewise, sustainable diet is introduced into this sustainable concept by adopting a lifestyle that reconciles meeting the nutritional needs of the current population and responsibility towards the environment without compromising the needs of future generations (Claasen et al., 2015).

Sustainable practices and Mediterranean diet

Different agroecological practices, such as the establishment of ecological farmer cooperatives or community organizations for green and circular agriculture, have emerged over the last decade, as an environmentally friendly agricultural approach, with social innovations aimed at ensuring a healthy and sustainable food supply to address concerns about food security while contributing to achieving the United

Nations Sustainable Development Goals (SDGs) (Claasen et al., 2015; FAO, 2021).

The Mediterranean dietary model constitutes a notable example of diet assuming the harmony between human health and environmental preservation (Serra-Majem et al., 2020). Indeed, the traditional diet of Mediterranean-type (MD) is recognized for its health benefits, widely documented in the literature since the first study of the seven countries (Besançon et al., 2000). The beneficial effects of MD on health, due to its nutritional composition, its respect for the environment, of the seasonality and biodiversity protection, have made MD and therefore of food system of Mediterranean countries, a sustainable diet model (Burlingame & Dernini, 2012; Serra-Majem et al., 2020) . The strong interconnection, in this model, of nutrition, local food production, biodiversity, culture and sustainability generates a lower impact on the environment. In addition, the notions of seasonality, fresh, traditional local and environmentally friendly products, culinary activities, variety of colors of fruits and vegetables were introduced at the same time as the main meals, conviviality and physical activity. It was therefore also included in the United Nations Educational, Scientific and Cultural Organization (UNESCO) world heritage list (Trichopoulou & Lagiou, 1997).

Transition from the Mediterranean dietary model

The MD is considered among the healthy diets recommended to combat non-communicable diseases (WHO, 2012). Moreover, consumers' awareness of the importance of adopting a healthy lifestyle on the environment and the impact of a healthy diet on strength of the immune system was also revealed during the Covid-19 pandemic crisis. Studies have indeed reported that adherence to a healthy diet, such as the MD diet, protected against Covid-19 infection (Perez-Araluce et al., 2022). However, the Mediterranean dietary model has also suffered increasingly amplified erosion in Mediterranean countries. Indeed, the current global transition including economic improvement, in addition to globalization accompanied by other factors such as food abundance and increased consumption have led to changes in food habits and quality with effects on nutritional status and health status (Serra-Majem et al., 2020). Furthermore, the Mediterranean model is threatened with being confronted with enormous challenges linked to climate change since Mediterranean countries are experiencing problems of water stress, a significant influx of inputs (pesticides, chemical fertilizers, etc.), and erosion of cultivable soils associated to the intensive human activities such as intensive agriculture that led to a loss of agricultural productivity and a breakdown of food systems (Iglesias et al., 2011). Added to this are the health problems that until now were at low prevalence compared to that in non-Mediterranean countries and which are currently increasing in this region, making this food system unsustainable (Iglesias et al., 2011).

On the other hand, the disruption of global supply chains created by various crises such as the pandemic and then the geopolitical conflict in Ukraine, has also shed light on the vulnerabilities of the global food system (Durand, 2023). This situation has prompted to rethink the food sovereignty of countries and direct consumption towards local production. This also placed emphasis on food production methods and the need to seek healthier options considering the sustainability of the food system while respecting the environment (Durand, 2023). On a global scale, the diet in the Mediterranean basin has also been affected by environmental problems compromising its sustainability. Safeguarding the latter requires taking into consideration the principles of sustainable development and adopting effective solutions for the long term (Serra-Majem et al., 2020).

Organic farming as a lever for sustainability

One of the sustainable and efficient agriculture models introduced as an aspect of agroecology is organic agriculture, which represents an alternative that respects the health of humans and the planet. Thanks to its standards and strict regulations, this agriculture is growing while gaining large shares of the global market and meeting the expectations of populations concerned about their health and that of the environment. It promotes health and well-being, the preservation of the environment, reduction of the carbon footprint, social equity, the local economy and it aligns with the concept of sustainable development (Bertrand et al., 2018).

As defined by the United States Department of Agriculture (USDA), organic agriculture means the

application of a set of cultural, biological, and mechanical practices that support the recycling of resources on the farm, promote the ecological balance and preserve biodiversity. This includes maintaining or improving soil and water quality, conserving wetlands, forests and wildlife, and refraining from the use of synthetic fertilizers, sludge purification, irradiation and genetic engineering (USDA, 2015).

Organic farming has been adopted by Mediterranean countries due to their favorable climate and the fact that the Mediterranean dietary model is close to the principles of sustainable agriculture. The adoption of this agriculture is made at different levels depending on the resources and means of each country. Indeed, Italy is one of the leaders of the organic market in Europe followed by Spain (Agence BIO, 2019).

The case of Morocco

Morocco, an African country on the southern shore of the Mediterranean basin, also became aware of the importance of organic agriculture from 1980.The first organic productions such as the cultivation of olive trees in the region of Marrakech and citrus fruits in the Benslimane region emerged in 1986.The organic sector was included in development strategies as in the Green Morocco plan (Bakkali, 2022; El Ghmari et al., 2022). In addition, the country is among the first African countries that adopted legislation specific to organic production set by the Moroccan government to regulate organic agriculture, including the law 39-12 relating to the organic production of agricultural and aquatic products. It has also established an 'BIO MAROC' label which allows it to produce organically for export (ONSSA, 2013). Furthermore, although Morocco faces enormous challenges to the sustainable development of organic agriculture, the demand for these products is continuously growing by Moroccan consumers who have become increasingly aware of the link between the quality of these products and health. Indeed, consumers are ready to invest more to guarantee healthier and more nutritious diet. This trend is also confirmed by the significant growth experienced by the market for organic infant products in Morocco, given the awareness of the benefits of organic farming among parents concerned about the origin and quality of the foods offered to their children. (Moussaid & Kharouaa, 2024).

Indeed, the choice of food of the Moroccan population is often oriented towards local products, locally called 'Beldi' products that they consider better than products from conventional agriculture judged on qualitative or taste aspects (Jazi & Kasmi, 2022).

Nevertheless, the development of sustainable organic agriculture within Morocco currently remains insufficient (Jazi & Kasmi, 2022) and the consumption of organic foods, also called "bio", remains modest or even low in the population. Knowledge of the situation and the determining factors which promote or prevent the consumption of organic products would therefore be of crucial importance for the development of organic agriculture in Morocco.

From this perspective, this work consists of a survey to understand the current situation and the evolution of the relationship and perception of the Moroccan population, with regard to the consumption of organic foods. More specifically, this involves collecting data on the way in which organic products are defined in the local context, the frequency of their consumption as well as the drivers and constraints of consuming products from organic farming among the population of the agricultural province of El -Jadida.

Materials and methods

Sample and the Survey conduction

Our questionnaire aimed to collect data on the perception of organic products by Moroccan consumers, mainly in the province of El Jadida which is an agricultural community. The questionnaire was developed taking into account the research objectives, including questions on the definition of organic products, the frequency of consumption as well as the motivations and obstacles to the consumption of products from organic farming. Participants were contacted randomly.

The study was conducted online from May 31 to August 22, 2023. The sample is made up of people contacted randomly via social networks (WhatsApp, Facebook, Instagram) and via the personal and professional networks of the authors of the study. Participants were also encouraged to share the questionnaire link with their acquaintances and networks. Some participants, mainly fruit and vegetable sellers, were contacted directly to collect

information using a pre-established questionnaire. Questionnaires with incomplete responses were discarded after the survey closed. The size of the final sample reached and used in this study was 423 respondents.

The questionnaire used to collect information from respondents was distributed online using the Google Forms tool and included questions on socio-demographic characteristics, consumption determinants and the type of food produced from the Organic Agriculture.

Data collection

Information on participant characteristics included the place of residence, the monthly income, the age range, the education level, the employment status, and their knowledge concerning organic products. Information on consumption determinants included frequency of consumption, motivation to consume and disincentives to consume organic products. Information on the types of organic foods consumed in the food categories of starches, fruits and vegetables, dairy and meats was also recorded.

Two essential parts were considered in the survey questionnaire, a first part intended for individuals familiar with the organic concept or organic farming, and a second part intended for individuals who are not familiar with this concept. Two groups are then created from the respondents participating in the study, the group of respondents called "organic known" (n=232) that was familiar with organic farming, and the 2nd group bringing together participants who are not familiar with these products and called “organic unknown” (n=191). The individuals included in this 2nd group were entitled to an awareness video before completing part 2 intended for them in the questionnaire. The video was released online and aimed to highlight the difference between organic and conventional farming. The awareness program presented in this video addressed the evolution of human food consumption through the ages with a focus on conventional agriculture and the emergence of organic agriculture. The program also highlights the positive and negative aspects of these two types of agriculture. The language used in the video was the Moroccan dialect to reach a larger number of people of different education levels. In addition, respondents have the choice between the two languages, Arabic or French to complete the questionnaire, to facilitate the collection of qualitative and quantitative data.

Strengths and limitations:

The present survey, largely based on the online aspect, gave us many advantages, not only in terms of ease for data collection and accessibility, but also by guaranteeing anonymity, thus promoting strong honesty in the responses to the questions. However, these responses may be influenced by factors such as whether participants are in a hurry to complete the questionnaire. Additionally, this technology-based survey method excludes a portion of the population that is not familiar with technology or does not have access to the internet. This is why our survey was not exclusively carried out on the internet; it was supplemented by a face-to-face survey carried out mainly within local markets. Certainly, the survey provides good results, but it is essential to take into account these limitations when analyzing the results.

Ethical considerations

Survey participants are informed of the purpose of the survey and that the information collected will be anonymous, confidential and used for research purposes only. Respondents also volunteer to participate in this anonymous study and are asked to provide consent before starting to complete the questionnaire. They are also free to leave the survey at any time if they wish.

Results

The Tables 1 and 2 and the Figures 1-8 present the data obtained in the present study. As shown in Table 1, among a total number of 423 respondents representing the final study sample, the majority were women (59.1%), resided mainly in urban areas (97.3%) and mostly students (59.5%). Also, 27.4% were professors, 25.7% workers, 21.0% employees and 1.7% were the retired and 12.8 unemployed people. The most represented age group (63.8%) was mainly that of 20 and 40 years old, followed by the group of those between 40 and 60 years old (20.0%) then the age group under 20 years old (15.3%) and finally, the respondents over 60 years old represent only 0.7% of the sample.

Table 1. Characteristics of the study sample (n=423) from El Jadida province (May 31 to August 22, 2023).

Characteristics		Number	%
Area of residence	Urban :	412	97.30%
	Rural	11	2.60%
Gender	Females	250	59.10%
	Males	173	40.80%
Age range	Under 20 years old	65	15.30%
	Between 20 and 40 years old	270	63.80%
	Between 40 and 60 years old	85	20.00%
	Over 60 years old	3	0.70%
Education level	High School	7	2.70%
	Baccalaureate	75	29.70%
	Baccalaureate+2yrs	58	23.00%
	Baccalaureate+3yrs	59	23.40%
	Baccalaureate+5yrs	33	13.00%
	Baccalaureate+>5yrs	20	7.90%
Professional Activity	Workers	44	25.70%
	Professors	47	27.40%
	Employees	36	21.00%
	Unemployed	22	12.80%
	Retired	3	1.70%
	Other professional activities	19	11.10%
Income per month (MAD)	< 4000	10	6.90%
	4000 - 8000	60	41.90%
	8000 - 15000	65	45.40%
	>15000	8	5.50%

Based on the response to the central question of the questionnaire consisting of whether the participants “know about organic farming?”, the respondents were classified into two distinct groups of individuals, those who know it and those who do not know it, named respectively "organic known" and "organic unknown".

The answers to the specific questions in each part of the questionnaire aimed to better raise awareness and understand the habits and perspectives of organic food consumption of each group.

The data obtained revealed that just over half 54.8% (232) of the participants were aware of organic farming, while 45.1% (191) said they had no idea.

Sensitization

This section presents the results regarding the answers to the questions asked to the participants of the group who were unfamiliar with organic products, which represent 191 of the total participants (423), named "organic unknown" after the broadcast of the awareness video. The results regarding the definition of organic by the participants in this group as presented in Figure 1, shows that 38.5% consider organic foods to be equivalent to "Beldi" or local products, a proportion of 52.6% associate them with health, 33.8% link them to quality, 18.2% perceive them to contribute to well-being, 19.2% declared them expensive, while 29.1% consider them in relation to the environment.

Table 2. Results of the survey conducted in the province of El Jadida, from May 31 to August 22, 2023.

Results		N	%
Definition of organic by "organic unknown" participants (n=191)	Health	101	52.80%
	Well-being	35	18.30%
	Quality	65	34.00%
	Environment	56	29.30%
	Expensive product	37	19.30%
	Beldi	74	38.70%
Motivations for consuming products from organic farming by "organic unknown" participants (n=191).	The preservation of the environment	88	46.00%
	Maintaining and improving your health	118	61.70%
	Participation in the development of animal welfare	18	9.40%
	The quality of the products (taste, nutritional, etc.)	63	32.90%
	None	1	0.50%
Factors that can slow down purchases of organic products by "organic unknown" participants (n=191).	Very expensive	161	84.20%
	Insufficient choices	25	13.00%
	Lack of confidence (are they really organic products?)	68	35.60%
	Difficulty finding these products	63	32.90%
	Appearance	5	2.60%
Varieties of organic food products consumed by 214 participants who are already familiar with organic foods.	Meats	79	36.90%
	Fruits and vegetables	132	61.60%
	Dairy products	124	57.90%
	Starchy products (bread, pasta, rice, etc.)	127	59.30%
	I don't know	9	4.20%
	Other	6	2.80%
The ways in which participants of "organic known" (n=232) recognize organic products.	Packaging indications (certifications, brands, labels…)	111	51.80%
	Trust in sellers	24	11.20%
	Does not contain preservatives or pesticides	15	7.00%
	Purchasing from farms	28	13.00%
	Not sure	24	11.20%
Reasons for consuming products from organic farming by participants that consume organic products (n=214).	They are more delicious in terms of aroma and flavor	127	59.30%
	Their production does not use pesticides	100	46.70%
	They may provide benefits for human health	175	81.70%
	They are less polluting for the environment	73	34.10%
	They are more nutritious	143	66.80%
	They help local farmers	63	29.40%
Reasons likely to slow down the consumption of products from organic farming by participants that consume organic products (n=214).	Because their prices are generally more expensive than conventional foods	183	85.50%
	Because they are the same as those produced conventionally	11	5.10%
	Because they are not for sale near me or my workplace	112	52.30%
	Because these products are less beautiful	28	13.00%
	Because I don't think they meet all food safety standards	25	11.60%

Places to buy organically grown food according to "organic known" participants (232 participants).	Supermarket	129	55.60%
	Local grocery store	52	22.40%
	Online, with commercial platforms	12	5.10%
	Organic shop	39	16.80%
	Go directly and in person to the producer	130	56.00%
	Online, with the producer's platform	7	3.00%
	I don't know	10	4.30%

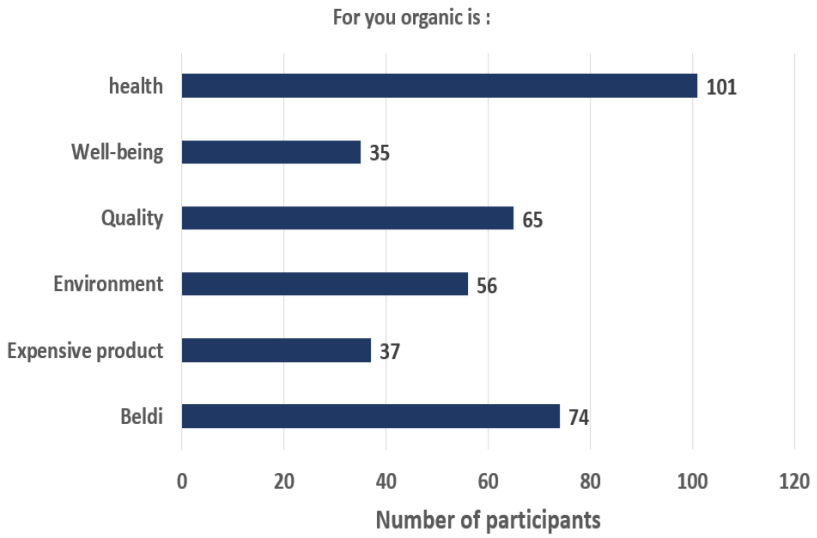

Figure 1. Definition of organic by "organic unknown" participants (n=191) from the province of El Jadida, from May 31 to August 22, 2023.

Regarding their willingness to consume organic agricultural products in the future, the results reveal that a significant percentage (48.9%) of respondents from the "organic unknown" group stated that they consider consuming these products in the future, while 7.8% have no intention of changing their consumption habits. However, a large proportion of participants, representing 43.2%, remain undecided about consuming organic products

The results concerning the reasons or motivations to adopt an organic diet by the "organic unknown" participants are presented in Figure 2. This figure shows that the vast majority of these participants (61.4%) associate the consumption of organic food with better health, 45.8% believe it contributes to environmental preservation, 32.8% consider that organic products surpass those from conventional agriculture in terms of quality, and 9.3% link organic consumption to animal welfare.

Data regarding the limiting factors likely to discourage participants from opting for organic food consumption are presented in Figure 3. According to these results, the main reason given by the majority of respondents (83.8%) was the high cost of these products. Next comes the lack of confidence among producers of organic products regarding effective compliance with the standards in force for organic products, representing a percentage of 35.4% of participants' responses. The third reason given by 32.8% of respondents is the difficulty of obtaining or finding organic products locally. Then, the limited choice of products offered by producers is declared by 13.0% of participants. Finally, the appearance of organic products constituting a minor obstacle was only declared by 2.6% of those questioned as part of the present survey.

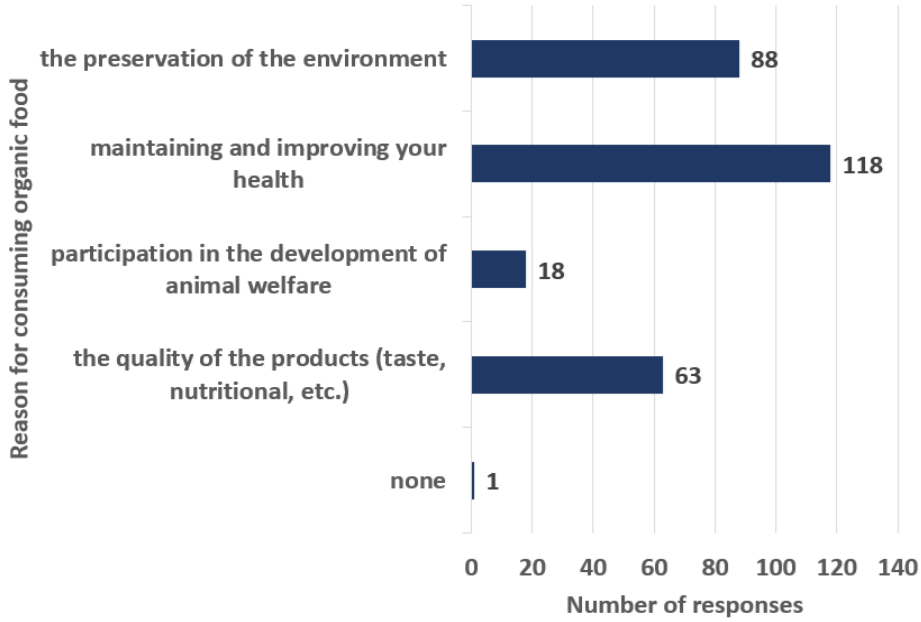

Figure 2. Motivations for consuming products from organic farming by "organic unknown" participants (n=191) from the province of El Jadida, from May 31 to August 22, 2023.

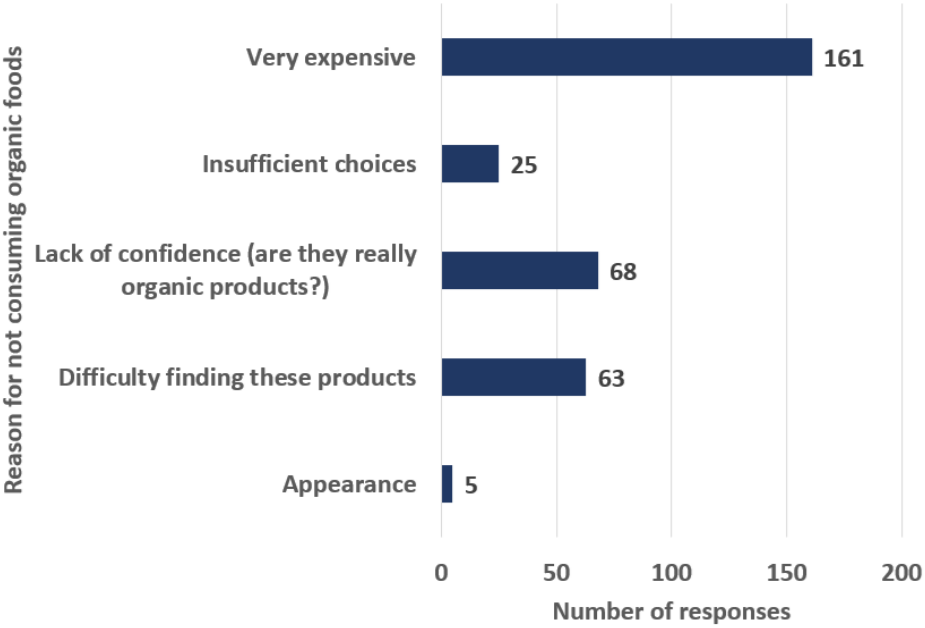

Figure 3. Factors that can slow down purchases of organic products by "organic unknown" participants (n=191) from the province of El Jadida, from May 31 to August 22, 2023.

Occasional consumption of organic

In this part of the study, the main objective of the results obtained is to provide a better understanding of the consumption habits of products from organic farming. They consist of responses to questions from the part of the questionnaire which exclusively targeted participants familiar with organic farming representing 54.8% of the sample surveyed, or 232 individuals. These results reveal that 86.2% of participants of this group, qualified as "organic known", have declared that

they occasionally consume organic products and only 6.0% consume them regularly while 7.7% declared that they do not consume them at all.

Regarding knowledge of the difference between organic food products and local products, commonly called "Beldi" in Moroccan dialect, the results showed that 66.3% of participants declared considering the term "Organic" as being synonymous with "Beldi" (local or terroir), while only 33.6% responded that the concept of "Organic" products differs from that of “Beldi” products.

Concerning the consumption habits of participants who consume organic products, representing 214 out of the total 423 participants, in terms of types of organic food products, including fruits and vegetables from organic farming come in 1st place, with 61.6% of those surveyed having declared a propensity to consume these products. Starchy foods come in 2nd position with 59.3%, then dairy products whose consumption is planned in the future at 57.9% and finally meat consumption was declared by 36.9% of the participants (Figure 4). However, 4% of the participants were unsure about what they would consume in terms of organic products, while 2.8% said they would use organic oils, honey and mint.

Regarding how consumers (214) recognize or determine whether the products they purchase are organic, the study results shown in Figure 5 reveal that more than half of study participants (approximately 51.8%) reported relying mainly on packaging indications, such as certifications, brands or labels like AB or Bio. Additionally, only about 11.2% of respondents expressed trust in sellers or suppliers, while 7.0% said they believe a product is considered organic if it does not contain preservatives or pesticides. Additionally, 13.0% knew the organic origin of their product because they purchased it from farms selling organic or “beldi” (local) products. Finally, 11.2% admit to not being sure of the true organic nature of their products.

The motivations underlying the consumption of organic foods among participants familiar with the organic concept (214) are summarized in Figure 6. Among these, concern for health was cited by 81.7% of participants, followed by nutritional quality mentioned by 66.8% and the taste of organic food products which comes in third position (59.3%). Other motivations were also declared by the participants of this group and concerned the desire to avoid harmful products such as pesticides (46.7%) and the impact on the environment expressed by 34.1%. Additionally, 29.4% of respondents mentioned using these foods to support local farmers.

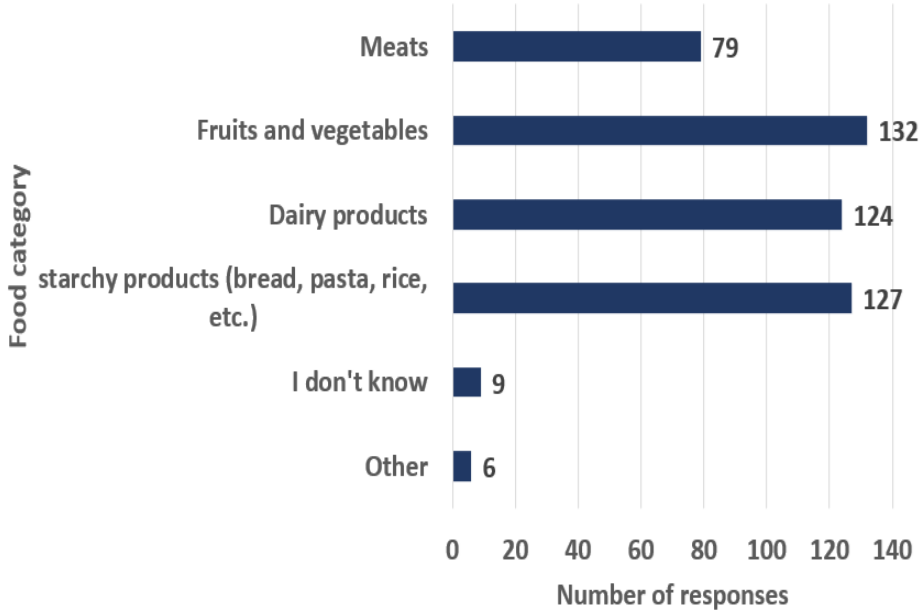

Figure 4. Varieties of organic food products consumed by 214 participants who are already familiar with organic foods in the province of El Jadida, (May 31 to August 22, 2023.

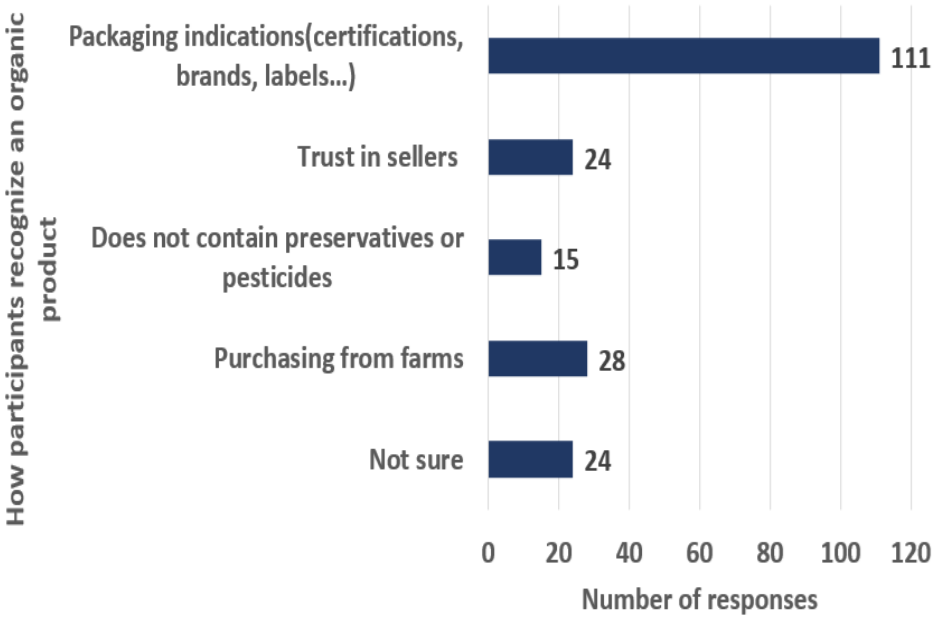

Figure 5. The ways in which participants of "organic known" group (n=232)

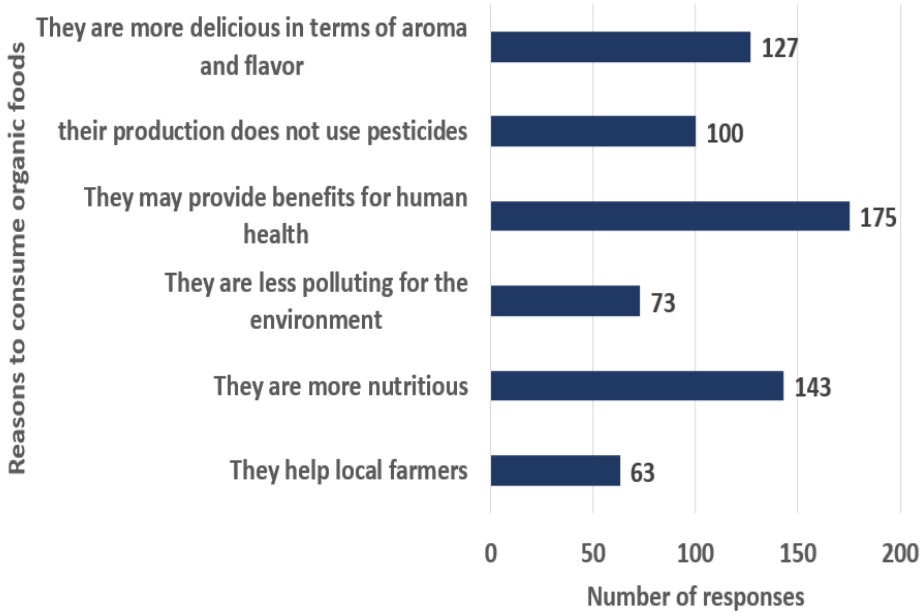

Figure 6. Reasons for consuming products from organic farming by participants that consume organic products (n=214).

The Figure 7 shows that the consumption of food products from organic farming by the participants (214) however remains moderate. The results of Figure 7, based on the declarations concerning the factors which slow down the purchase and consumption of these products by the participants, show that for the majority of them (85.5%) the high cost of these products represents the main reason. The non-availability of these products near the participants' place of residence was also mentioned by 52.3% of them, coming second, followed by the apparent aspect and appearance deemed unattractive by 13.0%. In

addition, 11.6% of the participants mentioned the non-compliance with health standards by these products and 5.1% believe that these products do not sufficiently distinguish themselves from conventional products.

As shown in Figure 8, among the consumers of organic farming products (214), the most favorable places to make purchases are supermarkets for a percentage of 55.6% and directly from producers for a percentage of 56.0%. Next come grocery stores (22.4%), followed by stores specializing in organic products (16.8%), as well as online purchases (8.1%).

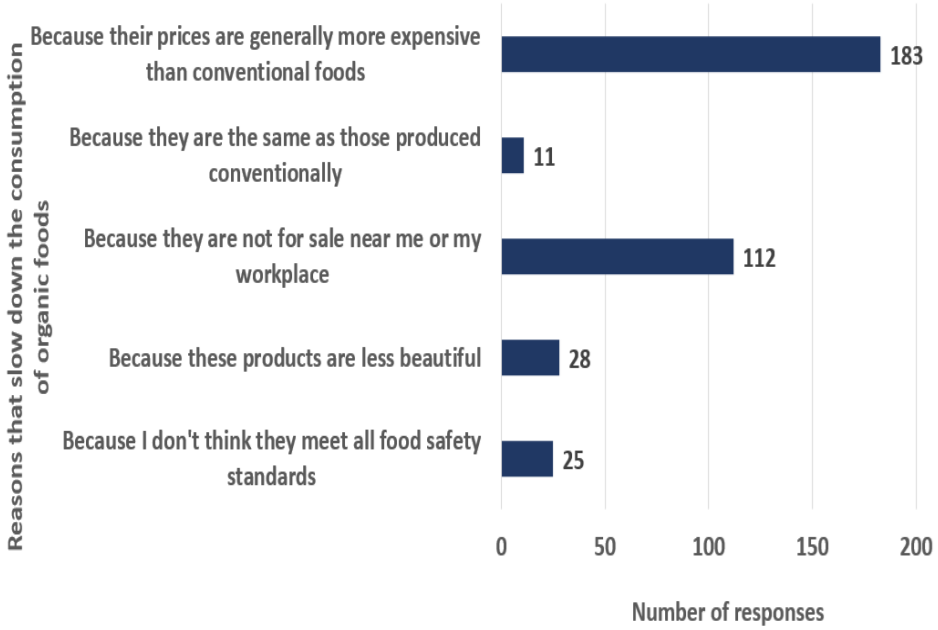

Figure 7. Reasons likely to slow down the consumption of products from organic farming by participants that consume organic products (n=214).

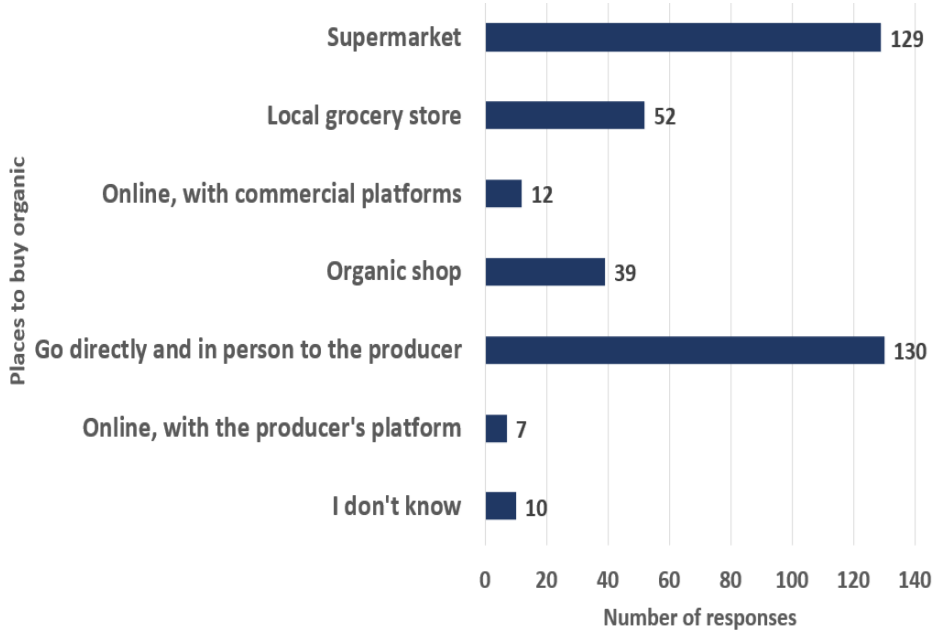

Figure 8. Places to buy organically grown food according to "organic known" participants (n=232).

Among respondents in the "organic known" group (232), the majority (85,7%) say there are few places to find organically grown products, while only 6,4% believe there are enough places to get these products and 7,7% have no idea of their availability.

Concerning the evaluation of knowledge of the label established to identify national organic products, named “BIO MAROC”, the survey results show varied responses among the 232 participants, the majority (52.1%) having declared not to know the label “BIO MAROC » against 24.1% of participants who recognize this. Furthermore, 15.0% say they are aware of the existence of the “ORGANIC MAROC” label but have never had the opportunity to see it during their purchases.

The opinion of the participants interviewed, on the impact of the major health crisis that the world has recently faced, namely the COVID-19 pandemic, on the consumption habits of organic products was also sought. The results showed that around 46.9% of those surveyed said they were not sure whether the consumption of organic farming products had increased after the crisis. However, 47.4% of those deemed 'organic known' believe that society began to place more value on local and organic products after the pandemic.

Discussion

This work sheds light on the motivations and obstacles to the consumption of food products from organic farming within the Moroccan population. The results showed that more than 50% of participants are aware of products from organic farming, half of which consume them and among the latters 81.7% declared “health” as the main motivation for this consumption. On the other hand, 85.5% cited the high price as the main obstacle to organic consumption. This work also made it possible to notice that 66.3% of the participants tend to confuse organic with local products also called 'Beldi' products. The study data reported in this article made it possible to assess Moroccans' knowledge of organic farming and their level of consumption of its products. The approach adopted included two parts in a survey with, on the one hand, individuals familiar with the concept of organic food and on the other hand to raise awareness among those who are not, through a video highlighting the advantages and disadvantages of organic farming compared to conventional farming.

As in other countries, organic agriculture in Morocco is of crucial interest. Currently, it constitutes an essential lever for the transformation of food systems in order to contribute to sustainable development. In Morocco, the agriculture sector represents 14% of GDP and accounts for 40% of the active population (Bakkali, 2022). Historically, the country has, since the 1960s, seen its economy and its social fabric flourish. This economic growth has been accompanied, however, by major concerns, notably the reduction of rural life and space associated with a gradual disappearance of local products directly from the land (Motib et al., 2020). This phenomenon is attributable to climate change, the rise of industrialization, the emergence of large commercial outlets, modernization in parallel with these factors the appearance of new diseases and the increase of certain health ailments. These problems have often led to a change in agricultural practices, which have become more intensive and characterized by the use of dangerous chemicals, directly impacting the sustainability of the food system.

In this regard, Morocco, a Mediterranean country, has favored, in its future orientations, agriculture, particularly organic farming, considered as an opportunity to achieve sustainable development ensuring the prosperity of the three main areas of society, namely, the economy, the social and the ecosystem (Capone et al., 2012). The country began the practice of organic farming in 1986 in certain regions, notably in the Marrakech region with the cultivation of olive trees and in Benslimane with citrus fruits. The country has since achieved its first organic harvests and continues to display organic production, mainly dedicated to export. Furthermore, this orientation is now more framed by laws and standards, in this case law 39-12 of January 16, 2013, relating to the organic production of agricultural and aquatic products and its BIO MAROC label (Bakkali, 2022; ONSSA, 2013).

Everyone is aware that conventional agriculture has the capacity to feed a large population that organic farming does not have. Nevertheless, one of the distinctive assets of the latter is to offer prospects for sustainable development and to contribute to the preservation of biodiversity (Bertrand et al., 2018).

Furthermore, the orientation of organic agriculture mainly towards export is linked to increased demand and valorization by importing countries (AFC, 2020), in addition to the high level of awareness among populations about the benefits of these foods both for health and on the environment. Paradoxically, this valorization leads to an increase in their expensive cost for local populations who thus resort to cheaper available products (Boulahoual & Gaber, 2016). Filling the lack of data on the situation of consumption of organic products in the Moroccan population as well as on the determinants which facilitate and those which hinder this consumption is of crucial interest. In the present study some elements of responses to these questions are provided.

The data collected in the present study, from respondents unfamiliar with organic food "organic unknown", revealed that a large part of the participants in this group tend to confuse organic products with local products locally named “Beldi”. Furthermore, the majority of these participants expressed their intention to integrate products from organic farming into their diet. Their main stated motivation was the benefits of these products on their health. However, they recognize that barriers such as high prices, lack of diversity of organic products at points of sale and distrust of farmers and producers of these products could limit their adoption of sustainable consumption of Organic Products. Furthermore, the lack of control over food products quality from organic farming has also aroused a certain mistrust on the part of consumers. This explains the tendency of participants not to favor organic products to the detriment of those from conventional agriculture.

Confusion between organic and local or “Beldi” (terroir) products also existed among the group declared to be familiar with organic products, "organic known" group. Indeed, the results show that most of the participants in this group actually confuse these two terms and only consume organic products occasionally. The reasons for consuming organic products as mentioned by the participants were mainly the health benefits, better quality in terms of nutritional value of these products as well as the taste and the lack of chemicals and harmful products such as pesticides. In addition, organic foods consumed occasionally in this group were mainly composed of organic fruits and vegetables. These products constitute an important food group of the Mediterranean diet and basic ingredients of culinary preparations widely consumed by the Moroccan population. The introduction of these foods when their origin is organic rather than conventional agriculture would have a beneficial impact on health (Baudry et al., 2019). However, the high cost and unavailability of these organic products are considered essential obstacles to their consumption by most individuals who are familiar with these foods ("organic known" group). Thus, it is noted that most individuals prefer to find out about organic products directly from the producers in the first place, or in supermarkets when shopping for these products. In addition, a large majority affirmed that there were few places that offer products from organic farming. This constitutes another among the many reasons mentioned which slow down the large consumption of organic products. The other reasons are similar to those noted in the first axis of the "organic unknown" group, which gives certain coherence to our study.

The data from this study also reveal the reasons explaining the lack of organic products on the Moroccan market. Indeed, organic agriculture in Morocco occupies a significant place and benefits from particular attention through the establishment of specific laws and regulations and measures whose objective is to protect both producers and consumers of foods from organic farming. In this context, the label 'BIO MAROC' was also established, to identify national organic products (Bakkali, 2022). However, these measures are rather focused on products intended for export. Among the reasons given for the unavailability of organic products, the most main is that a large part of local production is intended for the international market (AFC, 2020).This finding is supported by the statements of a large proportion of participants who are not aware of the ‘BIO MAROC’ label. Further, the few people who claim to know this label say they have never seen it when shopping. This confirms the legitimate thesis mentioned, namely that the majority of organic production is intended for export.

In this survey, knowing the extent of an urgent situation namely the covid crisis, on organic consumption was also evaluated. According to the results found, most respondents declared that during

this crisis, organic products were valued by society thanks to their better quality in relation to their nutritional value content. However, this increase in organic consumption was prevented, especially with the rise in prices following the economic crisis caused by COVID and aggravated by the geopolitical conflict between Ukraine and Russia.

Conclusion

This work reports that awareness of the positive impact of organic food on health and the environment begins to make itself known among consumers in Morocco. They nevertheless remain convinced that the prices of organic foods are excessively high. The data also shows that the population insists on the need to establish strict control to guarantee the availability of organic farming products in addition to ensuring compliance with the standards and regulations that govern them.

This study also drew attention to the importance and need to raise awareness about organic farming among individuals. Indeed, the information conveyed by the video used for this purpose in this research work, although modest and restricted, managed to reach a considerable part of the participants. More particularly, in the current context that the planet is experiencing with global warming, the broadcast of this video also made it possible to raise awareness of the interest in consuming products from organic farming, not only to improve their health but also to the preservation of the environment. Introducing the content of this video into a youth education program could have more impact.

The present survey also revealed the conviction of most of the respondents that “Bedi” and organic are synonymous and that it is enough to consume products from the countryside to adopt a sustainable diet. The present data also show that it is necessary to inform consumers to shed light on the difference between organic products and local products or “Bedi” within the Moroccan population. Efforts must be made in this direction, both to show the importance of organic and to eliminate the confusion between organic and “Bedi”.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Evaluation of the nutritional education program for the prevention of non-alcoholic fatty liver disease in people with obesity in Morocco

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RESUMEN

La obesidad y la enfermedad del hígado graso no alcohólico (EHGNA) crecen exponencialmente y amenazan el desarrollo mundial. En Marruecos la situación también es problemática y constituye un reto creciente para los servicios sanitarios. En la actualidad, no existen tratamientos aprobados para la enfermedad del hígado graso no alcohólico relacionada con la obesidad. Sin embargo, la pérdida de peso y una ingesta alimentaria adecuada pueden mejorar la enfermedad hepática (histología). Para ello es necesario concienciar a la población sobre el alcance de este problema de salud, lo que puede garantizarse mediante la sensibilización y la educación en materia de nutrición y estilo de vida saludable. Una educación nutricional bien realizada es un elemento clave para la prevención de la EHGNA. El objetivo de este trabajo es evaluar el impacto de un programa de educación sobre parámetros clínicos y metabólicos en personas obesas con o sin EHGNA en Marruecos. Método: se realizó un estudio de intervención en una consulta médica de gastroenterología de la ciudad de Marrakech (Marruecos). Se llevó a cabo un programa de educación nutricional de seis meses mediante la adopción de la dieta mediterránea y la práctica regular de actividad física en 210 participantes obesos. Los profesionales sanitarios realizaron un seguimiento de todos los participantes durante dicho periodo y se evaluaron parámetros clínicos y metabólicos al inicio y al final del programa educativo. Resultados: de los 210 participantes diagnosticados como obesos, 131 tenían EHGNA y 79 no presentaban esta patología. Tras los seis meses de educación nutricional los participantes mostraron mejoras significativas en los parámetros antropométricos, en la alanina aminotransferasa, el aspartato aminotransferasa, la gamma-glutamiltransferasa, los triglicéridos, el colesterol de lipoproteínas de alta densidad y el colesterol total. Conclusión: la educación nutricional es una vía eficaz para el tratamiento de la EHGNA relacionada con la obesidad.

Palabras claves:

EHGNA
Obesidad
Alimentación
Programa de educación nutricional

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ABSTRACT

Obesity and non-alcoholic fatty liver disease (NAFLD) are growing exponentially and threaten global development. In Morocco, the situation is also problematic and constitutes a growing challenge in health services. Currently, there are no approved treatments for obesity-related nonalcoholic fatty liver disease. However, weight loss and adequate food intake can improve liver disease (histology). This requires awareness among the population about the extent of this health problem which can be ensured through sensitization and education in terms of nutrition and healthy lifestyle. Well-conducted nutritional education is a key element for the prevention of NAFLD. The objective of this work is to evaluate the impact of an education program on clinical and metabolic parameters in obese people with or without NAFLD in Morocco. Method: An interventional study was conducted in a gastroenterology medical practice in the city of Marrakech (Morocco). On 210 obese participants, a six-month nutritional education program was carried out through the adoption of the Mediterranean diet and regular physical activity. A follow-up of all participants for six months was carried out by health professionals. Clinical and metabolic parameters were assessed at the beginning and at the end of the education program. Results: Among the 210 participants diagnosed as obese, 131 had NAFLD and 79 were without this pathology. After six months of nutrition education, participants showed significant improvements in anthropometric parameters, alanine aminotransferase. aspartate aminotransferase, gamma-glutamyltransferase, triglycerides, high-density lipoprotein cholesterol, and total cholesterol. Conclusion: Nutrition education is effective way for the treatment of obesity-related NAFLD.

Keywords:

NAFLD
Obesity
Food
Nutritional education program

Introduction

The World Health Organization (WHO) defines obesity as an abnormal or excessive accumulation of fat that can impair health (WHO, 2022). Its global prevalence in 2016 is 13% (Janssen, Bardoutsos & Vidra, 2020). Obesity has also been associated with an increased risk of non-alcoholic fatty liver disease (NAFLD) (Li et al., 2016). This disease occurs in 60 to 95% of obese patients in all countries of the world (Angulo, 2002). In Morocco, the prevalence of obesity was estimated at 33.6% by a prospective study carried out in 2000 by the Moroccan Ministry of Health. The prevalence of obesity-related NAFLD is estimated at 83.5%, between 2000 and 2017 (Liba & Belahsen, 2022). NAFLD is the chronic liver disease that generally refers to excessive accumulation of triglycerides without excessive alcohol consumption (less than 20 or 30 g/day for women and men, respectively) (Vernon, Baranova & Younossi, 2023) or other liver diseases such as viral, autoimmune, metabolic or drug-induced diseases (Fabbrini, Sullivan & Klein, 2010). In the context of the global transition including the epidemiological, demographic and nutritional transitions experienced by countries including Morocco, diet is one of the risk factors incriminated in the predisposition to obesity-related NAFLD (Ghoshal, Goel & Quigley, 2020; Janssen, Bardoutsos & Vidra, 2020; Vernon, Baranova & Younossi, 2023). Indeed, a diet rich in red and processed meats, sugar and refined grains, saturated fats, dairy products and sugary drinks is at the origin of an excessive calorie intake which can promote an accumulation of triglycerides and fats in the liver (Ludwig et al., 2018). Several studies have also reported the obesogenic effect of daily consumption of large amounts of added sugars (Choi & Diehl, 2008; Madero et al., 2011; Goran et al., 2013). This effect is associated with the breakdown of the feeling of satiety generated by the ingestion of fructose (Campos & Tappy, 2016). According to the WHO (2015), sugars should represent less than 10% in the daily diet. Similarly, high energy intake as well as consumption of refined grain products, pastries and sweets are associated with NAFLD, independent of changes in body weight (Jia et al., 2015). In addition, high consumption of animal protein and total fat, soft drinks and snacks have also been associated with NAFLD (Bray, 2010).

Biologically, obesity-related NAFLD is associated with elevated serum liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) (Miyake et al., 2003; Wieckowska, McCullough & Feldstein, 2007). Previous studies have reported that GGT is an independent predictor of NAFLD (Angulo, 2002; Hossain et al., 2016). Moreover, GGT could be a simple and useful biomarker for the accumulation of visceral and hepatic fat (Hossain et al., 2016) and that visceral adiposity is associated with NAFLD (Kim et al., 2016). Thus, the hepatic enzymes ALT, ASAT, and GGT are commonly used markers for hepatic steatosis and the increase in their levels reflects liver damage. In addition, other factors have been associated with obesity and could be used to predict NAFLD. These are waist circumference (WC), body mass index (BMI) (Zheng et al., 2012; Kim et al., 2016), triglycerides (TG) (Li et al., 2020; Xie et al., 2021), total cholesterol (TC)/high density lipoprotein (HDL) cholesterol (Chen et al., 2019), HDL cholesterol, and low density lipoprotein (LDL) cholesterol (Ren et al., 2019). Therefore, weight loss, liver enzyme adjustment, and obesity-related indices including BMI, WC, TG, GGT, HDL, LDL, and CT are predictive of NAFLD improvement (Zheng et al., 2012). However, there is no official consensus on how to treat NAFLD currently (Wieckowska, McCullough & Feldstein, 2007). Nevertheless, as a healthy diet, the Mediterranean diet (MD), which is also the type of diet in Morocco, is referenced as being beneficial for the prevention and improvement of NAFLD (Trichopoulou et al., 2014). It has indeed been reported effective in the treatment of obesity and its comorbidities (Casas, Sacanella & Estruch, 2014), in reducing LDL-cholesterol, and total cholesterol (Trichopoulou et al., 2003; Serra-Majem et al., 2019), and to have a role in the prevention of oxidative stress (Jomova & Valko, 2013), considered an environmental risk factor for obesity-related NAFLD (Angulo, 2002; Janssen, Bardoutsos & Vidra, 2020).

Adopting MD would therefore reduce NAFLD while promoting weight reduction and adjustment of liver enzymes. The protective effect of MD against health problems and non-communicable diseases was

indeed attributed to the virtues of its food composition associated to its significant content in antioxidants, anti-inflammatories, hepato-protectors, hypolipidemics and chemopreventives (Martirosyan, 2013; Trichopoulou et al., 2014). Among these components, polyphenols, anthocyanins, carotenoids, flavonoids, glucosinolates, isoflavonoids, limonoids, omega-3 and 6 fatty acids, phytoestrogens, phytosterols, probiotics, resveratrol and terpenoids have been cited (Ortega, 2006; Trichopoulou et al., 2014; Meybeck et al., 2017).

The traditional MD is characterized by a high consumption of vegetables, fruits, legumes, nuts, unrefined whole grains, a high consumption of olive oil as the main source of dietary fat, a moderate consumption of fish and seafood, low to moderate consumption of dairy products, and low consumption of red and processed meats (Jomova & Valko, 2013). The beneficial effects of MD, both on health and on the environment, made of it a model of sustainable diet (Trichopoulou et al., 2003; Casas et al., 2014; Serra-Majem et al., 2019). Moreover, physical activity (PA) is also prescribed to patients by the Haute Autorité de Santé (HAS) (Stamatakis et al., 2019). The HAS methodological guide published on October 17, 2018 and relating to the prescription of physical activity, provides useful information to guide patients towards appropriate PA (Stamatakis et al., 2019). Energy expenditure by the body depends on the intensity, duration, level and type of physical activity or sedentary lifestyle (Hogan, 2014; Vaara et al., 2019; Leung & Wong, 2022). PA includes walking, stairs, cycling, housekeeping, DIY, gardening, and work or school activities, physical exercises and sports activities (Hogan, 2014; Stamatakis et al., 2019). A sedentary lifestyle is characterized by a low energy expenditure and includes all activities performed at rest in a seated or lying position (watching television, reading, writing, working on a computer, moving around in a motor vehicle) or static standing (Stamatakis et al., 2019). Since physical activity is accessible to everyone, it can be practiced at different levels of intensity. The World Health Organization recommends to practice a minimum of 2h30' endurance physical activity of at least moderate intensity at a frequency of 3 to 5 times a week (Stamatakis et al., 2019; Vaara et al., 2019). Physical activity is assessed by the duration, the type of each activity, and also the time spent daily watching television, reading, writing, working on a computer or driving a motor vehicle (Stamatakis et al., 2019).

In this perspective, the general objective of this work was to evaluate the impact of an education program on clinical and metabolic parameters in obese people with or without NAFLD in Morocco. More specifically, this program aims to provide participants in the study with theoretical and practical knowledge enabling them to make lifestyle changes beneficial to their state of health.

Methodology

This interventional study was conducted in a gastroenterology medical practice in the city of Marrakech (Morocco), on a sample of 210 adult patients with a BMI≥ 25, with or without NAFLD. The minimum age was 19 years and the maximum 98 years. The operational objectives focused on nutrition education planning, implementation, and evaluation (Table 1). Table 2 shows the main characteristics of the studied sample.

Objectives and conduct of the education program stages

The first goal was to reduce body weight by 5 to 10% in six months. The proposed activities emphasize the importance of adopting a healthy lifestyle in the management of NAFLD with or without obesity. It is about the adoption of healthy behaviors such as, adherence to the Mediterranean diet and the removal of industrialized foods, good chewing, respect for sleep schedules and the reduction of smoking as well as the practice of physical activity.

The second objective emphasizes the adoption of the Mediterranean diet and regular physical activity. The activities explain the importance of a balanced diet and the clarification of doubts.

The third objective is to check the progress of the patient in terms of diet modification and weight reduction, as well as the patient's motivation to pursue the healthy lifestyle. Also, the activities focused on checking progress and keeping motivation high and explaining possible reasons, in cases of no progress being made for the patient.

The fourth objective was to motivate the patient to maintain a positive state of mind regarding a

permanent change in lifestyle. The activities consist of the incentive to lose weight for the improvement of NAFLD.

The fifth objective was to analyze the progress made by the patient during the six months of self-management, and to motivate him/her to maintain the change in behavior by recognizing personal successes.

Study protocol

The health examination was carried out by a specialist in gastroenterology. The diagnosis of NAFLD was made by ultrasound. At the first visit to the gastroenterology medical office, weight and height were measured with light indoor clothing, without shoes. Waist circumference was measured as the horizontal circumference passing through the position of the navel. Liver enzymes ALT, ASAT, GGT, and obesity indices including TG, TC, LDL and HDL were measured in dedicated analysis laboratories. Patients an-swered to questionnaires on dietary habits and physical activity (PA).

This last was as-sessed by the self-questionnaire on physical activity or AQAP questionnaire (in French "Auto-questionnaire sur l'activité physique"), which is a valid an easy-to-use tool for individual assessment of PA (Vol et al., 2011). All patients included in the study re-ceived individual nutritional instruction in accordance with the national nutrition pro-gram (MS, 2016). The aim is to detect negative behaviors to be corrected through per-sonalized prescription based on patients' needs and expectations and to set self-monitoring and behavior changes in patients, consolidating positive, and improving neg-ative behaviors. They also agreed to present their analysis reports before and after the nutrition education. A plan is developed in which the objectives, activities, tools, loca-tion and duration of each nutrition education session are set out. The details of each step are illustrated in Table 1.

Study variables

Anthropometric data included age (years), height (m), weight (kg), body mass index (BMI) (kg/m2) and waist circumference (WC) (cm). Overweight was defined as a BMI between 25 and 30 kg/m2, and obesity when the BMI was≥30 kg/m2. Waist circumference defined as high if WC≥88 cm in women or WC≥102 cm in men (Leung & Wong, 2022). These parameters along with biochemical analyses such as ALT, ASAT, GGT, TG, CT, LDL and HDL were used to monitor the health status of the participants. Table 2 shows the main characteristics of the studied sample.

Statistical analysis

All statistical analyzes were performed using SPSS (Statistical Package for Social Sciences) Windows, version 25.0. Quantitative variables were presented as means ± standard deviations (SD), while quantitative variables were presented as frequencies and percentages. The parametric test on paired sample made it possible to conclude that there were statistically significant differences between the means of the parameters before and after nutritional education (Table 3). Differences were considered statistically significant for p-values less than 0.05. Non-parametric tests were also used for statistical analysis. The Mann-Whitney tests was applied to compare means between the group studies. The level of rejection of the null hypothesis was set al 0.05 or 5% (Table 4).

Results

The present study involved a final sample of 210 patients who were divided into two groups, NAFLD (n=131) and non-NAFLD (n=79), of which 8% were overweight with a BMI between 25 and 30, and 92% were obese with a BMI ≥30. The latter are divided into 62% of patients with NAFLD and 38% without NAFLD. The minimum age was 19 years and the maximum 98 years. The percentage of NAFLD in obese women was higher than in obese men. Among patients with NAFLD, 23% were male and 77% were female. In the patients without NAFLD, 15% were male and 85% female.

Table 3 compares the anthropometric and biochemical variables of the sample before and after nutritional education. The mean values of weight, BMI, WC, ALT, ASAT, TG, GGT, LDL cholesterol, total cholesterol, and HDL cholesterol were higher in the obese group with NAFLD (n=131) than in the obese group without NAFLD (n=79). After the nutritional education program, the anthropometric and

biochemical variables improved in the obese groups with NAFLD or without NAFLD

Table 1. Nutrition education planning for people with or without NAFLD.

Goals	Activities	Tools	Duration	
Session 1	Establishment of the patient's consent.	Pre-established questionnaire on obesity and its comorbidities, in particular NAFLD, and the need to change the problem.		
Emphasize the goal of weight reduction in six months to aid recovery from NAFLD.	Identification of the patient's needs, expectations and health problems as a basis for his motivation. Administration of a questionnaire on eating habits.	Questionnaire on dietary habits. Tape-Meter.	1hour	The 1st medical visit to the gastro-enterologist doctor.
Emphasize the goal of weight reduction in six months to aid recovery from NAFLD.	Introduction to NAFLD and obesity and its comorbidities. Explanation of the importance of the Mediterranean diet. Advice on the adoption of healthy behavior. Taking anthropometric measurements. Exchange of telephone numbers.	Personal scale.		
Session 2 Focus on the Mediterranean diet and physical activity.	Patient motivation for adherence to MD. Clarification of doubts. Explanation to patients of parameters Weight, ALT, AST, HDL Cholesterol, LDL Cholesterol, Total cholesterol, GGT.	Questions answers. Analysis report Recipe booklet inspired by the Mediterranean diet pyramid.	1/2hour	The 2nd visit to the Doctor as a gastro-enterologist.
Session 3 Motivate the patient to pursue a healthy lifestyle (Mediterranean diet, physical activity).	Motivation maintaining. Explanation of possible reasons for the patient. in cases no progress is made. Providing of a new diet plan based on the patient's current needs (weight loss or maintenance). Setting new goals of weight loss and exercise to patients	Questions answers. Recipe booklet inspired by the Mediterranean diet pyramid.	1/2hour	The 3rd visit.
Session 4 Motivate the patient to maintain a positive mindset regarding a permanent lifestyle change.	Maintenance of motivation. Explanation of possible reasons for the patient. in cases where no progress is made. Reaffirmation of weight loss in improving NAFLD.	Questions answers.	1/2hour	The 4th visit.
Session 5 Analyze the progress made by the patient during the six months of self-management. Motivate the patient to sustain the behavior change by acknowledging personal successes to date.	Final evaluation of the patient's progress. Anthropometric measurements: Weight, BMI, Waist circumference. Analysis of blood balance after nutritional education.	Questions answers. Results of the patient's biological examinations. Tape-Meter. Personal scale.	1hour	The 5th visit.

Table. 2. Characteristics of the studied population.

Variables		With NAFLD n=131(62%)	Without NAFLD n=79 (38%)
Age		44±11.86	40±12.59
Gender	Men	23%	15%
	Women	77%	85%
Weight (kg)		29.23±107.38	103.87±24.59
BMI (kg/m^2)		40.40±10.29	40.71±8.22
WC (cm)		115.92±20.03	116.66±18.90

NAFLD : Non Alcoholic Fatty Liver Disease; BMI: Body Mass Index; WC: Waist Circumference.

Table 3. Effects of nutritional education on anthropometric and biochemical parameters in people with obesity-related NAFLD.

	With NAFLD (n=131)			Without NAFLD (n=79)		
Variables	**Before**	**After**	**t**	**Before**	**After**	**t**
Weight (kg)	107.38±29.23	101.70±28.96	6.1	103.87±24.59	98.45±21.92	4.62
BMI (kg/m^2)	40.40±10.29	37.83±10.31	23.14	40.71±8.22	38.00±7.96	20.36
WC (cm)	115.92 ±20.03	106.94±22.16	7.149	116.66±18.90	108.14±21.53	6.57
Total Cholesterol g/L	1.83±0.29	1.7±0.31	11.35	1.83±0.27	1.65±0.26	10.22
LDL Cholesterol g/L	1.54±0.15	1.30±0.17	15.95	1.52±0.15	1.3±0.20	11.6
HDL Cholesterol g/L	0.42 ±0.71	0.49±0.09	-11.84	0.42 ±0.80	0.48±0.09	-9.31
TG g/L	1.71±0.51	1.30±0.50	20.06	1.60±0.43	1.20± 0.46	18.49
ALT UI/L	32.22±19.29	18.44±11.67	28.23	42.90±6.31	22.99±4.98	21.3
ASAT UI/L	37.52± 13.62	31.52±13.62	E	33.83±10.33	27.88±10.33	E
GGT UI/L	51.33±16.66	42.55±16.16	17.75	34.95±13.80	27.82±11.49	13.99

The Table values are means ± standard deviation of ALT. AST. BMI. TC. GGT. HDL. HDL and LDL. A paired sample t-test was used to assess differences before and after nutrition education, $p<0.05$. E: standard error of the difference is zero.

Table 4. Differences of the variables in patients without NAFLD versus those with NAFLD.

Variables	Without NAFLD (n=79)	With NAFLD (n=131)
Weight (kg)	5.42±2.67	5.67±0.27
BMI (kg/m^2)	2.7±1.2	2.57±1.26
WC (cm)	8.59±11.52	8.98 ±14.41
Total Cholesterol g/L	0.17±0.15	0.14±0.14
LDL Cholesterol g/L	0.22±0.17	0.24±0.17
HDL Cholesterolg/L	-0.06±0.06	-0.07 ±0.06
TG g/L	0.40±0.19	0.42±0.21
ALT UI/L	5.42±10.12	5.6±2.34
ASAT UI/L	5.87±0.05	5.97±0.94
GGT UI/L	7.12±4.4	8.78±5.64

The differences of the parameters in patients with NAFLD versus those without NAFLD was respectively: 5.67± 0. 27 vs. 5.42±2.67 (p= 0.34) for weight; 2.57± 1.26 vs. 2.7± 1.2 (p=0.24) for BMI; 8.98±14.41 vs. 8.59±11.52 (p=0.39) for WC; 0.14±0.14 vs. 0.17±0.15 (p=0.04)) for Total Cholesterol; 0.24 ±0.17 vs. 0.22±0.17 (p= 0.44)) for LDL Cholesterol; 0.42±0.21 vs. 0.40±0.19 (p=0.63) for TG; 5.6± 2.34 vs. 5.42±10.12 (p=0.93) for ALT; 5.97±0.94 vs. 5.87±0.05 (p=1) for AST; 8.78±5.64 vs 7.12±4.49 (p=0.02)) for GGT and -0.07 ±0.06 vs -0.06±0.06 (p=0.17)) for HDL cholesterol. The non-parametric Mann-Whitney U test shows that the difference of these parameters means between the two groups NAFLD and without NAFLD is statistically significant for total cholesterol (p=0.04) and GGT (p=0.02) (Table 4).

For physical activity (PA), sedentary behavior is a major determinant of health status that also affects weight status. The majority of the present study participants (70%) spend more than 5 hours of their daily life watching television or in front of a computer screen. 1% spend an hour reading or writing and 20% move in a mobile vehicle. The most mobilizable physical activities over the long term are 30% active travel, 10% walking, 0% cycling or sports activities, and 90% find it difficult to climb stairs. After the 6-month nutritional education program, 90% of the participants practiced walking and active movements, and decreased the time in front of the television and the computer screen. The daily time spent in front of a screen decreased in average of 2 hours for participants. Also, the participants' weight decreased by 5.67±9.95 kg in NAFLD and 5.42±10.36 kg in non-NAFLD.

Discussion

This study showed that nutritional education was effective in improving the weight and metabolic status of obese patients with or without NAFLD. After the six-month nutritional education period, a significant decrease in the values of the anthropometric and biochemical variables measured was indeed demonstrated in the present studied obese patients with or without NAFLD. The significant reduction was in weight, BMI, waist circumference, ALT, AST, TG, GGT, LDL cholesterol and total cholesterol; an increase in HDL cholesterol was also observed. However, there was no significant difference in the paired mean of these parameters between the NAFLD group and the non-NAFLD group, except total cholesterol (p=0.04) and GGT (p=0.02). These results were in agreement with several studies that have demonstrated a positive impact of nutritional education in the treatment of obesity, insulin resistance and NAFLD (Elias et al., 2010; Esposito et al., 2011; Jia et al., 2015). In the present study, the education program led to a weight reduction of 5.67 kg ±9.95 in obese with NAFLD and 5.42 kg ±10.36 in non-NAFLD obese participants. Weight reduction in obese people is an important part of the management of patients with chronic liver disease, particularly NAFLD. This result confirms those reported in other studies showing an association of weight loss with a decrease in NAFLD, an improvement of hepatic enzyme profile and serum insulin levels as well as the quality of life (Burgert et al., 2006; Larson-Meyer et al., 2008). It is also reported that no specific diet is shown to be superior in improving NAFLD in the absence of weight loss (Burgert et al., 2006). This is also consistent with those studies reporting the treatment of patients with NAFLD with a combination of diet and exercise with a 5% body weight loss, with these patients showing reduced aminotransferase levels.

Thus, the components of nutrition education followed in the present study included a combination of diet and physical activity. Regarding diet, since Morocco is a Mediterranean country, nutritional recommendations for obese patients with or without NAFLD were based on adherence to the Mediterranean diet by respecting the different food sources dictated by the guidelines of the food pyramid (Trichopoulou et al., 2014; Serra-Majem et al., 2019). This is, an intake of complex carbohydrates, less than or equal to 50% of the total energy intake with a share of simple carbohydrates less than 10%. The food sources of complex carbohydrates are cereals. However, the amount of fructose consumed, rather than the total amount of sugars, is also reported by several studies to have a greater impact on the risk of NAFLD (Ouyang et al., 2008). Indeed, excessive and chronic consumption of fructose is likely to promote liver function, alter the oxidation of fatty acids, induce endoplasmic stress, and contribute to liver inflammation through the production of uric acid and gut-derived endotoxins (Ouyang et al.,

2008). Additionally, high dietary fructose intake has also been linked to impaired gut microbiota, with a shift towards depletion of beneficial microbial species (Lambertz et al., 2017). When fructose is consumed with a high-fat diet, mitochondrial function in the liver is further impaired (Souza et al., 2022). The results of another study further showed that a fructose intake below normal may be protective against the development of NAFLD in obese adolescents, as opposed to a high fructose intake compared to normal, leading to a risk increased NAFLD (Ilich et al., 2020). Each gram of fructose consumed daily by obese patients aged 14 years was associated with a 9% increased risk of NAFLD at 17 years (DiStefano & Shaibi, 2021). The consumption of fructose should therefore not be excessive.

A protein intake of less than or equal to 0.8g/kg body weight per day is recommended and should include good quality protein. Concerning the recommended lipid intake, it should not exceed 30% of the total energy intake (TEI), with a composition containing monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) mainly present in foods of plant origin including oil olives -the central element of the Mediterranean diet-, and oily fish. Among the other dietary recommendations, an intake of unprocessed foods rich in fiber (that induces satiety) recommended intakes are 25g minimum and 30g optimum, including 15g of soluble fibers. Fiber, the main source of which is plants, is known for its role in blood sugar regulation and reducing cholesterol absorption (Larson-Meyer et al., 2008).

Nevertheless, a balanced diet is not achieved in a single meal but rather from one meal to another and over several days by a combination of foods with nutritional values. The interest in using certain food equivalences is to consume a variety of foods, while providing the body with essential, diversified and complementary elements. Our results were in agreement with those of an American study that evaluated the effect of a 6-month nutritional intervention on non-alcoholic fatty liver disease, using an intervention protocol comparable to that of the present study (Elias et al., 2010). This latter study showed that the nutritional intervention applied to 31 NAFLD patients as exclusive treatment was effective in the treatment of non-alcoholic fatty liver disease with a loss of at least 5% of the initial weight.

The strengths and limitations of the study

This study adopted a personalized approach through a balanced diet aimed at gradual weight loss while being a participatory approach to inculcate self-monitoring. It was based on a six-month healthy lifestyle nutritional intervention program developed for obese patients with or without NAFLD, and led by a healthcare professional, trained in subject nutrition and education, and finally assessed by a gastroenterologist. The content is established on the basis of a literature review and on the national nutrition program, Morocco. Although this intervention seems simple and cost-effective in the short term, ensuring its long-term success rate requires more in-depth long-term research. Bias is that patients tend to overestimate or underestimate food intake.

Conclusion

Obesity-related NAFLD is a more common cause of chronic liver disease worldwide and is a growing public health problem. Lifestyle intervention is currently a mainstay of therapy. A 6-month nutritional education program, tailored to each patient according to their personal needs and expectations and the socio-cultural context, has led to a significant decrease in average weight values, BMI, WC, ALT, ASAT, TG, GGT, LDL cholesterol, total cholesterol, and a significant increase in HDL cholesterol. This program was based on the principle of reducing fat mass and not lean mass, implying respect for the main principles of a balanced diet which consists of eating at regular times. Eat all food groups to avoid monotony, reduce salt, sugar and trans-fat intake, eat enough vegetables/legumes, increase fiber intake, encourage fish consumption, limit consumption of processed products, avoid snacking, drink plenty of water and engage in regular physical activity. After the 6-month nutrition education program, 90% of participants engaged in walking and active movement, and reduced television and computer screen time. Daily screen time decreased by an average of 2 hours for participants. Additionally, participants' weight decreased by 5.67 ±

9.95 kg for NAFLD and 5.42 ± 10.36 kg for non-NAFLD.

Therefore, this study demonstrated that a correct diet combined with weight loss was effective in improving the clinical course of patients with obesity-related NAFLD. A limitation of our study was the difficulty patients have in adhering to a lifestyle intervention. Larger, longer-term studies are needed to confirm the sustainability of the benefits of a nutrition education program.

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Ethical considerations

The researcher asked for participants' approval if they agreed to take part and follow the nutrition education program in order to improve their health, the participants signed the informed consent. The researcher also guaranteed the respect of the participants' rights to anonymity and confidentiality. Exclusions criteria were identified as genetic disease, endocrine problem. and chronic alcohol consumption or other causes of steatosis and people who did not agree to participate in this study.

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Alimentación y estado nutricional de población rural masculina de Yaguajay, Sancti Spíritus, Cuba

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RESUMEN

Introducción: La alimentación y el estado nutricional son esenciales para la calidad de vida de los trabajadores rurales. Objetivo: Describir la frecuencia de consumo de alimentos y el estado nutricional de una muestra de población rural masculina de Yaguajay, Sancti Spíritus, Cuba. Métodos: En enero de 2024 se realizó un estudio descriptivo transversal en 30 campesinos. Se aplicó una encuesta de frecuencia de consumo de alimentos, se realizaron mediciones antropométricas y se evaluó la composición corporal, analizándose los estadísticos descriptivos y las correlaciones entre distintas variables. Resultados: La edad promedio de la muestra fue de 47,1 años. Los principales alimentos consumidos fueron arroz, frijoles, viandas hervidas, pan, café, carne de cerdo y pollo, con incorporación moderada de procesados. El índice de masa corporal promedio fue de 25,1 kg/m^2, con predominio de normopesos y sobrepesos. La media del porcentaje de grasa fue de 24,3 y 42,2 para el % de masa muscular. No se detectaron asociaciones entre la edad y el resto de las variables. Existieron correlaciones significativas entre el porcentaje de grasa y de músculo, el peso y el IMC. Conclusiones: La dieta en enero de 2024 se basó en alimentos locales, exhibiendo un patrón mixto que reflejó una transición nutricional hacia la diversificación y el consumo de productos procesados entre los campesinos evaluados. A este hecho se suma una elevada prevalencia de sobrepeso, por lo que resulta fundamental realizar estudios adicionales que examinen en profundidad cómo estos cambios afectan a largo plazo la salud y calidad de vida de los trabajadores rurales.

Palabras claves:

Alimentación
Estado nutricional
Composición corporal
Población rural

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ABSTRACT

Introduction: Food and nutritional status are essential for the quality of life of rural workers Objective: To describe the frequency of food consumption and nutritional status of a sample of male rural population of Yaguajay, Sancti Spíritus, Cuba. Methods: A cross-sectional descriptive study was conducted in January 2024 in 30 peasants. A food consumption frequency survey was applied, anthropometric measurements were taken and body composition was evaluated. Descriptive statistics and correlations were analysed. Results: The mean age was 47.1 years. The main foods consumed were rice, beans, boiled viands, bread, coffee, pork and chicken, with moderate incorporation of processed foods. The average body mass index was 25.1 kg/m^2, with a predominance of normal weight and overweight. The mean fat percentage was 24.3, and 42.2 for muscle mass percentage. No associations were detected between age and the rest of the variables. There were significant correlations between fat and muscle percentages, weight and BMI. Conclusions: The diet in January 2024 was based on local foods, exhibiting a mixed pattern that reflected a nutritional transition towards diversification and consumption of processed products among the farmers evaluated. In addition to this fact, there was a high prevalence of overweight, so it is essential to conduct further studies that examine in depth how these changes affect the long-term health and quality of life of rural workers.

Keywords:

Diet
Nutritional status
Body composition
Rural population

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Introducción

La ocupación desempeña un papel crucial en el estilo de vida de las poblaciones humanas. Tal es así que se reconoce la influencia de factores estresantes para la salud y calidad de vida de quienes realizan labores agrícolas (Riise, Moen y Nortvedt, 2003). Aspectos socioeconómicos, culturales y ambientales, como la disponibilidad de servicios de salud y la estacionalidad, impactan el entorno laboral, el nivel de vida y la nutrición de los trabajadores rurales (SafeWork-ILO, 2000). En estos grupos, se han identificado riesgos personales y financieros relacionados con la producción, el mercado y las políticas institucionales, con consecuencias para su bienestar físico y mental (Komarek, De Pinto y Smith, 2020; Alicandro et al., 2021). Por su parte, las prácticas alimentarias inadecuadas están asociadas con un deterioro de la salud y la calidad de vida, siendo uno de los principales factores de riesgo global para la muerte prematura y la discapacidad (Instituto para la Medición y Evaluación de la Salud y Red de Desarrollo Humano-Banco Mundial, 2013). Además, la nutrición influye en la aparición de enfermedades, afecta la productividad laboral, el desarrollo humano y el de las naciones (Macdonald et al., 2002).

Diversas investigaciones indican que las lesiones y enfermedades no se distribuyen uniformemente en la población general. Sin embargo, a pesar de su reconocida exposición a diversos factores, bien con influencia protectora o de riesgo para la salud (Karttunen y Rautiainen, 2013), los trabajadores rurales agrícolas han sido habitualmente menos estudiados. Sólo en las últimas décadas se han realizado distinciones respecto a la seguridad y la salud de los trabajadores agrícolas (Frank et al., 2004), por lo que se han convertido en un grupo de investigación relevante. Hay que señalar que la ruralidad por sí misma no conduce necesariamente a desigualdades en cuanto a la salud de los individuos. Sin embargo, si se reconoce la presencia de factores agravantes como las desventajas socioeconómicas, la menor disponibilidad de servicios y los mayores niveles de riesgos, con influencias provocadas por el ambiente, el transporte o la ocupación (Aljassim y Ostini, 2020). Dichos factores han sido definidos por la Organización Mundial de la Salud (OMS) como determinantes sociales de la salud (Gozzer, 2020). Estos incluyen todas aquellas circunstancias en las que las personas nacen, crecen, trabajan, viven y envejecen, y explican la mayor parte de las desigualdades sanitarias registradas en las poblaciones rurales (Soler, Moreno y García, 2020). A pesar de ello, algunos autores señalan la ausencia de perspectivas comparativas de la dimensión rural/urbana, o dentro de los propios territorios rurales, así como el vacío de investigaciones relacionadas con las desigualdades en materia de educación y salud en Latinoamérica, particularmente en Cuba (Hidalgo, 2020).

La dispersión geográfica y el difícil acceso de las comunidades rurales dificulta la inclusión en las investigaciones biomédicas de trabajadores rurales agrícolas. Otro factor importante es que, desde una perspectiva demográfica, la ruralidad ha ido disminuyendo sostenidamente desde mediados del siglo pasado. Algunos informes indican que solo una de cada cinco personas de Latinoamérica vive en el ámbito rural, lo que supone una menor representatividad que puede contribuir a disparidades en cuanto a los servicios de salud, el acceso a mejores oportunidades y condiciones de vida (Gozzer, 2020). Así, Corral y Pría (2017) han señalado que son necesarios los estudios de salud que tengan en cuenta la estratificación de territorios según sus condiciones de vida y situación socioeconómica, ya que esto supone un instrumento útil para el diseño de estrategias y acciones integrales según las necesidades concretas de los territorios, y la determinación de las desigualdades sociales en salud.

En el municipio de Yaguajay, provincia de Sancti Spíritus, ubicada en el centro norte de Cuba, se han realizado investigaciones previas con el objetivo de caracterizar la dieta y el estado nutricional de los trabajadores rurales de la zona. En 2018, Vázquez y colaboradores evaluaron el estado nutricional y la composición corporal de campesinos y pescadores ocasionales del citado municipio. Posteriormente, en 2023, Valdés y colaboradores estudiaron los cambios en la frecuencia de consumo de alimentos y el estado nutricional en 16 campesinos residentes en la comunidad rural La Picadora, perteneciente a dicho municipio. En el mismo año, Ramenzoni et al. (2023) describieron los efectos de la transición agrícola, tras el cierre de las centrales azucareras, en la diversificación

de la dieta de los campesinos de Yaguajay. Sin embargo, en estas investigaciones, el consumo de alimentos fue evaluado con encuestas de frecuencia semanal, y el estado nutricional por medio de antropometría. Con el propósito de profundizar en estos estudios, la presente investigación amplió el registro de información mediante encuestas sobre el consumo mensual en un mayor número de alimentos, e incluyó la bioimpedancia para el análisis de la composición corporal. Por ello, este estudio tiene como objetivo describir la frecuencia de consumo de alimentos y evaluar el estado nutricional de una muestra de población rural masculina de Yaguajay, durante el mes de enero de 2024.

Materiales y Métodos

Contexto, diseño y participantes

Con una superficie total de 1.055,60 km², el municipio de Yaguajay es el único territorio espirituano con costas en los mares del norte de Cuba. Está situado entre los 21°, 06′, 00″ y 21°, 28′, 00″ de latitud norte y los 78°, 57′, 00″ y 80°, 35′, 00″ de longitud oeste (Figura 1). Se caracteriza por ser un territorio agroindustrial, en dónde se desarrolla la actividad pecuaria, forestal y de cultivos varios, además de la acuicultura (Portal del ciudadano de Sancti Spíritus, 2023). Se realizó un estudio descriptivo, transversal y observacional en enero de 2024. Mediante un muestreo intencional se estudiaron 30 hombres vinculados a labores agrícolas, utilizando como criterio de inclusión ser adultos residentes en este municipio y que aceptaran participar voluntariamente en el estudio. Se excluyeron aquellos que no se dedicaban a la agricultura como principal fuente de ingresos, y los que mostraban discapacidades físicas o mentales que les impidieran responder a los cuestionarios, o que se les realizaran las mediciones antropométricas. Los campesinos estudiados realizaban al menos una sesión diaria de labores agrícolas de siembra, cosecha, atención a cultivos (generalmente no mecanizadas), ganaderas, constructivas, mecánicas o domésticas. Las actividades mecánicas eran básicamente de reparación de medios de transporte e instrumentos de trabajo, y las domésticas incluían la preparación y cocción de alimentos, la atención a animales y plantas en el hogar o a los terrenos aledaños.

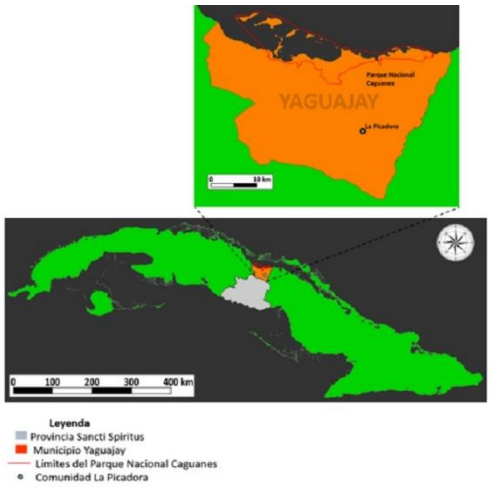

Figura 1. Mapa de Cuba con provincia de Sancti Spíritus, municipio Yaguajay, comunidad rural La Picadora y límites del Parque Nacional Caguanes. Cortesía de Mark Besonen.

Recolección de datos y variables

Se registró la edad en años cumplidos de cada participante. Se aplicó una encuesta de alimentación donde, mediante una entrevista estructurada, los investigadores formularon preguntas y anotaron las respuestas para determinar la frecuencia de consumo diario, semanal y mensual de 65 alimentos; éstos fueron previamente seleccionados por ser tradicionalmente utilizados en las encuestas aplicadas por el Centro de Nutrición e Higiene de los Alimentos, del Instituto Nacional de Higiene, Epidemiología y Microbiología de Cuba. En la encuesta se hicieron distinciones en cuanto al consumo de azúcar, sola o añadida a la leche, yogurt, jugos de frutas cítricas y no cítricas; esto se debe a que en la investigación de carácter nacional realizada previamente por Porrata (2009), se identificó el gran gusto por lo dulce de la población cubana, lo que implica añadir cantidades excesivas de azúcar no solo a los postres caseros, sino también a batidos, jugos naturales, leche y café. Se incluyó además el consumo de viandas, término utilizado en Cuba para hacer referencia a los tubérculos, raíces feculentas, frutas (como algunas variedades de plátanos) y hortalizas (como la calabaza) (Juárez, 2013). Mediante una balanza tipo InBody H20N y un tallímetro portátil

marca SECA se midieron el peso (kg) y la talla (cm), y se calculó el Índice de Masa Corporal (IMC=kg/m^2), cuya clasificación se realizó según los puntos de corte de la FAO (en Shetty y James, 1994): <16,0 Desnutrición Energética Crónica III; 16,0-16,99 Desnutrición Energética Crónica II; 17,0-18,49 Desnutrición Energética Crónica I; 18,5-24,99 Normopeso; 25,0-29,99 Sobrepeso I; 30,0-39,9 Sobrepeso II; ≥ 40,0 Sobrepeso III (Shetty y James, 1994). Cabe mencionar que las categorías de "Sobrepeso I" y "Sobrepeso II" en esta clasificación corresponden a las categorías actualmente definidas por la Organización Mundial de la Salud (OMS) como "Sobrepeso" (IMC entre 25,0 y 29,9) y "Obesidad" (IMC≥30,0), respectivamente (OMS, 2024).

Para el análisis de la composición corporal se utilizaron los datos sobre la grasa corporal y la masa muscular esquelética, proporcionados por un dispositivo de impedancia bioeléctrica tetrapolar (balanza InBody H20N); se empleó el análisis de multifrecuencia segmentaria directa (DSM-BIA), que mide por separado la resistencia a las corrientes eléctricas alternas del tronco, los brazos y las piernas. El porcentaje (%) de grasa corporal estimado por la balanza InBody H20N incluye la grasa corporal esencial y de almacenamiento, y el fabricante establece en el manual de uso que el rango estándar para el sexo masculino se sitúa entre el 10-20%. Asimismo, con la masa muscular y el peso corporal obtenidos a través de la balanza InBody H20N, se calculó el porcentaje (%) de masa muscular esquelética como resultado de la división de ambas variables, multiplicado por 100.

Análisis estadísticos

Se construyó una matriz de datos en Microsoft Excel para su posterior análisis en el software IBM SPSS Statistic, versión 21. Los resultados de la encuesta de alimentación se resumieron en una tabla, indicando el porcentaje de la frecuencia de consumo diario, semanal y mensual. Se calcularon los promedios, la desviación estándar y los valores mínimos y máximos de las variables cuantitativas para la totalidad de la muestra. La distribución (normalidad) de dichas variables se comprobó mediante la prueba Shapiro-Wilks. El peso (p=0,306), la talla (p=0,065), el IMC (p=0,435), el % de grasa corporal (p=0,785) y la masa muscular esquelética (p=0,411) seguían una distribución normal, pero no así la edad (p=0,036). Para el estudio de las asociaciones entre las variables numéricas se empleó la correlación de Pearson en aquellas que cumplieron los supuestos de los métodos paramétricos. En caso de incumplimiento, la correlación se analizó mediante el método no paramétrico de Spearman. Se calcularon las correlaciones parciales utilizando el peso como variable control, debido a su influencia en las variables antropométricas estudiadas. Una correlación se consideró significativa con un p-valor ≤0,05; el grado de asociación entre dos variables se clasificó como débil si el valor del coeficiente de correlación era ≤0,05, moderado entre 0,5-0,59, fuerte entre 0,6 y 0,79 y muy fuerte si el valor era ≥0,8.

Consideraciones éticas

El estudio fue aprobado por el Consejo Científico de la Facultad de Biología de la Universidad de La Habana (Cuba), y se realizó siguiendo los principios de la Declaración de Helsinki. Se obtuvo el consentimiento informado de todos los participantes antes de su inclusión en el estudio (Asociación Médica Mundial, 2013).

Resultados

Los porcentajes de la frecuencia de consumo de los 65 alimentos evaluados en la encuesta se resumen en la Tabla 1. Los alimentos están ordenados según el porcentaje total de individuos que los consumieron, con columnas que detallan la frecuencia de consumo diario, semanal o mensual. Se identificó una preferencia por alimentos básicos y accesibles como el arroz, frijoles, pan, café y viandas hervidas con un consumo casi universal. Estos fueron incluidos en la dieta por el 93,3% al 100% de los participantes, que además registraron elevados porcentajes de consumo diario. Las proteínas animales fueron consumidas con menor frecuencia diaria y mayor dependencia de patrones semanales o mensuales. Por su parte, el aceite de girasol y la manteca de cerdo fueron los principales tipos de grasa consumidos. El aceite tuvo un consumo diario notable y en la manteca destaca la frecuencia semanal.

Tabla 1. Distribución del porcentaje de la frecuencia de consumo de 65 alimentos, consumidos por campesinos de Yaguajay en enero de 2024.

Alimento	Sí consume %	% Diario	% Semanal	% Mensual
Frijoles	100	63.3	30	6.7
Arroz	100	93.3	3.3	3.3
Pan	100	93.3	6.7	0
Café	100	93.3	0	6.7
Carne de cerdo	96.7	0	46.7	50
Otros vegetales	96.7	36.7	33.3	26.7
Pollo	93.3	0	30	63.3
Viandas hervidas	93.3	56.7	26.7	10
Viandas fritas	93.3	16.7	36.7	36.7
Aceite de girasol	90	33.3	10	3.3
Queso blanco	86.7	16.7	33.3	36.7
Manteca de cerdo	86.7	23.3	20	3.3
Plátano (fruta)	83.3	13.3	40	30
Carne de res	76.7	0	23.3	53.3
Huevos	76.7	10	36.7	30
Vegetales en hoja	76.7	23.3	23.3	30
Leche de vaca fluida	73.3	40	3.3	30
Picadillo de res	70	0	20	50
Pizza	70	3.3	26.7	40
Azúcar para leche	66.7	36.7	6.7	23.3
Refrescos instantáneos	66.7	23.3	16.7	26.7
Mantequilla	63.3	6.7	23.3	33.3
Jugos de frutas no cítricas	63.3	3.3	36.7	23.3
Ron	63.3	13.3	16.7	33.3
Perro caliente	60	0	16.7	43.3
Azúcar para jugos de frutas no cítricas	60	3.3	36.7	20
Pastas y fideos	56.7	0	3.3	53.3
Frutas cítricas	56.7	3.3	33.3	20
Pescado	53.3	0	13.3	40
Batidos de frutas con leche	50	3.3	23.3	23.3
Embutidos	50	0	13.3	36.7
Galletas saladas	50	6.6	26.7	20
Cerveza	46.7	0	26.7	20
Dulce en almíbar	46.7	0	10	36.7
Hígado	43.3	0	6.7	36.7
Yogurt natural	40	0	20	20
Azúcar para yogurt	40	3.3	20	16.7
Mayonesa	36.7	10	13.3	16.7
Refrescos gaseados	36.7	3.3	6.7	26.7
Croquetas	33.3	0	6.7	26.7
Jugo de frutas cítricas	33.3	0	20	13.3
Azúcar para jugos de frutas cítricas	33.3	0	20	13.3
Caramelos	33.3	0	6.7	26.7
Otras frutas no cítricas	30	0	10	20
Dulces de harina	30	3.3	13.3	13.3
Tamal	26.7	0	3.3	23.3
Helado	23.3	0	6.7	20
Miel de abeja	23.3	3.3	3.3	16.7
Azúcar sola	23.3	0	3.3	20
Picadillo de cerdo	20	0	16.7	3.3
Otras carnes	20	0	3.3	16.7
Otros dulces	20	0	6.7	13.3
Picadillo de soya	16.7	0	0	16.7
Hamburguesas	16.7	0	3.3	13.3
Harina de maíz	16.7	0	0	16.7
Galletas dulces	16.7	0	6.7	10
Yogurt de soya	10	0	3.3	6.7
Otros quesos	10	0	6.7	3.3
Leche condensada	6.7	0	6.7	0
Polvo de leche descremada	6.7	0	3.3	3.3
Queso crema	6.7	0	3.3	3.3
Queso fundido	6.7	0	3.3	3.3
Vino	6.7	0	3.3	3.3
Polvo de leche entera	3.3	0	0	3.3
Aguacate	3.3	0	3.3	0
Turrones	3.3	0	0	3.3

Con respecto a los lácteos, resulta notable que, aunque la leche de vaca fluida y el queso blanco resultaron ampliamente aceptados, su consumo diario no fue alto. Dentro de los alimentos que fueron consumidos por el 50% o poco más de la mitad de los campesinos se encuentra el batido de frutas con leche, los embutidos, pescados, pastas y fideos. Sobre ellos, la encuesta evidenció patrones de consumo mayoritariamente semanales o mensuales, lo que refleja que, aunque son aceptados por una parte considerable de la población, su inclusión en la dieta no es regular. Con menor frecuencia, entre el 40% y el 36,7% de los campesinos respondió positivamente al consumo de yogurt natural, hígado, cerveza, dulces en almíbar, mayonesa, croquetas y jugo de frutas cítricas. Finalmente, menos del 30% de los entrevistados consumieron helado, otros dulces, picadillo de cerdo, picadillo o yogurt de soya, leche condensada, polvo de leche descremada, queso fundido y polvo de leche entera. Los resultados muestran que la dieta en el mes de enero estuvo centrada en alimentos con una alta dependencia de productos locales, combinados en menor medida con productos procesados.

La Tabla 2 presenta el perfil antropométrico y de composición corporal de los campesinos estudiados, incluyendo medias, desviaciones estándar y rangos de valores (mín-máx). Dado que la muestra tiene un amplio espectro etario, que oscila entre 23 y 65 años, las características antropométricas presentan una notable variabilidad, reflejada en las desviaciones estándar y en los valores mínimos y máximos de las mediciones. El IMC promedio (25,1 kg/m²), aunque cercano al límite superior del normopeso, sitúa a la población en la categoría de sobrepeso. La dispersión del IMC, que va desde 17,2 hasta 38,6 kg/m², revela una significativa heterogeneidad en el estado nutricional de esta muestra. Esta diversidad también se manifiesta en la composición corporal, con porcentajes de grasa que varían desde el 10,4% hasta el 40,8%, y una masa muscular de entre el 34,0% y el 48,9%, sugiriendo distintos patrones de adiposidad y desarrollo muscular entre los participantes.

La Figura 2 representa la distribución de la clasificación del IMC. El análisis de los porcentajes permite identificar que la muestra estuvo compuesta con igual prevalencia, por individuos normopesos y sobrepesos (tipo I y II), con escasa representación de la desnutrición energética proteica.

Tabla 2. Perfil antropométrico y de composición corporal de la muestra analizada.

Variables	Muestra total (n=30)	
	Media ±Desviación Estándar	Mínimo-Máximo
Edad años	47.1±13.1	23-65
Peso (kg)	73.2±15.8	50.3-116.1
Talla (cm)	170.5±6.7	152.6-187.3
IMC (kg/m²)	25.1±4.8	17.2-38.6
% de grasa corporal	24.3±7.9	10.4-40.8
% de masa muscular	42.2±4.2	34.0-48.9

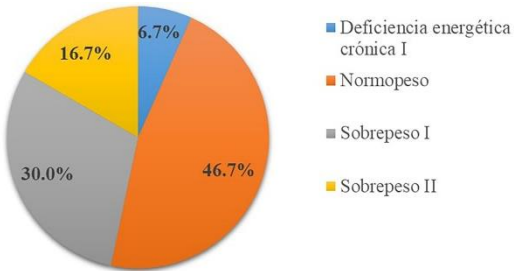

Figura 2. Distribución porcentual de la clasificación del índice de masa corporal (IMC) en una muestra de campesinos (n=30).

Con el propósito de proporcionar una mejor comprensión de las relaciones entre variables relevantes dentro de la población de estudio, se utilizó el análisis de correlación de Pearson o el de Spearman, según el caso. La Tabla 3 muestra la ausencia de correlaciones entre la edad y el resto de las variables estudiadas. El peso tuvo una correlación significativa pero débil con la talla, mientras que mostró correlaciones significativas y fuertes con el porcentaje de grasa corporal, el de masa muscular y el índice de masa corporal (IMC). Se observó que un mayor peso se asociaba con un incremento en el porcentaje de grasa corporal, una disminución en el de masa muscular y valores más altos de IMC, reflejando una fuerte interrelación entre estas variables. El IMC, por su parte, mostró una correlación muy fuerte y positiva con la grasa corporal y una correlación muy fuerte y negativa con el porcentaje de masa muscular, relación que se mantuvo incluso después de ajustar los resultados considerando el peso como variable de control. De

manera similar, el porcentaje de grasa corporal presentó una correlación fuerte, negativa y significativa con el porcentaje de masa muscular, y una correlación fuerte, positiva y significativa con el IMC, que se mantuvieron consistentes tras el ajuste por peso. Por último, el porcentaje de masa muscular tuvo una correlación fuerte, negativa y significativa con la grasa corporal y con el IMC, incluso al incluir el peso como variable de control.

Tabla 3. Asociación entre edad, variables antropométricas y de composición corporal obtenidas mediante impedancia eléctrica.

Variables			**Talla (cm)**	**% Grasa corporal**	**% Masa muscular corporal**	**Índice de masa corporal**	**Peso (kg)**
Ninguna variable a controlar	Edad años	Correlación	-0.053	0.19	-0.022	-0.071	-0.159
		p-valor	0.780	0.921	0.907	0.709	0.403
	Talla cm	Correlación	1.000	-0.134	0.206	0.080	0.431
		p-valor		0.480	0.276	0.673	**0.017***
	Peso kg	Correlación	0.431	0.774	-0.710	0.933	1.000
		p-valor	0.017	**0.000****	**0.000****	**0.000****	
	% Grasa corporal	Correlación	-0.134	1.000	-0.993	0.910	0.774
		p-valor	0.480		**0.000****	**0.000****	**0.000****
	% Masa muscular corporal	Correlación	0.206	-0.993	1.000	-0.868	-0.710
		p-valor	0.276	**0.000****		**0.000****	**0.000****
	Índice de masa corporal	Correlación	0.080	0.910	-0.868	1.000	0.933
		p-valor	0.673	**0.000****	**0.000****		**0.000****
Variable control Peso kg	% Grasa corporal	Correlación	-0.818	1.000	-0.994	0.826	
		p-valor	0.000**		**0.000****	**0.000****	
	% Masa muscular corporal	Correlación	0.806	-0.994	1.000	-0.810	
		p-valor	**0.000****	**0.000****		**0.000****	
	Índice de masa corporal	Correlación	-0.988	0.826	-0.810	1.000	
		p-valor	**0.000****	**0.000****	**0.000****		

****** La correlación es muy significativa (p≤0.01); ***** La correlación es significativa p≤0.05.

Discusión

La encuesta de frecuencia de consumo aplicada a 30 campesinos de Yaguajay, ha proporcionado una visión general de su alimentación durante el mes de enero de 2024. Los resultados indican una dieta en la que predominan los productos locales, complementados ocasionalmente con alimentos procesados o de menor accesibilidad. Este patrón refleja una inclinación hacia la autosuficiencia y el uso de recursos disponibles en la zona, con una incorporación moderada de alimentos procesados y de aquellos que resultan menos accesibles por factores tales como la estacionalidad, las limitaciones económicas o las preferencias culturales.

Los alimentos básicos identificados en el mes de enero fueron: arroz, frijoles, viandas hervidas, pan y café, observaciones que están alineadas con la dieta campesina tradicional cubana (González y Núñez,

1995). Además, la carne de cerdo y pollo fueron las principales fuentes de proteína animal, el consumo de manteca de cerdo fue parte de la dieta de más del 80% de los encuestados, y se constató la inclusión habitual del aceite vegetal para cocinar. Más del 60% de los participantes declararon haber consumido carne de res, huevos, vegetales en hoja, leche de vaca fluida, perro caliente, pizza, jugos de frutas y refrescos instantáneos, resultados que evidencian una diversificación de la dieta tradicional campesina e indican la incorporación de alimentos procesados y de conveniencia.

La persistencia de dietas tradicionales basadas en productos locales, pero con tendencias a la inclusión de alimentos externos también se ha observado en otros estudios en la región del Caribe. Tal es el caso de Colombet et al. (2021) quienes analizaron una muestra representativa de guadalupeños y martiniqueños, e identificaron 4 patrones coexistentes en las Antillas francesas, marcados por un contraste generacional, que parecen sugerir una transición nutricional en curso. La introducción de alimentos industriales y ultra procesados en comunidades rurales ha sido señalada por el riesgo de desplazamiento de los tradicionales y su efecto en la disminución de la calidad de la dieta, con consecuencias negativas para la salud. Algunos autores han alertado sobre este fenómeno en países del continente. México es un claro ejemplo de transición nutricional y el deterioro de sus indicadores de salud lo demuestra (Domínguez et al., 2021). Por su parte, Silva et al. (2022) observaron en comunidades rurales de las regiones Centro-Oeste, Nordeste y Amazonia de Brasil, que el mayor acceso a las economías de mercado y a alimentos de supermercado cambiaron las condiciones de subsistencia de las comunidades rurales, con compromiso para la agricultura tradicional y la soberanía alimentaria.

En el ámbito biomédico, resulta preocupante el hecho de que la sustitución de alimentos tradicionales por procesados haya sido asociada con un aumento de enfermedades crónicas. Lane et al. (2021) en una revisión sistemática y meta-análisis hallaron que el consumo de procesados estaba asociado con mayor riesgo de sobrepeso, obesidad abdominal, mortalidad, síndrome metabólico, depresión, enfermedades cardiometabólicas, fragilidad, síndrome del intestino irritable, dispepsia funcional y cáncer en adultos, mientras que también se relacionó con síndrome metabólico en adolescentes y dislipidemia en niños.

En 2007, Vargas señaló que en la alimentación de las poblaciones existían alimentos básicos, primarios, secundarios y periféricos. Según este autor el alimento básico suele satisfacer las necesidades de energía en la dieta, ofrece la posibilidad de preparar variados platillos y bebidas, ha sido domesticado y tiene un fuerte arraigo cultural. Por su parte, el alimento primario se considera acompañante del básico, ambos se combinan en la cocina y a menudo resultan nutricionalmente complementarios (Vargas 2007). Un buen ejemplo en el caso de los campesinos de Yaguajay es el consumo de arroz como alimento básico y el frijol como primario, donde su combinación permite un aporte de lisina y metionina que mejora la calidad nutricional total de la dieta. (Young y Pellett, 1994; Pellett, 1996; FAO, 2013). En cuanto a los alimentos secundarios y periféricos, se incluyen aquellos que aportan diversidad a la dieta y cuya representatividad oscila en función de factores tales como la estacionalidad, la disponibilidad, la solvencia económica y las preferencias personales (Vargas, 2007). En la población rural estudiada, a estos grupos de alimentos podrían pertenecer aquellos que mostraron gran variación individual y que fueron consumidos por el 50% o menos de la población. A pesar de la menor representatividad de los mismos, su estudio puede ser importante a largo plazo, debido a que alimentos que en un inicio fueron el centro de la dieta, pueden ser sustituidos por otros periféricos, y de este modo reflejar los distintos cambios socioculturales por los que transita una población. Este fenómeno ha sido descrito por su relación con la transición nutricional que actualmente afecta a numerosos países, en especial aquellos de medianos y bajos ingresos, en dónde alimentos tradicionales son reemplazados por otros de menor valor nutricional, de más fácil acceso y menor costo, pero relacionados con el desarrollo de obesidad y la mayoría de las enfermedades crónicas no transmisibles (Baker et al., 2020).

El presente estudio incluyó campesinos dentro de un amplio rango de edad, entre los 23 y 65 años, identificándose que, en esta muestra, los años cumplidos no fueron un elemento determinante en el estado nutricional y la composición corporal. Dicha afirmación se sustenta en la ausencia de correlación

entre la edad y el porcentaje de grasa y músculo, el peso corporal o el IMC. La literatura registra que, de forma general, muchos de los cambios en la composición corporal se asocian con el envejecimiento, en el que se produce una pérdida de masa muscular y ósea y un aumento de la masa grasa total (Fatyga-Kotula et al., 2022). Sin embargo, la evidencia sugiere que factores adicionales a la edad juegan un papel crucial sobre la composición corporal, como el dimorfismo sexual (Więch et al., 2021), el deterioro cognitivo y la soledad (Iwasaki et al., 2021), la práctica de ejercicio físico y la ingesta energética (Methenitis et al., 2022). En este sentido, la ausencia de correlaciones entre la edad con el IMC y la composición corporal en la muestra de campesinos, podría estar influenciada por las actividades físicamente demandantes a las que se encuentran vinculados debido a su ocupación laboral. Los individuos encuestados, independientemente de su edad, realizan en menor o mayor medida labores agrícolas, mecánicas, constructivas, domésticas y ganaderas, distribuidas en una jornada laboral que comienza en el campo en horas tempranas de la mañana.

En cuanto a la clasificación del IMC, aproximadamente la mitad de la muestra presenta sobrepeso. Este resultado concuerda con los datos aportados por las Encuestas Nacionales de Factores de Riesgo realizadas en Cuba, que revelan que el exceso de peso (sobrepeso global) se ha incrementado en la población cubana de forma exponencial sin diferencias según su ubicación geográfica (Varona, Gámez y Díaz, 2018).

Con respecto a la composición corporal, la media del porcentaje de grasa de la muestra total fue de un 24,3%, superior al rango estándar definido por el fabricante de la balanza InBody H20N para el sexo masculino (entre10% y 20%). Esta cifra indica que en general hay un exceso de grasa corporal. Aunque con la limitación del tamaño de muestra analizado en este trabajo, dichas observaciones coinciden con estudios de carácter nacional que indican que, en Cuba, el exceso de peso y la adiposidad se han incrementado sin diferencias significativas por lugares de residencia, tanto en zonas urbanas como rurales de las regiones occidente, centro y oriente (Díaz et al., 2022). Los riesgos del incremento de la adiposidad en el organismo han sido ampliamente descritos. Un alto porcentaje de grasa corporal, sobre todo de tejido adiposo intraabdominal, puede desempeñar una función intermediaria clave en el desarrollo de la resistencia a la insulina y la posterior diabetes *mellitus* tipo 2. En la actualidad se conoce que la grasa intraabdominal se comporta como un órgano endocrino activo, ya que produce adiponectina, leptina, resistina e interleucinas, que desempeñan un papel crucial en la regulación del apetito y la energía. Una producción desequilibrada de estos factores debida a una masa grasa intraabdominal aumentada probablemente contribuye a los trastornos metabólicos relacionados con el peso (Han et al., 2019).

Debido a las conocidas limitaciones del IMC respecto a su capacidad para describir la composición corporal, se calcularon correlaciones para analizar las asociaciones entre variables. Los resultados indicaron que, en la muestra estudiada, el aumento de peso e IMC fueron indicadores adecuados del incremento del porcentaje de grasa corporal y la disminución del porcentaje de masa muscular. Además, existió una relación inversa, en la que aquellos campesinos con un mayor porcentaje de grasa corporal exhibieron un menor porcentaje de masa muscular, y viceversa, observaciones que se mantuvieron aun controlando el peso. Este hecho coincide con estudios que afirman que, en la población general, el porcentaje de grasa corporal está positivamente relacionado con el IMC. Al respecto, Bradbury et al. (2017) reportaron, en población británica, una fuerte correlación entre el IMC y el porcentaje de grasa corporal tanto en mujeres (r=0,85) como en hombres (r=0,79). Se ha demostrado que esta relación cambia en grupos de población "especiales", como los jugadores de fútbol americano o los boxeadores de peso pesado (Han et al., 2019); por ello, el IMC puede resultar útil para predecir el incremento de masa muscular en población deportiva y de masa grasa en población general. Estas observaciones parecen justificar su uso para identificar sobrepeso y obesidad en estudios epidemiológicos a gran escala, con la necesidad de complementarse con mediciones que permitan obtener información sobre el compartimento muscular.

Este estudio ha abordado, en una primera aproximación, los patrones dietéticos y el estado nutricional de una población rural masculina de Yaguajay (Cuba), la cual pertenece a un grupo

demográfico frecuentemente subrepresentado en las investigaciones biomédicas. Pese a las limitaciones, como el tamaño reducido de la muestra y el diseño transversal, los resultados obtenidos ponen de manifiesto la necesidad de profundizar en los factores sociales, económicos y ocupacionales que influyen en la salud rural, promoviendo investigaciones futuras que incorporen análisis más amplios y perspectivas longitudinales.

Conclusion

La dieta de los campesinos de Yaguajay, en el periodo estudiado, se basa en alimentos locales, exhibiendo un patrón mixto que refleja una transición nutricional hacia la diversificación y el consumo de productos procesados. A este hecho se suma una elevada prevalencia de sobrepeso, por lo que resulta fundamental realizar estudios adicionales, que examinen en profundidad cómo estos cambios afectan a largo plazo la salud y calidad de vida de los trabajadores rurales de nuestro país.

Conflicto de intereses: Los autores declaran no tener conflicto de intereses.

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Intolerancia a la lactosa en una población nativa de la costa oeste atlántica africana. Historias evolutivas y barreras genéticas

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RESUMEN

Se ha estimado que alrededor del 65% de los humanos sintetizan la lactasa intestinal después de terminar el periodo de lactancia. La persistencia de la enzima lactasa, fenotipo LP, constituye un rasgo biocultural y un ejemplo de selección positiva y evolución convergente. La geografía genética del fenotipo entre la población humana mundial no es aleatoria. Las frecuencias más altas se encuentran en Europa y, particularmente, entre sus poblaciones más septentrionales, con una variación clinal negativa hacia el Mediterráneo. En otras áreas continentales no-europeas, como las africanas, algunas de sus poblaciones nativas, cuyos modos de subsistencia tradicional han estado basados en la producción y el consumo de leche, comparten el rasgo LP, aunque asociado a una fuerte regionalización. El presente trabajo tiene como objetivo la caracterización de un conjunto de variantes dentro del gen *MCM6* las cuales regulan, a su vez, al gen *LCT*, el cual codifica la lactasa en una muestra perteneciente a la población de Guinea Ecuatorial. Todos los individuos mostraron el alelo ancestral para los marcadores *-13910*C/T*, *-22018*G/A*, *-13915*T/G*, *-14010*G/C* y *-13907*C/G*. La ausencia de individuos persistentes a la lactasa es congruente al observado en otras muchas poblaciones africanas sub-Saharianas de la misma área. Un análisis detallado de las variaciones geográficas mostradas por los marcadores moleculares relacionados con la tolerancia a la lactosa dentro de África ha permitido detectar diferentes barreras al flujo de genes, como un reflejo del impacto del rasgo LP sobre la definición de la estructura genética de sus poblaciones nativas.

Keywords:

LCT
MCM6
Human genetic diversity
Equatorial Guinea
Positive selection

ABSTRACT

Around 65% of humans synthesize intestinal lactase after ending breastfeeding period. Lactase persistence, LP phenotype, constitutes a biocultural trait and an example of positive selection and convergent evolution. Frequencies of LP phenotype in the world are non-randomly distributed. The highest values are found in Europe, particularly, in northern Europe, with a negative gradient toward the Mediterranean. In other non-European continental areas, such as Africa, some of their native populations, whose traditional livelihoods have been based on the production and intake of milk, LP trait is present and associated to a strong regionalization. The present study aims to characterize molecular variants in the *MCM6* gene –which regulates, in turn, its neighbor *LCT*, responsible for encoding the enzyme lactase– in a sample of African natives from Equatorial Guinea. The genotyped sample only showed ancestral alleles for the markers: *-13910*C/T*, *-22018*G/A*, *-13915*T/G*, *-14010*G/C* and *-13907*C/G*. The non-detection of lactase persistence seems to be a coherent finding when comparisons are made with other surrounding sub-Saharan African human populations. Exceptions are found in some African ethnic groups from Eastern and Central Africa, with ancestral and deep-rooting cultural habits in shepherding and milk consumption. Analyses of geographical variations related to lactose tolerance across Africa has allowed to identify different barriers to gene flow. The observed scenario represents a signal of how the LP trait has been able to influence the human genetic structure within the continent.

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Introducción

Las poblaciones humanas se han caracterizado por su gran capacidad de adaptación frente a diferentes condiciones climáticas, ambientes específicos y otras presiones selectivas (Rees, Castellano y Andrés, 2020). Desde un punto de vista evolutivo, uno de los efectos de la selección natural ha sido el mantenimiento e incremento, en frecuencias variables, de determinadas variantes alélicas que confieren una ventaja a los individuos portadores, en relación a otras que carecen de esa condición. Este escenario constituye un ejemplo de selección positiva, la cual actúa sobre los fenotipos e implica una mayor adaptación en términos de supervivencia y capacidad reproductiva entre los individuos que conforman una población dada (Harris y Meyer, 2006).

Numerosos rasgos fenotípicos están ligados a procesos adaptativos locales. Por ejemplo, en el caso de la pigmentación de la piel, se han identificado una serie de genes como el *SLC24A5, SLC45A2* entre otros, que han mostrado claras evidencias de selección positiva en relación con la intensidad de exposición a la radiación ultravioleta a la que están sometidas muchas y diversas poblaciones humanas continentales (Harding et al., 2000; Beleza et al., 2013; Feng, McQuillan y Tishkoff, 2021). Asimismo, la presencia de modificaciones en la fisiología de las hemoglobinas y de las células eritrocitarias como respuesta al *Plasmodium*, patógeno causante de la enfermedad infecciosa malaria, ha sido motivo de una atención preferente y continuada, por sus claros efectos selectivos, en el África sub-Sahariana, Mediterráneo y sureste asiático (López et al., 2010; Hedrick, 2011; McManus et al., 2017; Kariuki y Williams, 2020).

Sin embargo, no solo las características ambientales u otros factores han podido conducir el cambio evolutivo, sino que el propio comportamiento humano podría haber actuado también como motor de dicho cambio. En esta línea, las importantes modificaciones en la dieta que tuvieron lugar en muchas sociedades humanas con la llegada del Neolítico (≈11.000 BP), acompañadas por la irrupción y difusión de la agricultura primero y, más tarde, por la domesticación de especies animales, representa un verdadero paradigma de co-evolución entre *genes* y *cultura*. De este modo, el uso de la leche de origen animal en la alimentación humana habría sido el desencadenante del mantenimiento de particulares variantes genéticas responsables de la expresión continuada de la enzima "*lactasa"* en el intestino delgado a lo largo de la vida de una persona. Esta condición es conocida como "persistencia de la lactasa" (*Lactase Persistence*, LP) (Gerbault et al., 2011). La tolerancia a la lactosa es la capacidad de una persona para digerir la lactosa, el principal azúcar de la leche. La enzima lactasa transforma la lactosa en los dos metabolitos que conforman ese disacárido: glucosa y galactosa (Ingram et al., 2009).

Se ha estimado que ~65% de los humanos adultos sintetizan correctamente la lactasa intestinal (Itan et al., 2010). Los valores más elevados del fenotipo LP se encuentran entre las poblaciones europeas más septentrionales (~90-98%) (Ingram, Liebert y Swallow, 2012). En otras áreas continentales no-europeas, algunas de sus poblaciones nativas cuyos modos de subsistencia tradicional han estado generacionalmente basados en la producción y el consumo de productos lácteos, también comparten esa particularidad.

El gen de la lactasa, conocido como *LCT* (*florizina-hidrolasa*), está localizado en el brazo largo del cromosoma 2 en humanos (2q21). Es interesante señalar que el fenotipo LP tiene su base genética en el gen *MCM6,* situado en sentido 5' (*upstream*) del gen *LCT*, el cual codifica un componente del complejo de mantenimiento de mini-cromosomas, componentes esenciales para la iniciación de la replicación del DNA (Jensen et al., 2011). El mosaico de variantes genéticas que alberga el gen *MCM6* incluye, en su mayor parte, polimorfismos de un único nucleótido o *Single Nucleotide Polymorphisms* (SNPs), distribuidos por todo ese gen o en genes adyacentes también ubicados en el cromosoma 2. Esos SNPs se encuentran en el intrón 9 y 13 del *MCM6* y son los responsables de potenciar la unión de los factores de transcripción al promotor, conduciendo a la expresión del gen *LCT* y, por tanto, el fenotipo LP (Ranciaro et al., 2014). Asimismo, estas variantes alélicas poseen carácter dominante (Ingram et al., 2009). En consecuencia, bajo el paraguas fenotípico LP coexisten diferentes mutaciones como la *-14010 G/**C**, -13915 T/**G**, -13910 C/**T**, -13907 C/G y -22018 G/**A***, con una particular

geografía genética, lo que permite inferir un origen evolutivo independiente. Este interesante escenario ha hecho identificar al rasgo LP como un claro ejemplo de evolución convergente (Campbell y Ranciaro, 2021).

Los estudios de ADN antiguo (ADNa) de restos esqueléticos humanos de diferentes cronologías están aportando interesantes resultados acerca de la presencia de mutaciones específicas asociadas al rasgo LP, así como de sus edades evolutivas asociadas. En poblaciones cazadoras-recolectoras pre-neolíticas europeas (Ségurel y Bon, 2017) no han sido detectadas variantes genéticas derivadas relacionadas con la tolerancia a la lactosa. Con estos enfoques, Itan et al. (2009) sugirieron que la variante *-13910***T*** (marcador LP *europeo* por excelencia) habría alcanzado una incidencia apreciable en la Europa Central y en los Balcanes durante el periodo Neolítico. Más tarde, los estudios desarrollados en África y Oriente Próximo por Ranciaro et al. (2014) sobre la presencia y frecuencia de mutaciones dentro del gen *MCM6*, sugirieron que: *i*) la variante *-13910***T*** podría haber llegado a África desde Europa hace ~5.000-12.000 años BP y, que la *-13915***G*** (marcador de la *Península de Arabia*) habría podido surgir en el Este de África hace 3.000-6.000 años BP. Entre las poblaciones africanas se ha detectado la mutación *-13.907***G*** con una más bien alta presencia en el este y regiones centrales sub-Saharianas, con una edad evolutiva ~5.000 años BP. A *-14.010*C,* reconocida como una variante *africana,* se la ha relacionado con una mayor profundidad temporal (~ 6.000-7.000 años BP) (Tishkoff et al., 2007).

La mayoría de los estudios sobre la tolerancia a la lactosa en el continente africano se ha centrado regularmente en poblaciones nativas asentadas en su franja geográfica más oriental (Etiopía, Kenia, Tanzania y Somalia) (Ranciaro et al., 2014; Macholdt et al., 2015; Hassan et al., 2016). Por el contrario, en el África central y la occidental atlántica las investigaciones desarrolladas sobre la antropología molecular del fenotipo LP son bastante escasas. Algunos resultados publicados han puesto en evidencia las muy bajas frecuencias o la no existencia de individuos tolerantes a la lactosa (Mulcare et al., 2004; Ingram et al., 2009; Jones et al., 2015).

El presente estudio tiene como objetivo central el análisis genético-molecular del rasgo LP en una muestra de población nativa ecuatoguineana, través de la caracterización de cinco variantes genéticas dentro del gen *MCM6*. El impacto de los contactos históricos coloniales prolongados que se han producido entre la población autóctona de Guinea Ecuatorial con las de Europa occidental, fundamentalmente, ha podido dejar huella en el genoma de la población huésped (receptora) a través del consiguiente flujo génico. Las frecuencias geográficas observadas de las cinco mutaciones analizadas en otras poblaciones de Europa, África, y del Mediterráneo, junto con la incidencia del fenotipo LP registrada en esas grandes áreas geográficas, han sido utilizadas para identificar, en su caso, patrones de variación espacial y posibles barreras al flujo de genes.

Materiales y Métodos

Población de estudio: geografía, demografía e historia

Guinea Ecuatorial forma parte del contexto geográfico del Golfo de Guinea, en la costa oeste africana. Curiosamente, se trata de un territorio al que no se le ha considerado como un componente más dentro de las ambiciosas investigaciones desarrolladas en las dos últimas décadas dirigidas a conocer la magnitud de la diversidad genética humana contemporánea dentro de África (Choudhury et al., 2020; Fortes-Lima et al., 2024). De acuerdo con el censo de 2023, el tamaño demográfico (N) de Guinea Ecuatorial es de 1.600.000 habitantes (Instituto Nacional de Estadísticas de Guinea Ecuatorial; https://inege.org/). Sin embargo, en la década de los 1980, el país registraba un número de habitantes sensiblemente menor (~289.000, https://ourworldindata.org/) lo que nos estaría indicando que a lo largo de las últimas décadas hasta la actualidad se ha producido un auténtica explosión demográfica.

Desde un punto de vista geográfico-administrativo, Guinea Ecuatorial comprende una región continental y una insular (Figura 1). La última incluye a las islas de Bioko y Annobón. Las ciudades más relevantes son Malabo, capital del país, situada al norte de la isla de Bioko (la antigua *isla de Fernando Poo*) y Bata, centro económico de la región continental de Guinea Ecuatorial. La población nativa de este

territorio africano presenta un alto interés para ser estudiada en un marco antropológico, por los grupos étnicos que alberga (los *Fang* y los *Bubis*) (Bolekia, 2003; Gelabert et al., 2019; Turbón, 2020). El origen de estas etnias parece tener un sustrato Bantú (África central).

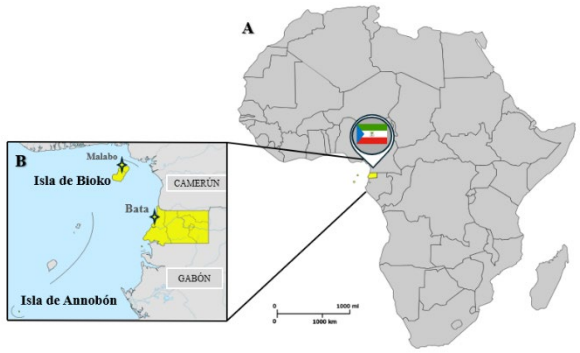

Figura 1. Localización geográfica de Guinea Ecuatorial dentro de África (**A**) y la composición de su territorio continental e insular (**B**).

Históricamente, Guinea Ecuatorial ha mantenido fuertes lazos de conexión con la Península Ibérica tanto por parte de Portugal como de España, con presumibles implicaciones biológicas. Desde finales del siglo XV hasta 1778, el reino de Portugal tomó el control de la isla de Bioko, aunque hubo algunas otras incursiones europeas como fue el caso de los Países Bajos. Las relaciones comerciales entre Guinea Ecuatorial y Portugal estuvieron centradas principalmente en el tráfico de esclavos y en las plantaciones de caña de azúcar. Entre 1777 y 1778, en virtud de los Tratados de San Ildefonso y de El Pardo, el dominio portugués finaliza para dar paso a la posesión de los territorios del Golfo de Guinea por parte de la Corona de España. En 1968, Guinea Ecuatorial alcanza su independencia, pero ha seguido manteniendo estrechas relaciones bilaterales con España. Una prueba de ello es el haber conferido al español la condición de ser una de las lenguas oficiales del país.

Proceso de muestreo y genotipado de las variantes genéticas (SNPs) ligadas a la tolerancia de la lactosa (LP)

La muestra poblacional analizada consistió en 26 individuos nativos de Guinea Ecuatorial (54% varones, 46% mujeres) con una edad promedio de 31.46 ± 9.38 años. Todos los participantes dieron su consentimiento informado y aportaron información puntual sobre sus orígenes familiares y etnia a la que pertenecían (81% *Fang*, 19% *Bubis*). Las muestras biológicas fueron de saliva, se tomaron en la capital de Malabo (octubre 2018) y fue utilizado el kit *Oragene OG-500* (DNA Genotek Inc.). Una vez estabilizadas las muestras salivares (~2 ml), se procesaron para la obtención de su ADN mediante el kit *prep IT L2P-5* (DNA Genotek Inc.) siguiendo los protocolos recomendados por el fabricante. Durante el proceso de extracción, en dos de las muestras no fue posible extraer su material genómico, por lo que el tamaño total de individuos genotipados fue de n= 24.

La tecnología TaqMan, basada en una PCR a tiempo real (RT-PCR), fue la utilizada para el genotipado de las cinco variantes genéticas seleccionadas del gen *MCM6*. Las correspondientes reacciones se llevaron a cabo en un equipo *StepOne* (*Thermo Fisher Scientific*). Para el genotipado de los polimorfismos: *-22018 C/**T*** (rs182549), *-14010 G/**C*** (rs145946881), *-13915 T/**G*** (rs41380347) y *-13907 C/**G*** (rs41525747) se diseñaron *de novo* los ensayos correspondientes (*Custom TaqMan SNP Genotyping Assays*). En el caso del ensayo para la caracterización del SNP *-13910 C/**T*** (rs4988235) estaba ya prediseñado por la empresa (ref. C_2104745_10, *Thermo Fisher Scientific*). Las lecturas de los tres genotipos posibles se realizaron directamente en el equipo *StepOne*, mediante el *StepOne software*. En aquellos casos en los que fue posible, se utilizaron controles positivos para los tres genotipos potenciales, empleando para ello muestras de genotipos conocidos de individuos con otras procedencias geográficas disponibles en nuestro Laboratorio de Antropología Molecular.

Análisis genéticos y estadísticos de los datos.

Los genotipos observados junto a las frecuencias de los alelos “derivados” para cada variante genética, se incorporaron a una base de datos general, la cual se ha venido actualizando hasta el presente por este equipo de investigación para análisis genéticos, estadísticos y de variaciones espaciales del rasgo LP y sus bases moleculares (ver Calderón et al., 2019). La base de datos mencionada incluye hasta el momento

presente 140 poblaciones y más de 19.000 individuos (no mostrada en el presente trabajo, y disponible bajo petición a los autores). A partir de esta amplia y detallada fuente de información se generaron mapas de interpolación de frecuencias (*surface maps*) mediante el programa ArcGIS Pro (Esri), utilizándose el método de interpolación *Inverse Distance Weighting* (IDW), el cual asigna a los puntos desconocidos en el espacio un promedio ponderado de los valores disponibles en aquellos puntos conocidos mediante una función de ajuste (Setianto y Triandini, 2015).

También se llevaron a cabo análisis de estructura genética. Para ello, se aplicó el procedimiento multivariado, PCA (*Principal Component Analysis*) mediante la librería *factoextra* de R (Kassambara y Mundt, 2020). Esta metodología tuvo un doble marco de desarrollo: a) el conjunto global de las poblaciones que conforman nuestra base de datos, y b) el continente africano, en particular. Asimismo, la exploración de posibles barreras al flujo de genes dentro de África, sobre la base de los patrones de variación geográfica mostrados por los marcadores LP entre las poblaciones nativas, ha sido realizada mediante el programa *BARRIER v. 2.2* (Manni, Guérard y Heyer, 2004). Para la visualización de las barreras más robustas desde un punto de vista estadístico, se aplicó una técnica de re-muestreo (*bootstrap*) empleando cuatro matrices de distancias distintas (distancias Euclídeas, de Manhattan, de Canberra y de Minkowski). La existencia de posibles diferencias significativas en la composición genética subyacente al rasgo LP entre aquellas poblaciones separadas por las barreras identificadas, fueron contrastadas mediante un Análisis Molecular de la Varianza (AMOVA) usando el programa ARLEQUIN 3.5 (Excoffier y Lischer, 2011). El AMOVA es un procedimiento jerárquico el cual permite explorar la diferenciación genética poblacional, mediante el estadístico *F* de Wright y sus parámetros (componentes): F_{ST}, F_{CT} y F_{SC}.

Resultados

La Tabla 1 muestra las frecuencias genotípicas observadas, sobre la base de los cinco SNPs relacionados con el rasgo LP. La totalidad de los individuos que conforman la muestra ecuatoguineana analizada, presentaron el alelo ancestral en homozigosis, un resultado que implicaría la ausencia del fenotipo tolerante para digerir la lactosa y similar a lo encontrado en la mayor parte del África sub-Sahariana.

Dentro de un marco comparativo, los resultados obtenidos en el presente estudio han sido contextualizados junto a los de otras poblaciones continentales. Las poblaciones utilizadas, códigos, referencias y orígenes geográficos están disponibles, siempre que esta información sea oficialmente requerida. Los patrones de variación espacial (*surface maps*) del rasgo LP (Figura 2) a nivel de la población humana mundial y utilizando la información fenotípica actualizada, paraleliza a la publicada en el clásico trabajo de Itan et al. (2010). El mapa de contorno nos presenta dos "*hotsposts*" o áreas definidas por sus altas frecuencias. Una de ellas está localizada en la Europa septentrional y la otra en la Península de Arabia. La primera registra valores extremos en relación al fenotipo tolerante a la lactosa [Noruega (97%), Reino Unido (96%), Suecia (94.1%)] mientras que esas proporciones son más moderadas, aunque muy representativas, entre las poblaciones de la Península de Arabia (83.30 %, como valor promedio). En Europa, el

Tabla 1. Genotipos observados en la muestra de población nativa de Guinea Ecuatorial analizada para cada uno de los marcadores que condicionan el fenotipo LP (lactasa persistente). Para cada variante genética (SNP), el alelo derivado se muestra en negrita.

SNPs	Fenotipos LP					
	Homozigoto ancestral	**Intolerante a la lactosa**	**Heterozigoto**	**Tolerante a la lactosa**	**Homozigoto derivado**	**Tolerante a la lactosa**
*-13910*T*	CC	24	C**T**	0	**TT**	0
*-13907*G*	CC	24	C**G**	0	**GG**	0
*-13915*G*	TT	24	T**G**	0	**GG**	0
*-22018*A*	GG	24	G**A**	0	**AA**	0
*-14010*C*	GG	24	GC	0	**CC**	0

fenotipo LP presenta una clara variación clinal negativa N/S, desde el área escandinava a la mediterránea, donde alcanza valores medios alrededor del 30-50%. En Asia, las frecuencias poblacionales LP son regularmente muy bajas e incluso nulas, como es el caso de China y Japón. Entre la población nativa de Mongolia las proporciones de este carácter son ~14%.

Cuando se hace referencia al continente africano sobre el rasgo LP, sus frecuencias registradas son normalmente bajas (<20%, como valor promedio) en relación a las observadas en la vecina Europa, aunque la geografía genética de poblaciones tolerantes/no-tolerantes LP dentro de África revela escenarios muy heterogéneos. En el Magreb más atlántico, sus poblaciones nativas alcanzan proporciones LP>30% [Saharauis (45.24%); Bereberes de Marruecos (33.50%); Argelia (36.34%)]. Un patrón muy similar es observado en el noreste continental (incluyendo el cuerno de África), [Sudán (8.16%); Etiopía (27%); Kenia (43.28%); Tanzania (30.46%)].

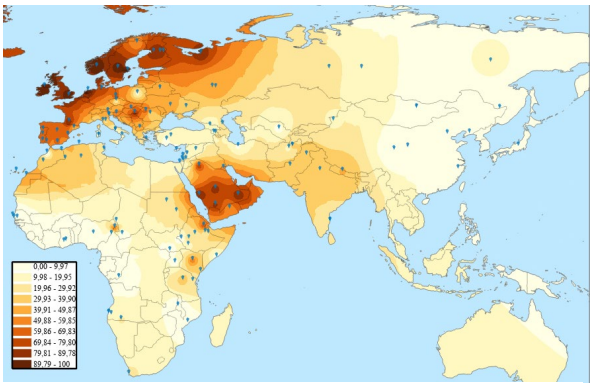

Figura 2. Patrones de variación geográfica del fenotipo LP (*lactase persistence*) entre poblaciones humanas del Viejo Mundo. Los marcadores azules indican la ubicación geográfica de las muestras poblacionales utilizadas para la construcción de los mapas de contornos (*surface maps*).

En lo que se refiere al mosaico de variantes (SNPs) relacionadas con el fenotipo LP, es importante señalar que, desde el punto de vista de la genética de poblaciones, el marcador *-22018 G/**A*** parece estar focalizado al sur del continente europeo, con un peso destacable entre las poblaciones norteafricanas occidentales (Argelia y Marruecos) (Calderón et al., 2019). El alelo derivado *-22018**A*** se encuentra en marcada asociación con su variante hermana *-13910**T*** (Ranciaro et al., 2014). Sería interesante enfatizar que la mutación *-13910 C/**T*** fue el primer polimorfismo relacionado con la tolerancia a la lactosa investigado a nivel poblacional (Enattah et al., 2002).

En la Figura 3 se presentan conjuntamente los mapas de contorno referidos a los otros cuatro polimorfismos caracterizados en el presente trabajo, lo que nos permite visualizar que las señales moleculares relacionadas con la condición LP arrojan geografías muy distintivas. Por ejemplo, el alelo *-13910**T,* el marcador antropogenético LP europeo por excelencia, exhibe un gradiente negativo N/S tal y como como ha sido mencionado más arriba (ver Figura 3A). En esta línea, es interesante destacar su apreciable representación de esta variante a lo largo del Magreb. Más allá de la franja norteafricana, entre la población nómada-ganadera de los *Fulani* del Camerún en el África occidental, la incidencia de individuos tolerantes a la lactosa es bastante apreciable (36%). Un resultado que ha sido interpretado como una consecuencia de los contactos de poblaciones norteafricanas con sus vecinas del Sahel (Ingram et al., 2006; Ranciaro et al., 2014).

El marcador *-13915**G* parece estar asociado a poblaciones de la Península de Arabia [Arabia Saudí (58.90%), Yemen (26%) Kuwait (25%)] observándose, además, un claro solapamiento entre el fenotipo LP y las visibles variaciones geográficas de esta variante molecular a lo largo y ancho del territorio peninsular (Figura 3B). Es interesante observar el paralelismo que revelan los polimorfismos *-13907**G* y *-13915**G* en términos de sus patrones de variación geográfica (Figuras 3B y 3C), una observación que puede haber sido causada por los movimientos poblacionales que han tenido lugar en la historia humana reciente entre el cuerno de África y la Península de Arabia. Curiosamente, la mutación *-13915**G* está presente en poblaciones nativas asentadas alrededor del Estrecho de Bab el-Mandeb, el cual separa el cuerno de África de la Península Arábiga [región de Afar (Etiopía) (27%), Sudán (11%)]. También, las variantes *-13907**G*

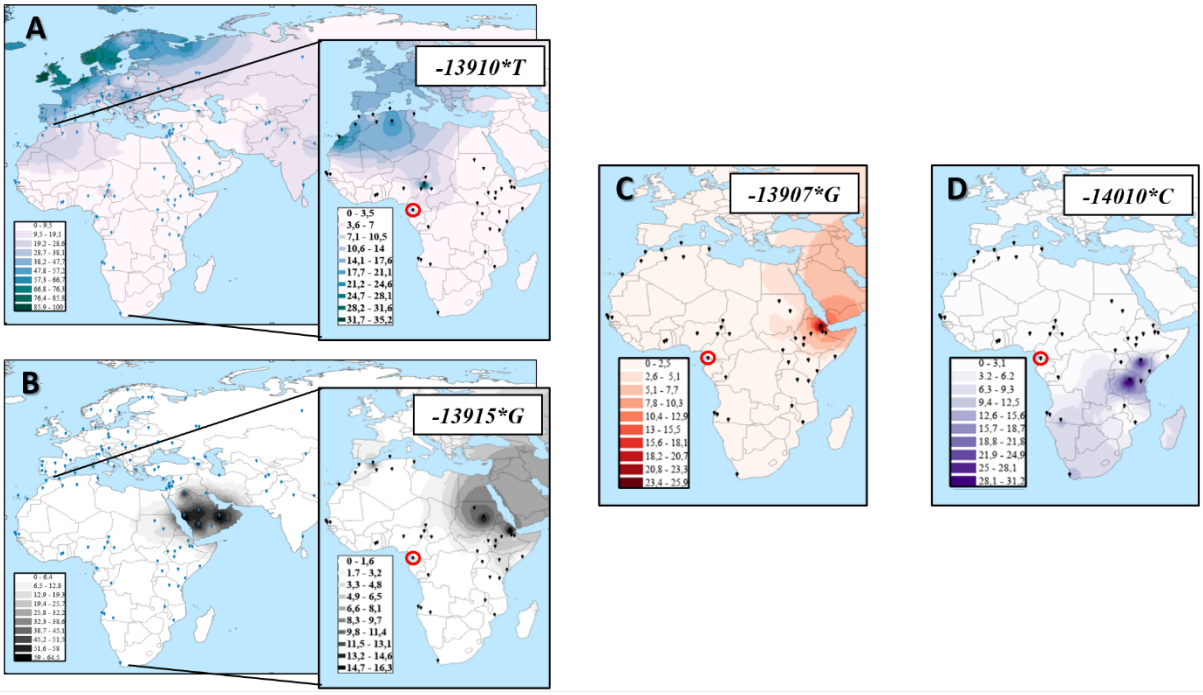

Figura 3. Mapas de contornos continentales mostrando las variaciones espaciales correspondientes los alelos derivados que identifican a las variantes genéticas *-13910*T* (**A**), *-13915*G* (**B**), *-13907*G* (**C**), y *-14.010*C* (**D**), relacionadas con el fenotipo LP.

y *-14010*C* son identificadas como marcadores antropogenéticos de poblaciones particulares sub-Saharianas, localizadas en el este africano: Kenia (27%) y Tanzania (31%) (ver Figura 3C y 3D).

Asumiendo la fuerte regionalización de las variantes genético-moleculares asociadas al rasgo LP en el continente africano, dicha información ha sido utilizada con objetivos de análisis de estructura genética a diferentes niveles. El PCA construido sobre la base de datos global de poblaciones continentales (Figura 4A) nos revela claramente una estructuración geográfica asociada a los marcadores LP caracterizados en el presente estudio. Los dos primeros componentes explican el 49% de la varianza observada. El PCA, nos revela que las variables *-13910*T* y *-22018*A* condicionan la topología de las poblaciones europeas utilizadas en el análisis y, en menor medida, otras del sudoeste asiático. Las orientaciones de ambos grupos de muestras poblacionales están dirigidas en sentido negativo del componente principal 1 (PC1). En este marco, sería interesante recordar la fuerte dependencia existente entre estas dos variantes genéticas, mencionada anteriormente. El resto de las poblaciones continentales (africanas, arábigas y del sudoeste asiático) se localizan en la dirección positiva del PC1. Curiosamente, el PC2 parece estar correlacionado con la mutación *-14010*C*, la cual alcanza sus más altas frecuencias en dentro de África. El mismo argumento se puede trasladar a la *-13915*G* para las poblaciones de la Península de Arabia y otras próximas del suroeste de Asia. La topología de la muestra de Guinea Ecuatorial (código 14, presente estudio), se posiciona coherentemente en el centroide, dado que la frecuencia de los alelos derivados en esta población es, en todos los casos, de 0. Idénticas localizaciones dentro del espacio bidimensional se refieren a algunas poblaciones asiáticas (en amarillo) las cuales comparten la condición de ser mayoritariamente no-persistentes para la enzima lactasa.

Cuando se hace referencia específicamente el continente africano, el PCA denota que los dos primeros componentes absorben el 65% de la varianza total (Figura 4B). Uno de los resultados más destacados que emergen del análisis multivariado lo representan las poblaciones norteafricanas del Magreb, las cuales aparecen agrupadas en el tercer cuadrante, en el sentido negativo del PC2. Su topología parece estar muy influenciada de nuevo por la alta presencia en el

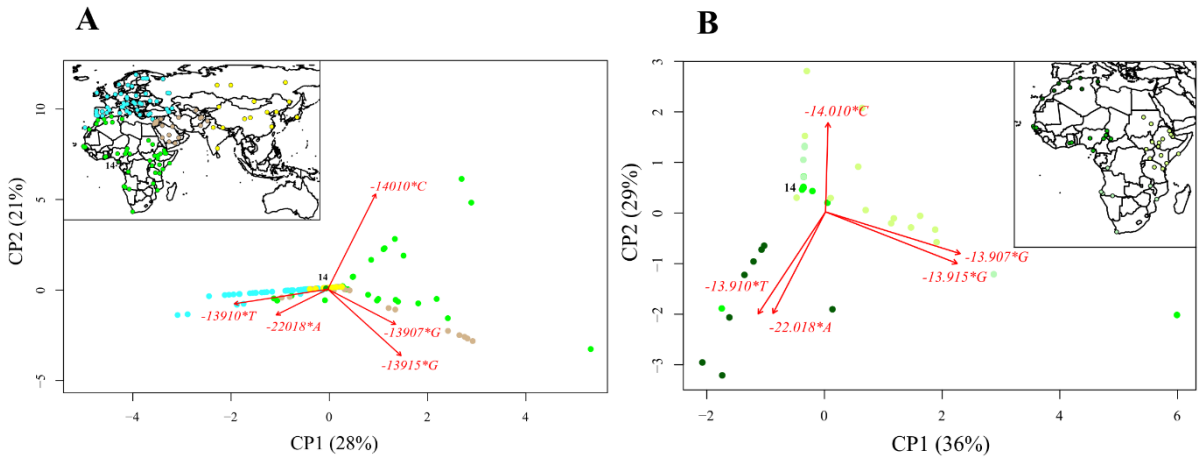

Figura 4. **A**) PCA basado en las frecuencias relativas de diferentes marcadores moleculares relacionados con la expresión fenotípica del rasgo LP en Europa (azul), África (verde), suroeste asiático/Oriente Medio/Cáucaso (ocre) y Asia (amarillo). **B**). PCA construido utilizando la misma información anterior pero referida a las poblaciones africanas, exclusivamente. Dichas poblaciones se representan en tonos variables de color verde, indicando el Norte, Oeste, Este y Sur de África, Las muestras utilizadas en el análisis multivariado están representadas con los códigos numéricos designados en la base de datos general.

genoma de estas poblaciones del marcador LP europeo *-13910*T* siempre acompañado del *-22018*A*. Este ejemplo de clusterización o de agrupamiento puede constituir una evidencia de barrera una geográfica-genética respecto a otras poblaciones hermanas norteafricanas asentadas más al sur. En la Figura 5 se presentan las cinco barreras (A-E) inferidas desde las variaciones geográficas del fenotipo LP en África. La primera de esas barreras (A), separa el Norte de África del África sub-Sahariana. La segunda (B) permite dividir distinguir una zona oriental y otra occidental y traza, aunque de manera ligeramente desplazada hacia el oeste, el corredor del valle del Rift y las zonas de sabana del Serengueti. Las tres otras barreras restantes (C, D y E) permiten visualizar patrones más específicos los cuales podrían estar relacionados con la representatividad diferencial de algunos marcadores moleculares LP restringidos a algunas poblaciones nativas del este de África.

El análisis molecular de la varianza (AMOVA), el cual es jerárquico en esencia, ha permitido evaluar el grado de significación genética de dichas barreras (Tabla 2). Los resultados revelan la existencia de diferencias significativas *dentro* de las poblacionales africanas (índice F_{ST}) y *entre* poblaciones dentro de los grandes grupos geográficos establecidos (F_{SC}). Cuando

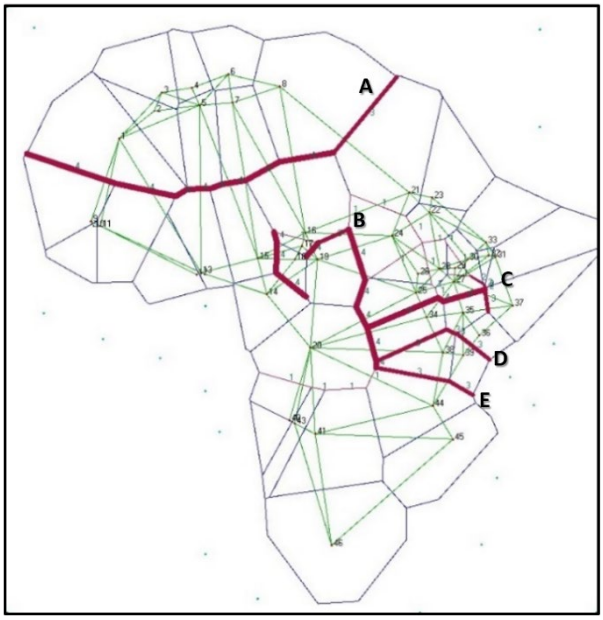

Figura 5. Barreras al flujo génico (color rojo) detectadas a partir de las distancias genéticas estimadas entre poblaciones africanas. Este análisis se basa en las frecuencias de los alelos derivados correspondientes a los marcadores (SNPs) genotipados en el presente estudio los cuales que confieren la condición, a la persona portadora, de ser tolerante a la lactosa.

se hace referencia al análisis entre grupos geográficos (F_{CT}), los cuales incluye un numero variable de

poblaciones por grupo establecido, la extensa barrera geográfica detectada entre el Norte de África (Magreb)/zona del Sahel con el resto del continente, nos permite desvelar que los patrones genéticos que emergen desde los marcadores moleculares LP dentro de África son significativamente diferentes a ambos lados del desierto del Sáhara (F_{CT}: 0.082, *p valor*<0.001). Es interesante enfatizar de nuevo que ese alto valor estimado del parámetro F_{CT} podría ser interpretado por la notable presencia del marcador europeo *13910*T* en el África septentrional desde Túnez hasta Marruecos. La otra barrera que recorre una buena parte del África oriental/central en dirección N/S (barrera B) podría ser explicada por los efectos causados por el valle del Rift y su geografía, apartando significativamente (F_{CT}: 0038, *p valor*<0.05) a las poblaciones de la costa atlántica, donde las frecuencias de alelos persistentes para cualquiera de los marcadores moleculares LP genotipados son próximas a 0 o nulas, y la vertiente oriental, donde algunas de sus grupos étnicos registran valores relativamente apreciables de las variantes *14010***C***, *-13907*__G__* "*africanas*" y la *-13915*__G__* "*arábiga*".

Tabla 2. Índices de fijación de Wright obtenidos mediante el análisis de la varianza molecular (AMOVA), tomando como fuentes de información las frecuencias observadas de los marcadores del gen *MCM6* que regulan la síntesis de la enzima lactasa (gen *LCT*) en grupos poblacionales africanos.

Barreras genéticas	Índices de fijación					
	F_{ST}	% varianza	F_{CT}	% varianza	F_{SC}	%varianza
Sáhara (A) Norte de África (N=8) vs. resto de África (N=37)	**0,210*****	78,97	**0,082*****	8,18	**0,140*****	12,85
África oriental/central (B) África oriental (N=21) vs. occidental (N=12)	**0,192*****	80,79	**0,038***	3,89	**0,159*****	15,32
África oriental 1 (C) África NE (N=13) vs. SE (N=8)	**0,154*****	84,62	0,029 (ns)	2,93	**0,128*****	12,45
África oriental 2 (D) Uganda, Kenia y Somalia vs. Tanzania	**0,082*****	91,72	-0,019 (ns)	-1,92	**0,100*****	10,20
África oriental 3 (E) Tanzania vs. S de África (N=6)	**0,226*****	77,42	**0,194***	19,38	**0,039*****	3,20

**p <0,05; **p <0,01; ***p <0,001*

Discusión

La geografía genética del fenotipo LP entre la población humana mundial no es aleatoria, siendo la latitud el factor utilizado para explicar su distribución en Europa; en África, sin embargo, los patrones de variación espacial LP parecen ser más complejos en su interpretación. La localización geográfica de Guinea Ecuatorial (costa oeste atlántica africana) puede ser una variable importante para entender la ausencia de alelos derivados de ancestría africana (*-13915*__G__* y *-14010*__C__ y*) relacionados con la condición LP. Históricamente, la actividad pastoril no ha sido una práctica tradicional de la población nativa ecuatoguineana, la cual ha disfrutado de otros recursos como fuentes de alimentación, como la caza, la pesca y la agricultura de productos locales (Bolekia, 2003). Estudios arqueológicos han permitido inferir que el pastoreo y

los cuidados de rebaños por parte de poblaciones que viven en las regiones más áridas del este, centro y sur del continente africano, representarían hábitos culturales que se habrían introducido entre los 4.500 o 3.500 años BP (Tishkoff et al., 2007).

En ese contexto, sería interesante resaltar la ausencia de la variante *-13910*T* (*marcador europeo* LP por excelencia) en la muestra de población nativa ecuatoguineana analizada. Este resultado permitiría rechazar una de nuestras hipótesis planteadas: la esperada detección de señales genéticas de otros continentes vecinos, Europa, como consecuencia del sostenido e importante pasado colonial del país. En este punto, debemos destacar que el presente estudio se basa en una muestra reducida (n=24), y en un muestreo localizado exclusivamente en la localidad de Malabo, por lo que no podría descartarse la existencia de acontecimientos de flujo génico entre esta población africana atlántica con otras no-africanas y, preferentemente, europeas. En la historia reciente de Guinea Ecuatorial, las uniones mixtas han sido (y continúan siendo) frecuentes. Estos patrones de cruzamientos, como una expresión más del flujo génico, se han distinguido tradicionalmente por estar compuestos de mujeres ecuatoguineanas y varones de procedencia europea (Aixelà-Cabré, 2013). El escenario citado se ha podido contrastar a través de marcadores moleculares de la región no-recombinante del cromosoma Y (NRY). El ejemplo lo encontramos en el haplogrupo paterno R1b-M269 distinguido como europeo (González et al., 2013) y cuya presencia en Guinea Ecuatorial ha sido interpretado como un resultado de las mezcla biológicas ocasionadas.

Tomando como referencia los mas que remarcables patrones de variación geográfica mostrados por las variantes LP dentro de África, los análisis estadísticos-genéticos desarrollados en el presente estudio han puesto en evidencia la presencia de dos grandes y fuertes barreras al flujo génico, marcando significativas heterogeneidades genéticas interpoblacionales. Los perfiles de esas barreras parecen ser muy correspondientes a las detectadas desde el uso de otros marcadores de diferentes regiones del genoma humano (Gomez, Hirbo y Tishkoff, 2014; Schlebusch y Jakobsson, 2018; Vicente y Schlebusch, 2020).

Una de esas barreras sería de carácter longitudinal (el desierto del Sáhara) y, la otra, latitudinal (el valle del Rift), aunque con un cierto desplazamiento geográfico hacia el África central. La barrera que separa el Magreb y el África sub-Sahariana, habría limitado, presumiblemente, los movimientos poblacionales Norte-Sur y recíprocamente. Una notable e interesante ausencia de marcadores africanos sub-Saharianos (*-14010*C* y *-13907*G*) se observa entre las poblaciones del Magreb. En consecuencia, se puede decir con fundamento que el Norte de África habría estado especialmente relacionado con la vecina Europa y, sobre todo, con sus poblaciones más meridionales. Este hecho nos estaría indicando que el mar Mediterráneo habría supuesto una barrera mucho más permeable al flujo de genes que el desierto del Sáhara. En clara coherencia con lo expuesto, la marcada incidencia del marcador europeo *-13910*T* entre las poblaciones nativas del Magreb, sobre todo, en aquellas localizadas en el extremo geográfico más occidental, sería un claro testimonio de esos contactos. Los continuos y recurrentes movimientos poblacionales, que han tenido lugar entre ambos lados del Mediterráneo occidental, jalonado por el Estrecho de Gibraltar (~12 km), se habrían producido con diferentes profundidades temporales en la historia evolutiva humana reciente. Por ejemplo, se han puesto de manifiesto numerosos paralelismos entre las industrias Epipaleolíticas del sur de la Península Ibérica (Magdaleniense) y el Norte de África (Iberomauriense) así como en los procesos de Neolitización a ambos lados del Estrecho de Gibraltar (Linstädter et al., 2012). Los numerosos acontecimientos históricos, incluyendo el período Romano y la conquista musulmana (711-1492) de la Península, habrían reforzado sensiblemente los intercambios poblacionales entre el Magreb e Iberia.

La segunda barrera geográfica identificada en nuestros análisis parece seguir una orientación paralela al trascurso del valle del Rift, una fractura geológica localizada en el este de África con una extensión ~5.000 km, en dirección norte-sur. Es justamente en las poblaciones de África oriental asentadas en zonas áridas de Etiopía, Kenia, Somalia, caracterizadas por sus prácticas ancestrales pastoriles, donde se concentran las frecuencias más altas del rasgo LP a través de sus variantes *-14010*C* y*-13907*G*. Otra de

las barreras genéticas detectadas en esa área geográfica parece estar sustentada por el marcador arábigo -*13915*G*, el cual se concentra casi exclusivamente en la costa del Mar Rojo y en el cuerno de África.

Estas evidencias y otras observadas, aunque con un impacto no tan evidente, nos estarían indicando que la presencia del rasgo LP con frecuencias >5% en poblaciones especificas africanas, podría explicarse a través los efectos de una selección natural positiva, proceso evolutivo que permitiría entender la detección en el Este de África de variantes responsables de la persistencia de la lactasa: *-14010*C*, *-13907**G, las cuales podrían haber emergido *in situ* en el continente.

Asimismo, desde otros resultados que emergen del presente estudio ellos podemos inferir que los patrones de variación espacial del fenotipo LP y de sus marcadores moleculares asociados estarían muy influenciados por la geografía de las poblaciones, por los movimientos migratorios internos y por hábitos culturales inherentes a poblaciones antropológicas específicas. Algunos de estos factores, pueden ser razones causales de escenarios de variaciones geográficas clinales. El modelo de migración que mejor parece explicar esos gradientes es el del "*modelo de aislamiento por distancia",* el cual nos señala que un cambio lento y gradual de las frecuencias alélicas puede ser una consecuencia de la distancia geográfica entre poblaciones (Handley et al., 2007).

El hecho de que un mismo fenotipo pueda ser debido a diferentes causas genéticas, como es el caso del LP, constituye un caso paralelo al de la paradigmática adaptación molecular humana ejemplarizada en la enfermedad de la malaria, una enfermedad infecciosa de primer orden y endémica en el continente africano y, especialmente, en áreas y poblaciones sub-Saharianas. La selección natural, la cual ha actuado como hilo conductor de los efectos de la malaria en esos territorios y poblaciones de África y en otros del Viejo y del Nuevo Mundo, ha provocado cambios, durante generaciones, en las frecuencias alélicas de varios genes relacionados con la hemoglobina y los eritrocitos, como vías de entrada del *Plasmodium* en el organismo. Estos acontecimientos genéticos observados en poblaciones africanas, entre otras, como respuesta a una presión selectiva en combinación con una oportunidad ambiental, se puede trasladar al caso del rasgo LP como otro ejemplo de adaptación humana y de evolución convergente.

El presente estudio ha intentado aportar más evidencias sobre diversidad genética y adaptación cultural en África, subrayando la complejidad de las interacciones entre genética, geografía, cultura y ambiente en la evolución de fenotipos adaptativos humanos.

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Polygenic Risk Score for coronary artery disease and stroke in European populations

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RESUMEN

Las enfermedades cardiovasculares componen la principal causa de muerte en el mundo, particularmente en Europa, donde existen ciertas disparidades entre países en cuanto a su impacto. Además de un componente ambiental, en esta enfermedad interviene un componente genético asociado a varios genes, cada uno con un pequeño efecto. Gracias a los análisis de riesgo poligénico es posible cuantificar el riesgo genético a poseer cierta enfermedad o rasgo a partir de ciertos marcadores genéticos. En este estudio se ha comparado el riesgo genético de diferentes poblaciones europeas a contraer dos tipos de enfermedades cardiovasculares: la enfermedad de las arterias coronarias y los accidentes cerebrovasculares. Los resultados mostraron que solamente el riesgo genético para la enfermedad de las arterias coronarias mostraba una diferencia significativa entre los valores medios de las distintas poblaciones. Además, se investigó la posible correlación entre el riesgo genético de ambas enfermedades y la cantidad de variantes de origen Neandertal presentes en cada individuo. A pesar de que la correlación resultaba estadísticamente no significativa, sí hay un indicio de que haya una relación con el riesgo genético a la enfermedad de las arterias coronarias. Finalmente, tras comparar el riesgo genético con la situación de hospitalización en cada país para las enfermedades cardiovasculares se concluye que, a pesar del efecto ambiental, es posible identificar la relación entre el componente genético y la enfermedad, resultando de interés para estudios evolutivos, así como clínicos.

ABSTRACT

Cardiovascular diseases are the main cause of death worldwide and particularly in Europe, where there are some disparities across the countries regarding its impact. In addition to an environmental component, this disease has also a genetic component by which many variants in different genes, contribute with a small effect each. Thanks to Polygenic Risk Scores it is possible to quantify the genetic risk for a certain illness or trait. In this study, we compared the genetic risk of different European populations for two types of cardiovascular diseases: coronary artery disease and stroke. The results showed that populations presented differences in genetic risk only for coronary artery disease. Moreover, we also explored for both traits the possible correlation between risk scores and the number of Neanderthal variants in the individuals. Although overall we did not find found any statistically significant correlation, some clue indicates there could be a correlation with the genetic risk score for coronary artery disease. Finally, after comparing the genetic risk scores with the hospitalization situation for cardiovascular diseases in each country, we conclude that, despite the possible effect of environmental factors, it is possible to identify a genetic component for the disease, which is relevant not only for evolutionary studies but also for clinical purposes.

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Introduction

Recently, the World Health Organization (2020) estimated that the top 2 leading causes of death were a) ischaemic heart disease (IHD), also known as coronary artery disease (CAD), and b) stroke. CAD consists in the formation of an atherosclerotic plaque in the vessel lumen which hinders blood and oxygen flow to the myocardium (Shahjehan and Bhutta, 2023). Meanwhile, stroke happens when brain cells die due to a problem with blood and oxygen supply (Centers for Disease Control and Prevention, 2023). These diseases have caused 8.9 million and over 6 million deaths respectively in 2019, representing together 27% of world's total deaths, and the number of deaths for both diseases has kept increasing since the year 2000 (World Health Organization, 2020).

According to Townsend et al. (2021), CVD has remained the most common cause of death in Europe since the early 1980s. Previous studies have reported that CVD kills nearly 4 million people in Europe every year (44% of all deaths), with IHD accounting for 44% of these CVD deaths, and stroke accounting for an additional 25%. Disparities, however, are found across the continent, with large differences in current age-standardized and crude death rates for CVD among countries (Townsend et al., 2021).

Despite advances in risk prediction, most people's first sign of CVD risk is already a late event such as a stroke or myocardial infarction. Thus, when identifying biomarkers and risk factors that might add information to clinical risk factors, genetics seems to be a good path (Abraham, Rutten-Jacobs and Inouye, 2021). As Hüls and Czamara (2019) explained, since most complex diseases are influenced by several genes, each having only a small effect on its own, polygenic approaches, due to their genome-wide nature, often explain a greater proportion of the variance in complex traits than is possible with single variant approaches. The most popular polygenic approach is weighted polygenic risk scores (PRS) or genetic risk scores (GRS) (Hüls and Czamara, 2019). They integrate information from many common single nucleotide polymorphisms (SNPs) and provide a quantitative metric of inherited risk (Patel and Khera, 2023). This is done by weighted sums of risk alleles of many SNPs. Particularly for complex diseases, PRS are statistically powerful to test for marginal genetic effects and gene–environment interaction effects, as well as to predict individual trait values or risks of disease (Hüls and Czamara, 2019). Both CAD and stroke possess a multifactorial genetic basis, with independent genetic variants conferring small differences to the risk of both diseases (Chauhan and Debette, 2016; Patel and Khera, 2023).

As Abraham, Rutten-Jacobs and Inouye (2021) say, CVD PRSs seem to perform as well as or better than individual traditional risk factors and even being independent of clinical risk scores. This happens since genome-wide summary statistics (GWASs) have uncovered many genetic associations which implicate the role of novel biological pathways unexplained by traditional risk factors. Therefore, PRS that are based on GWAS tend to capture a broad range of genetic effects in a way that they are not so biased regarding to the biological pathways (Abraham, Rutten-Jacobs and Inouye, 2021).

What's more, in populations of European ancestry, a genome-wide association study of Neanderthal-introgressed alleles showed a wide range of associations with pathological outcomes, such as myocardial infarction and coronary atherosclerosis, among others (Simonti et al., 2016; Koller et al., 2022). Thus, a possible link between CVD and the Neanderthal introgression component is worth investigating.

Consequently, the aim of this study is to analyse whether different European populations show different PRS to CAD and stroke, as well as to study if the differential amount of introgressed Neanderthal DNA could represent an additional genetic risk. We hypothesize that the if CAD and stroke have a genetic basis, European populations in 1000 Genome Project (Auton et al., 2015) may show different genetic risk to these diseases. Based on the observations described above, we also hypothesize that the possible differential Neanderthal component could also be playing a role in this risk. We hope that elucidating this could have both an evolutionary and a clinical value

Methodology

To obtain PRS values for CAD and stroke, we first obtained from the NHGRI-EBI GWAS Catalog

(Sollis et al., 2022) a series of SNPs significantly associated to these diseases (p-value $<10^{-5}$). The search was done in December 2022, finding 3247 and 608 associations for CAD and stroke respectively. Then, those associations were filtered by the ancestry of the studied individuals, selecting those which had individuals with European ancestry, whether entirely or partially. Cases in which the articles did not provide ancestry data were also included, unless any clue could indicate no European ancestry individuals were present. Next, associated polymorphisms which were not SNPs (i.e., indels or repetitions) or SNPs for which the risk allele was unknown, were excluded. Moreover, repeated SNPs, indels or variants on the sexual chromosomes or mtDNA were also excluded, as well as those SNPs which had more than two alleles. Summary statistics of these SNPs were downloaded from the same NHGRI-EBI GWAS Catalog (Sollis et al., 2022) in December 2022 for trait EFO_0000712 and trait EFO_0001645.

Later, to perform a quality control (QC) of the data, we proceeded as described by Choi, Mak and O'Reilly (2020). In the absence of MAF value in GWAS, the frequency for European population was obtained from dbSNP (https://www.ncbi.nlm.nih.gov/snp/). Any SNP not found in the original article or found out to be an error was also discarded.

Genotypic population data was obtained from the 1000 Genomes Project (Auton et al., 2015), for the following European populations: Northern Europeans from Utah (CEU), Finnish in Finland (FIN), British from England and Scotland (GBR), Iberian population in Spain (IBS) and Tuscans from Italy (TSI), and a standard QC was applied (Choi, Mak and O'Reilly, 2020). Genomes were Downloaded from 1000 Genomes FTP on 08-06-2016. Since these populations were assembled as GRCh37/hg19 while SNPs were in GRCh38/hg38, a transformation was performed for SNPs using the webpage https://genome.ucsc.edu/cgi-bin/hgLiftOver. PRS calculation was accomplished by means of PLINK v1.90 b7 64-bit (Purcell et al., 2007). A clumping process was applied, requiring that the associations had a linkage disequilibrium (r2) lower than 0.1; 250 kb of distance and without restricting any SNP according to its p-value (threshold=1) (Choi, Mak and O'Reilly, 2020).

To calculate the Neanderthal introgression into these European populations, a total of 137853 SNPs identified previously as of Neanderthal origin were extracted (Vernot and Ackey, 2014; Gunz et al., 2019). Of these, those SNPs that were also present in the Yoruba in Ibadan (YRI) population were identified and removed. Finally, an estimate of Neanderthal ancestry was assessed by scoring 0, 1 or 2 if each locus was homozygous for the ancestral allele, heterozygote or homozygous for the derived, Neanderthal allele, respectively.

Statistical analyses were done by using R-Studio 4.1.1 (RStudio Team, 2020) and R commander 2.9-2 (Fox, 2005, 2017; R Core Team, 2023; Fox and Bouchet-Valat, 2024). First, a Shapiro-Wilk test was performed to test the normality of the data. Then, an ANOVA and Kruskal-Wallis test were performed to study the differences among the groups. Finally, the Pearson correlation coefficient between the estimated Neanderthal ancestry component and PRS was calculated. Further, QC summary statistics SNPs were filtered by using a Linkage Disequilibrium and MAF threshold of 0.2 and 0.1, respectively, to perform a PCA.

Finally, after analysing the differences of PRS results for both diseases in every population, we proceeded to compare the actual cardiovascular disease incidences as well as some health indicators. They were obtained from Eurostat database (https://ec.europa.eu/eurostat/data/database), selecting for the filtering all ages as well as both sexes from 2014, as all populations were present in the same study. The searched items were: 1) "Hospital discharges by diagnosis, in-patients, per 100 000 inhabitants", using "Diseases of the circulatory system (I00-I99)"; 2) "Causes of death - standardised death rate by NUTS 2 region of residence", for "Diseases of the circulatory system (I00-I99)"; 3) "Population structure indicators at national level", using "Median age of population"; 4) "Body mass index (BMI) by sex, age and educational attainment level", marking "obese" and "All ISCED 2011 levels"; 5) "Daily smokers of cigarettes by sex, age and educational attainment level", using "total" and "All ISCED 2011 levels"; 6) "Frequency of heavy episodic drinking by sex, age and educational attainment level", using "never or not in the last 12 months"; 7) "Frequency of fruit and vegetables

consumption by sex, age and educational attainment level", using "All ISCED 2011 levels and At least once a day" for both fruits and vegetables.

The databases World of Science (WOS) and PubMed were used for bibliographical research. The following keywords were used: coronary artery disease, cardiovascular disease, stroke, polygenic risk score or genetic risk score. These key words were integrated in the search bar by using "AND". Preferentially, we considered reviews from the last ten years. Moreover, we focused on journals with an impact index higher than 3 or belonging to the first 2 quartiles. In addition, articles cited in the bibliography of those references were also considered. Other sources such as Eurostat (https://ec.europa.eu/eurostat) and the Centers for Disease Control and Prevention (https://www.cdc.gov/index.htm) were also used.

Results and discussion

The list of SNPs after performing the QC along with their characteristics are available at Tables S1 and S2 (see Supplementary data). After filtering, the number of SNPs used for PRS calculation was 429-437 for CAD and 101-103 for Stroke (Table S3, see Supplementary data), as not all populations had information for all SNPs.

The two highest CAD PRS average scores correspond to CEU and TSI, while GBR and FIN had the two lowest values (Figure 1A).

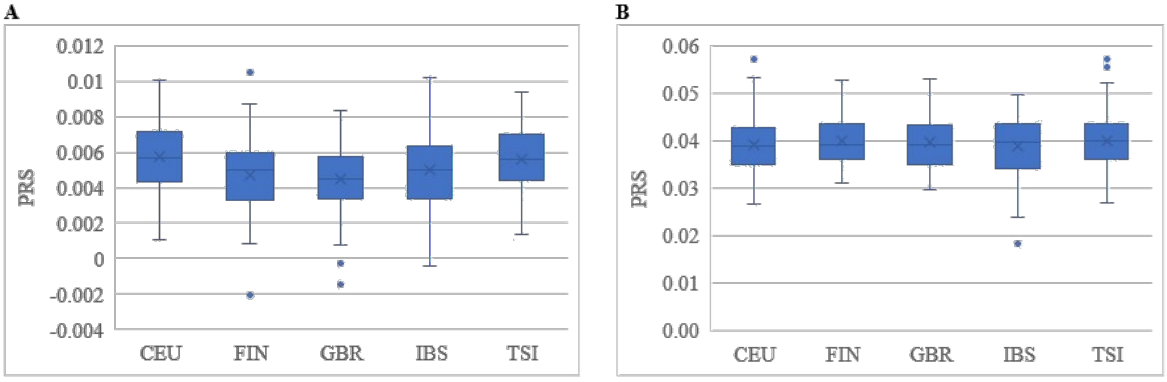

Figure 1. Box plot of PRS values (Y axis) for each population (across the X axis) for CAD (A) and stroke (B). Note that the scale for Y axis of both graphics is not the same. Mean values, marked as an x in the graphic, are 0.00578, 0.00472, 0.00451, 0.00498 and 0.00561 for CEU, FIN, GBR, IBS and TSI, respectively, for CAD. For stroke PRS, they are 0.0393, 0.0399, 0.0397, 0.0289 and 0.04, respectively. Standard deviations for CAD PRS are 0.00203, 0.00198, 0.00179, 0.00214 and 0.00178 for CEU, FIN, GBR, IBS and TSI, respectively. For stroke PRS standard deviations are 0.00548, 0.00486. 0.00577, 0.00603 and 0.00599, respectively.

According to the ANOVA (Table 1), these two groups (CEU-TSI vs GBR-FIN) were significantly different pairwise. Meanwhile, IBS with an intermediate risk score only showed significant difference with CEU. For stroke, the differences among populations were not significant for both ANOVA (Figure 1B, Table 1) and Kruskal-Wallis test (p score=0.8508). PRS scores for each individual are available at Table S4 (see Supplementary data). Differences in CAD and stroke PRS values may be due to difference in overall Odds Ratio of SNPs as well as different frequencies of risk alleles in populations.

We compared these results with actual data found in Eurostat (n.d.) (Table 2). The selected countries are thought to be a good proxy for 1000 Genome populations analysed: FIN for Finland, GBR for the UK, IBS for Spain, TSI, although being Tuscans, for Italy, and CEU for Germany, the Netherlands, Sweden, Norway and Denmark, as all these countries conform the Northern European region.

Table 1. P-values of ANOVA pairwise for difference among the European populations for CAD and stroke PRS. Values lower than 0.005 (Bonferroni correction for multiple tests for a nominal alfa 0,05 and 10 tests) are marked with 3 asterisks (* p-value <0.05; ** p-value <0.01; *** p-value < 0.005).

		FIN	GBR	IBS	TSI
CAD	**CEU**	0.00133***	< 0.001***	0.02828*	0.97213
	FIN		0.94603	0.86981	0.00957**
	GBR			0.43283	< 0.001***
	IBS				0.12612
Stroke	**CEU**	0.913	0.98	0.993	0.877
	FIN		0.999	0.697	1
	GBR			0.863	0.997
	IBS				0.630

Table 2. PRS values and actual data about the situation in 2014 for European countries that better represent the analysed populations Eurostat (n.d.). *Value for Toscana. **Data from 2019, since there was no information in 2014.

Country	Denmark	Finland	Germany	Italy	Netherlands	Norway	Spain	Sweden	The UK
Average CAD PRS	0.005783	0.004719	0.005783	0.005614	0.005783	0.005783	0.004982	0.005783	0.004507
Average Stroke PRS	0.039253	0.039942	0.039253	0.0400013	0.039253	0.039253	0.038922	0.039253	0.039716
Hospital discharges per 100,000	1629.3	2647.8	3772.5	1904.8	1385.2	2013.3	1307.1	2052.8	1206.6
Deaths per 100,000	256.73	378.86	403.67	310.15 281.25*	271.86	272.74	244.99	338.37	265.26
Mean age (years)	41.3	45.6	42.4	44.7	42	39	41.8	40.9	39.9
Obesity rate (%)	14.4	17.8	16.4	10.5	12.9	12.6	16.2	13.4	19.8
Smokers rate (%)	12.3	11.6	15	17.4	17.2	12.5	22.2	8.7	13.7
No heavy episodic drinking (%)	27.5	37.3	39.9	83.6	75.9**	56	77.5	53.5	54.4
Eating at least 1 fruit per day (%)	53	44	47.3	70.9	41	54.1	66.7	46.8	62.8
Eating at least 1 vegetable per day (%)	44.1	45.4	34.1	61.9	31.3	55.7	44.6	52.1	65.5

Although BMI is suggested not to be a perfect predictor of body composition, as occasionally two people with similar BMI could have different fat concentrations, it is often the best available measure (Rychter et al., 2020). By looking at Table 2, we can appreciate an overall tendency that as the obesity rate increases, so do the death and hospital discharges rates. However, some discrepancies can be seen, such as the fact that Germany despite having lower obesity rates compared to Finland, still has higher values of death and hospitalizations. This phenomenon could be explained by the lower CAD PRS value that FIN population has, indicating that genetics would be a factor compensating these values. Another anomaly would be the case for TSI, which despite having the lowest obesity rate as well as having a better eating habit and lower alcoholism, still has average heath results. This could be also explained by their high CAD PRS, along with their higher average population age. The contrary happens with GBR population, which in

spite of having the highest obesity rate, has a pretty low death and hospital discharges rates. Something similar can be appreciated in the case of Spanish people, that have low health issues regarding cardiovascular diseases although having higher obesity and smoking rates. In this case, genetics would not be explaining much of that improvement since PRS values were average. Instead, it could be attributed to healthier eating behaviour as well as lower alcoholism.

However, there are the cases of Sweden and Norway which by having similar obesity rates, lower smoking, lower average age, and better eating habits than the Netherlands, still have worse health results. The higher alcoholism could be contributing to this result, but there might be more risk factors which have not been gathered in Table 2. Moreover, there could be differences in the genetic risk score for all Norther European countries that have not been considered since CEU population is a mixture of all. Another limitation is that the comparison is being made mixing CVD incidences and CAD PRS values, therefore information about other CVD PRS is being overlooked.

Regarding the Neanderthal Component Value (NCV), when it was correlated with each disease for all European individuals and individuals for each population, in none of them there was a significant correlation (Table 3). However, in the case of CAD when using all individuals the effect of NCV on PRS value the p score reduced considerably up to 0.0562.

Table 3. Pearson correlation coefficient (r) and p value for relationship between NCV and PRS values for CAD and stroke.

	CEU	FIN	GBR	IBS	TSI	Total
			CAD			
r	-0.0274	-0.0266	0.0004	-0.0273	-0.1255	-0.0852
p value	0.7878	0.7942	0.9971	0.78	0.1976	0.0562
			Stroke			
r	-0.1184	-0.0313	-0.0444	0.0207	0.0301	-0.0096
p value	0.2432	0.7598	0.6776	0.8325	0.7583	0.8307

This low p value (scarcely higher than 0.05) could mean that the NCV might have a significant effect on CAD PRS, but more individuals would be required in order to prove this hypothesis. When observing each population, TSI showed lower p score for CAD PRS than others. We speculate that this might be due to the following idea described by Anagnostou et al. (2022): Italy is a highly diverse population, with a great genetic distance between northern and southern groups, and in this line, the risk of developing CAD also varies among Northern and Southern Italy. On the one hand, in Northern Italy the cold temperatures could have enhanced natural selection on genes involved in adipogenesis (*WDPCP*), HDL concentration (*RNMTL1P2*), cholesterol and triglyceride levels (*RAB3GAP1* and *R3HDM1*), as well as the sensibility of cells to insulin (*TMEM163*), for which Neanderthal derived adaptative alleles have been associated with lower risk of having CAD. On the other hand, in Central and Southern Italy, to where TSI population would belong to, the high presence of ancestral alleles for some of these loci might have increased susceptibility to CAD (Anagnostou et al., 2022).

In this sense, according to Khrameeva et al. (2014) contemporary Europeans have an excess of Neanderthal-like sites in genes involved in lipid catabolic processes. Neanderthals might have acquired changes in the lipid catabolism processes which would have resulted beneficial, possibly changing involved metabolites concentrations along with the expression of enzymes, and therefore have spread into European ancestors. In this regard, some diseases, among them CAD, appear to show an enrichment in genes involved in lipid catabolism which contain an excess of Neanderthal-like sites. However, alternative hypotheses suggest that this situation might have not been produced because of Neanderthal introgression, but rather it could be due to the presence of those genes in the ancestors of Neanderthals and out-of-Africa humans and then the frequencies had increased in both

groups because of the prehistoric European conditions (Khrameeva et al., 2014). Although p score was not statistically significant, as previously said, this could be due to a being a small sample. Nonetheless, limitations intrinsic to the method to obtain the NCV must be considered, as only ascertained SNPs were used for its estimation.

Regarding the risk for stroke, it was suggested that some Neanderthal genes were likely to increase risk for stroke due to a blood coagulation disorder (Gibbons, 2016; Groß, 2019). However, in this study that association has not been shown, as the p value suggests that there is no correlation between stroke PRS and NCV. By contrast, CEU population showed a much lower p value than other populations, but still has a high p value.

When performing a PCA with the all the risk SNPs, it seems that the main variance is explained by a North-South geographical tendency (Figure 2). Finns are shown at the left end of axis PCA1, followed by the north-western European populations (GBR and CEU), and finally southern European populations (IBS and TSI) at the right end of axis PCA1. Meanwhile, on the second axis intra-population variance is mostly appreciated. The ellipses manifest a great overlap between GBR and CEU, and another one between IBS and TSI for the stroke and CAD informative SNPs (Figure 2).

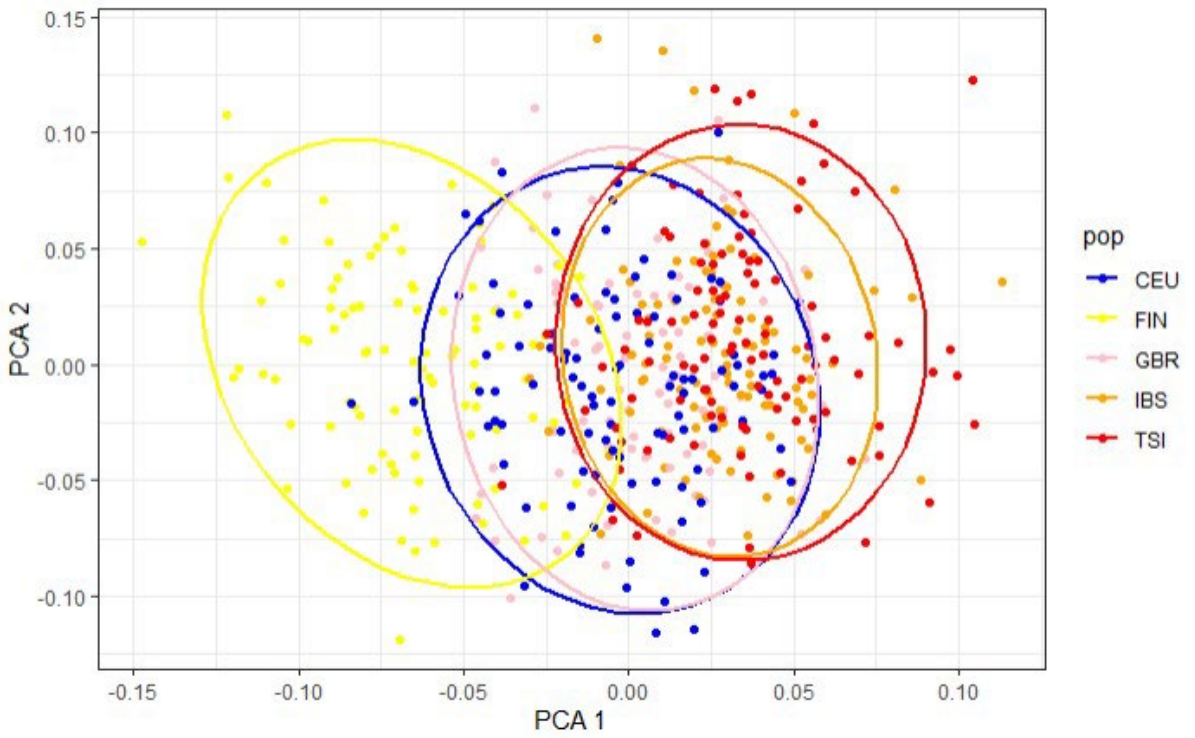

Figure 2. PCA constructed with CEU, FIN, GBR, IBS, TSI populations along with Russian population.

Finally, this study has several limitations. First, it has not been checked whether the chosen association p-value threshold ($p<10^{-5}$), standard in GWAS Catalog (Sollis et al., 2022), is the best for disease prediction by PRS. This could have been tested if a full GWAS study with the genotypes of control and cases as well as their phenotype would have been available. Second, only samples of 90-107 individuals were used, which could be limiting our power to detect relationships. Third, the fraction of the genetic variance not explained yet may still be substantial, so new informative SNPs might be discovered in the future that will allow a more precise inference of the underlying genetic risk. Finally, and most importantly, the poor transferability of PRS among people of different ancestries due to differences in linkage disequilibrium patterns, allele frequencies, heritability, and genetic architecture (Patel and Khera, 2023) makes it difficult to generalize the genetic risk of

certain disease obtained from GWAS data in one population to another population. However, there is consensus that PRS can be applicable within a continent, and consequently the results obtained in this work could be assumed to represent a reasonable inference.

Overall, we conclude that our first hypothesis can only be accepted for CAD, as stroke PRS values showed no difference among populations. Regarding the effect of Neanderthal component (second hypothesis), it cannot be completely discarded that there is a Neanderthal component involved in CAD genetic risk. Finally, research on this topic might be useful not only for evolutionary purposes, but also clinical ones, as this could indicate genetic risk of different populations and therefore, speed up preventive actions.

Supplementary data

https://docs.google.com/spreadsheets/d/1_AI-9POqbtLzwu7QiUlahY6KjAH63fx7FMHpLK1zDNY/edit?usp=sharing

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