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SUMARIO . SUMMARY . RESUMO

Editorial

From the Editorial Board of the journal MLS Health and Nutritional Research in the transfer of scientific knowledge in the field of health, nutrition, and food. We would like to thank the authors who have placed their trust in the journal since their contributions have allowed us to consolidate the journal and continue with its activity in our third year of life.

The first article deals with the; Valorization of food waste from tomato processin (SOLANUM LYCOPERSICUM); The consumption of Solanum Lycopersicum, commonly known as tomato, has increased in recent years. Therefore, the production and consequently the waste generated from it, too. The present work aims to determine whether there are significant differences between the different methods of valuation in the utilization of tomato food waste, as well as their practical application.

The following article discusses the; The role of chrononutrition in weight loss; Circadian clocks are closely related to nutrition. The number of meals, the timing of meals, and other parameters appear to influence a person weight and metabolism. The aim of this study is to gather scientific evidence that circadian rhythms influence weight loss.

From the field of food technology; Physical and nutritional aptitudes of eight type of potato" In this experimental study, measurements and evaluations of different parameters were carried out on eight types of potatoes, such as vitamin C content using the Indophenol method, dry matter by ash determination, calibers using ring ranges to determine their size, and they were classified between medium and large. In addition, the external appearance and coloration of the flesh was visually evaluated, differentiating between rounder or more oval shapes and white or yellowish colors. A tactile analysis was also performed to determine if the samples had a firm and consistent texture

The following article; The scientific overview of the status of biosimilars worldwide and their conception of biosimilarity versus their quality attributes; The attempt to bring biological treatments closer to the public, at a high cost, has driven the birth and growth of biosimilar drugs. Molecules whose production is focused on being copies of the active ingredients of drugs of biological origin classified as innovative. As they are biological molecules, the fact that they are copies of the active ingredient becomes complex, as small variations in their biochemical composition can affect their safety and efficacy.

Finally; Akkermansia muciniphila, a bacterium against obesity and its relationship with diet. Systematic review. The anaerobic bacterium Akkermansia muciniphila has demonstrated its role in regulating metabolism and markers of inflammation since its discovery. It is a Gram-negative bacterium classified within the phylum Verrucomicrobiae. It is recognized as a non-pathogenic bacterium, devoid of virulence factors and lacking significant interaction with the host leading to infection or disease.

Editor in Chief

Dr. Iñaki Elío Pascual

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VALORIZATION OF FOOD WASTE FROM TOMATO PROCESSING (SOLANUM LYCOPERSICUM)

VALORACIÓN EN EL APROVECHAMIENTO DEL RESIDUO ALIMENTARIO DEL PROCESADO DEL TOMATE (SOLANUM LYCOPERSICUM)

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ABSTRACT

The consumption of Solanum Lycopersicum, commonly known as tomato, has increased in recent years. As a result, the production and consequently the waste generated from it, as well. The present work aims to determine whether there are significant differences between the different methods of valuation in the use of tomato food waste, as well as its practical application. In order to find out, a bibliographic review was carried out through which a total of 52 articles published in the last five years were selected. Of the total, twelve focused on the extraction of carotenoids, ten on the extraction of phenolic compounds, four on pectin and seven on the formation of biogas, as methods for the valorization of the raw material. Although the food valorization of tomato is wide and little studied yet, it was observed that the extraction of phenolic compounds, pectin or lycopene represent a wide range of new possibilities with the use of emerging pretreatment methods such as high pressures, electric pulses or supercritical CO2. The conclusion is that even though more studies are needed, emerging methods are more effective for the extraction of active compounds from tomato.

Keywords:
Tomato valorization. literature review, bioactive compounds, food waste, food industry.

RESUMEN

El consumo de *Solanum Lycopersicum*, conocido comúnmente como tomate, se ha visto incrementado en los últimos años. Por ello, la producción y en consecuencia el desperdicio generado a partir de él, también. El presente trabajo pretende propiciar si existen diferencias significativas entre los distintos métodos de valoración en el aprovechamiento del residuo alimentario del tomate, así como su

Palabras clave:
Valorización del tomate. revisión bibliográfica, compuestos bioactivos, desperdicio alimentario, industria alimentaria.

aplicación práctica. Para poder conocerlo, se realizó una revisión bibliográfica a través de la cual se seleccionaron un total de 52 artículos publicados en los últimos cinco años. Del total, doce se centraron en la extracción de carotenoides, diez en la extracción de compuestos fenólicos, cuatro en la pectina y siete en la formación de biogás, como métodos de valorización de la materia prima. Aunque la valorización alimentaria del tomate es amplia y poco estudiada aún, se pudo observar que la extracción de compuestos fenólicos, pectina o licopeno suponen un gran abanico de nuevas posibilidades con el uso de métodos de pretratamiento emergentes como las altas presiones, los pulsos eléctricos o el CO2 supercrítico. Llegando a la conclusión de que aun observando la necesidad de mayor número de estudios, los métodos emergentes son más eficaces para la extracción de los compuestos activos del tomate.

Introduction

The consumption of fruits, vegetables and greens throughout history has always been high. During the last few years, due to various situations such as the search for health improvement, some vegetables, for example, the case of tomatoes, have seen an increase in demand. This increase in demand in turn implies a need to increase production, resulting in an increase in the waste generated as a result of its processing (1-4).

Due to this increase in food waste and other related issues, the European Union has approved various proposals such as the green pact or the 2030 agenda, some of which aim to promote the recovery of food by-products. (1,5,6).

Objectives

The general objective is to determine whether there are significant differences between the different methods of valuation in the utilization of tomato food waste, as well as their practical application. Therefore, the following specific objectives are proposed:

- Promote new emerging methods of recovery in the use of tomato waste.
- To establish whether there are significant differences between the different methods of by-product utilization.
- To demonstrate the different methodological characteristics for the use of tomato products.

The tomato industry

According to the FAO, in 2019, the amount of food waste worldwide of fruits and vegetables was 1300 million tons, being therefore, one of the sectors that generated the most waste, with up to fifty percent of waste during the periods between harvesting and food production (1,7,8). Consequently, in the 21st century there are important challenges linked to food safety and the need to reduce food waste at the global level, promoted by various organizations, including national governments and the FAO (1,4,6,12).

Globally, the growth of the Solanum lycopersicum industry has steadily increased in recent years, resulting in the production of a considerable amount of waste derived from the increase. The global increase in the production of said raw material between 2019 and 2020 was 2.4%, with a total of 38,282 million kilograms of tomato at industrial level. Food waste from this vegetable is large and has a negative environmental impact due to the processes involved in handling, conditioning and processing (5,7,9-12).

The reduction of materials derived from treatment and their use as new raw materials to obtain new value-added products is a change that must take place in order to obtain a circular economy and that such a level of global waste is not generated, assuming the valorization of most of the products. In addition, the demand for bioactive compounds among the population for their health benefits continues to grow every year, and they can be extracted from foods such as tomatoes. Among the main consumer demands are antioxidants, phenolic compounds and phytochemicals (1,4,6,8,10,12).

Tomato by-products

Tomato by-products can be classified according to the process in which the surplus is created or according to the industrial use of the by-product, so there are two main classifications: by-products according to their origin and by-products ac20cording to their industrial use (13-15)

Depending on the origin, three categories are distinguished: industrial processing by-products, harvest or post-harvest by-products and tomato crop by-products. As the name itself refers, they are related to the production process in which the tomato is found. It should be noted that the last group includes tomato crop by-products, which include tomato pruning residues, i.e. the vegetable matter eliminated throughout the life of the plant for proper development and the tomato plants at the end of their life cycle. When plants are no longer productive and are removed from the field, the by-products have different applications including animal feed, the creation of bioplastics, fertilization or energy generation (4,13,16,16,17).

According to the industrial purpose for which the product is intended, tomatoes are classified into five groups: human food, animal food, bioplastics formation, energy generation and soil improvement (13-15).

It is worth mentioning that the food industry is currently focused on the creation of functional ingredients or nutritional supplements for humans. Functional ingredients are used to enrich foods and beverages. The main compounds used are lycopene, dietary fiber and antioxidants. All these compounds can be extracted from the plant, from areas such as the husk and seeds, which are rich in bioactive compounds (3,13,18-21). In addition, among the most commonly used for valorization, others such as FC, pectin and the formation of bioethanol or biogas for use in the generation of electricity (2,4,7,12,12,22-24) are also noteworthy.

Valorization of carotenoids

Carotenoids are isoprenoid pigments that are synthesized in all organisms that photosynthesize. Carotenoid compounds are essential for the photosynthetic apparatus and perform a powerful role as antioxidants and light-harvesting pigments. (12,20,25,26).

Lycopene is a symmetrical triterpene molecule, composed of eight isoprene units. It is an important intermediate for the synthesis of important carotenoids. Among those with a higher amount of lycopene and being the main source of natural lycopene are tomato, watermelon, gac, Southeast Asian fruit, and grapefruit (8,19,25,27,28).

In the extraction methods, there are the traditional and the modern or sustainable methods of extraction. Traditional lycopene extraction has been extensively studied, in this method organic solvents are used. Studies such as that of Almeida et al. (12) use hexane and ethanol for the extraction of carotenoid compounds, others, such as the study by Górecka et al. (21) make use of some others such as methanol, acetonitrile or dichloromethane. These types of compounds are considered analytical grade solvents with which significant information on the extraction of compounds can be obtained. According to some studies (8,10,28), the use of traditional methodology is not optimal because of its low performance and efficiency in the process, requiring large amounts of solvent and time, this was done with organic solvents so that it could pass through the membranes. Therefore, alternatives have been sought that are less harmful to the environment and more efficient in the process of obtaining the compound (12,21,29).

Among the non-conventional methods, part of the literature reviewed (8,26,27,30) mentions electric pulses (PEF) as a pretreatment method for the extraction of lycopene and β-carotene, together with the use of solvents that have a low environmental impact, which translates into beneficial effects on membrane permeabilization and thus the uptake and recovery of the target compounds. This type of technique is mainly used on the peel of tomatoes, although it is not the only part where it is performed. The study by Coelho et al. also cites the use of ohmic technology for the extraction of bioactive

compounds. The use of this technology focused on heat production was used as a pretreatment prior to the achievement of the component (8).

On the other hand, methods such as high-pressure homogenization (HPH) (31), water-induced hydrocolloid complexation (WIHC) (25), ultrasound-assisted extraction (UAE), microwave extraction (MAE) and pressurized liquid extraction (PLE) (32) are cited.

Valorization of tomato pectin

Pectin is a branched heteropolysaccharide of galactose found forming the walls of plants. It is a compound with high food availability and low economic production requirements. In 2019, it was one of the most widely used biopolymers worldwide. Its main current uses are as a gelling agent, thickener and stabilizer in beverages and foods due to its physicochemical properties that make it capable of forming hydrogels. In tomatoes, it is found in the skin in higher concentrations and in the interior of the tomato in smaller amounts (20,22,33-35).

According to the most relevant studies of recent years, the extractive methods of this compound are, as in the case of carotenoids, of two types: traditional or non-conventional. Within the first group, there is the use of solvents as is the case in the article by Ninčević et al. (34) in which hydrochloric acid, sodium chloride or sulfuric acid are used to obtain pectin. Hydrolysis and subsequent extraction take place (20,22,33,34).

Among the non-conventional methods, some researchers (22,25,36) seek methods with which the use of solvents is less or even non-existent. On the one hand, there is the study in which five types of ultrasound are used: UAE, MAE, ohmic heating assisted extraction (OHAE), ultrasonic microwave assisted extraction (UAME) and ultrasonic ohmic heating assisted extraction (UAOHE). With all of them, the aim is to know which is the most efficient within the extractive techniques of the element in order to achieve, in addition, optimization and efficiency in the process (36). On the other hand, research such as that carried out by Pirozzi et al. (22) in which use is made of HPH and mild solvents in order to obtain an improvement in the obtaining yield (22). Finally, the study by Nagarajan et al.(25), mentioned in the previous section, where by means of WIHC, extraction was sought with the formation of a complex between carotenoids and pectin (22,25,36).

Valorization of phenolic compounds and antioxidants

FCs are one of the most common groups of secondary metabolites within the dietary phytochemicals belonging to plant tissue of plants. The concentrations of these compounds in the tomato plant are usually less than one percent of the dry weight of the plant, which is a very low percentage compared to other compounds. Phenolic compounds are considered essential because of the benefits to both the plant and humans. FC are part of the plant's defense against extreme temperatures or radiation, and in humans they have antioxidant properties that contribute to delaying the development of degenerative diseases (25,37,38).

Traditional methods are still used in the food by-products industry (12,39), seeking, as in the study by Almeida et al., the valorization of the compounds generated in the extraction as a possible source of biogas.

In emerging methods, the most commonly used recovery methods are those of the peel, seeds and all tomato waste without discrimination. For their recovery, methodologies such as high-voltage cold plasma (HVACP), freeze-drying, subcritical and

supercritical CO2 and some others already mentioned above, such as MAE, PEF, UAE, ohmic technology and HPH, are used. Certain protocols such as HPH, UAE or MAE continue to use solvents for extraction after this pretreatment, although milder (8,27,29,31,34,37-41).

Valorization for the generation of biofuels

The generation of biofuels and biogas is a pending task in the European Union, being a concept of almost zero waste reuse. According to the European Commission, the biorefinery is one of the most attractive proposals for waste recovery, promoting sustainable economic growth (42,43).

In addition, the amount of waste that can be used as raw materials is high. Initially, this food waste was deposited in landfills where it decomposed into liquid compounds and methane. The latter is the main compound that can be used for gas production, which is why several studies have focused on the potential of tomato as a biofuel generator (12,44-46). To generate it, anaerobic digestion (AD) of the food is necessary. On the use of the raw material, different results have been obtained due to the need to establish strategies in which the digestibility of the biomass is facilitated and the surface area of action of the microorganisms is increased. Pretreatments include thermal, physical, chemical or biological (4,12,43,47).

In general, the production of biofuels through the use of extractions and AD represents an effective and feasible solution to maximize the yield of high value-added compounds such as tomatoes, in addition to helping to reduce the environmental footprint (48,49).

Method

This article consists of a narrative review of scientific articles to determine the valorization in the use of tomato food waste from cultivation to consumption.

In order to carry it out, we proceeded to search for scientific articles on the area to be treated, in this case, the valorization in the use of Solanum lycopersicum. For the development of the state of the art, the review was started on January 09, 2023 and finalized on March 29, 2023, both publications and books of interest were consulted, as well as relevant international organizations in reference to product valorization and circular economy (1,6,9).

Likewise, it was taken into account that the items had an age, except in specific cases, of 5 years, i.e. the range used was from 2018 to 2023. Exceptions regarding their age were due to historical significance. The languages of the articles included in the review are English and Spanish. And the impact factor required in the studies was a minimum of one out of five. On the other hand, among the exclusion criteria are those that do not contribute to understanding the objective of tomato food recovery, invalid studies due to poor statistical analysis, erroneous execution design, and those prior to 2018 that lack historical relevance to be included (6,9,10,14).

The databases used in the search for articles, documents and studies are listed below, in order from most to least important in terms of their use.

1. Pubmed: As keywords used for the article search, the following terms were used in English:

- Tomato valorization "Tomato valorization". A total of 78 related documents were acquired from this search. For the screening of the articles, the established inclusion criteria were taken into account, although it is true that since the purpose of this search was to find the origins of this part of science as well as the history in this regard, after this search, the bibliography of the articles was searched until the first author was found. Therefore, a total of 15 articles were selected that were useful for the literature review process.

- Valorization of Solanum Lycopersium "Solanum Lycopersium valorization" A total of 38 results were found with the search, of which 2 of them helped in the writing of the article, although in the previous search articles were found that dealt with the subject together.

- Tomato waste "Tomato waste". A total of 352 articles were obtained, of which 4 were used for the literature review.

2. ScienceDirect: As keywords, the following terms were used in English:

- Tomato valorization "Tomato valorization": Out of a total of 1278 articles, 17 were used.

- Tomato by-products "Tomato by-products": A total of 54 articles were found in the search, 4 of which were part of the study.

- Biogas from tomato "Biogas from tomato": Of the 1246 articles found, 3 were used.

3. Scielo: As keywords, the following term was used:

- Tomato by-products: Of the 4 results obtained, only 1 was used for the article.

4. Internet. A Google search was conducted on aspects of food waste, circular economy and food recovery projects. Documents belonging to official scientific associations in health and industry, such as FAO, were consulted. The keywords used in these searches were "circular economy" and "food recovery". Six publications were selected, one of which was used to define terms and to complete and verify information provided by articles and documents.

Finally, a total of 52 articles were used for the literature review.

Discussion and conclusions

The valorization of tomato as a food from which carotenoids, CF and pectin are extracted, in addition to its use for the formation of biogas, can be an important strategy for reducing food waste and transitioning to a circular economy (4,20).

In relation to the use of Solanum lycopersicum as a raw material from which to extract lycopene, 12 articles address the subject directly, as shown in Table 1. Among the results obtained, there is a common similarity, in all those that have used emerging techniques, they bet on the use of these as the main methodology to obtain a greater extraction of lycopene. Supercritical CO2 (11,31) is presented as an emerging method

that needs to be investigated, due to its possible benefits in relation to not needing to use solvents, as well as HPH (31), which does not require subsequent extractions with solvents that can affect the environment.

Among the articles assessing traditional extraction (12,21,28,29,40), two of them find higher amounts of lycopene in tomato skin (28,29), while the rest extract it from tomato branches or biomass pruning (12,40) and from tomato paste rather than pomace (21). As for the most efficient method of extraction, neither the percentage of purity nor the most effective solvent for the process is specified.

The difference in the results obtained between the studies is mainly centered on the method used and the part of the tomato valorized, with greater use of in situ tomato and tomato pomace for lycopene valorization (11,31).

Table 1. Valorization for lycopene extraction

Study	Target	Methodology	Valued portion	Parameters	Results
Almeida et al. 2021 (12)	Investigate the valorization of tomato production residues.	Traditional (organic solvents)	Broken tomatoes, green tomato and tomato branches	SPME and GC/SM of CF, β-carotene and lycopene	Higher carotenoid extraction was obtained in tomato branches
Górecka et al. 2020 (21)	To know the lycopene content in fresh and dried tomatoes and tomato pomace, as well as in tomato paste at different harvesting times	Traditional (organic solvents)	Green tomato, ripe tomato, tomato paste and tomato pomace	Liquid chromatography, measured in mg/100 g dm	Higher content in tomato paste vs. tomato pomace irrespective of harvest time
Popescu et al. 2022 (11)	Identification of natural sources, extraction efficiency and evaluation of antioxidant activity	Supercritical CO_2	Diced tomato, tomato pulp and tomato seeds	Tomato matrices, extraction methods, green solvents and operating parameters	The highest amount of lycopene was extracted from tomato pulp. At 450 bar, 70 ºC and 11 kg/h, 016.94 ± 23.95 mg lycopene/100 g extracted were obtained.
Lazzarini et al. 2022 (28)	To valorize tomato pomace, a by-product composed of skin and seeds, by extracting carotenoids, especially lycopene and β-carotene	Comparison of SC*, freeze-drying and SANT*, in addition to traditional methods: H-A* compared to 2 greens: EA-EL*,M-AL.*, EA-EL*,M-AL.*, EA-EL*,M-AL	Tomato skins, tomato seeds and tomato pomace	Extraction with ethyl acetate to measure lycopene and β-carotene in µg/g of dry sample,	Tomato skin has more lycopene. The most effective way of its removal is with the use of EA-EL together with SANT.

Añibarro-Ortega et al. 2020(40)	To know the phenolic composition and bioactive properties of the primary by-products of the tomato plant	Ethanol with Folin-Ciocalteu solution and sodium carbonate, 30 min at 40ºC.	Aerial biomass after the end of the crop cycle and pruning mass	HPLC-DAD-ESI/MS^n for identification of phenolic acids and flavonoids	Increased amount of carotenoids and antioxidants in biomass produced from pruning tissue
Pataro et al. 2018 (30)	to evaluate PEF in combination with steam blanching of tomato fruits in tomato processing, to provide, in addition to increased energy efficiency of the peeling process and improvements in carotenoid recovery	Blanching with PEF and acetone	Tomato skin	Measurement by spectrophotometry at 470nm, 645nm and 662nm	The application of the combined treatment increased carotenoid content and power (37.9 mg/100 g fresh weight of tomato peels).
Coelho et al. 2019 (8)	Optimizing the extraction of BC from tomato by-products by OH and PEF	Comparison between O_HAE and PEF with the aid of 70% ethanol	Tomato peel and tomato seeds	Amount of lycopene measured in µg/gFW	O_HAE as the best technique for lycopene extraction.
Jurić et al. 2019 (31)	Investigate the potential of HPH processing of tomato peel in water for the recovery of intracellular compounds and the possibility of making the best use of by-products from the tomato processing industry	Comparison between HPH, organic solvents and supercritical CO_2	Tomato skin	CF measured in mg GAE/L and lycopene by UV-Vis spectra analysis (pellet-ethyl lactate curve) and subsequent analysis of pellets for lycopene absorption (mg/g)	56.1% lycopene extracted by HPH and without the need for any organic solvent compared to traditional organic solvents and supercritical CO_2.
Pataro et al. 2020 (26)	Influence of PEF at different field strengths (E = 1-5 kV/cm) and energy inputs (WT = 5-10 kJ/kg) on the recovery yield of lycopene in acetone or ethyl lactate from industrial tomato peel waste.	PEF	Tomato skin	Extraction rate and antioxidant power by HPLC	Increased extraction of bioactive compounds from tomatoes
Nagarajan et al. 2020 (25)	To evaluate the potential of carotenoid-pectin complexation in tomato pomace containing carotenoids and pectin.	WIHC vs. traditional methods	Tomato pomace	Use of complexation and traditional methods measured in mg carotenoid fractions/100 g tomato pomace	Recovery was 9.43 mg carotenoid fractions/100 g tomato pomace with WIHC.

Szabo et al. 2019 (29)	To evaluate the CF and carotenoid content of tomato peels	Traditional (organic solvents)	Skin, seeds and general pruning waste on 10 different types of tomatoes	Amount of lycopene in mg β-carotene/100 g DW and CF in mg/100 g DW	The highest amount of lycopene found in tomato skin from a local tomato with 5.31 ± 0.12 mg/100 g.
Nunes et al. 2022 (32)	Using non-conventional MAE and PLE techniques to recover bioactive compounds from tomato pomace	Comparison between MAE, PLE and traditional methodologies	Tomato pomace	Amount of lycopene measured in µg lycopene/g extract	Extraction with MAE showed the highest lycopene content (59.66 µg lycopene/g extract recovery of 66.93% compared to a standard technique with acetone.
Kehili et al. 2019 (50)	Optimization of tomato skin extraction by maceration	Maceration in refined olive oil (AOR)	Tomato skins	Amount of lycopene measured in mg/kg on dry basis	99.3% of the initial lycopene content was extracted using a biomass/oil ratio of 2.5% (w/v), at 80ºC and 400rpm agitation for 45 minutes, obtaining 35mg lycopene/kg AOR

* SC: Heat drying; SANT: Non-thermal air drying; H-A: Hexane-acetone; EA-EL: Ethyl acetate-ethyl lactate; M-AL: Methanol-Lactic acid

On the other hand, 10 articles address the issue of recovery for obtaining FC. The results obtained from tomato valorization for the extraction of FC can be seen in Table 2. The largest amounts extracted have been found in the skin and pulp of tomatoes (8,29,38-40).

As for the extractive methods used, emerging methods predominate over traditional ones, with greater quantities obtained with them (31). The results suggest that the use of OHAE, HPH and MAE could be the best option for tomato valorization and CF extraction (8,31,41). In the case of phenolic compounds, one of the studies in which traditional extraction is performed, 70% methanol is cited as the best extractive method among the existing ones (34).

Table 2. Valorization for the extraction of FC

Study	Target	Methodology	Valued portion	Parameters	Results

Almeida et al. 2021 (12)	Investigate the valorization of tomato production residues.	Traditional	Broken tomatoes, green tomato and tomato branches	SPME and GC/SM of of of CF, β-carotene and lycopene.	Higher FC extraction was obtained in green and broken tomatoes (not suitable for consumption initially)
Añibarro-Ortega et al. 2020 (40)	To know the phenolic composition and bioactive properties of the primary by-products of the tomato plant	Ethanol with Folin-Ciocalteu solution and sodium carbonate, 30 min at 40ºC.	Aerial biomass after the end of the crop cycle and pruning mass	HPLC-DAD-ESI/MS^n for identification of phenolic acids and flavonoids	Increased CF in biomass produced from pruning tissues
Coelho et al. 2019 (8)	Optimizing the extraction of BC from tomato by-products by OH and PEF	O_HAE and PEF with the aid of 70% ethanol	Tomato peel and tomato seeds	Amount of lycopene measured in µg/gFW	O_HAE as the best technique for CF extraction.
Jurić et al. 2019 (31)	Investigate the potential of HPH processing of tomato peel in water for the recovery of intracellular compounds and the possibility of making the best use of by-products from the tomato processing industry	HPH, organic solvents and supercritical CO_2	Tomato skin	CF measured in mg GAE/L and lycopene by UV-Vis spectra analysis (pellet-ethyl lactate curve) and subsequent analysis of pellets for lycopene absorption (mg/g)	32.2 % more CF extracted by HPH versus organic solvents and supercritical CO_2.
Bao et al. 2020 (37)	To examine the effect of HVACP on tomato pomace microstructure and correlate it with CF extraction	HVACP (Air, He, Ar and N_2)	Tomato pomace	Creation of analysis curve with spectrophotometry and conversion to mg GAE/gdm	Higher CF extraction (10% increase) with He and N_2 plasmas.
Szabo et al. 2019 (29)	To evaluate the CF and carotenoid content of tomato peels	Traditional (organic solvents)	Skin, seeds and general pruning waste on 10 different types of tomatoes	Amount of lycopene in mg β-carotene/100 g DW and CF in mg/100 g DW	The highest amount of CF was found in the tomato skin of a commercial hybrid tomato with 155 ± 2 mg/100 g.

Ninčević et al. 2020 (34)	To seek the use of tomato peel waste for the simultaneous recovery of high-value compounds	Traditional (organic solvents)	Dried tomato peel for the extraction of aagg-pectin and phenolic compounds-pectin	Amount of FC measured in mg/100 g, in 96% ethanol and pectins in g/L	The extraction of the CF together with the pectin does not oxidize, although its extraction is lower. The best solvent for extraction is 70% ethanol.
Arab et al. 2019 (38)	Develop methods to extract compounds of high commercial value	Subcritical CO_2	Tomato leaves	Amount of CF measured in mg (GAE) g-1and of flavonoids in mg Qe g-1	The FC obtained from tomato leaves, by CO_2 extraction at high pressure and without solvents achieved substantial improvements over traditional methods (contracted with bibliography).
Solaberrieta et al. 2022 (51)	Optimizing MAE and UAE of antioxidant compounds from tomato seeds using response surface methodology.	Comparison between MAE and UAE with ethanol support	Tomato seeds	MAE and UAE extraction parameters on total phenolic content (TPC) and antioxidant activity (DPPH) responses in mg GAE g TS-1.	MAE extracts showed higher total CF values compared to UAE (1.72 ± 0.04 and 1.61 ± 0.03 mg GAE g TS-1 for MAE and UAE, respectively)
Tranfić Bakić et al. 2019 (39)	Describe MAE as an innovative technique for the isolation of polyphenols from tomato peel waste	MAE with methanol at different times and Tª (22, 55 and 90ºC) (5-10 min)	Tomato skins	Parameters of kaemferol-3-O-rutoside, p-coumaric acid and chlorogenic acid derivative for CF quantification	Time is not a significant factor in the extraction of FCs, in terms of Tª and methanol: 50% methanol 25 °C; 70% methanol 55 °C or 50% methanol 90 °C for higher extraction.

* Identification of volatile compounds by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC/MS)

With regard to tomato pectin extraction, the present article focuses on four studies shown in Table 3. Three of the articles (22,34,34) suggest that innovative techniques are more effective in extracting the compounds, although one of the articles (25) does not observe significant differences between traditional and WIHC in particular.

The most effective emerging techniques are UAME (36) and HPH (22). Pectin extraction is extracted in half of the articles from tomato peel (34,36) and the other half from tomato pomace (22,25). One of the reviewed studies even shows similar pectin recovery with WIHC and traditional methods (25).

Table 3. Valorization for pectin extraction

Study	Target	Methodology	Valued portion	Parameters	Results
Sengar et al. 2020 (36)	Reducing the carbon footprint by extracting pectin from tomato peel	Comparison between 5 techniques: UAE, MAE, O_HAE, UAME UAO_HE	Tomato skin	Amount of pectin in g/kg of pectin, by galacturonic acid	UAME is the best technology in terms of yield and pectin quality compared to other technologies.
Ninčević et al. 2020 (34)	To seek the use of tomato peel waste for the simultaneous recovery of high-value compounds	Traditional (organic solvents)	Dried tomato peel	Amount of FC measured in mg/100 g, in 96% ethanol and pectins in g/L	The extraction of pectin-aagg or CF-pectin helps to avoid oxidation
Pirozzi et al. 2022 (22)	To achieve cellulose isolation for the valorization of value-added compounds contained in biomass	HPH with acid hydrolysis vs. traditional extraction with acetone	Tomato pomace	Cellulose and pectin measured in mg_{GAE}/g_{DM}	HPH promoted a 9% increase in extraction. It was even extracted without the need for the solvent.
Nagarajan et al. 2020 (25)	To evaluate the potential of carotenoid-pectin complexation in tomato pomace containing carotenoids and pectin.	WIHC vs. traditional methods	Tomato pomace	Use of complexation and traditional methods measured in mg carotenoid fractions/100 g tomato pomace	Recovery was of traditional pectin-carotenoids vs. WIHC is similar

Finally, the valorization for obtaining biofuels, for the article seven studies shown in Table 4 were taken into account. Most of the studies made use of tomato pomace (45,52) or a mixture of tomato and other compounds such as animal manure and other food residues (7,46,47). Of the seven studies, two of them performed prior extractions for the valorization of lycopene and other bioactive compounds (12,44).

Among the mixtures of compounds for biogas formation, olive pumice (OP), sheep manure (SM), corn stover and dairy manure give the highest biogas production (7.46). Among the emerging methodologies studied, UAE is particularly relevant (47.52).

Table 4. Valorization for the formation of biofuels

Study	Target	Methodology	Valued portion	Parameters	Results
Almeida et al. 2021 (12)	Extraction for biogas formation after the valorization of other bioactive compounds	Traditional	Broken tomatoes, green tomato and tomato branches	SPME and GC/SM of CF, β-carotene and lycopene	A higher methanol extraction was obtained in green and broken tomatoes, with a final amount contributed of 232-285 mL CH4/g
Tabrika et al. 2021 (7)	Direct DA (without prior valorization of other compounds)	Traditional	Tomato mixture with: OP, SM, chicken manure (CM) and sawdust.	Measurements of humic acid-type carbon (HAC) and fulvic acid-type carbon (FAC) concentrations	SM and OP are the most suitable raw materials for tomato waste composting.
Scaglia et al. 2020 (44)	Pre-valorization of lycopene by supercritical CO_2 and subsequent DA	Supercritical CO_2	Tomato peel and tomato seeds	Supercritical CO2 extraction technology in combination with anaerobic digestion, in % biodegradability	Supercritical CO_2 acts positively for the prior degradation of the fiber and its subsequent DA resulting in energy (better than corn-currently used to give biogas)
Hijosa-Valsero et al. 2019 (45)	Direct valorization by twelve different strains of bacteria with acetone-butanol-ethanol-ethanol-isopropanol fermentation (ABEI)	Traditional	Tomato pomace	Butanol and isopropanol measurements in g/L	*Kluyveromyces marxianus*, *Saccharomyces cerevisiae Ethanol Red®*, *S. cerevisiae Hercules* and *Lachancea thermotolerans* produced 20.1-21.7 g/L ethanol. According to these results, tomato pomace could be an interesting feedstock for ABEI biorefineries.
Mahmoodi-eshkaftaki et al. 2022 (47)	DA together with an AEU pretreatment at different powers and times	UAE	Tomato waste and cow manure	Measurements of hydrogen and methane, based on mg/g generated from volatile solids, carbohydrates and total solids	The UAE of 197.21 W, 21.47 min for mixtures with high amounts of tomato residues (>90 %) led to produce Bio-H_2 > 18 %vol and Bio-CH_4 > 2 %vol.

Girotto et al. 2021 (52)	Direct DA with UAE pretreatment, without extraction of biocompounds	UAE	Tomato pomace	Methane production in MJ/kg solid	The increase in methane production was not high enough to offset UAE's electricity needs
Li et al. 2020 (46)	Direct DA (without prior valorization of other compounds)	Traditional	Tomato peel together with corn or dairy manure	Amount of methane in L/kg of solid weight	Net energy production was achieved with the mixture of 24% corn stover, 36% dairy manure and 40% tomato waste: formation 379.1 L/kg $VS_{Methane\ feed}$

The general objective of the article is to determine whether there are significant differences between the different recovery methods for the use of tomato by-products, and it can be concluded that the greatest difference is found in the traditional methods compared to the emerging ones. Most of the traditional methods are still the most economically profitable, but this is not the case for the more environmentally friendly part, as they tend to generate larger amounts of environmentally harmful compounds. In addition, newer methodologies generally have the ability to increase the extraction of bioactive compounds from the food.

Among the emerging methodologies, PEF, MAE, UAE and supercritical CO2 as a necessary pretreatment prior to extraction stand out. These are the most studied of all the processes currently in existence. The main reasons are higher economic profitability and better extraction of target molecules. Among all the methods of utilization of by-products, it is necessary to emphasize the differences that exist not only in the utilization of the by-products, but also in the part valorized and in the measures used for the quantification of the by-product obtained, which makes it difficult to compare the studies.

As discussed throughout the paper, tomato by-products have many interesting and sustainable applications. The fact that tomato by-products contain high levels of anti-inflammatory and antioxidant compounds shows potential for further research. To focus on new proposals with the objective of achieving the optimization of this food. It is important to note that there are not yet many studies available that demonstrate the effectiveness of these innovative applications in practice.

To date, according to the studies, there is no extraction method that is more economically viable than synthetic production or traditional extraction, although according to the conclusions reached in them, this is closer to being achieved.

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Conflict of interest

There are no relevant conflicts of interest in this article.

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The role of chrononutrition in weight loss
El papel de la crononutrición en la pérdida de peso

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ABSTRACT

Keywords: number of meals, frequency, schedule and intermittent fasting

Circadian clocks and nutrition are closely related. The number of meals, the timing of consumption, and other factors can influence a person's weight and metabolism. The aim of this study is to gather scientific evidence that supports the idea that circadian rhythms affect weight loss. This is a bibliographic review that utilizes 56 references, with Pubmed being the most frequently used database, followed by Google Academic. The review discussion is divided into three categories: meal frequency, meal schedule, and intermittent fasting. Most studies agree that a greater intake of food should be consumed in the morning, but it's essential to note that the type of meals consumed during this time is also crucial for weight loss. Regarding the number of meals, there is more disagreement, so more research is needed. Finally, intermittent fasting appears to be effective for certain groups of people. After conducting a thorough study, we have concluded that there is a correlation between circadian cycles and body weight. However, further research with appropriate clinical studies is needed. It is advisable to consult a professional before making any sudden changes to your diet.

RESUMEN

Palabras clave: número de comidas, frecuencia de comidas, horario y ayuno intermitente

Los relojes circadianos están muy relacionados con la nutrición. La cantidad de comidas, el horario en el que se realizan y otros parámetros parece ser que puede influir en el peso y metabolismo de una persona. El objetivo de este estudio es recopilar evidencia científica de que los ritmos circadianos tienen influencia en la pérdida de peso. Se trata de una revisión bibliográfica. Se utilizaron 56 referencias. Siendo Pubmed la base de datos más utilizada seguido de Google académico. La discusión de los estudios encontrados se ha separado en: frecuencia de comidas, horario de comidas y ayuno intermitente. Con respecto al horario de las comidas, la mayor parte de los estudios coinciden en una mayor ingesta durante la mañana. Respecto al número de comidas hay mayor controversia por lo que tiene que haber mayor investigación. Por último, el ayuno intermitente, depende que grupo de personas parece funcionar muy bien. Tras haber hecho un profundo estudio, se llega a la

conclusión de la relación de los ciclos circadianos con el peso corporal. Aun así, se necesita mayor investigación con estudios clínicos adecuados. Lo primero, antes de realizar algún cambio brusco en la dieta, sería recomendable atender a un profesional para tener un seguimiento de lo realizado.

Introduction

Chrononutrition is a very controversial topic today. Chrononutrition is a concept directly related to metabolism, pancreatic function and hormone secretion (1**).** The timing of meals, the number and sequence of meals play a very important role at a physiological and hormonal level.

Recent research suggests that metabolic processes exhibit circadian rhythmicity (2). what are circadian rhythms? These are the physical, mental and behavioral changes that follow a 24-hour cycle. These natural processes respond mainly to light and darkness and affect most living things.

Circadian rhythms can be affected by: hormone release, temperature regulation, and regulation of eating habits and digestion. A very important aspect is the role of circadian rhythms in sleep patterns. This is regulated by the suprachiasmatic nucleus (SCN), which is said to be the central nucleus (3).

The chronotype is distinguished according to the individual's circadian rhythms (schedules, sleep habits, physical activity, etc.)

Different chronotypes can be found depending on the person:

Depending on the bibliographic source, chronotypes are classified somewhat differently. The most direct and simple way to classify it would be:

Morning chronotype. People with a very high cognitive level in the morning that decreases throughout the day

Evening. Their cognitive functions are very high in the evening, so they tend to go to bed late and get up late.

Intermediate chronotype. There is no predilection for either the morning or the afternoon-evening.

As mentioned, it is a topic that is being studied frequently in recent years, especially in the influence on health with respect to weight variation. There are studies (5) that support an increase in weight and obesity with an inadequate breakfast, comparing it with lipid metabolization. Changes have also been observed in the metabolism of some macromolecules such as lipids. Plasma triglyceride concentrations are elevated during the night so that the postprandial response after an evening meal is increased with respect to a daytime intake. So it could be said that there are genes related to lipid metabolism and circadian rhythms. Studies such as that of Watanabe et al (6) showed that people who sleep little (less than 7 hours a day), increased the production of ghrelin (increased appetite) and suppressed leptin (hormone responsible for the regulation of appetite and thermogenesis), resulting in an increase in intake and thus an increase in the patient's BMI.

The timing, number of meals and intermittent fasting is a critical determinant of metabolic health. Improving patient education and raising awareness of the metabolic implications of the timing of meals should be part of the tools available to healthcare professionals in the fight against the current obesity epidemic. Obesity and weight gain are a global health concern right now on the planet. The prevalence of obesity is increasing dramatically and this public health problem needs to be addressed. The WHO (World Health Organization) has published some relevant and worrying statistical data. Since 1975, obesity has tripled, and in 2016 more than 1.9 billion adults were

overweight or obese (7), a figure that continues to rise today

In Spain, 45.2% of children between 6 and 9 years of age are overweight or obese according to the ALADINO project (8).

Within the strategies for weight loss, some interventions have been seen that in principle would not require pharmacological treatment, which would be the control of dietary management and physical activity. In the purely food area, it is the individual's job to take care of his or her diet, although the authorities in each country or region can help by means of labeling, advertising regulations and other options. If necessary, the help of a dietitian nutritionist can be very helpful in guiding the patient and providing nutritional education.

On the other hand, as regards physical exercise, we will be guided by the recommendations of the WHO (12), which has divided by age the estimated minimum time of physical activity necessary to obtain an adequate state of health. (These are general recommendations, for people with a normal level of health, without conditions or diseases)

Weight loss often does not come from an intentional way on the part of the person, but multiple factors may be involved, and in many occasions it does not occur intentionally.

However, the causes (10) can be quite diverse for weight loss to occur, such as:

- Tumors of the digestive system. The most common are those of the pancreas and liver, producing a lower food consumption and leading to weight loss.
- Lung cancer.
- Diabetes mellitus, especially of sudden onset, due to problems of insulin resistance and glycolytic (glucose-related) problems
- Hyperthyroidism.
- Depression and mental illness. There is usually a decrease in intake, and with it comes a decrease in intake.

Chrononutrition, the science that studies the effect of food on our circadian system, and weight loss, are two concepts that are being studied in depth in recent years, due to their possible importance in improving our state of health. The main objective of this literature review is to check whether factors such as meal timing, frequency, or fasting at certain times have an impact on weight loss.

Objectives.

GENERAL OBJECTIVE

Gathering scientific evidence to see if circadian rhythms have an influence on weight loss

SPECIFIC OBJECTIVES

Establish the relationship of meal timing and number of meals to weight loss

Determine the effectiveness of correctly following circadian rhythms

To see if different eating patterns (intermittent fasting, time-restricted eating), are effective in weight loss.

Method

In this literature review, a study on chrononutrition and weight loss is conducted. PubMed and Google Scholar were mainly used as databases.

The search includes research studying both the relationship of circadian clocks and diet to beneficial and detrimental weight loss. We have tried to prioritize the search for clinical trials over other types of studies. However, meta-analyses, observational studies and others have also been used.

Specific inclusion and exclusion criteria were used: In terms of inclusion criteria:

Articles from indexed journals, journals with an impact factor > 1.5 years maximum of research, articles mostly in English (75%) and the rest in Spanish (25%).

Regarding exclusion criteria:

Titles that are not related to the subject to be studied, studies with insignificant or non-representative samples. On many occasions, the title may seem appropriate, but the content is not adequate and vice versa.

The search for items began in June 2023 and ended in August 2023. The databases used were:

-Pubmed. Keywords were used in the title and abstract. We used the Boolean operator "and. Together with Google Academic were the most used bases. Approximately 35 articles from this platform were used.

-Sciencedirect. Keywords were used in the title and abstract. The Boolean operator "and" was used. About 10 items used.

-Other sources:

- Internet. Performed a search mainly for definitions to give figures and numbers. The introduction part is also where these pages have been used. About 7 articles (e.g. National Melatonin Institute, WHO, UN...)

Table 1. Methodology of the work. Own elaboration

Part of the work	**Search strategy**	**Number of items used**	**Number of items found**	**Databases and other sources used**
Introduction	Weight loss	15	45	Google Scholar, Pubmed and internet sources
Meal frequency	Meal frequency and weight loss. The Boolean "and" Filter was used: Clinical trials mainly and 5 years old	4	140	Pubmed
Meal times	Weight loss and meal timing. The Boolean "and" was used. Filter: Clinical trials mainly and 5 years old	6	167	Pubmed

Intermittent fasting	Weight loss and meal timing. The Boolean "and" was used. Filter: Clinical trials mainly and 5 years old	6	210	Pubmed

Results and Discussion

Frequency, schedule and intermittent fasting

Table 2. Table on meal timing and weight loss. Own elaboration

Authors	Type of study	Weight loss efficacy
Ruge T et al (13)	It is an observational study. A survey of eating habits of 1504 people over 18 years of age was used to explore the times and places where people ate	According to the results of this study, people who ate meals later in the day had higher weight and BMI
Garaulet M al (14)	Randomized clinical trial studying the effectiveness of weight loss in 420 individuals. 51% of the subjects ate early and 41% ate late. Intake, energy expenditure, appetite hormones, etc. were studied.	Late eaters lost less weight and showed a much slower rate of weight loss P(=0.002) (Short time constraint)
Purslow LR et al (15)	6764 men and women aged 40 to 75 years at baseline, daily food monitoring for 2 years, and objective weight and height measurements at baseline and at the end of the project	The lowest BMI was found in people who consumed more food at breakfast and gained much less weight than those who did not eat breakfast
Sievert K et al (16)	Review and meta-analysis of randomized clinical trials published between 1990 and January 2018. Of the 13 trials examined, 7 examined the effect of eating breakfast on weight change	Small difference in weight favored patients who skipped breakfast and ate at later hours. (95% Confidence Interval 0.007 to 0.82)
McCrory MA et al (17)	Review of 3 scientific literature studies	They see no clear effect on weight loss based on meal timing

Most of the studies show satisfactory results when meals are taken mostly in the early hours of the day (until approximately 3:00 p.m.) (13-15)

However, not all studies were like this, they did not see significant differences in weight difference depending on the time of intake (16,17).

There is no certainty as to why it is better to eat earlier in the day. There are hypotheses of a possible slowing of metabolism when eating late in the day. Based on the relationship between circadian rhythms and weight loss, it seems that it may be due to the production of ghrelin (orexigenic hormone) according to research (13,15,17).

The regulation of food intake and energy balance is a complex process and is made possible by many endocrine signals, whereby the variables to be taken into account are very diverse.

The vast majority of the studies mentioned are trials, as they are the best way to test scientific theories, the empirical test. Meta-analyses are mentioned for their scientific quality in journals, although they have severe limitations. The number of studies analyzed are somewhat scarce, so that authors complain about the lack of clinical trials to demonstrate or clarify their effect (16,17,) in addition to the fact that the studies mentioned in this research are observational and therefore do not have the same certainty as randomized clinical trials.

The positive aspect of meta-analyses is the variety of conclusions and results of all the projects studied in order to be able to draw conclusions based on what has been studied (15). Garaulet M et al et al (14), in their clinical trials the follow-up and study time is relatively short. The time period of the investigations should be increased in order to obtain more accurate and objective results.

Table 3. Table on number of meals and weight loss. Own elaboration

Author	**Type of study**	**Weight loss efficacy**
Paoli A et al (19)	Meta-analysis	To obtain a complete picture of the physiological and health effects of meal timing and frequency, multiple lines of research must be integrated, and an exploratory review seems, in our opinion, to be the appropriate approach to understand, at a glance, the influence of fasting, meal frequency and timing on cardiovascular disease

Kahleova M et al (21)	Cohort study of about 50660 subjects	Weight loss to people who ate fewer meals. (1 or 2 meals > weight loss of subjects who ate more than 1 meal)
Ha K et al (22)	Meta-analysis concentrating Korean health and nutrition survey data from 27220 patients	Increased meal frequency appears to be associated with fewer metabolic diseases
Maukonen M et al (23)	Comparative study conducted in Finland on 1097 Finns and meal numbers were assessed with 48-hour dietary records	The conclusion of this study is a possible increase in obesity when nighttime eating is prioritized
Grangeiro ED et al (20)	Randomized clinical trial of 40 women divided into 2 groups. 3 meals per day and 6 meals per day	Weight changes were minimal, although glucose, ghrelin and other lipid parameters were satisfactory

There is much disparity in the frequency of meals and weight loss, when it comes to reaching conclusions. Studies (19,20,23) support that a greater number of meals is involved in increased weight loss. On the other hand, Khaelova M et al (21) through their cohort study, affirm that it is better to have around 1 or 2 meals per day, as well as Maukonen and Grangeiro ED et al (20,23) bet on more meals per day due to other variables different from body weight.

what mechanisms promote weight loss depending on the number of meals you eat?

Meal timing and frequency are usually accompanied, so finding articles that mention this individually is difficult and as some studies mention (22), they would have to be compared with papers and research dealing with intermittent fasting and meal timing.

As a result, finding clinical trials proved difficult.

Nutritionists do not emphasize in consultation the number of meals to be taken per day, perhaps due to the complications that the subjects may have to take meals because of their work or their different chores.

In addition to weight (22), a greater number of meals distributed throughout the day improves other cardiovascular and metabolic parameters. More research is needed on this as they are secondary results, so there may have been flaws in the research. In contradiction, they verify a better lipid profile with a lower meal frequency, contrary to the studies of Paoli A and Ha Kyungho Ha et al (19,22). There are people who for different reasons cannot eat all their meals during the day, either because of their work, ethical, moral or other issues. A very important limitation is the pathophysiological status of the individuals. Khaelova M et al (21), in their research included obese people, smokers, with normal weight, so the result may be biased. Or other studies in which only women are included (19), so that the results of the study can sometimes not be comparable, due to the physiological and anatomical differences of the individuals. As

previously commented Paoli A et al (19), in their conclusions, claim that to see if there is a weight loss due to meal frequency would require future lines of research and to search for more current articles.

Other limitations would be the time of the studies. Those mentioned in the table above are not very recent (19,22), so recent articles of good quality would be desirable.

As before, the certainty in some studies is somewhat lower (23), due to the way the study methodology is carried out, which in the case of the Finnish comparative study, uses a 48h reminder to record dietary habits.

Table 4. Intermittent fasting and weight loss

Author	**Type of study**	**Weight loss efficacy**
Schubel R et al (29)	12-week randomized controlled trial, 3 groups (Group 1 deficit 75%, Group 2 deficit 20% and Group 3 no intervention)	Some weight loss in the fasting group, but it was not significant. In glucose levels, there was a difference in the non-fasting group.
Catenacci VA et al (25)	Randomized pilot study in 8 weeks of supervision. 2 groups. (Group 1 with no intake restriction and group 2 with a caloric restriction of 400 kcal per day)	Somewhat greater weight loss in people who were fasting.
Headland ML et al (30)	1-year randomized parallel trial. 3 groups (2 groups with continuous restriction and 1 group with intermittent fasting)	The weight loss results were: - 6.6 kg for the continuous restriction group, 5.1 kg for the group combining intermittent fasting and Fasting and 5 kg for the normal group.
Lowe DA. et al (28)	Randomized clinical trial of 116 overweight adults divided into 2 groups (Group 1 had only 3 meals and Group 2 had intermittent fasting)	The primary outcome sought was weight loss. The results do not show effective results in intermittent fasting.
Mandal et al (31)	Randomized controlled trial conducted in 101 obese and overweight adults with prediabetes	Intermittent fasting on alternate days was shown to have greater weight loss benefit than intermittent fasting.
Fiastuti Witjaksono et al (32)	Randomized clinical trial conducted in Jakarta, Indonesia. He was divided into a group that fasted twice a week and another group that did not fast.	Improvements in weight loss in the alternating fasting group.

Since intermittent fasting has been studied so recently, the number of 5-year studies is notorious, especially clinical trials, which are the most valuable in the field of medical sciences.

Studies (24,27,28) support the use of intermittent fasting for weight loss, but some, such as Jashmed H et al (26), do not mention weight loss in numerical terms, as does Headland ML et al (30), mentioning a somewhat greater difference, although not significant enough to affirm categorically the greater benefit of intermittent fasting with respect to normal caloric restriction.

Even so, intermittent fasting seems to have better results than a normal diet in terms of weight loss, in most cases, since lipid aspects and with glucose levels and so on, it would have to be studied more closely, and therefore this dietary tool is not recommended for some people.

In addition to weight loss, parameters such as glucose appear to undergo changes with intermittent fasting. Fasting glucose is lower during fasting; logically, as our glycogen reserves are used up, it can be recommended for diabetics, although more research is needed (29)

Another issue is whether alternate fasting or continuous fasting is better. Clinical trials (31,33), advocate alternate day fasting while (25) advocate intermittent fasting, in which fasting can sometimes be skipped. Although preached with weight loss, clinical trials such as the one of

Lowe D.A. et al (28**)**, mentions weight loss, but there is no decrease in BMI or lipid parameters so it may not be such a beneficial result because when weight is lost and it is not fat, it is usually water and body compounds. Other studies such as that of Headland ML et al (30) also show weight loss, but there is no significant difference with respect to the other groups in the study, whose weight loss is greater than in the case of fasting.

One of the most important limitations, as described for the other studies, is the pathophysiological situation of the subject. Many of the trials used are performed on obese patients, so comparing them to overweight or normal weight individuals is quite difficult, as the manner of weight loss with such heavy subjects is somewhat irregular and personal.

Another limitation with intermittent fasting is the fasting schedule. That is, if it is a 16 :8, 8:16 etc., (16 hours of fasting, 8 hours of intake and 8 hours of fasting and 16 hours of intake, respectively). Most studies (25,27,29,30) do not mention the hours, as it also happens with alternate fasting, except for one research, which specifies the alternate fasting of 5:2 (29).

It is very difficult to find research that compares alternate and intermittent fasting in a single study to see which may be more suitable. Most of them were compared with a calorie-restricted diet (25,30,32). Alternate fasting is a type of fasting, which is performed sporadically or, rather, usually several days of fasting and then stop fasting for a few days.

Conclusions

This literature review analyzes the role of chrononutrition in weight loss structured in different blocks: frequency, schedule and fasting, through a literature review of about 50 studies, both national and international.

Emphasizing the studies analyzed, several conclusions can be drawn regarding meal frequency, meal timing and intermittent fasting. Regarding meal timing and weight loss, a large number of studies conclude that a higher intake earlier in the day is related to greater weight loss, although there are studies that do not support this hypothesis.

Continuing with the frequency of meals and weight loss, it is worth mentioning

that this is a more controversial topic than the previous one. Many studies look at meal frequency in conjunction with meal timing. There is more diversity of conclusions when studying meal frequency, one of the most frequent being that differences in parameters such as glucose, cholesterol or triglycerides can be seen when larger meals are consumed per day rather than weight loss benefits.

Finally, intermittent fasting, which is a way of eating that has become well-known in recent years. For a nutritionist, it should not be the first option to give because of its possible difficulty of adaptation for the individual. Most of the research found differences with respect to a normal diet with the same calories, especially in people who are overweight or obese. Even so, it is a diet for a specific population group that can cope well with fasting. In addition, it would be necessary to study how effective alternate or intermittent fasting is compared to continuous fasting.

Some of the aspects to be improved would be: to develop clinical trials with a large sample size. Studies should be conducted with a similar methodology to be as objective as possible. Nutritional education to the general population. Incentivize governments and public entities to invest in research and achieve greater studies over time.

As a final conclusion, the frequency, timing and number of meals are involved in weight loss on numerous occasions, without knowing the specific mechanism.

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The scientific overview of the status of biosimilars worldwide and their conception of biosimilarity versus their quality attributes

El panorama científico del estado de los biosimilares a nivel mundial y su concepción de la biosimilaridad frente a sus atributos de calidad

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ABSTRACT

The attempt to bring closer biological treatments, with high cost for citizens, has boosted the birth and growth of biosimilar drugs. Molecules whose production is focused on being copies of the active ingredients of drugs of biological origin catalogued as innovative. As they are biological molecules, the fact of being copies of the active ingredient becomes complex, as small variations in their biochemical composition can affect their safety and efficacy. Unlike innovators, whose marketing rationale is aimed at the safety of the drug through clinical studies, the basis for being marketed under safe conditions, however, biosimilar drugs focus on ensuring that their quality attributes are as close as possible to the molecule they are intended to replace. For this reason, by studying the critical quality attributes and the modulators that affect them, it is possible to establish a classification of these attributes that will allow harmonization of the biosimilar concept. The attributes that characterize the molecules are antagonistic or complementary to each other, making it possible to establish a range of acceptance that allows the development of a system for grading the comparability between innovators and biosimilars, bringing the concept, which to date has been theoretical, closer to a quantitative aspect. But always taking into consideration fundamental aspects such as the incidence of laboratory error in its assessment. Therefore, based on a harmonized model of the concept of the quality attribute, this should be reformulated towards a term that unifies the concept with its intrinsic error, so that it can be assessed in a harmonized way.

RESUMEN

Palabras clave:
biosimilares, atributos de calidad, armonización

El intento de acercamiento de tratamientos biológicos, con elevado coste para los ciudadanos, ha impulsado el nacimiento y crecimiento de los medicamentos biosimilares. Moléculas cuya producción está enfocada a ser copias de los principios activos de los medicamentos de origen biológico catalogados como innovadores. Al ser moléculas biológicas, el hecho de ser copias del principio activo se hace complejo, pues pequeñas variaciones en su composición bioquímica pueden afectar a su seguridad y eficacia. A diferencia de los innovadores, cuyo razonamiento de comercialización está dirigido a la seguridad del medicamento mediante estudios clínicos, base para ser comercializado en condiciones seguras, sin embargo, los medicamentos biosimilares, se centran en que sus atributos de calidad sean los más próximos a la molécula que pretenden sustituir. Por ese motivo, mediante el estudio de los atributos críticos de calidad, y los moduladores que le afectan, es posible establecer una clasificación de los mismos que permitan la armonización del concepto biosimilar. Los atributos que caracterizan a las moléculas son antagonistas o complementarios entre sí, permitiendo establecer un rango de aceptación que permita el desarrollo de un sistema de graduación de la comparabilidad entre innovadores y biosimilares, acercando el concepto hasta la fecha teórico, a un aspecto cuantitativo. Pero siempre tomando en consideración aspectos fundamentales como la incidencia del error del laboratorio en su valoración. Por lo que, basándose en un modelo armonizado del concepto del atributo de calidad, este debe ser reformulado hacia un término que unifique el concepto con su error intrínseco, de manera que pueda ser valorado de forma armonizada.

Introduction

In the last decade, biosimilars have experienced exponential growth, transforming the treatment paradigm for various diseases (1).

They are biologic drugs that have become increasingly popular worldwide (2) and are similar to other biologic drugs already on the market, known as reference biologics. However, unlike these, biosimilars are not identical in terms of molecular structure (3) and aim to provide more affordable and accessible alternatives to reference biologics (4). These drugs have been shown to be equally effective and safe compared to reference biologics in numerous clinical studies.

Currently, biosimilars are available in many countries around the world, but their regulations are diverse and in many cases diffuse. The European Union has been a pioneer in the approval of biosimilars since 2006 (5), and has established a solid regulatory framework to guarantee their quality, efficacy and safety. Other countries, such as the United States and Japan, have also developed their own regulatory frameworks for the approval of biosimilars. One of the most important milestones for biosimilars was the approval of the first biosimilar by the World Health Organization (6).

In terms of therapeutic indications, these drugs cover a wide range of therapeutic areas, such as oncology, rheumatology and diabetes. These drugs have been shown to be effective in treating a variety of diseases and conditions, making them an attractive option for many patients and healthcare professionals.

However, despite advances in the field of biosimilarity, there are still challenges to be overcome. One of the main challenges is public education and awareness about biosimilars, as many patients and healthcare professionals may still have doubts or concerns about the quality and efficacy of biosimilars compared to reference biologics (5).

In addition, access to biosimilars may vary from country to country due to national regulations and policies. Some countries have implemented policies of automatic substitution of reference biological products for biosimilars, which has contributed to greater use and access; however, in other countries, the adoption of biosimilars may be slower due to legal or economic barriers (7).

Based on this, this article aims to critically examine the scientific status of biosimilars, highlighting regulatory progress and the evolution of research in this area, relying on statistics as a means of establishing a definition of the term biosimilar with greater standardization.

Current status of biosimilars in the world..:

1. Global Regulation:

The regulatory frameworks of the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) have been key pillars in the development of biosimilars. As mentioned above, the global regulation of biosimilars is constantly evolving and varies between different regions and countries. However, there are certain key aspects that are considered in the regulation of biosimilars worldwide (8).

In general, international regulators consider the comparability, quality, efficacy and safety of biosimilars to ensure that these drugs are equivalent to reference biologics. Some of the key elements of the global regulation of biosimilars are as follows (9):

a. Benchmarking: Regulators require comparative studies between biosimilars and reference biologics, which include analyses of physicochemical, functional, pharmacokinetic and pharmacodynamic characteristics. This is essential to demonstrate that biosimilars are similar in terms of structure and function to reference biologics.

b. Clinical studies: Biosimilars must also undergo comparative clinical studies that demonstrate their efficacy and safety compared to reference biologics. These studies may include Phase III clinical trials or bioequivalence studies, depending on the requirements of each national or regional regulation.

c. Pharmacovigilance: Regulators emphasize the importance of adequate pharmacovigilance to monitor and evaluate the side effects and long-term safety of biosimilars once they are on the market. This is done through the monitoring and analysis of data on the safety and efficacy of biosimilars.

d. Change of manufacturer: The global regulation also addresses the switching of biosimilar manufacturers and the need to demonstrate equivalence between the different versions of the drug in terms of quality, efficacy and safety.

e. Labeling and nomenclature: The regulation also includes requirements for the labeling of biosimilars, ensuring that patients and healthcare professionals can clearly identify the medicines and distinguish them from reference biologics. In addition, the adoption of an appropriate and distinctive nomenclature for biosimilars is considered important.

While there are international guidelines and regulations for biosimilars, each country or region has its own approach and approval process that must be scrupulously followed by pharmaceutical companies. Some regions, such as the European Union, the United States and Japan, have developed specific regulations and more comprehensive regulatory frameworks for drugs in this category. However, a greater effort is still required to achieve greater harmonization and convergence in the global regulation of biosimilars, since qualitative criteria prevail in their definition.

2. Scientific Developments:

Scientific progress in the characterization and manufacture of biosimilars is essential to guarantee their quality and efficacy.

The scientific development of biosimilars is a steadily growing field, driven by technological advances and scientific knowledge in molecular biology, genomics and biotechnology. The following are some highlights of the current scientific development of biosimilars (10,11):

a. Analytical characterization: Thorough characterization of biosimilars is essential to demonstrate their similarity to reference biologics. Advanced molecular biology techniques, chromatography, mass spectrometry and protein folding and aggregation analysis are used to evaluate the structure, purity and biological activity of biosimilars.

b. Modeling and simulation: Computational modeling and simulation are used in the development of biosimilars to predict and optimize pharmacokinetic and pharmacodynamic properties. This helps to establish the development strategy and identify the critical characteristics of biosimilars.

c. Preclinical studies: Preclinical studies play an important role in the evaluation of the toxicity and biological activity of biosimilars. In vitro and in vivo studies are conducted to demonstrate the similarity between biosimilars and reference biologics.

d. Clinical studies: Comparative clinical studies are crucial to establish equivalence in terms of efficacy, safety and immunogenicity between biosimilars and reference biologics. These studies usually involve patients with specific diseases and evaluate clinical and pharmacokinetic parameters.

e. Innovations in production: The production of biosimilars has undergone significant technological advances, which has improved the quality and efficiency of their manufacture. For example, improvements in cell culture processes, purification and

formulation have allowed for greater reproducibility and consistency in the production of biosimilars.

f. Precision medicine: Precision medicine, which is based on the identification of specific molecular characteristics of patients, is also influencing the development of biosimilars. Approaches such as biosimilar drugs tailored to a specific biomarker profile are being investigated, which could further improve efficacy and safety in the treatment of diseases.

In general, the scientific development of biosimilars continues to evolve with the aim of improving the quality, efficacy and safety of these drugs, through advances in analytical characterization, modeling and simulation, preclinical and clinical studies, innovation in production and the use of precision medicine.

3. Adoption and Challenges:

Despite successes, widespread adoption of biosimilars faces persistent challenges. Despite advances in the field of biosimilars, there are still several challenges that will need to be addressed in the future. These challenges include (12):

a. Change of mentality and education: One of the main challenges is to change the mentality and educate both healthcare professionals and patients about biosimilars. Many still have doubts or concerns about the quality, efficacy and safety of these drugs compared to reference biologics. Increasing awareness and understanding is essential to ensure wider adoption and confidence in biosimilars.

b. More consistent regulations and policies: While many countries have established regulatory frameworks for the approval of biosimilars, there is still some variability among national regulations and policies. It is important to promote greater harmonization and consistency in the evaluation and approval of biosimilars, which would facilitate their entry and access globally.

c. Economic sustainability: As biosimilars enter the market, they may provide more affordable options for patients and healthcare systems. However, due to the costs associated with the production and development of biologic drugs, there is still a need to address the economic sustainability of biosimilars. This involves balancing drug prices to ensure affordability and maintaining investment in research and development.

4. Strengthening the supply chain and quality: The quality and integrity of biosimilars are essential to ensure their efficacy and safety. It is important to strengthen the supply chain (13) and maintain high quality standards in the manufacture, storage and distribution of these drugs. This implies establishing and maintaining effective quality control and regulatory oversight mechanisms.

5. Development of new formats and technologies: As the field of biosimilars continues to evolve, it is also important to invest in research and development of new formats and technologies (14). This includes the formulation of biosimilars in different presentations, such as tablets or inhalers, to provide additional options and convenience to patients and reduce the need to visit medical centers or the training required for administration. New technologies also include the development of molecules that allow several diseases to be treated in a single administration, including research to develop molecules with specificity for two or three different antigens, and thus obtain more specific treatments or broaden the range of action (14).

6. Search for a standardized element of the biosimilar concept: The current definition of biosimilar used to standardize the concept of biocomparability between an innovative drug and a generic drug is reinforced by the fact that these are highly complex molecules, and therefore the comparison between them must be made from a qualitative

point of view. The available studies on how to catalog biosimilars always present a comparison between the different attributes on a case-by-case basis (15-18).

If individualized studies of quality attributes are grouped together for globalized comparison, it is possible to establish comparability as a key concept.

From a statistical point of view, and based on the numerous individual studies of quality attributes (19), it is possible to establish whether these attributes are complementary or antagonistic.

One of the main difficulties encountered by regulatory agencies when approving a dossier of a drug that claims to be biocomparable to a reference drug is that the analytical results of the different critical quality attributes (CQA) are presented on an individualized basis. This way of characterizing a biosimilar may cause loss of knowledge or a masking of knowledge by not discussing the complementarity between the same quality attributes together with other CQA. This classification of attributes is intended to ensure that the drug can be interchanged, without any harm to the patient, the treatment with the innovator, maintaining the health benefit, but reducing the cost of treatment or, on the contrary, negatively impacting the outcome of treatment. It is necessary to understand that a biosimilar drug, or any biological drug, may present health risks that are highly variable in both time and effect (8,20)

It is easy to argue that the different attributes independently may be comparable against an innovator drug, but these same attributes found in the different comparisons attached to the approval dossiers (21), do not facilitate understanding for the patient or for untrained personnel.

Therefore, the industry must move towards an evolution of the concept of biosimilar, which will allow the establishment of a clear and understandable definition for every individual. This can provide answers to several of the problems that have been raised throughout the article, such as awareness and education, as well as the definition itself.

One of the simplest forms of presenting any information is the graph (22), as it is the statistical concept demanded by industry and regulatory agencies, and requires little training to elucidate the result provided. Since attributes can exhibit complementarity and antagonism, it is an easily presentable form of comparability and should be part of the definition of the biocomparable antibody. This article establishes the methods for the valuation of the quality attributes, since they form the main structure of the definition.

Method

In order to address the definition of biosimilar and achieve harmonization of the definition, the classification of biosimilars into different phases must be taken, the subject of this article being the analytical or preclinical phase, as it is the most relevant when submitting pre-approval dossiers to any regulatory agency or to clients.

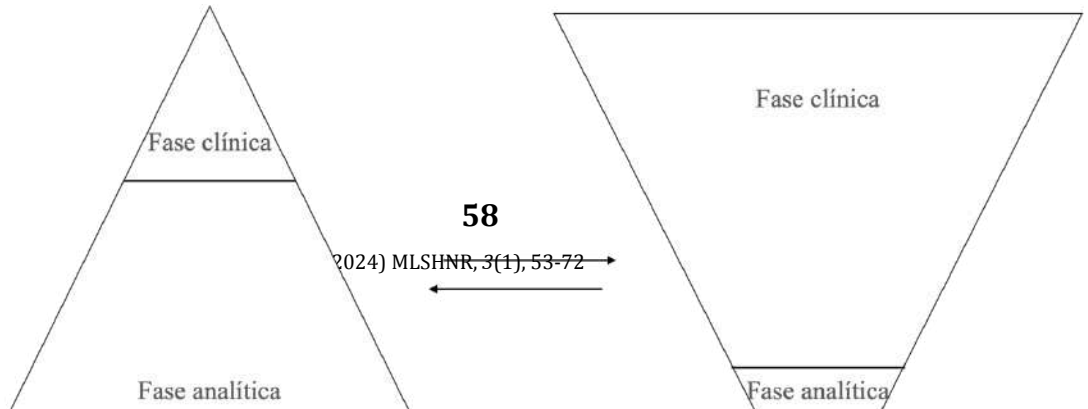

Figure 1. Approach to the development of a biosimilar vs. an innovator

As can be seen in Figure 1, the analytical or preclinical phase is the most relevant phase when establishing a biosimilar, since, as indicated in the most prestigious international guidelines, the fact of being analytically similar for some or analytically comparable for others is a compelling reason for approval. This concept forms part of the basis of the methodology as it is part of the hypotheses identified. For this reason, the starting point of the methodology is the quality attributes. Since the starting point of the quality attributes is so broad, the objective of this article is to delimit the classification of the attributes according to their criticality and the rest of the variables that directly or indirectly influence the quality attributes themselves and how to value them.

A. Quality attributes

From the guidelines published by the agencies, it can be concluded that, for similarity and comparability, they include the common attributes of potency, biological activity and function. But then we find separate structure, properties and stability, with influence on similarity and immunogenicity and pharmacology on comparability. All together should determine a biosimilar.

Based on the terminology provided by Kwon et al. (23) on the critical quality attributes and characteristics that positively and negatively influence the structure of an antibody indicated by BWG (12), a basic equation can be developed that brings together all the aspects required by the agencies and by science.

The authors cited above classify the quality attributes under a 3-level ranking, determining which, according to their criteria, are considered critical. Relying mainly on the functionality of the characteristic indicated in their writings, they divide the attributes into seven major groups, implying their structure and nature.

- Primary protein structure.
- Higher order structures.
- Variants loaded.
- Mass variants.
- Oligosaccharides.
- Biological activity.
- Content.

These attributes are given a score of three levels of criticality compared to their biological or clinical function.

It is true that the attributes indicated are the most relevant in a monoclonal antibody, and it is true for any of its variants that may be developed in the future, but the agglutination in only three levels causes overestimation of the criticality of some attributes and underestimation of others, since the three levels are associated to high, medium and low. And it is true that the worst case is an immunogenicity derived from the molecule, but it should not be at the same level of an attribute that influences a mode of action, since from studies performed in the laboratory by *in vitro* or *in vivo* techniques, sufficient information is obtained to determine the extent of the influence of that attribute.

In response to one of the issues raised, namely the importance of attributes, it is necessary to redefine the criticality of the attributes that currently exist in the field of mAbs (monoclonal antibodies), since they do not exclusively influence one factor. An

attribute such as amino acid sequence cannot be included only with influence on biological potency. The amino acid sequence, classified as level 3 criticality by the studies of Kwon et al. (23), it cannot be evaluated together with the rest of the quality attributes because it implies an underestimation of the true complexity. Changes in specific regions of the amino acid structure of the protein can completely modify its conformational structure by charge interactions, even changing the function produced, indicating that it can not only affect potency, but can become a potential immunogenic attribute or simply destabilize the protein. For this reason, it was determined that the study of this attribute should be extracted from the group of critical quality attributes and, in particular, the weight should be placed on the regions determining complementarity (CDR), since these are the most variable regions and the ones directly related to complementarity. These regions are identified in protein databases such as UniProt and can be automatically identified (24). But in the case of biosimilars, being copies of an original molecule, these regions are already pre-sequenced, and it is only necessary to perform a comparative BLAST-type analysis.

The comparative analysis of these characteristics in particular can determine if it presents a complementarity against the same antigen, or if it is a new function for the protein, which would be due to the fact that it is a new molecule. (25) Thus it is determined that not all quality attributes can be compared under the same umbrella, nor under the same classificatory characteristics. It is important to establish a higher level of characterization and to establish groups that are not based exclusively on their function, but on all the interactions they have in a living environment.

From the body of information it is understood that it is necessary to re-classify the critical quality attributes, from a broader view, starting from the knowledge bases provided by Kwon and collaborators or those published by BWG at the Fimea conference 2017.

In such a way that the relationship of an analytical variable, versus antagonist or enhancer, represent a complex biological system through the results of the laboratory analysis itself.

Results

From the study of the quality attributes, a series of characteristics or variables of relevance were obtained when assessing the importance of the different characteristics of the biosimilars and, therefore, what relevance should be applied when classifying their comparability. These attributes can be grouped into the following groups:

A. General quality variables

The general quality attributes can be broken down into the following classification:

- Lower order (primary to tertiary structure):
  - Amino acid sequences: They define the primary structure of the protein. From the initial research study of this parameter, the need to work on this variable independently from the rest of the parameters was obtained due to the influence it has on the rest of the parameters. The variable is controllable by international databases and will be compared

by BLAST. Even so, it will be subject to the matrix criterion that will be developed during the subsequent phases.

- N-terminal pyroglutamate: This variable should initially be studied independently to obtain the scope of its results, techniques with which results can be obtained, influence of the analyst's work, etc
- C-terminal lysine: as with the previous variable, the influence of this variable and the techniques with which results are obtained should be studied. In turn, its influence on the structure of the antibody and its effect on other non-structural variables must be identified.
- Disulfide bridges: They are involved in the definition of the tertiary structure of the protein. Their influence on protein stability will be studied independently.
- Other free amino acids: The stability of this variable in the structure of a monoclonal antibody will be studied.

- Higher order (Quaternary structure): It will be evaluated whether the study of this variable is relevant in the final packaging of a biosimilar, knowing its amino acid structure.
- Variants of loads: Of the variables mentioned below, the analysis techniques used will be studied and the quantification of those identified as qualitative will be assessed.
  - De-amidation.
  - Isomerization.
  - Oxidized forms.
  - Sialized forms.
- Mass variants: As the variables of variant loads, the quantification of techniques that by their nature are qualitative in nature will be obtained and a comparison will be made with respect to their influence on structure, function and immunogenicity.
  - Aggregates.
  - Fragmentations or degradation products.
  - Truncated forms.
  - Monomers.
  - PEGylations.
- Oligosaccharides: As with other variables that make up the critical quality attributes, their influence on the biosimilar concept and, in turn, the influence of the techniques applied to obtain consistent data should be studied in matrix form. All values are available from field studies for different molecules.
  - Fucose or galactose.
  - Non-human glycans.
  - Glycans *High* manosas.
  - Non-glycosylated forms.
- Other post-translational modifications: Changes in the protein during different phases of its development can modify the initial design of an antibody, being a fundamental part in determining comparability and biosimilarity. For this reason, these variables must be studied under the criticality matrix and their influence by the human factor when obtaining results. Since these are qualitative variables, their values will be quantified by normalizing them in order to be able to compare all the critical data.

- Phosphorylation: addition of phosphate groups to the antibody.
- Deamidation: removal of an amino group from the antibody.
- Oxidation: initial modification of amino acid side chain groups by ROS and subsequent conversion to carbonyl and other derivatives.
- Glycation: modification of amino groups by the action of reducing sugars.
- Glycosylation: addition of carbohydrates to an antibody.
- Sulfation: addition of a sulfur trioxide group.
- Isomerization Succinylation: transformation of one molecule into another.

- Glycan forms: They have an identified immunogenic effect, but of different influence depending on their nature. In turn, they can act on the structure and function of proteins.
  - Manosa: Increases the elimination of antibodies and acts on biological functions.
  - Fucose: They have a direct influence on biological functions, improving ADCC and *binding* if found in lower amounts.
  - Galactose: In the case of exposure, it increases the clearance of antibodies.
  - GlcNAc: They influence the elimination and biological functions of antibodies. Special relevance if bisecting.
  - NANA sialic acid: It has anti-inflammatory activity and is critical in the elimination of fusion proteins. Their influence on mAbs will be studied.
  - NGNA sialic acid: It interferes with the biological functions of antibodies and is immunogenic in humans.
  - Galalpha-3Galbeta1-GlcNAc-R: It is highly immunogenic in humans and produces anaphylaxis.
- Biological activity: this is the name given to the set of variables whose results are obtained from *in vitro* experimentation.
  - ADCC: From this variable we obtain a potency result that is part of the performance of the drug in a living organism. This indicates the ability to neutralize the antigen.
  - ADCP: From this variable we obtain a potency result that is part of the performance of the drug in a living organism. Compatibility with the rest of the variables and the scope will be studied.
  - CDC: From this variable we obtain a potency result that is part of the performance of the drug in a living organism.
  - Apoptosis: The scope of this variable should be studied since not all drugs have the same function. It will be studied how to assess in case of not having influence on all antibodies.
  - *Binding*: Variable that highlights the binding capacity against an antigen, its influence on the antibody structure and its effect against potency values will be determined.
  - Union FcgR: It responds to binding specificity criteria and is directly related to *binding* and potency. The scope of this variable in a biosimilarity study should be clarified.

- Union C1q: Variable relating one of the functions of antibodies in living complement-binding systems. Being a characteristic not present in all antibodies, its relevance and how to include it in the final equation developed during the research will be studied.
- Union FcRn: It also responds to binding specificity criteria and is directly related to *binding* and potency. The scope of this variable in a biosimilarity study should be clarified.

- Impurities:
  - Host impurities: Since this is one of the characteristics that all parenteral pharmaceutical products have in common, we will study whether its inclusion is relevant when determining whether a monoclonal antibody is a biosimilar. Within this variable are:
    - hcDNA, which refers to the DNA fragments of the host cell that produces the monoclonal antibody. It is measured in particles per billion by PCR and its values must be negligible to be approved. Its elimination occurs in the different stages of purification of a monoclonal antibody.
    - Insulin: a molecule necessary in the development of a culture, but which may not be present in the final formulation.
    - Protein A: Protein used in the first chromatographic columns in the purification of an antibody due to its high affinity to regions of the antibody and used to remove the remaining residues from a culture. Treatment of these columns to remove the antibody may result in the release of protein A and is immunogenic. Therefore, it is eliminated in subsequent treatments.
    - HCP: proteins that are part of the structure and metabolism of host cells. The values of this variable should be negligible in the final form of a biological drug.
  - Leachables/Extractables: impurities that have an adjuvant effect on folding. Its influence and the techniques that detect it should be studied, since they are valued in the critical quality parameters.
- Protein concentration: This variable will be studied in the influence of the clinical phase, since the concentration that the drug must have in market format must be identical to that of the innovator to be compared. It is a value included in the technical dossiers of the products, as well as in the medical vademecums.

B. Quality variables by structure

- Variable region attributes: This variable will be studied together with the parameters detected during the investigation that are directly related to this region of an antibody.
- Attributes constant region: The relevance of this region of the antibody for the characterization of a biosimilar will be studied. Quality analyses will be determined for this region in order to establish the critical quality attributes.
- Physicochemical characterization: This variable will be part of the critical quality attributes characterization matrix. Different levels will be determined by means of statistical techniques or Tier ranking.

- Biological and functional characterization: This variable will be used for the development of the critical quality attributes matrix. Its value will be determined by statistical methods or Tier ranking during the development of the research.

C. Other non-structural quality variables

- Osmolarity: the concentration of the protein in the solution and the rest of the components that make up the buffer in which the active ingredient is embedded can act on the rest of the quality variables, making this comparison indispensable. In proteins where the composition may be comparable, buffer changes may cause the properties to be altered.

From the study of the individual quality attributes and their analytical techniques, it was found that there is an intrinsic error in the techniques and in the laboratories themselves, which are not considered as part of the relevance of the attributes from a statistical point of view:

- Laboratory error: This variable will be developed based on the available methodologies and adapted to the needs of the analysis and development laboratories of the biotechnology industry under GxP methodology. It will act as a modulator of the analysis of each critical quality parameter selected and based on the techniques used to obtain them.

Within this variable, it was found that they should be assessed from two points of view:

- Method errors: The difference between methods that have automation versus methods that are mainly manual or whose critical analysis steps depend on human interaction will be studied
- Uncertainty of the method: The systematic error or bias present in the design of the analytical techniques or in the instrumentation used to assess the attributes will be studied, as opposed to the random error resulting from unpredictable causes and from working with population samples.

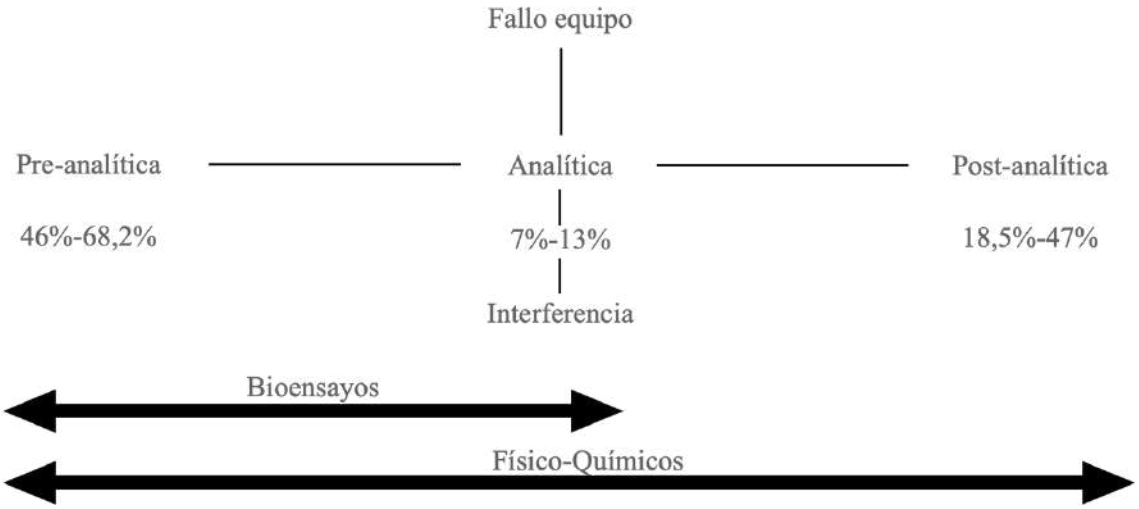

Figure 2. Errors in laboratory tests.

The study of the classification of the quality attributes has been developed taking into account the three main normative guidelines established for the assessment of the biosimilarity of a monoclonal antibody. Since the consideration of biosimilarity must be accompanied by comparability, these attributions have been taken into account at the time of the assessment.

The guidelines established by the major regulatory agencies with international prestige establish qualitative principles for assessing the statistical relevance of the quality attributes of a monoclonal antibody. Taking into account that these agencies have similar regulations and that they are recognized by other agencies and associations as the basis for establishing the internal standards of their respective countries, the study began with a comparison of the major standards and their points in common. The World Health Organization establishes in its international guidelines the following principles as critical points for the establishment of biosimilarity of a monoclonal antibody:

- The physicochemical properties including the structural properties of the antibody
- The biological activity of the antibody
- Impurities present in the antibody preservation medium
- The immunochemical properties of the antibody against the target organism
- and technical specifications of innovative antibody

For their part, the European Medicines Agency and the *Food and Drug Administration* of the United States establish certain attributes for physicochemical properties as critical, in particular, when establishing the characteristic of biosimilarity. Among these characteristic attributes would be the structure and function of the antibody, impurities, class and subclass, amino acid structure, N- and C-terminal amino acids, disulfide bridges, carbohydrate content and other post-translational modifications that may influence the structure of the antibody. *In vitro* assays carry great weight in most agencies such as those mentioned above, since binding capacity and binding strength of antigen and antibody form a major part of *in vitro* studies for biosimilarity. Another common point presented by all the agencies is that the purity of the antibody, in terms of its composition, the presence of impurities in its medium and the absence of contaminations are critical in patient safety.

It can be concluded that in order to determine the biosimilarity of a monoclonal antibody, it is not only possible to opt for a statistical quantification of the relevance of each attribute measured in the Certificates of Analysis (CoA) and which therefore form part of the final specifications of the monoclonal antibodies, but also to have the attributes widely recognized by government agencies so that the equation, and therefore the ITEM of quality attributes that is being developed, is widely recognized and admitted.

The study of the quality attributes item was developed taking into account the three fields analyzed in the methodology. Within these fields are the quality attributes recognized by the scientific literature, the quality attributes considered critical by international regulatory agencies for the establishment of biosimilarity and biocomparability, and the technical specifications of marketed monoclonal antibodies for which relevant documentation is available.

The following results were obtained for the quality attributes recognized by the scientific literature.

The studies developed to establish the critical quality attributes in monoclonal antibodies intended to be a biosimilar antibody are based on establishing qualitative criticality to their different attributes. In the studies of Kwon et al. (23), a score of 3º of confidence is established for the primary structure and higher structures, charged variants, mass variants, oligosaccharides, biological attributes and content. the different degrees of criticality granted by this score for the different impacts that may be suffered on the biological or clinical function of the drug itself do not take into account the analytical methods by which this criticality can be determined by carrying out a more detailed study of each of the attributes that belong to the classification developed by Kwon et al (23), it can be seen that the same impact on the biological or clinical function is attributed a different criticality even though the same analytical technique can yield specific results.

Table 1 shows the comparison between the variables to be taken into account for a score of the criticality of a quality attribute versus an impact and the analytical method most commonly used in the pharmaceutical industry to obtain specific results.

Table 1. Relationship of quality attributes and their criticality from a qualitative point of view.

Quality attribute	Criticality	Impact on biological/clinical function	Analytical method
Structure 1st			
Amino acid sequence	+++	Power	
N-terminal Pyroglutamate	++	*In vivo* pharmacokinetics	Peptide mapping, Edman degradation
C-terminal lysine elimination	+	No influence on biological activity *in vivo*	
Bisulfate bonds	+++	Power	
Higher order structure	++	Potency and receptor-antigen binding	FT-IR spectrophotometry, fluorescence, circular dichroism
Variants loaded			
De-rolled shapes	+	Biological activity *in vitro*	Liquid chromatography under IEX, IEF, CE, HPAEL-PAD, Mass techniques.
Oxidized forms	++	Immunogenic aggregates	
Sialized forms	+++	*In vivo* thinning or clearing	
Mass variants			
Aggregates	+++	Immunogenicity	Liquid chromatography under SEC, Mass, HIC techniques. SDS-PAGE
Truncated shapes	+	Biological activity	
Monomers	+	Biological activity	
PEGilations	+++	*In vivo* clearance	
Oligosaccharides			
Fucose or galactose	+++	Influence on Fc-effector activity	HPAEL-PAD, Liquid Chromatography (LC), Capillary Electrophoresis (CE), Mass Spectrometry (LC-MS)
Non-human glycans	+++	Immunogenicity	
High-mannose glycans	++	Immunogenicity	
Non-glycosylated forms	++	Influence on Fc-effector function, ADCC and clearance *in vivo*	
Biological activity			
ADCC	+++	Mode of action	Cell-based assays, ELISA, SPR
ADCP	++	Mode of action	
CDC	+++	Mode of action	
Apoptosis	++	Mode of action	
Antigen-Antibody Binding	+++	Mode of action	
Union FcγR	++	ADCC	
Union C1q	++	CDC	
Union FcRn	++	*In vivo* clearance	
Content	+++	Pharmacokinetics	UV-Vis spectrophotometry

Note: Consideration of critical quality attributes in the assessment of analytical comparability of biosimilar products (23)

As can be seen in the table above, the classification of quality attributes in terms of their criticality is based on their impact on biological or clinical function. None of the studies carried out to test the criticality of the quality attributes of monoclonal antibodies in the organism, whether by in vitro, in vivo or in silico methods, take into consideration the different interactions that may occur between the quality attributes themselves, the medium in which the drug itself is embedded, and the problems associated with the performance of laboratory tests.

The pharmaceutical industry establishes critical quality attributes against the target antigen profile. This profile is catalogued in a specific study called QTPP (*Quality Target Product Profile*). It should be considered a compelling reason why the assays included in the QTPP should have greater weight than other assays that do not influence this profile.

It is also necessary to consider the presence or absence of anti-drug antibodies, known as *anti-drug antibodies* (ADAs), as part of a detailed study of the critical quality attributes for monoclonal antibodies.

During the development of the concept of the quality variable item or critical quality attribute coined in this dissertation work, the need to classify the different attributes used in the fields of biosimilarity assessment and comparability of a monoclonal antibody against its reference molecule versus the attributes themselves was observed. Table 2 breaks down which attributes favor biosimilarity and which attributes favor comparability.

Table 2. Classification of attributes with respect to their biosimilarity or comparability

Demonstrate biosimilarity	Demonstrate comparability
Primary structure	Analytical studies
Higher order structures	Non-clinical studies
Immunochemical properties	Pharmacological clinical studies (PK/PD)
Attachment to the receiver	Clinical safety
Stability	Clinical efficacy
Biological function	Immunogenicity
General properties of the antibody	
Excipients	

On the other hand, during the study of the different quality attributes that were to be called items for the evaluation of the definitive critical quality attributes in the conception of the biosimilar concept, it was required to establish, in comparison with the studies prepared by the European Medicines Agency, the U.S. Medicines Agency and pharmaceutical industry associations such as Fimea, regions within the superior structure of the monoclonal antibody that influence each of the characteristics and attributes.

For this reason, when faced with a simplified structure of the antibody, it is established which attributes are of greater relevance in the case of the absence or presence of modifications in the biosimilar antibody compared to the innovator antibody.

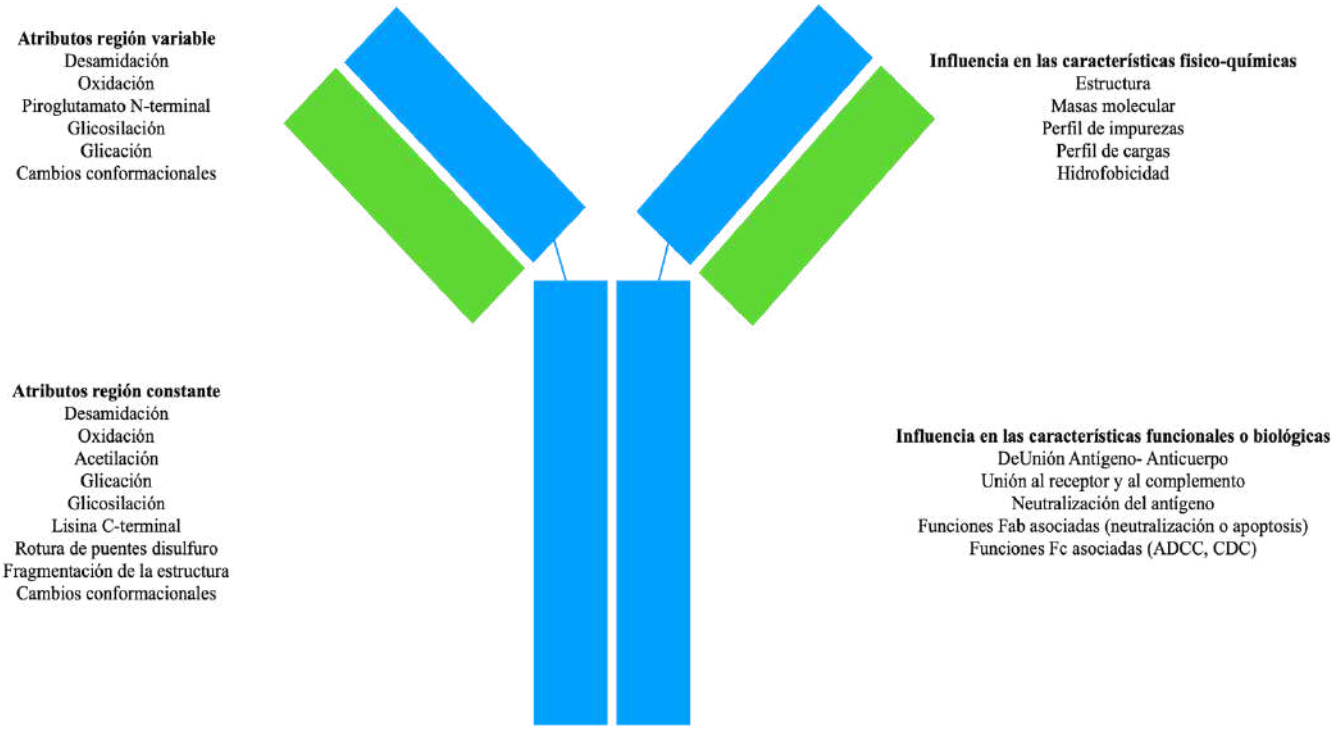

Figure 3. Antibody-attribute relationship

Therefore, it can be concluded that the variables are currently studied individually and not considered as a whole within the definition of biosimilar. A new structure of the definition should be adopted to bring together all the concepts, the proposed unit being the quality ITEM, since it should be composed of the quality attribute and the laboratory error.

Discussion and conclusions

The current status of biosimilars reveals a constantly evolving scientific landscape, supported by regulatory advances and technical developments that have enabled the simplification of analytical techniques as well as the representation of results. Definitions, whatever the concept, must allow a clear and precise understanding

of what is being explained. In the case of biosimilars, the current definition presents a qualitative basis that allows free interpretation of the results for both pharmaceutical laboratories and regulatory agencies. This range of possibilities, radically opposed to the conception of a definition, does not allow the biosimilar term to be understood without prior knowledge of the subject. In turn, the definition is focused on the characterization of critical quality attributes on an individual basis. The error in the conception of the current definition of biosimilarity stems from its comparison to a generic drug, which can be directly compared by therapeutic dose and active ingredient. In contrast, in biosimilarity, the drug interacts with a living organism and it is part of this living organism that performs the therapeutic functions, which requires not only pharmacological but also immunological factors.

Scientific advances have made it possible to establish a rigorous connectivity between the critical attributes and biological functions of a living organism. For this reason, the concept of biosimilarity must go through the process of restructuring to the concept of item promulgated in this document, since it must be assessed in a globalized manner and not in an individualized manner. The restructuring of the definition of biosimilar should not stop exclusively at the critical quality attributes, but should take under the term item, other variables of great relevance for the conception of a definition of greater accuracy and quantifiable basis, to establish in a single sentence the reality of the functioning of a biological drug in an organic system with which it interacts.

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Physical and nutritional properties of eight types of potatoes
Aptitudes físicas y nutricionales de ocho tipos de patata

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ABSTRACT

In this research, measurements and analyses of different characteristics were carried out on eight types of potatoes. The vitamin C content was analysed by the Indophenol method, the dry matter content was determined by ash determination, the sizes were measured with ring ranges to determine their size and classified into medium and large sizes. In addition, the external appearance and colouring of the flesh was visually judged by differentiating between rounder or oval shapes and white or yellowish colours. A tactile analysis was also carried out to determine whether the samples had a firm and consistent texture. Among the results obtained, it was found that the Monalisa potato had the largest size of 67 mm, the Gallega potato was the only one with a white flesh colour and the Gallega and Agria potatoes were the only ones with large and deep eyes. The Valderredible potato excelled, as it had the highest values in terms of dry matter content, vitamin C and presented a firm and consistent texture, which makes it a favourable choice in terms of quality.

Palabras clave:
patatas, materia seca, almidón, vitamina C, calibre.

RESUMEN

En este estudio experimental, se realizaron mediciones y evaluaciones de diferentes parámetros en ocho tipos de patatas. Se analizó el contenido de vitamina C utilizando el método del Indofenol, se determinó el contenido de materia seca mediante la determinación de cenizas, se midieron los calibres utilizando rangos de anillas para determinar su tamaño y se clasificaron entre medianas y grandes. Además, se evaluó visualmente el aspecto exterior y la coloración de la carne diferenciando en formas más redondas u ovaladas y colores blancos o amarillentos. También se realizó un análisis táctil para determinar si las muestras tenían una textura firme y consistente. Entre los resultados obtenidos, se encontró que la patata Monalisa presentó el mayor calibre de 67 mm, la patata Gallega fue la única que con un color de pulpa blanco y las patatas Gallega y Agria fueron las únicas que presentaron ojos grandes y profundos. La patata Valderredible destaco, ya que tuvo los valores más altos en cuanto a contenido de materia seca, vitamina C y presento una textura firme y consistente, lo que la convierte en una opción favorable en términos de calidad.

Introduction

The potato Solanum tuberosum L. is a herbaceous plant that produces tubers for vegetative propagation. Tubers, commonly called potatoes, are protrusions of the subway stem, also called rhizome or subway stolon (1). It is one of the most important food crops for food security, with high levels of production and consumption worldwide, mainly in developing countries. Its importance is due to its high yield, high cost efficiency and good nutritional value containing an important source of essential nutrients (2). In addition, potatoes are very versatile, due to their multiple culinary uses. They can be cooked, fried, dehydrated or used as ingredients in processed foods. This made potatoes a staple food in many cultures, having a much higher production growth than other tubers (2). Its production is widely distributed in more than 160 countries and its cultivation area reaches 19 million hectares (3). In addition, it is estimated that it provides food for more than one billion people. It is one of the most important crops worldwide, with a production of more than 376 million tons (3).

1.1 Health Benefits of Potatoes

Potatoes provide significant amounts of various vitamins, minerals and phytochemicals. Potatoes are rich in calcium, potassium, magnesium, phosphorus, and several B vitamins. A cooked potato provides a significant amount of potassium and magnesium, which are important for cardiovascular and muscular health, providing 544 mg of potassium/100 g and 27 mg of magnesium/100 g, which represents 12% of the recommended adequate intake of potassium (4). Potatoes are an important source of vitamin C, containing an average of 20 mg per 100 g, accounting for about 20% of dietary intake in Europe (5). Vitamin C or chlorogenic acid is present in potato as carotenoids and phenolic compounds (PC) (6). The amount of carotenoids varies between 50 and 100 µg per 100 g fresh weight in white-fleshed potatoes and 2000 µg per 100 g fresh weight in orange to yellow-fleshed potatoes (6), these confer antioxidant capacity (7). Potatoes have been shown to scavenge 94% of hydroxyl radicals due to their flavonoid and flavone content, which have a high oxygen radical scavenging capacity (8). Potato skins are particularly rich in phenolic compounds and anthocyanins, especially in the outer 1 mm layer of the skin (9). These compounds are known for their beneficial health effects, including reducing the risk of chronic diseases such as cancer and cardiovascular disease (CVD) (10). FCs reduce CVD risk factors by reducing platelet activity, reducing anti-inflammatory effects and protecting against oxidation (10). Notably, the composition of phenolic acids and anthocyanins in potato was found to vary depending on the potato and the place of cultivation, suggesting that these compounds may be influenced by environmental factors (11). Therefore, it is important to consider the origin and type of potato.

There are more than 4,000 varieties worldwide that have significant differences in the contents of macronutrients and micronutrients, so attention must be paid to biodiversity (12)therefore, it is important to pay attention to biodiversity. The 4,000 varieties are the result of thousands of years of evolution, where multiple hybridizations have taken place between different species of plants of the Solanaceae family. The first potatoes were cultivated some 6,000 - 10,000 years ago in the Andes mountain range (13). Subsequently, they spread throughout Latin America and after the arrival of

Columbus in 1492, they spread to Europe and finally to the rest of the world. Over the past 150 years, farmers have tried to develop potato cultivars that are earlier maturing, smoother tubers, more disease resistant, and of better quality from a processing point of view by hybridizing between different varieties, resulting in many varieties (14).

1.2 Main characteristics of potatoes

In the composition of the potato, the carbohydrate content stands out, being one of the vegetables with the highest caloric content (88 kcal/100 g of potatoes), mostly in the form of starch and a small proportion as glucose, fructose and sucrose (15). A medium potato (148 g) contains 4 g of high quality protein, having a particularly good amino acid balance, as it contains recommended amounts of 4 of the 9 essential amino acids, and is the only staple food that meets the recommended level of lysine (16).

Variety, size, color, texture, dry matter and vitamin C content are the main quality parameters in potatoes, conditioning their use and acceptability in the market (17)and their acceptability in the market. Harvest time and post-harvest storage are critical as they have a direct impact on quality (18). The dry matter content of potatoes represents a criterion of nutritional or energetic value, mainly because the dry matter content corresponds to between 60% and 80% of the starch content (19). 80% of the weight of the potato is water, while the rest (20%) is dry matter, of which starch makes up 60-70% (20). Dry matter content is a key quality determinant in potatoes because it influences the flavor, aroma, texture, shelf life and cookability of potatoes (21).

1.3 Potato quality parameters

Potatoes are classified by size or caliber, which will be given by the length in millimeters of the side of the grid of a square mesh. Depending on the size range, potatoes are considered small if they have a diameter of less than 30, medium if they have a diameter of 30 to 60 mm and large if they are larger than 60 mm. Texture is the resistance of the tuber to an applied force and varies according to the cellular structure of the potato (22). The texture will depend on the interaction of several factors such as the structure and composition of the cell wall, whether the potato is raw or cooked, the starch content, and the shape and size of the starch granules (22). Texture is a quality determinant as firmness is an important textural attribute in potatoes because firmness is used to assess quality and freshness during storage and marketing (23). The hardness and firmness of raw potatoes decrease progressively with post-harvest storage as the starch is converted to reducing sugars, over time the reducing sugars accumulate and the starch is depleted (24).

The color of the skin can vary from white to violet red, this is due to the type of pigments present in the cells of the periderm (25). In general, except in the case of certain varieties, most of the varieties destined for the market are grouped into white, yellow and red skinned tubers. The texture of the skin, the shape and the presence of eyes can be distinctive features that may or may not attract consumers, however it is known that consumers tend to associate a skin of bright and uniform color and shape, without spots or discolorations, with greater freshness, indicating an optimal state of maturity and quality (26). As for the color of the internal matter (medullary tissue and reserve vascular parenchyma), generically known by the term "flesh," they are usually grouped into two blocks: white and yellow flesh (27). In each case, it can be accompanied by a second term

referring to the shade (creamy, light or dark). The color of the tuber peridermis does not determine the intrinsic quality of each variety, but it does influence consumer preference for quality (28). According to different studies, the culinary quality of potatoes is often associated with the color of the peridermis (29). Potatoes with uncolored peridermis generally have low dry matter contents. On the contrary, potatoes with yellow peridermis would be recommended for processing, due to their high dry matter content and low concentrations of reducing sugars (30).

The objective of this study is to analyze and describe "Monalisa", "Gallega", "Valderredible", "Agria", "Spunta", "Baraka", "Jaerla" and "Kelly" potatoes, measuring size, flesh color, external appearance, dry matter and vitamin C content.

Method

2.1 Evaluation of physical and nutritional aptitudes

From November 2021 to February 2022, 8 types of potatoes were evaluated, taking 30 samples of each type. The potatoes evaluated were: "Monalisa" 30 units, "Gallega" 30 units, "Valderredible" 30 units, "Agria" 20 units, "Spunta" 20 units, "Baraka" 20 units, "Jaerla" 20 units and "Kelly" 20 units.

To determine size square rings of the diameter corresponding to the potato to be measured were used, with a range of diameters from 20 mm to 80 mm, with 5 mm intervals. To characterize the physical aptitudes began with an evaluation of flesh coloration, which was carried out by 4 trained evaluators by 4 trained evaluators, who used a reference color scale with the following options: whitish, yellow, and intense yellow. The same panel of assessors made a description of the external appearance of the potatoes, distinguishing whether the samples were round to oval, elongated oval or rounded. Finally, they made a discriminative classification, differentiating the samples that presented deep and large eyes, and the samples that had a firm and consistent texture.

To characterize the nutritional properties, the vitamin C content was analyzed using the Indophenol method (31). This titration method based on the redox reaction between ascorbic acid and 2,6-dichlorophenolindophenol gave the amount of vitamin C present in potatoes. Dry matter was measured using a gravimetric method for ash determination (32). The procedure consisted of subjecting the samples to drying by heating, reaching a temperature of between 103 and 105 °C for one hour, and then calcining the sample in a muffle at a temperature of 550 °C until grayish ashes were obtained, thus obtaining the dry matter.

2.2 Vitamin C analysis by the 2,6-dichlorophenolindophenol titration method

The process began by extracting ascorbic acid from the potato sample using an oxalic acid solution. Vitamin C oxidizes very easily, so to prevent oxidation of the before titration add metaphosphoric acid to the sample (33). Then, titration was performed with 2,6-dichlorophenolindophenol solution, the reagent was added dropwise with a burette until a very pale pink color persisted in the sample added (33). The amount of reagent consumed was recorded during the titration. The use of 2,6-dichlorophenolindophenol as a reagent allowed an accurate and sensitive measurement of ascorbic acid concentration,

since in the presence of ascorbic acid an observable color change is generated which facilitates quantitative determination (33). This method is suitable for the determination of vitamin C due to its ability to measure the amount of ascorbic acid present in a sample. Ascorbic acid is an active form of vitamin C and has reducing properties that allow its detection and quantification using redox reactions (34). Ascorbic acid (C6H8O6) is oxidized to dehydroascorbic acid (C6H6O6), while DCPIP or iodide is reduced to DCPIPH2 or iodide, respectively (35). This method uses standard solutions and standard curve, which allows the precise quantification of the ascorbic acid concentration.

For the preparation of the sample, 100 g of potato were peeled and cut into pieces, which were crushed to obtain a homogeneous sample. 1 g of potato was weighed and placed in a beaker, into which 10 mL of metaphosphoric acid was added and allowed to stand in the dark for 10 minutes to allow extraction of the ascorbic acid. After this time, a Kitasate flask and a vacuum pump were used to filter the sample, thus obtaining a clean extract. This filtered extract was then transferred to a 100 mL volumetric flask and volumetrized to a final volume of 100 mL using distilled water. For the ascorbic acid stock solution, 2 mg of ascorbic acid was dissolved in 100 mL of water, creating a concentration of 2 mg/100 mL. From this stock solution, different standard solutions were prepared, e.g., for solution I, 50 mL of the ascorbic acid stock solution, 10 mL of metaphosphoroacetic acid, and made up to 100 mL with distilled water were added to a final volume of 100 mL. Similar processes were followed for solutions II, III, IV and V.
In the Titration of the standard solutions, a burette was filled with the titrating solution of 2-6-dichlorophenolindophenol. Then, titration was performed for each standard solution, slowly adding the DCPIP titrating solution until the sample acquired a very pale persistent pink color. The volume of DCPIP consumed in each titration was recorded for each standard solution, using these data to construct the standard curve, representing the volume of reagent consumed versus ascorbic acid concentration. In the Titration with 2,6-dichlorophenolindophenol, a burette was used to titrate the potato sample, adding the DCPIP solution dropwise until the sample turned a very pale persistent pink color. The volume of DCPIP solution used was recorded, and this data was used to calculate the concentration of vitamin C in the potato sample.

2.3 Determination of ashes by gravimetric method

Total ash analysis was used as the basis for determining the dry matter content of foods (36). The total ash method consisted of incinerating a precise and weighed sample of the food in a crucible resistant to high temperatures, using a muffle at temperatures between 500 and 600°C (58). During incineration, the organic matter decomposed and was transformed into carbon dioxide (CO2) and water vapor, while the constituent minerals remained in the form of oxides, sulfates, phosphates, silicates and chlorides. The amount of ash obtained after incineration is related to the content of inorganic minerals present in the sample. By subtracting the weight of ash from the initial weight of the sample, the weight of organic matter in the potato can be obtained. By dividing this weight by the initial weight of the sample and multiplying by 100, the percentage of dry matter in the potato can be calculated (36).

For moisture percentage determination, the empty crucible was preheated in an oven at 103 °C for 1 hour and cooled in a desiccator. The empty crucible was weighed on an analytical balance (m0). The potato was peeled and cut into pieces to be mashed to have a homogeneous sample (100 g). 5 g of potato sample (m1) was weighed and placed

in the crucible. The crucible with the sample was placed in an oven at a temperature of 100-105 °C for 1 hour. After cooling in a desiccator, the crucible with the residue was weighed. For the determination of the ash percentage, the crucible with the sample was calcined in a muffle at a temperature of 550 °C until white or grayish ashes were obtained. The crucible was cooled in a desiccator and weighed (m2). The percentage of ash was calculated using the formula: % ash = [(m2 - m0) / m1] × 100, where m0 is the mass of the empty crucible and m1 is the mass of the sample.

Results

The mean values obtained from the size analysis of each type of potato are shown in Table 1. The results showed that all the potatoes were of different and varied sizes, with a difference of 13 mm between the largest and the smallest. Monalisa presented the maximum size with an average of (67 mm), followed by Baraka (63 mm) and Gallega (62 mm). These three are of large category since they have a caliber greater than 60 mm. The rest of the potatoes are considered medium, with sizes between 52 and 59 mm. None of the potatoes measured were less than 30 mm in size, so none of them are small potatoes. Once the results of the samples were available, the mean dispersion was calculated to see if the value obtained was representative for the type of potatoes evaluated. Kelly, Jaerla and Baraka potatoes showed the lowest dispersion around the mean with a value very close to 1. Galician and Valderredible potatoes are of moderate dispersion with 1.5 to 1.8. Finally, Monalisa and Spunta showed a high dispersion around the mean with values of 2.5 and 2.8. Indicating that, with the exception of the last case, the results of the average size for each type of potato that were obtained from this study are representative of the size of each type of potato.

Table 1 *Mean values of size, dry matter and vitamin C of each potato sample*

Potato	Caliber (mm)	Dry Matter (%)	Vitamin C (mg/100g)
Galician	62 (+/-8,26)	19,09 (+/-2,64)	20,54 (+/-1,05)
Monalisa	67 (+/-8,06)	14,88 (+/-2)	15,81(+/-1,09)
Valderredible	59 (+/-8,2)	22,48 (2,69)	24,91 (+/-2,23)
Sour	56 (+/-6,04)	21,58 (+/-1,16)	29,62 (+/-3,33)
Spunta	52 (+/-5,72)	20,11 (+/-2,14)	21,82 (+/-1,63)
Baraka	63 (+/-3,17)	21,81 (+/-1,82)	17,92 (+/-1,63)
Jaerla	53 (+/-5,15)	19,7 (+/-2,56)	20,26 (+/-1,16)
Kelly	58 (+/-4,97)	17,02 (+/-2,09)	20,35 (+/-1,16)

The potatoes with a predominance of yellow color were Monalisa, Baraka and Kelly, since all the samples evaluated showed a yellow color in the flesh. Spunta presented 45% of samples with a yellow color in the flesh. The potatoes with a predominance of intense yellow color were Agria, Valderredible and Jaerla, with 100%, 94% and 65% of samples with intense yellow color, respectively. The Galician potato was the only one with a predominance of whitish color, with 89% of the samples having this color. Spunta and

Jaerla potatoes obtained less definitive values of 45% and 65%, respectively, which means that there may be variation in flesh color in these varieties.

The rounded shape is the most common, representing between 40% and 60% of the samples. Elongated oval and round to oval are also present in different proportions, ranging from 20% to 50% in most of the potatoes evaluated. It was observed that Agria, Baraka, Jaerla and Monalisa potatoes had a higher proportion of potatoes with rounded shape. Valderredible and Spunta potatoes showed an equal distribution between round and elongated oval shapes. Kelly potatoes were distinguished by having more potatoes with an elongated oval shape. Finally, the Galician potato showed a balanced distribution between round and rounded shapes. After taking all the measurements and analyzing whether there was any relationship, they determined that there is a strong relationship between volume and diameter. Monalisa, Valderredible and Kelly potatoes have a majority of elongated oval potatoes, which were considered the largest in diameter. These same potatoes occupied the first, second and fourth largest sizes among the potatoes evaluated. The same could be said for Jaerla and Agria potatoes, which have a majority of round potatoes, which were considered to be the smallest in diameter. These occupy the second and fourth smallest sizes according to their caliber in mm. In this case, this study also found a moderate relationship between diameter and volume.

Gallega and Agria potatoes were the only ones with deep, large eyes. The Galician potato, of the 30 potato samples, 5 samples or 16.67% of the potatoes were found to have deep and large eyes, while 25 samples or 83.33% of the potatoes did not have these characteristics. The Sour potato was an interesting case since, in the study of the 20 samples, it was found that 50% of the potatoes had deep and large eyes, while the other 50% of the potatoes did not. This indicates that there is some variability in the characteristic of deep and large eyes within this potato. Monalisa, Spunta, Baraka, Jaerla, Kelly and Valderredible potatoes did not have any samples with deep, large eyes, these results indicate that these potatoes tend to have a smoother, more uniform appearance.

It was determined that Monalisa and Baraka potatoes do not tend to have a firm and consistent texture. While Valderredible, Spunta and Kelly potatoes had a high proportion of samples with firm and consistent texture. Finally, Gallega, Agria and Jaerla potatoes showed an equal distribution between firm and consistent and non-firm and consistent samples, indicating that these potatoes may have different textures.

The potatoes with the highest dry matter percentage according to their mean were Valderredible potatoes with 22.50%, Baraka with 21.81% and Agria with 21.77%. On the other hand, the potatoes with the lowest percentages were Monalisa with 14.88 and Kelly with 17. To check whether the mean value of dry matter percentage of each type of potato is representative, a mean deviation analysis was performed. Gallega and Monalisa potatoes showed the greatest variability and dispersion of dry matter percentages with values of 2.86 and 4.1 respectively, although this is considered a moderate mean deviation. Agria, Spunta and Kelly potatoes presented a low mean deviation, showing the highest consistency in the data with 1.25, 1.79 and 1.80 respectively. This indicates that the mean values obtained can be considered representative of the dry matter content of the potato types analyzed. A lower starch content (16-18%) results in waxy potatoes; whereas floury potatoes have a higher starch content (20-22%). Under this criterion, of the 8 types of potatoes analyzed in this study, Monalisa and Kelly would be considered waxy and the rest floury.

Agria, Valderredible and Spunta potatoes showed values of 29.62 and 24.91, 21.82 mg/100 g of sample respectively, having high vitamin C contents. While Monalisa and Kelly have very low vitamin C values, with 5 and 3 mg/100g less than the average of 20 mg/100g, having 14.88 and 17.02 respectively. with an average value of 20% Vitamin C per potato. This study confirms this data as an average value for different potato cultivars, highlighting Agria, Valderredible and Spunta potatoes that exceed the average. Monalisa and Kelly's potato, potato below average. Taking into account how much the average vitamin C content varies according to potato type, it is important to analyze whether the results of this study are representative for each type of potato. For this reason, a mean deviation analysis was performed, in which it was found that Spunta, Baraka and Jaerla potatoes presented a low dispersion, with values very close to 1 indicating that these potatoes had the same amount of vitamin C mg/100 g fresh weight consistently. The rest of the results obtained for the other types did not deviate much from the mean, having moderate values of less than 2, so they can be taken as representative data of the percentage of vitamin C for each potato.

Discussion and conclusions

The results obtained are used to classify the potatoes evaluated within the quality ranges established above (37)being: small: <=30 mm, median: 30 - 60 mm and large: >60 mm. An effort was made to find studies or databases that had information on the sizes in millimeters for the types of potatoes evaluated, in order to compare results, but none could be found, since they did not give specific values in mm. For this reason, this study is useful to have a measurement in mm of the potatoes evaluated. This is determined by genotype, harvest conditions, post-harvest and ripening time. Leading to the thought that a variety of other genetic, environmental and agricultural factors, including genotype, seed size, plant density in a crop and number of stems per plant affect the growth and yield of different potatoes.

The potatoes with predominant yellow color were Monalisa, Baraka and Kelly, since all the samples evaluated showed a yellow color in the flesh. Spunta presented 45% of samples with a yellow color in the flesh. The potatoes with a predominance of intense yellow color were Agria, Valderredible and Jaerla, with 100%, 94% and 65% of samples with intense yellow color, respectively. According to these results it can be seen that the color of the flesh of each potato changes according to its type. This is determined by genotype, harvest conditions, post-harvest and ripening time. It is important to note that the higher the color intensity, the higher the amount of carotenoids present. This could explain, for example, why Agria and Valderredible potatoes, which have predominantly deep yellow flesh, have the highest vitamin C values. Similarly, Gallega, which is usually white in color, is the third potato with the lowest vitamin C content. This could explain, for example, why Agria and Valderredible potatoes, which have predominantly intense yellow flesh, have the highest vitamin C values. Similarly, the Gallega, which is usually white in color, is the third potato with the lowest vitamin C content. However, more rigorous and specific tests on each sample would be needed to establish a direct relationship.

This study confirms this data as an average value for different potato cultivars, highlighting Agria, Valderredible and Spunta potatoes, which exceed the average vitamin C value of potatoes, which is 20%, according to a group of researchers in Spain in 2002 (38). The study consisted of analyzing the loss of Vitamin C in 5 different types of potatoes, changing factors such as access to different gases or changing temperatures. Although before subjecting the potatoes to the different changes, they noticed that their potatoes had an average of 19.7 mg/100g of vitamin C per potato.

It was observed that Agria, Baraka, Jaerla and Monalisa potatoes had a higher proportion of round potatoes had a higher proportion of potatoes with rounded shape. Valderredible and Spunta have an equal distribution between round and elongated oval shapes. Kelly potatoes are distinguished by having more potatoes with an elongated oval shape. Finally, the Galician potato shows a balanced distribution between round and rounded shapes.

There are few studies on eyes in potatoes, but a study in the American Journal of Potato Research in 2022 reports that they facilitate the exchange of gases between the atmosphere and the interior of the potato (39). Research is presented that demonstrates interactions between the presence of eyes and certain diseases, although it is not very clear. The European Potato Journal, for example, published a study in 1965 announcing that no obvious relationship was found between eye structure and susceptibility to infection in relation to skin and eye structure, since in the nine potatoes examined, no significant differences in susceptibility to disease were shown (40). This would indicate that the presence of deep-set and large eyes is more related to consumer preferences at the time of purchase. This explained the 2008 U.S. study, which said that a product with good appearance, uniform size and shape will be preferred by most consumers and will have a greater sales appeal (41).

Valderredible, Baraka and Agria potatoes showed above average dry matter content, making them favorable choices for culinary and industrial uses. A high dry matter content is one of the most important values when choosing a potato. One of the first studies to determine the cooking quality of potatoes, conducted in 1937 by the official American potato magazine, states that the quality of potatoes is directly associated with the dry matter content, so that a high dry matter content of 25% is associated with "good quality" and a low dry matter content of 15% is associated with "poor quality" (42). Other studies of the time found similar results, noting that after chemical and cooking tests they concluded that good cooking quality is closely associated with high starch and dry matter content and low nitrogen content (43). However, nowadays, this issue is no longer so widespread, since a potato with a low dry matter content may have other nutritional or culinary qualities and can be used and exploited in a different way. For example, good quality can be given by high vitamin C contents, as is the case of Agria, Valderredible and Spunta potatoes have high vitamin C values, with values of 29,62mg/100g and 24.9mg/100g, 21.82mg/100g, respectively.

Gallega, Agria and Jaerla potatoes showed an equal distribution between firm and consistent and non-firm and consistent samples, indicating that these potatoes may have different textures, which may be explained by post-harvest storage and storage during marketing, which has a considerable effect on the texture of a potato (44). The longer the time and the extreme temperatures the starch is converted into reducing sugars, resulting in loss of texture and therefore quality.

In conclusion, this study analyzed eight types of potatoes and described their physical and nutritional characteristics. It was found that potato size is not related to the number of planting days, but rather to genetic, environmental and agricultural factors, such as genotype, environment or soil type.

Valderredible, Baraka and Agria potatoes showed above average dry matter content, making them favorable choices for culinary and industrial uses. Agria, Valderredible and Spunta potatoes presented high levels of vitamin C, which is important considering that its content is reduced after cooking or processing. As for potato flesh color, it was observed that potatoes with an intense yellow color, such as Agria and Valderredible, showed the highest vitamin C values. However, flesh color is not a reliable indicator of dry matter content or antioxidant capacity. In terms of shape and size, a possible relationship between potato diameter and size was found. Elongated oval potatoes, such as Monalisa, Valderredible and Kelly, were the largest. Gallega and Agria potatoes were the only ones with large and deep eyes. However, this did not affect their appearances negatively. Finally, the Valderredible potato stood out in terms of dry matter content, vitamin C and firm and consistent texture, making it a favorable choice in terms of quality.

See further research should be further research should be conducted in order to validate the present findings in larger settings and by including a larger number of samples. In addition, the implementation of controlled studies is recommended to examine the representativeness of the characteristics obtained for each variety. Additional studies on each quality parameter should also be carried out individually. It is noteworthy that the lack of studies on the characteristics of the different potato varieties has been identified, so it is important to carry out studies such as the present one with the aim of updating potato catalogs in the European context. It should be noted that recent potato research has been predominantly conducted in the Americas and Asia, highlighting the relevance of extending these studies to the European region to gain a more global perspective.

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Akkermansia muciniphila, una bacteria contra la obesidad y su relación con la dieta. Revisión sistemática

Akkermansia muciniphila, a bacteria against obesity and its relationship with diet. Systematic review

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RESUMEN

Palabras clave:
Akkermansia muciniphila, obesidad, intervención dietética

La bacteria anaerobia *Akkermansia muciniphila* ha demostrado su papel en la regulación del metabolismo y los marcadores de inflamación desde su descubrimiento. Es una bacteria Gram negativa que se clasifica dentro del filo Verrucomicrobiae. Es reconocida como una bacteria no patógena, desprovista de factores de virulencia y carente de una interacción significativa con el huésped que conduzca a la infección o enfermedad. Forma parte del microbioma intestinal humano y su mayor concentración se encuentra en los individuos de peso normal. Se realizó una revisión sistemática para analizar intervenciones clínicas dietéticas que examinan la asociación entre el fenotipo o estado de obesidad y la concentración de *A. muciniphila* en la microbiota intestinal, después de modificaciones nutricionales específicas en pacientes humanos con sobrepeso. La búsqueda de artículos se realizó utilizando Pubmed y Clinicalkey como motores de búsqueda. La terminología booleana ((*Akkermansia muciniphila*) and (obesidad)) and (intervención or nutrición or dieta or nutriente) se utilizó para seleccionar artículos relevantes para nuestra investigación. De los 301 artículos originales identificados, solo se seleccionaron aquellos que involucran intervenciones dietéticas en humanos. Los resultados indican que el aumento de *A. muciniphila* (ya sea mediante suplementación directa o intervención dietética) se asoció con efectos beneficiosos como disminución de la inflamación, reducción del riesgo cardiovascular, aumento de la sensibilidad a la insulina y reducción de los niveles de colesterol. En conclusión, se necesitan más intervenciones en seres humanos para determinar los beneficios y riesgos del aumento de las concentraciones de *A. muciniphila*.

ABSTRACT

Keywords:

Akkermansia muciniphila, obesity, dietary intervention

The anaerobic bacterium Akkermansia muciniphila has demonstrated its role in regulating metabolism and markers of inflammation since its discovery. It is a Gram-negative bacteria that is classified within the phylum Verrucomicrobiae. It is recognized as a non-pathogenic bacteria, devoid of virulence factors and lacking a significant interaction with the host that leads to infection or disease. It is part of the human intestinal microbiome and its highest concentration is found in individuals of normal weight. A systematic review was performed to analyze clinical dietary interventions examining the association between obesity phenotype or status and the concentration of A. muciniphila in the intestinal microbiota, after specific nutritional modifications in overweight human patients. The search for articles was carried out using Pubmed and Clinicalkey as search engines. The Boolean terminology ((Akkermansia muciniphila) and (obesity)) and (intervention or nutrition or diet or nutrient) was used to select articles relevant to our research. Of the 301 original articles identified, only those involving dietary interventions in humans were selected. The results indicate that increasing A. muciniphila (either through direct supplementation or dietary intervention) was associated with beneficial effects such as decreased inflammation, reduced cardiovascular risk, increased insulin sensitivity, and reduced cholesterol levels. In conclusion, further interventions in humans are needed to determine the benefits and risks of increasing A. muciniphila concentrations.

Introduction

Global curiosity surrounding anaerobic bacteria *Akkermansia muciniphila* has spiked in the last decade. Gut microbiota is closely related to overall human health, providing a physical and chemical barrier between pathogenic bacteria and enterocytes, as well as aiding in cellulose digestion, formation of vitamins, such as vitamin K, thiamine, riboflavin and vitamin B12. As well as, maintaining normal levels of inflammation and having an impact on the immune system especially during neonatal life (1,2).

Multiple interventions have been following gut microbiota changes after certain dietary interventions. For example, diets based on fiber, fats, or milk products such as fermented milk modulate the gut microbiota levels for each specific bacteria. What's more interesting is the consumption of breast milk in infants and the direct impact in regulating gut microbiota. Receiving human oligosaccharides, some bacteria including *A. muciniphila* are able to use them as an energy source using some enzymes, contributing to an increase in their concentration levels. *Akkermansia* also has a beneficial effect in the production of short chain fatty acids, which have been proven to improve metabolic functions (3).

Since its discovery in 2004, multiple studies have described its behavior surrounding dysbiosis in patients with obesity, diabetes, metabolic syndrome, among other diseases (4). Higher concentrations of *A. muciniphila* have been associated with the activation of CREBH (cyclic adenosine monophosphate (cAMP)-responsive element-binding protein H) transcriptional factor that regulates triglycerides metabolism in the gastrointestinal tract, protecting it from hyperlipidemia and hypertriglyceridemia (5). Additionally, *A. muciniphila* is associated with the regulation of adipocyte differentiation and, moreover, its abundance is related to a reduction in visceral fat and body weight in trials involving mice (3-4,6-7).

Previous studies had shown the significance of *A. muciniphila* in metabolic functions and its inverse correlation with obesity (4,8-9). The most intriguing studies are those that involve dietary interventions, either with prebiotics, food components or supplementation with *A. muciniphila*, since their results show the potential to become a novel approach to obesity management (10). However, most of the available information has animals as subjects of study, without replicating its methodology on humans (4, 11).

There are very few studies conducted with human subjects that evaluate the fluctuations of *A. muciniphil*a related with dietary interventions (7). Hence, it is important to review the current available literature in order to understand utterly the role of nutrition in the abundance of the gut microbiome and its effects on obesity biomarkers.

Our aim is to evaluate the correlation between dietary interventions in patients with obesity and the abundance of *A. muciniphila*; and its relationship with inflammation markers.

Methods

The chosen databases were PubMed and Clinical Key. The inclusion criteria for our study were: Study type being a dietary intervention or supplementation of *Akkermansia muciniphila*, randomization of patients, original language English, time of publication between 2016 and 2023, human patients with overweight and/or obesity, and

measurements of the abundance of *Akkermansia muciniphila* before and after the intervention as one of the measured variables. The exclusion criteria for our study were: Pharmacological or surgical interventions, systematic reviews, meta-analysis, cross sectional and disclosure studies, patients included in the study having non-related diseases and articles with expressed conflicts of interest. The Boolean terminology used for the search was: ((Akkermansia muciniphila) AND (obesity)) AND (intervention OR nutrition OR diet OR nutrient). These terms were selected in order to retrieve the articles that breach the subject of *Akkermansia muciniphila* and obesity and also included dietary interventions in the subjects. Date filters were applied to exclude articles that were published prior to 2016.

The results of this search in each database yielded the following number of articles. PubMed 257 articles relating to the search were found. Clinical Key yielded another 44 articles relating to the search. Two articles appeared in both databases. In total, 301 unique articles regarding *Akkermansia muciniphila*, obesity and interventions were found. After applying the exclusion criteria a total of 25 articles were reviewed. The search began on 15 June 2021 and concluded on 13 July 2023.

To collect reports data, a spreadsheet was made, and the reviewers summarized each individual article. The data included in the spreadsheet was: purpose of the trial, how *Akkermansia muciniphila* was directly affected by the intervention, patient's characteristics, method used on the intervention and metabolic measurements used. This information guided us towards comparing the available information from different studies and the fluctuations on the abundance of the bacteria.

Not all interventions employed the same obesity measurement markers. Besides these we decided to collect any marker that was compared before and after the dietary intervention and that demonstrates a modification increasing or decreasing relating to *A. muciniphila.* Also, time intervals manage a range from a minimum of one month.

In order to ascertain bias-free articles, a conscious search of a conflict-of-interest disclaimer was made. Added to this, a final search of Google Academic was made to be certain that no article that could be included in this review was ignored.

The chosen articles were summarized in a spreadsheet in order to compare the results of different nutritional interventions. Qualitative data was sorted out and our findings will be explained further. Bias was not found within the chosen articles, and no other articles were found on Google Academic that could be included in our study.

Results

Microbiology

Akkermansia muciniphila is a gram negative strictly anaerobic bacteria, taxonomically classified within the phylum *Verrucomicrobiae,* with a characteristic lack of motility. Morphologically, the bacterium features an oval-shaped cell with an axial diameter ranging from 0.6 to 10 μm. Cultivated under suitable conditions, *A. muciniphila* showcases diverse growth patterns, appearing as solitary cells, pairs, short chains or forming conglomerates. The genomic architecture of *A. muciniphila* MucT strain is characterized by a singular circular chromosome of 2.66 mbp. Further investigation divided *A. muciniphila* into three species phylogroups, however MucT is the most studied strain (12).

A prominent characteristic of *A. muciniphila* is its exceptional metabolic versatility. Specifically, it demonstrates a remarkable ability to thrive in environments enriched with

gastric mucin, utilizing this complex glycoprotein as a source of essential carbon, energy and nitrogen. This adaptive metabolic strategy not only underscores the ecological significance of the bacteria, but also implies potential implication in host-microbiome interactions (12) .

Regarding specific requirements for its growth, *A. muciniphila* thrives optimally at a temperature of 37°C and a pH of 7.5, without the need for exogenous vitamins. The strict anaerobic nature of the bacteria emphasizes its preference for environments devoid of oxygen, introducing an additional layer of complexity to its in vitro cultivation (12-13).

Akkermansia muciniphila has been documented to establish colonization within the human gut through a symbiotic relationship, typically initiated in the early stages of life, potentially within the first year of life. Noteworthy traces of this bacterium have been identified in human milk, implying a potential mode of transmission from mother to infant. Consequently, detectable quantities of the bacterium could be observed in the gastrointestinal tract of newborns (8, 13).

The abundance of *A. muciniphila* exhibits a distinctive pattern across the human lifespan. In healthy adults, this bacterium constitutes a noteworthy proportion, accounting for approximately 1 to 4% of the total gut microbiota, emphasizing its integral role within the complex microbial environment contributing to overall host health. However with advancing age, there is a reported decrease in the abundance of *A. muciniphila* within gut microbiota, suggesting a potential correlation between the aging process and its concentration on the microbiome. The mechanisms and implications of this decline in elderly populations warrant further investigations, to properly comprehend the dynamic interplay between *Akkermansia muciniphila* and the hosts physiological changes over the course of time (12-13).

Akkermansia muciniphila is acknowledged as a non-pathogenic bacterium, devoid of virulence factors and lacking a significant host interaction leading to infection or disease. Instead, disturbances in the abundance of this bacterium have been linked to certain pathological conditions, emphasizing its potential role as a biomarker or contributing factor to disease states such as obesity, type 2 diabetes mellitus, inflammatory bowel disease, atopy, and autism[12]. Even in diseases like cancer, it has been found that immunotherapy in combination with *A. muciniphila* administered as probiotic through microbiota transplantation has the potential to achieve better clinical results (12).

Its role in the microbiome is to maintain mucosal integrity, influence on metabolic processes and regulate immune responses, because of its anti-inflammatory properties. These characteristics are fundamental to maintain a healthy human microbiota (12-13).

Mechanisms of action

It was first postulated that the mechanism of action of *A. muciniphila* could be related to the endocannabinoidome, the endogenous system related to regulation of appetite, metabolism and inflammation. However, new hypotheses suggest that *A. muciniphila* has a positive effect on two endocannabinoidome lipids (1-Palmitoyl-glycerol and 2-Palmitoyl-glycerol), which in turn activate peroxisome proliferator-activated receptor alpha (PPARα) which could be one of the mechanisms for producing the benefits further described (14).

A focal point of investigation pertains to *A. muciniphila's* role in sustaining the intestinal barrier. According to Bian, et al (2019), the Amuc_1100 protein derived from *A. muciniphila* demonstrated a noteworthy reduction in inflammatory cell populations within the colon. This reduction targeted macrophages, cytotoxic T lymphocytes, and key

inflammatory cytokines such as TNF-a, IL-1a, IL-6, IL-12, MIP-1a, GCSF, and CXCL1. Beyond the colon, a decrease in CD16/32+ was observed in the spleen and lymph nodes of the mesentery, signifying broader immunomodulatory effects within extracolonic regions (15).

A recent investigation by Qian, et al. (16) has unveiled that Amuc_2109, an enzyme actively secreted by A. muciniphila, exhibits a mitigating impact on DSS-induced colitis in mice. This enzymatic intervention is characterized by increased expression of tight junctions (TJs) and at the same time, a reduced expression of NLRP3 inflammasome. Interestingly, the protective nature of viable *A. muciniphila* against colitis is dependent on NLRP3 activation. Notably, the regulatory role of NLRP3 in maintaining intestinal homeostasis has been previously delineated, evidenced by increased susceptibility to experimentally induced colitis in NLRP3-/- mice. Furthermore, administration of *A. muciniphila* has been shown to induce the proliferation of intestinal stem cells, while simultaneously enhancing the differentiation of Paneth and goblet cells in both the small intestine and colon. This phenomenon is observed in both healthy mice and those with gut damage induced by radiation and methotrexate.

Added to the former, the study establishes a correlation between the favorable effects of *A. muciniphila* in the intestinal tract and increased levels of acetic and propionic acids in the cecal content of treated mice. This elucidates the bacterium's contribution to tissue repair in the intestinal mucosa, implicating short-chain fatty acids (SCFAs) as crucial players in this reparative process. Thus, the study underscores the multifaceted role of *A. muciniphila* in intestinal health, spanning from immune modulation to the promotion of tissue repair through the production of SCFAs (16-17).

In the investigation of Ashrafian, et al. (18) regarding murine high-fat diet (HFD)–induced intestinal dysbiosis, the outer membrane vesicles derived from *Akkermansia muciniphila* demonstrated a capacity to enhance the intestinal mucosal barrier function. This was evident through augmented expression of tight junctions and IL-10, coupled with the inhibition of inflammatory markers within the colon .

Moreover, in Chelakot, et al. (19) *Akkermansia muciniphila's* outer membrane vesicles exhibited the ability to mitigate intestinal permeability, elevate tight junction expression via AMP-activated protein kinase (AMPK), suppress TLR-4 and interferon-alpha (IFN-α) expression, and enhance TLR-2 expression and IL-4 production in Caco-2 cell lines in vitro. This comprehensive exploration unveils the diverse mechanisms by which *Akkermansia muciniphila's* outer membrane vesicles contribute to intestinal health, encompassing mucosal barrier fortification, immune modulation, and regulatory effects on cellular signaling pathways.

Akkermansia muciniphila as a supplement

Considering the background of existing studies in mice and the benefits of *A. muciniphila* concentration, the simplest way to study its effects on the body is by supplementation. This bacteria can be ingested either live or pasteurized at 70°C for 30 min. The effects of ingesting live vs pasteurized *A. muciniphila* were extremely similar, including lowering fasting insulin levels, improving insulin sensitivity, lower white blood cell levels, lower total levels of cholesterol, all of these markers are related to levels of LPS which the supplementation of *A. muciniphila* reduced. However similar they may, ingesting the pasteurized version of *A. muciniphila* instead of the live sample lowered the activity of the enzyme dipeptidyl peptidase-IV, that decreased activity is related to improved glucose modulation, reduced cardiovascular risk and lowered levels of inflammation (7).

Druart et. al (20) have concluded that pasteurized *A. muciniphila* presents no genotoxicity nor subchronic toxicity, making it a safe food ingredient according to the FDA and EFSA guidelines on toxicology for non absorbable ingredients .

Pasteurized supplementation has shown its viable capacity to regulate hepatic molecules associated with the expression of atherosclerosis in mice. Specifically, gen Fmo3 from the liver reduced its expression after the intervention with pasteurized bacteria, subsequently reducing metabolites linked to thrombus development. However, it has been observed that the mechanisms responsible for reducing pro-inflammatory conditions and thrombus generation in lab mice may not precisely replicate those found in the bloodstream of human patients (21). Consequently, doubts have arisen regarding these mechanisms for reducing atherosclerosis through the hepatic route.

The effects of dietary interventions and supplements in A. muciniphila concentration

The available scientific evidence supports the influence of dietary interventions and the consumption of distinct nutrients on the concentration of *Akkermansia muciniphila.* The evidence supports the idea that adhering to a Mediterranean diet, having an elevated fiber intake, and operating under a caloric deficit in overweight individuals, resulting in an increase of the concentration of *A. muciniphila* in fecal material (20) .

Derrien et al (13) proved that food components like conjugated linoleic acid, polyamines, pectine, fructooligosaccharides, corn starch, fermentable oligosaccharides disaccharides monosaccharides polyols (FODMAP), have a beneficial effect increasing the abundance of the bacteria. A follow-up study in which an augmentation of fiber consumption, for the most part FODMAPS, in an overweight and obese population for 6 weeks, resulted in an increase in the abundance of *A. muciniphila* in fecal material .

Several clinical interventions had been conducted to show the correlation of Mediterranean diet and the augmentation of *A. muciniphila* (3,21). A study targeting Mexican men with metabolic syndrome involved the supplementation of polyphenol resveratrol, a naturally found compound in Mediterranean diet foods such as cranberries and grapes. This intervention has been observed to induce a notable enhancement in glucose homeostasis, consequently leading to an increase in the concentration of *A. muciniphila* in the fecal samples of the participants (22).

In another intervention conducted in 2021, a total of 210 hypercholesterolemic subjects were prescribed with a daily consumption of 85 grams of oats; which are rich in phenolic compounds known to increase the fecal excretion of cholesterol. Over a course of 45 days, this dietary intervention resulted in an increase of the fecal concentration of *A. muciniphila* and a significant reduction in serum levels of total cholesterol and LDL (23).

Additionally, a separate study in 2020 (3), showed that a traditional Mediterranean diet reduced insulin resistance in individuals and improved their serum glucose levels. A possible correlation exists with the potential involvement of dietary fiber and butyric acid and their effects on improving the gut metabolism. Notably, the improvement of glucose metabolism coincided with an elevation in the concentration of *A. muciniphila* in the studied population.

Another clinical intervention was conducted by Tagliamonte et al. (21) in which an overweight population was administered a Mediterranean diet, increasing their consumption of fruits, vegetables, fish and olive oil. After a four-week intervention, the fecal levels of *A. muciniphila* were increased, which can be attributed to the dietary change.

Through the implementation of a caloric deficit diet, where the daily caloric intake was reduced from 2400 kcal/day to 1680 kcal/day in men and from 2100 kcal/day to

1470 kcal/day in women, the gut microbiome of overweight participants was restored. The ingested calories were provided from nutritious food such as fruits, vegetables, fiber and local produce. Coincidentally, the dietary changes were similar to the ones on a traditional Mediterranean diet. Over the course of six months, this dietary intervention resulted in a significant increase in the abundance of *A. muciniphila* in fecal material (24).

Obesity and its effect on A. muciniphila concentration

Although the etiology of obesity is complex and multifactorial, it is well known its main cause is the consumption of excessive energy through dietary intake, disproportionate to the individual's energy loss rate via metabolic and physical activity (25).

High-fat diets, consisting of excessive intake of fats and refined carbohydrates, are not only linked to obesity and metabolic diseases, but also to the gut microbiota since they have been associated with dysbiosis and its reduction of intestinal bacterial diversity, disruptions in intestinal membrane integrity and permeability, increased LPS transfer to the bloodstream and systemic inflammation (26).

An example of high-fat diet-induced dysbiosis is the generation of the "obese microbiome", which has been typified as a reduced percentage of the *Bacteroidetes* phylum and an equivalent increase in the percentage of the *Firmicutes* phylum in the gut microbiota (27); this altered ratio of *Bacteroidetes:Firmicutes* was present at baseline in 12 obese people who went through two types of low-calorie diets interventions, after which the ratio was restored and the increased abundance of *Bacteroidetes* was correlated with the percentage of body weight loss (28).

Another type of obese microbiome was the decrease in *Bifidobacterium spp.* abundance and the increase in Clostridia XIV and Enterobacteriales in mice fed with a high-fat diet (9); the reduction in bifidobacteria alters the intestinal membrane integrity because that type of bacteria maintains and improves intestinal barrier function as well as prevents the passage of toxins to the bloodstream (26). In addition, studies in humans have associated higher intake of monounsaturated fatty acids with lower levels of *Bifidobacteria spp* and only slightly higher levels of *Bacteroides spp.;* and *in vitro* studies have found that high levels of polyunsaturated fatty acids, like linolenic, arachidonic and α-linolenic acids at up to 10-40 $\mu g/ml^{-1}$, can inhibit both mucus adhesion and growth of all tested *Lactobacillus* strains (29-30); hence, demonstrating how high-fat diets diminish the concentration of beneficial bacteria.

More studies in human subjects have proven this high-fat diet induced dysbiosis by demonstrating that the *Bacteroidetes:Firmicutes* ratio alongside LPS levels, of up to 7.8 EU/ml, are elevated in patients with obesity. Interestingly, other studies have shown healthy human subjects that are exposed to a 5-day high-fat diet intervention have an enhanced abundance in detrimental bacteria such as *Firmicutes* and *Proteobacteria* [31]. Thus showing how a high-fat proinflammatory environment has an effect in the gut microbiota.

All these examples of high-fat diet-induced dysbiosis can be attributed to *A. muciniphila* since mucin-degrading bacteria are highly influenced by high-fat diets (26). The latter can be proven by data of several studies that increased the abundance of *A. muciniphila* through dietary interventions but whose participants, at baseline, had a very low percentage of *A. muciniphila* in stool samples due to their obesity phenotype and their lifestyle habits of consuming a high-fat diet (3,6-7).

The theorized mechanisms by which chronic consumption of high-fat diets induces dysbiosis, thus decreasing the concentration of beneficial bacteria in the gut like *A.*

muciniphila, are thought to be two (31). The first one consists in alterations in lipid metabolism-related genes that cause an overflow of fat from the diet to the distal small intestine, the higher concentrations of fatty acids in this location of the intestine are hypothesized to have an antimicrobial effect, reducing the bacterial diversity and altering the *Bacteroidetes:Firmicutes* ratio, characteristic of the obese microbiome (32-33). The second one is related to changes in the host bile composition since high-fat diets increase the production of bile acids, which raises the number of species capable of metabolizing them and lowers the abundance of other bacterial species due to the bile acids' antimicrobial activity (34).

Now that a high-fat diet induced dysbiosis environment is established, a cascade of inflammatory pathways is unraveled which leads to adipose tissue dysfunction, ultimately promoting the obese phenotype. What is proposed is that Toll-like receptors (TLRs), specially TLR4, are constantly activated in dysbiosis by a high amount of LPS-producing Gram Negative bacteria, causing the ongoing activation of nuclear factor kappa β (NF-κB)-dependent transcription factors for proinflammatory cytokines such as IL-1β, IL-18, IL-6, IL-33, tumor necrosis factor α (TNFα) and interferon-gamma (IFNy), all of which contribute to colonic inflammation (31).

Moreover, the persistent activation of these pathways maintain a chronic inflammatory state that damages the intestinal epithelial barrier. This integrity is jeopardized by a decrease in tight-junction proteins like zonulin, occludin, and claudin-1/5 which allow LPS, cytokines and bacteria to get into the bloodstream, causing endotoxemia, and translocate to peripheral targets such as the adipose tissue. This exact translocation is what impairs adipose tissue, hence perpetuating the obese phenotype (31). However, further research is needed to fully understand the exact pathophysiological mechanisms by which high-fat diets, thus an obesity phenotype, induce gut dysbiosis in humans and lowers the abundance of *A. muciniphila* specifically.

Table 1. Type of interventions in obesity individual and effects in *Akkermansia muciniphila* levels

Author	Study type	Country	Year	Relevant finding
Dao, et al (6)	Non randomized trial	France	2016	There was a decrease in *A.muciniphila* abundance in a high genes group related to the bacteria after the nutrition intervention period, but it remained consistently and significantly higher then the low genes group.
Roshanravan, et al (35)	Randomized double-blind trial	Iran	2017	A group of 60 patients with obesity and diabetes were divided in 4 groups of supplements intervention: sodium butyrate, inuline, both and placebo. After 45 days, both supplements groups have an increase in *Akkermansia muciniphila* group.
Deppomier, et al (7)	Randomized, double-blind	United States	2019	The abundance of *A.muciniphila* was similar between 3 groups of patients at baseline. Whereas the supplementation significantly increased by 1.7 to 2.6 Log the quantity of *A.muciniphila* recovered in the feces of Pasteurized *A.muciniphila*

				and Alive *A.muciniphila* groups, and not in placebo.
Payahoo, et al (36)	Randomized double blind trial	Iran	2019	From 60 obese patients, the trial proved that the group that were receiving Oleoylethanolamide supplementation for 8 weeks have an increase of *Akkermansia muciniphila* abundance compared to the placebo group, through a quantitative real-time PCR.
Walker, et al (22)	Randomized, placebo controlled clinical trial	United States	2019	*Akkermansia muciniphila* abundance increased in 11 caucasian subjects out of 28 subjects in total with metabolic syndrome after a 35 day administration of 1 g orally of polyphenol resveratrol twice daily. Insulin sensitivity and glucose homeostasis also improved in these subjects.
Guevara-Cruz, et al (37)	Randomized double-blind trial	Mexico	2020	Interestingly, five genera, including Paraprevotella, Suterella, Anaeroplasma, Akkermansia and Oscillospira, were increased in the Gestein intervention groups, representing 41% of all genera of the gut in contrast to the 7%–10% represented in the placebo group.
Tagliamonte, et al (21)	Randomized and controlled trial	Italy	2021	A dietary intervention consisting of a switch from a Western diet to a tailored Mediterranean diet in 82 subjects with overweight and obesity increased the fecal abundance of *Akkermansia muciniphila*.
Vitale, et al (3)	Randomized, controlled, parallel group designed study	Italy	2021	Improved postprandial glucose metabolism and insulin sensitivity was reported in 16 subjects with overweight and obesity after following a 8 week isoenergetic Mediterranean diet intervention. These changes were accompanied by an increase in the abundance of *A. muciniphila*.
Jie et al (24)	24-week dietary intervention cohort	China	2021	83 participants (29 with overweight, 43 with obesity and 11 with normal weight) underwent a 6-month dieting program in which fecal microbiome data was analyzed. *A. muciniphila* abundance was reported to be increased after the dietary intervention.

Discussion

The proinflammatory state of obesity and the concentration of *A. muciniphila* are closely intertwined. However, as the exact mechanism of action remains unclear, it is uncertain whether the decline of *A. muciniphila* is a predisposing factor for increased inflammation or if the inflammatory state of the body leads to a reduction of the bacteria. Nevertheless, it is well-established that dysbiosis significantly influences inflammation within the microbiome (29-30). Further colonization has gathered attention from the pediatric care after knowing human milk capacity to transfer bacteria from the mothers gut, that initiates new approaches that symbiosis and the microbiome could play an important role in proinflammatory conditions since the transmission of mother's own microbiome to its child (38).

Regardless of this uncertainty, the increment of *A. muciniphila,* whether through direct supplementation, prebiotic consumption, or dietary changes, confers beneficial effects to overweight individuals. Increased levels of *A. muciniphila* in the gut microbiota have been associated with reduced cardiovascular risk, improved insulin sensitivity, lower cholesterol levels, reducing proinflammatory endotoxins (TLR and TLR4) and cytokines (IL-6, TNF, C-reactive protein), improved barrier function decreasing its permeability, reduce anthropometric measurements such as waist-to-hip ratio and subcutaneous adipocyte diameter, weight loss, and an overall healthier metabolic status (25). With all of these revolutionary studies that should be done could finally understand completely the exact mechanisms that *A. muciniphila* impacts in all these metabolic factors including if there's a direct correlation in atherosclerosis prevalence besides the improvement in cardiovascular health and health parameters.

All these benefits have been documented in studies on obesity, where patients initially exhibited low levels of *A. muciniphila* in their stool samples, reflecting the dysbiosis commonly observed in obese individuals. The chronic consumption of high-fat diets induces dysbiosis through antimicrobial effects, such as fatty acid deposition in the distal small intestine or increased bile acid production, promoting the growth of pathogenic bacterial strains and selectively inhibiting beneficial bacteria. This leads to the formation of proinflammatory microbiota frequently observed in obese patients. It is possible that patients with obesity have a lower baseline concentration of this bacterium due to their obese phenotype, although further studies are needed to establish a significant correlation.

However, by correcting this dysbiosis through dietary interventions, individuals can reach the associated benefits, including improved cellulose digestion, reduced inflammation, and enhanced synthesis of vitamins such as Vitamin K and B12.

It is important to note that most of these health benefits in patients with obesity have been achieved through dietary interventions involving modifications such as a polyphenol-rich diet, hypocaloric diets, Mediterranean diet, and fiber-rich diets, rather than direct supplementation with the bacteria. We believe that dietary modifications have the potential to serve as a remarkable therapeutic approach for treating obesity by increasing the abundance of beneficial bacteria like *A. muciniphila*, as dietary changes may be more accessible to patients rather than direct supplementation with *A. muciniphila*, whether in live or pasteurized form. We also believe that dietary modification could be strategic for changing genetic transmission of obesity By implementing a diet based on

the approaches that increase *A. muciniphila,* breast feeding ,with enough presence of the bacteria in mother´s microbiome, could make new generations to stop transmitting the complete genetic sequence of obesity DNA information, through healthy epigenetic factors of a healthy lifestyle.

Conclusions

In conclusion, the relationship between the proinflammatory state of obesity and the concentration of *Akkermansia muciniphila* (*A. muciniphila*) in the gut microbiota is complex and not fully understood. *A. muciniphila* is a strict anaerobic gram negative bacteria that possess an outstanding metabolic versatility, which provides the human being a symbiotic relationship that offers intestinal barrier integrity protection and anti-inflammatory based benefits that aids in several metabolic processes and even immune responses. Additionally, since it is a non-pathogenic bacterium devoid of virulence factors it lacks a significant host interaction pathway that could lead to disease.

Quite opposite, disturbances in its percentage within the gut microbiome have been linked to pathological conditions, and the abundance of *Akkermansia muciniphila* has an inverse correlation with the augmentation of inflammation markers and an "obese" microbiome. Despite the exact mechanisms for this are not completely understood, new hypotheses have arisen, and one of them suggests that *A. muciniphila* lowers obesity biomarkers by having a positive effect on two endocannabinoidome lipids and synthesizing the Amuc_1100 protein which reduces inflammatory cell populations within the colon.

Adjustments on dietary intake of nutrients that increase the ampleness of *A. muciniphila* in the gut microbiome will create a favorable environment for the augmentation of cellulose digestion; thus reducing the risk of metabolic diseases and its further complications.

It is worth noting that most of the health benefits observed in obese patients have been achieved through dietary interventions rather than direct supplementation with *A. muciniphila*. Dietary modifications offer a more accessible therapeutic approach for treating obesity as they can be easily adopted by patients. Further research is needed to establish a significant correlation between the baseline concentration of *A. muciniphila* and obesity. Nonetheless, dietary modifications hold great potential for increasing the abundance of beneficial bacteria like *A. muciniphila* and can serve as an effective strategy in the treatment of obesity.

Conflict of Interest

There are no conflicts of interest

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