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Research Article

Orthostatic response in patients with type 2 diabetes mellitus evaluated through acceleration photoplethysmogram

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ABSTRACT

Introduction: One of the striking complications of diabetes mellitus is arterial circulatory dysfunction. The 30:15 ratio is an orthostatic index commonly used to diagnose circulatory alterations in diabetic patients with a long evolution. Indices obtained from the second derivative of photoplethysmogram (SDPPG) or acceleration photoplethysmogram (APG) characterize arterial pathological changes. **Aim**: To compare the cardiovascular response of non-diabetic subjects to active standing *versus* that of type-2 diabetes (DM2) patients using APG indices. **Methods**: Digital photoplethysmography (PPG) was obtained from healthy subjects (n = 15, age ± SD, 44.6 ± 7.2 years) and DM2 patients (n = 15, age ± SD, 48.3 ± 7.9 years). The 30:15 ratio, b/a, d/a, and APG-AI, all APG-based, of the participants were calculated and compared at baseline, 15 and 30 s. **Results**: Comparison of the 30:15 ratios between groups did not show a significant difference. No significant differences were observed between the APG indices in the two groups in the baseline period. However, d/a decreased, and APG-AI increased significantly at beat 30 after active standing in non-diabetic subjects. Values of APG indices in DM2 patients did not show significant changes. **Conclusion**: The results suggest that APG indices could be used to detect early vascular dysfunctions in DM2 patients.

Key words: orthostatism; acceleration photoplethysmogram indices; type 2 diabetes; arterial stiffness.

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RESUMEN

Introducción: Una de las complicaciones de la diabetes mellitus es la disfunción circulatoria arterial. El coeficiente 30:15 es uno de los índices ortostáticos que se emplean para diagnosticar alteraciones circulatorias en diabéticos con evolución prolongada. Por otra parte, se emplean índices de la segunda derivada del fotopletismograma (SDPPG) o fotopletismograma por aceleración para caracterizar cambios patológicos de la función arterial. **Objetivo:** Comparar la respuesta cardiovascular a la bipedestación activa de sujetos sanos *versus* sujetos con diabetes tipo 2 mediante los índices de la SDPPG. **Métodos:** Se tomaron registros fotopletismográficos digitales (PPG) en sujetos sanos (n = 15, edad ± DE, 44.6 ± 7.2 años) y sujetos con diabetes tipo 2 (n = 15, edad ± DE, 48.3 ± 7.9 años). Se calcularon los coeficientes 30:15, los índices b/a, d/a y SDPPG-IE en cada participante, basados en los componentes de la onda del SDPPG, y se compararon en el período basal y a los segundos 15 y 30. **Resultados:** Los coeficientes 30:15 de ambos grupos no mostraron diferencias significativas. Respecto a los índices SDPPG, no se observaron diferencias significativas entre los dos grupos en el periodo basal. Sin embargo, d/a disminuyó y SDPPG-IE aumentó, ambos en el latido 30 y de manera significativa después del ortostatismo activo en el grupo de sujetos sin diabetes. Los valores de los índices SDPPG en el grupo con diabetes tipo 2 no mostraron cambios significativos. **Conclusión:** Los resultados sugieren que los índices de la SDPPG pueden ser usados para detectar de manera temprana disfunciones vasculares en pacientes con diabetes tipo 2.

Palabras clave: ortostatismo; índices de la segunda derivada del fotopletismograma; diabetes tipo 2; rigidez arterial.

INTRODUCTION

Type 2 diabetes mellitus (DM2) causes high morbidity, mortality, and high socioeconomic costs; it is estimated to affect more than 300 million people by 2030.[1] Cardiovascular autonomic neuropathy as part of diabetic neuropathy is a frequent complication of types 1 and DM2 and leads to high mortality and morbidity.[2, 3]

Various tests are used to diagnose the cardiovascular component of cardiovascular autonomic neuropathy, such as heart rate variability, heart rhythm disorders, and the Ewing test.[4] One of the best studied parameters of the Ewing test is the heart rate response to standing up or 30:15 ratio.[5] A continuous recording of the heartbeat is obtained from when the subject stands up after lying down to obtain RR intervals. The 30:15 ratio is the coefficient of the RR intervals at beat 30 and beat 15, both with the subject already standing. A 30:15 ratio of ≥ 1.04 is considered normal.

The 30:15 ratio is also considered a criterion of the afferent (baroreceptor) and efferent parasympathetic function of the cardiac autonomic nervous system.[6] Standing up from a supine position produces an integrated reflex response of the cardiovascular system, including changes in heart rate and blood pressure.[6] This response to circulatory stress elicited by standing up requires the proper functioning of diverse and complex mechanisms of the cardiovascular system. The autonomic nervous system (ANS) is the primary mechanism of the immediate responses to changes in position, and the renin-angiotensin-aldosterone system, which is also a component of this response, acts in the longer term.[7]

Standing up from supine causes significant hemodynamic changes, such as decreased blood return to the heart, cardiac output, and blood pressure. The reduction in the pressure on the baroreceptors causes parasympathetic inhibition, and a compensatory sympathetic activation increases the heart rate and systemic vasoconstriction. Therefore, there is a change in the systolic and diastolic blood pressures.[8]

The acceleration photoplethysmogram (APG) is an optoelectronic tool that measures and records changes in the blood volume in a part of the body. Photoplethysmography (PPG) signals are applied in healthcare, including clinical, physiological, and vascular assessment, and research contexts as autonomic function.[9] The second derivative of photoplethysmogram (SDPPG) or APG has characteristic traces that provide additional elements and a more sophisticated analysis of the PPG.[10] The APG distinguishes five sequential waves called a, b, c, d, e, and f. From the relative heights of these waves, various ratios are obtained, such as b/a, d/a, and (b-c-d-e)/a or aging index (APG-AI), which are related to various physiological and pathophysiological variables such as age[11, 12], the compliance of the carotid artery[13], blood pressure[14], coronary artery disease[15], and the presence of atherosclerotic disorders.[16] In the early stages of DM2, the cardiovascular indices do not show significant changes, and it is until the stage of frank diabetic neuropathy that the 30:15 ratio decreases its values.[17] Therefore, the purpose of this work was to determine whether the second derivative indices show changes in DM2 patients under orthostatic stress, even though diabetic neuropathy is not present yet.

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METHODS

Two study groups were formed: one with 15 healthy subjects and another with 15 patients diagnosed with DM2, an evolution of ≤ 6 years, and oral glycemic control. All the participants were normotensive (blood pressure < 120/80 mmHg) at the time of the study; none had total serum cholesterol values > 200 mg/dl or electrocardiogram abnormalities. No subject had heart disease or was taking antihypertensive medication. The Toronto Clinical Neuropathy Score (TCNS) questionnaire was applied to DM2 patients to rule out the presence of polyneuropathy[18], while a physical examination was also carried out to rule out lower limb abnormalities as sensitivity, strength, and abnormal ankle reflex. Subjects were studied fasting and abstaining from caffeine, alcohol, and tobacco use 24 h before the study. The Ethics Committee of the Biological and Health Sciences Division at the Iztapalapa Unit, Autonomous Metropolitan University approved the study. Additionally, the ethical principles for medical research with human beings of the World Medical Association (WMA) of the Declaration of Helsinki (2013) were followed, and all participants gave their written informed consent. The recordings were made in a laboratory with a temperature of 23 °C ± 1 °C. All subjects were allowed to rest and acclimatize for at least 30 min before the start of the recordings.

PPG registration. The PPG detects changes of light absorption by hemoglobin, which reflects changes in blood flow volume. A photoplethysmography transducer (TSD 200; BIOPAC Systems, Goleta, CA, USA) that transmits infrared light at 860 ± 90nm was used to obtain the PPG (Figure 1). The transducer was placed on the index finger of the right hand of each experimental subject. The frequency response of the photoplethysmograph was flat at 10 Hz. The PPG curve with a frequency of 200 points per second was recorded through a 12-bit analog-digital converter. Acknowledge v3.8.1 (MP100; BIOPAC Systems, Goleta, CA, USA) was used as analysis platform.

SDPPG registration. The SDPPG was obtained using Origin v7.5 (Microcal Software, Inc., Northampton, MA, USA). The d2PPG/dt2 or APG wave typically comprises five distinct waves from a to f. The APG indices are calculated using the relative height of the positive or negative inflections. The values of b wave and d wave are normalized to a wave, then the ratios b/a and d/a are obtained. The APG-AI is defined as the quotient of the algebraic sum /b-c-d-e waves normalized with a wave value 10 (see Figure 1). The APG indices of the basal values in each group were compared versus the periods corresponding to beats 15 and 30.

30:15 ratio. To obtain the 30:15 ratio, the participant remained in supine position for a stabilization period of 10 min. The photoplethysmographic recording lasted 2 min. Each participant stood up unaided at the end of the first minute of recording.

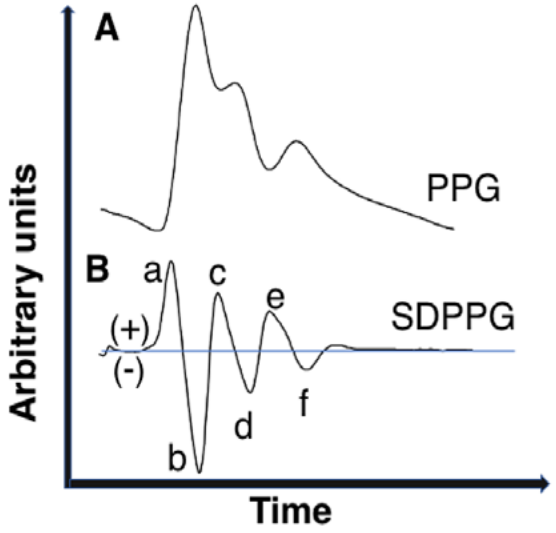

FIGURE 1. Representative trace of the digital volume pulse and second derivative of photoplethysmogram (SDPPG).

The SDPPG or acceleration photoplethysmogram wave is made up of five components from wave a to e: positive waves a, c, and e; negatives b and d. The b/a ratio is calculated with the quotient of relative values b and a; the d/a ratio is calculated with the quotient of relative values of waves d and a. The aging index (APG-AI) was defined as the quotient of the algebraic sum of (b-c-d-e) divided by a.

The zero start time of the analyses was considered when the participant was fully standing. Once the person stood up, the RR intervals were measured at beats 15 and 30; the RR30/RR15 ratio or 30:15 ratio was calculated using these values. Values 30:15 > 1.04 were considered normal, those between 1.01 and 1.03 were limits of normality, and values < 1.00 were considered abnormal.[17]

STATISTICAL ANALYSIS

The records of the subjects were plotted using Plot2 (Michael Wesemann, Berlin, Germany, 2019). The interpolated data of the set of 15 subjects in each group were averaged and the kinetic curves of the RR intervals were obtained. Baseline values were compared with those corresponding to beats 15 and 30 using a two-tailed t-test. Data are reported as mean ± standard deviation (SD) and analyzed using SPSS Statistics v22.0 (Chicago, USA). Data are presented as mean ± SD. The chosen level of statistical significance was $p < 0.05$.

RESULTS

The characteristics of healthy subjects and DM2 patients are shown in Table 1. The demographic and physiological data were not significantly different between the two study groups. None of the DM2 patients obtained ≥ 5 points in the TCNS questionnaire.

Index 30:15

The comparison of the 30:15 ratio of healthy subjects (1.125 ± 0.13, mean ± SD) versus that of DM2 patients (1.139 ± 0.07, mean ± SD) showed no significant differences (p = 0.7802).

SDPPG indices

Figure 1 shows typical traces of the PPG or volume of the digital pulse and SDPPG. Table 2 shows the APG indices in healthy subjects at baseline and beats 15 and 30. The d/a index was significantly reduced, and the APG-AI significantly increased at beat 30 in both cases, p = 0.02 and p = 0.04, respectively.

The SDPPG indices in DM2 patients at baseline and beats 15 and 30 are shown in Table 3. Comparing the three periods, none of the indices showed a significant difference concerning the baseline values.

DISCUSSION

The noteworthy findings of this study were that the 30:15 ratios did not show significant differences between groups. Before the active standing test, the APG indices evidenced no significant differences between the two groups in the b/a, d/a, and APG-AI indices. However, d/a decreased, and APG-AI increased significantly in healthy subjects at beat 30 after standing up. On the other hand, the APG indices in DM2 patients did not show significant changes in the periods corresponding to beats 15 and 30 as compared to the baseline values.

The d/a index is related to vasoactive drugs[10] and metabolic syndrome[20]; it has also been proposed as a predictor of cardiovascular events.[19] The d wave occurs in the late systolic phase of PPG and is strengthened by the retrograde wave from the periphery. Therefore, the d/a ratio correlates significantly with the central augmentation index and represents the

Table 1. Demographic and clinical data of the study groups.

Demographic and clinical parameters	Controls	Type 2 diabetes patients
n (women)	15 (4)	15 (9)
Age (years ± SD)	44.6 ± 7.2	48.3 ± 7.9
Diastolic pressure (mmHg)	123 ± 12.2	128 ± 14.1
Systolic pressure (mmHg)	74.1 ± 9.2	76.3 ± 10.5
Heart rate (beats/min)	74.5 ± 8.2	75.4 ± 9.4

Data are mean ± standard deviation.

Table 2. Indices of second derivative of photoplethysmogram wave in basal periods and at beats 15 and 30 after active standing in healthy subjects.

Index	Basal	Beat 15	Beat 30
b/a	–0.81 ± 0.11	–0.78 ± 0.12	–0.74 ± 0.14
d/a	–0.17 ± 0.15	–0.24 ± 0.14	–0.39 ± 0.12*
APG-AI	–0.86 ± 0.28	–0.79 ± 0.21	–0.62 ± 0.16*

Data are mean ± standard deviation. * p < 0.05; APG-AI = aging index defined as (b-c-d-e)/a; b/a = quotient of the relative values of the height of b-wave and a-wave; d/a = quotient of the relative values of the height of d-wave and a-wave.

Table 3. Second derivative indices of the photoplethysmogram wave at baseline and at beats 15 and 30 after active standing in type 2 diabetes patients.

Index	Basal	Beat 15	Beat 30
b/a	–0.73 ± 0.10	–0.70 ± 0.15	–0.67 ± 0.14
d/a	–0.23 ± 0.15	–0.27 ± 0.14	–0.33 ± 0.12
APG-AI	–0.74 ± 0.28	–0.69 ± 0.19	–0.64 ± 0.16

Data are mean ± standard deviation. * p < 0.05; APG-AI = aging index defined as (b-c-d-e)/a; b/a = quotient of the relative values of the height of b-wave and a-wave; d/a = quotient of the relative values of the height of d-wave and a-wave.

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structural and functional properties of the systemic arterial tree, including peripheral circulation.[20]

The rate of pressure increase is also related to central arterial pressure.[21,22] The d/a index is associated with the reflected wave component of the aortic arterial pressure; then, the d/a index is an indirect clue of arterial stiffness.[19] Consequently, the d/a index reflects the functional properties of peripheral circulation and is also related to peripheral vascular resistance.[20] Furthermore, the APG-AI index correlates with age[10] and can help to assess vascular aging and atherosclerotic disease.[23,24] The absence of changes in d/a and APG-AI indices here observed in DM2 patients under orthostatic stress may indicate an initial lacking response due to arterial stiffness.[25,26]

CONCLUSIONS

In conclusion, the comparison of 30:15 ratios of healthy subjects against those of type 2 diabetes patients did not show significant differences. However, the acceleration photoplethysmogram aging and d/a indices showed differences between groups at beat 30. Our results suggest that circulatory disturbances anticipate other components of diabetic neuropathy. Trials with a more significant number of participants and people with diabetic neuropathy will establish whether acceleration photoplethysmogram indices can be used in the early detection of diabetic neuropathy.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest regarding the content of this manuscript.

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Review Article

Effect of different bacteria on the biodegradation of polyurethane

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ABSTRACT

Polyurethane has been used and over-exploited worldwide in the manufacture of different goods, but it is hard to break down and represents an important contaminant due to its accumulation when discarded. Recent research findings have shown that several bacteria and their enzymes can biodegrade various plastics, such as polyurethane. In this review, we sought to group, analyze, and relate the techniques used by different bacterial species to biodegrade polyurethane that have been identified in different studies by searching databases, such as PubMed, Web of Science, and Scopus. Different species of proteobacteria, actinobacteria, and endobacteria biodegrade polyurethane by oxidation and hydrolysis to obtain carbon and nitrogen sources. Changes as weight loss, tensile strength, and chemical and surface changes were observed in polymer properties, showing that biological technologies have a direct impact on polyurethane by modifying the molecule in different ways.

Key words: bacteria; biodegradation; poly(ether urethane); biotechnology.

RESUMEN

El poliuretano se usa y sobreexplota alrededor del mundo en la fabricación de diversos productos, sin embargo, es un material de difícil degradación y, por su acumulación, un contaminante importante al ser desechado. Los resultados de investigaciones recientes demuestran el potencial de diferentes bacterias y sus enzimas para biodegradar diferentes plásticos, como el poliuretano. En esta revisión se buscó agrupar, analizar y relacionar las técnicas utilizadas por diferentes especies bacterianas para biodegradar poliuretano, identificadas en diferentes estudios, buscando en bases de datos como PubMed, Web of Science y Scopus. Se encontraron diferentes especies de proteobacterias, actinobacterias y endobacterias que biodegradan el poliuretano por oxidación e hidrólisis para obtener fuentes de carbono y nitrógeno. Se observaron cambios como pérdida de peso, fuerza de tensión y cambios químicos y de superficie en las propiedades del polímero, mostrando que las tecnologías biológicas tienen un impacto directo sobre el poliuretano al modificar la molécula de diferentes maneras.

Palabras clave: bacterias; biodegradación; poli(éter uretano); biotecnología.

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INTRODUCTION

Polymer biodegradation is widely applied as a solution to plastic pollution. A higher biodegradation efficiency has been observed in studies where individual strains are isolated and consortia of microorganisms with degradation capacity are used, as they eliminate potentially toxic intermediates in the environment. Therefore, they are a promising tool for the degradation and bioremediation of plastic waste.[1] About 140 million tonnes of plastics, synthetic polymers, are consumed annually as packaging materials, and the number increases continuously day by day. Polyurethanes (PUs) are synthesized polymers containing polyethers or polyesters (polyols), diisocyanates, diols or diaminescan and a carbamate group as a linker. They constitute an important group among synthetic polymers in terms of their widespread application and unique properties. They have been used in the materials sector as constituents of mattresses, shoe soles, chairs, and even elements for refrigerators and panels, where they are found as coatings, supports, and foams. All those applications make PU the polymer with the biggest production.[2,3] Plastics are highly chemically and biologically resistant and have a high resistance to stretching. For that reason, they are used in medicine and different industrial products, such as foams, adhesives, construction materials, fibers, and coatings.[4] PUs have become a fundamental material in human life because their versatility allows them to be used as a substitute material to create several products. For that reason, the consumption of this plastic has increased worldwide and, therefore, its accumulation at the end of its useful life.[5] In 2014, an accumulation of 4.4 million tonnes of PU was registered in Europe alone. This is an alarming amount since it is known that PU degradation takes hundreds of years. The real problem is that there is no correct method to carry out its disposal.

The current options are chemical and mechanical recycling as well as incineration; however, none of these methods are effective or safe for the environment.[6,7] It is known that bacteria, both natural and genetically modified, degrade contaminants into simpler and less toxic forms, transforming them into other compounds. In natural and contaminated sites, the interaction of microbial communities with a metabolism regulated to obtain carbon and energy has garnered interest in environmental biotechnology and research. The latter focuses on the biodegradative capacity and adaptability of their metabolic processes to make use of chemicals found in the environment.[8] PU can undergo different alterations, starting with changes at molecular level, reflected in chemical and physical changes.

The aim of this review is to find and group the processes by which different types of bacteria carry out their effective biodegradation. With this information, these biological technologies can be considered a potential alternative solution for PU contamination.

METHODS

Databases PubMed, Web of Science, and Scopus were used for the search of scientific information. Boolean operators and the keywords biodegradation OR biological degradation AND microorganisms OR bacteria AND plastic OR polyurethane NOT bioplastic OR biodegradable plastic were used. Using the filters, we obtained theses and articles from 2015 to date, in Spanish o English, published in systematic review or journals.

Inclusion criteria:

- Bacteria with faster growth rate
- Bacteria with a high rate of PU degradation in short periods
- Articles published in 2015 and on
- Direct biodegrading activity on PU
- Basic Research Articles
- Articles in English and Spanish

Exclusion criteria:

- Non-pathogenic bacteria
- Articles published before 2015
- Yeast or fungal analysis
- Review articles
- Meta-analyses

RESULTS

Microbial degradation of polyurethane

The degradation of PU has been investigated, including changes in its properties through biological reactions caused by microorganisms that cause its loss of function and lead to the degradation of the material.[8] An example of the above are bacteria, as presented by Oceguera-Cervantes, et al.[3] They discovered *Corynebacterium spp*., *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, and *Bacillus subtilis* use PU as a carbon and nitrogen source when strains are grown in PU media supplemented with yeast extract or glucose. On the other hand, in their study on microbial degradation in plastic, Peng Y, *et al.*[9] leave aside bacteria as an entity and focus on identifying bacterial enzymes for process optimization. The latter are responsible for degrading PU and products of this biodegradation. The authors also mention experimentation with aliphatic polyester and polyester PU under the activity of microorganisms through the hydrolytic breaking of the ester or urethane bonds in PU structures. Bacterial degradation, also used in other biotechnological processes, has been demonstrated in food production; the lipase and hydrolases secreted by *Pseudomonas chlororaphis* act over the PU.[4]

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Biodegradation mechanism

The environment in which the PU is found will determine the degradation mechanism since the presence of microorganisms and even acidic, alkaline, or oxidative conditions are potential degraders of this polymer. Microbial communities can adapt to the presence of different components and use them as sources of energy and growth, since they can degrade polymers into intermediate products by enzymatic systems.[6,10] These degradative enzymes produced by microorganisms have been classified into intracellular and extracellular enzymes, where we find both oxidative and hydrolytic action, in which in the PU hydrolysis mechanism a series of steps have been observed in the presence of hydrolase type enzymes, where depolymerization of the long carbon chains of the plastic polymers is carried out causing a decrease in molecular weight and viscosity and the rupture of all the chains. Other microbial enzymes with a similar mechanism have been discovered, such as laccases, peroxidases, lipases, esterases, and cutinases.[9,10]

Polyurethane biodegradation by different bacteria

Bacteria have the function of making the transformation and flow of nutrients to the soil and, therefore, the environment. It has been shown that the decomposition of different complex polymers by bacteria allows the release of carbon and nutrients. These degradative bacteria are both gram positive and gram negative, including *Pseudomonas*, *Burkholderia gladioli*, and *Bacillus subtilis* species (Table 1) and their activity was measure in contaminated media.[4,9,10]

PU degradation is directed by carbon catabolic controls, using two types of lipases (one encoded by the *pueE* gene and the other encoded by the *pueB* gene), to obtain dispersal growth.[11] *Pseudomonas putida* is reported to work in this way, obtaining a high degradation of PU in a couple of days.[9] A significant activity in the degradation of PU is also observed by enzymes from *Pseudomonas spp*. and *Bacillus spp* with esterase activity. In comparison with these two types of enzymatic reactions, lipase activity has a greater significance when obtaining the data analyzed by Nuclear Magnetic Resonance (NMR) and Infrared (FT-IR) Spectroscopy.[12] Stepien AE, et al.[4] performed biodegradation tests with *Pseudomonas denitrificans* with commercial PUs.

The changes in the chemical structure of the polymer were evaluated using methods as mass loss analysis to assess the degradation process during the incubation of the polymer in the bacterial environment. The activity is related to the oxidative action of enzymes released by the microorganisms into the polymer. According to Glaser JA[13], *Pseudomonas otitidis* biodegrades PU by an enzymatic reaction that causes depolymerization. It has urease enzymes that degrade urea linkages as well as proteases and esterases that hydrolyze ester bonds to depolymerize PU.[9] Although the individual biodegradation of each bacterium is effective, an efficient process can be obtained by using the activity of two or more bacteria together (consortium). For example, the urethane bonds in the polyester immersed in the PU are broken by fusing the polyamidase from *Nocardia farcinica* with a receptor of the polymer found in a polyhydroxyalkanoate depolymerase from *Alcaligenes faecalis*.[14] The biodegrading activity of

Table 1. Bacteria proven to biodegrade polyurethane. Contaminated sites-samples from contaminated sites (e.g., landfill/dump sites, activated sludge, contaminated soils, etc.). PU (polyurethane) biodegradation.

Polymer	Sample origin	Phylum	Bacteria	References
PU	Contaminated	Firmicutes	*Bacillus sp. AF8* *Bacillus subtilis*	(4)
PU	Contaminated	Proteobacteria	*Pseudomonas chlororaphis, P. denitrificans ATCC 19244*	(4)
PU	Contaminated	Proteobacteria	*Burkholderia gladioli, Pseudomonas otitidis, P. putida*	(9)
PU	Contaminated	Firmicutes	*Bacillus subtilis*	(17)
PU	Contaminated	Actinomycetes	*Thermomonospora curvata*	(15)
PU	Contaminated	Actinomycetes	*Saccharomonospora viridis*	(16)
PU	Contaminated	Actinobacteria	*Nocardia farcinica*	(14)
PU	Contaminated	Proteobacteria	*Alcaligenes faecalis*	(14)

actinomycetes *Thermomonospora curvata* DSM4318312 and *Saccharomonospora viridis* AHK 190 is produced by hydrolyzing enzymes. They break the polyester bonds by adding a water molecule that reacts chemically with the macromolecule. The activity of these enzymes occurs at moderate temperatures.[15,16] In a recent publication, Schmidt J, et al.[17] used polyester hydrolases—previously described—such as LC-cutinase, TfCut2, Tcur1278, and Tcur0390, to demonstrate the biodegradation of PU by this type of enzymatic activity. The tests were carried out over long periods of time and at elevated temperatures, resulting in significant weight loss in the tested media.[17] *Thermobifida*-derived cutinases performed the highest biodegradation of PU due to their nonspecific nature, producing up to 78 different substrates by lipolysis.[18]

Experimental conditions modify the degradation activity

The microbial metabolism requires certain conditions for its degradation activity, so it is necessary to know the growth media, temperature, and activity time of the bacteria.[19] In some of the experiments, a pre-treatment is applied to increase the bioavailability of the polymers. The materials are washed with distilled water or ethanol, which align the polymer structure and change the polarity of the material to non-polar, causing certain structures to break and be retained by other molecules forming clathrate. Alternatively, materials are exposed to high temperatures or UV radiation. This last pre-treatment causes abiotic degradation, weakening the polymer structure and thus promoting the biodegradation process.[20-23]

Growth conditions

The growth and survival of bacteria with degradation activity are mainly affected by temperature. High levels of temperature during experiments show that an increase in the metabolism of microorganisms also requires an increase in environmental temperature and, therefore, in plastic degradation.[24] Other growth factors of the bacteria reported by the investigations are pH and the culture medium, where often the bacteria were grown *in vivo* and the most used was the mineral salt medium, as well as Bushnell-Haas or the liquid basal medium free of carbon and some with added glucose, where the bacteria are forced to use the carbon of the plastics and thus have a greater biodegradation of the plastics.[24,25]

Common changes in the polymers during degradation

Molecular Weight loss

The alteration in the physical properties of the polymer during its biodegradation is reflected as molecular weight reduction in which it is mainly demonstrated as percentage weight reductions calculated from the difference between the initial polymer weight and the weight after the exposure to bacteria that achieved biodegradation. The PU molecules change their bones and release compounds leading to a weight loss of the molecules. These observations were typically combined with another method, such as surface changes and/or FT-IR (Fourier Transform Infrared) spectroscopy.[26]

Tensile strength

The changes in tension originate from the breaks between PU molecules, resulting in a polymeric matrix with a lower order in the crystalline domains and fewer cross-links compared to the original material. Subsequently, Young's modulus decreases and the material becomes more brittle and less stiff.[27] Changes in plastic tensile strength are typically calculated using a tensile machine (e.g. INSTRON 5566) and determined in megapascals (MPa), as the percentage loss in tensile strength, elongation at break point (percent) or ultimate tensile strength (i.e. the stress the material experiences when extended to break point).[28]

Surface changes

In 60% of the studies, surface alterations (cracks, pores, and holes) in plastics were assessed by noticeable changes in the *before* and *after* SEM (Scanning Electron Microscopy) images. SEM image analysis determined the degradation of PU by bacteria and evidenced the formation of biofilms occurred within 15 days; the HDPE (High Density Polyethylene) films were cracked and developed holes when incubated with *Klebsiella pneumoniae*.[20] Atomic force microscopy is used to characterize the surface of relatively flat, solid and semi-solid samples. The technique provides morphological information in 3D from topographic images at a nanometric scale. It also provides surface parameters as roughness and distribution (homogeneity) of particles on various materials, such as plastic sheets.[29]

Chemical changes

Polymers undergo chemical changes at the time of biodegradation. FT-IR is the most used method (60.9% of the studies) to evaluate this type of chemical changes. The spectra prove chemical changes because they demonstrate the intensity variations of the carbonyl bands due to changes in the double or triple bonds or the methyl groups that show as flat-spectra with different ratio peaks. The analysis of this technique relies on the fact that most molecules absorb in the infrared region. This absorption corresponds specifically to the vibration modes of the different bonds present in the analytes

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and provide detailed information on the chemical structure of the polymer. Because of its accuracy and rapidity, FT-IR is especially common for PU degradation analysis. It is a non-destructive method: The sample is recovered without damage after the analysis. To assess superficial biological degradation, surface analysis can be easily performed.[30] As time progresses, chemical changes continue according to biodegradation that is taking place: decrease of carbonyl groups and an increase in the number of unsaturated hydrocarbons resulting from the conversion of the carbonyl groups. Another method to evaluate these changes is through the thermal profiles using Thermal Gravimetric Analysis (TGA), obtaining the degradation curves product of the digestion of the hydrocarbon skeleton, where there is a modification in the molecular weight results in a low molecular weight. Analyzing the culture medium is another way to detect changes with HPLC (High Pressure Liquid Chromatography) to seek for intermediate molecules of degradation,[30] and due to the accessibility of this.

DISCUSSION AND FUTURE PERSPECTIVES

The review of the information shows the need for improving the research by comparing tests between degrading bacteria and their plastic-enzyme activity in the near future. This will allow to focus on their effectiveness as a biodegradation technology, considering time and yield. Similarly, further studies should be developed without PU pre-treatment and under natural environmental conditions. These observations will lead to the use of specific bacteria in different ecosystems. It is necessary to identify and characterize depolymerases, because they lead the biodegradation procedure and over expression of these enzymes might have a greater benefit.

CONCLUSION

Polyurethanes are versatile polymers with highly variable structures, chemical compositions, formulations, morphologies, and shapes. Due to accumulation, they have become a severe environmental and social issue, so innovative approaches have been developed to reduce these persistent contaminants. Some of the most relevant innovations are the reports on their biodegradation by microorganisms or their enzymes isolated from polyurethane-degrading environment. This biological treatment was transformed into a technology to reduce plastic waste. These biological technologies have direct impact on the biodegradation mechanisms and kinetics by modifying physical characteristics of PU.

Several authors have reported different kinds of bacteria that degrade polyurethane or its plastic-degrading enzymes. Most of them are relevant to the research of biological technologies aimed at reducing polyurethane contamination. The bacteria that biodegrade polyurethane are from different phylum, like protobacteria, actinobacteria, and firmicutes; each one has different biodegradation properties that confer different processes, time, speed, growth conditions, among others to make the molecule decomposition. Degradation occurs when the main molecule undergoes changes in its bonds, making it weaker or releasing molecules; therefore, the structure and chemical properties are affected and it is possible to measure them by looking for the changes in the molecule before and after the process; in the same way, the activity of the bacteria is measured to see its efficiency in the degradation process and its characteristics. The research showed that authors measured this activity according to tensile machine measuring, FT-IR spectroscopy, surface changes method, TGA, or HPLC. Structural and chemical changes evidenced the deterioration of the polymer under experimental conditions since natural environmental conditions were not reported. However, the experiments were performed with degradation pretreatment of the polyurethane molecules to promote enzymatic degradation in the structures.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS

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Review Article

mRNA-based COVID-19 vaccines: a new age

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ABSTRACT

The development of vaccines based on mRNA technology involves more than a decade of hard work and important advances. Many clinical trials are underway to test these vaccines for the treatment and prevention of infections and diseases, such as cancer, cytomegalovirus, Ebola, hepatitis C virus, human immunodeficiency virus, influenza, malaria, rabies, and Zika. However, after the COVID-19 pandemic in 2020, it played a leading role in an important race to develop therapeutic strategies, mainly a vaccine, against the disease. mRNA technology allows the quick and safe creation of vaccines and large scale production. There are currently mRNA vaccines against COVID-19 (Pfizer-BioNtech® and Moderna®) that have received the emergency use authorization of regulatory entities, including the FDA in the USA, the EMA in Europe, and many others, in the process of obtaining clinical data so that they are available in a short time. On the other hand, phase 3 clinical trials continue their course. In preliminary analyses, remarkably high levels of efficacy have been reported, reaching around 95% effectiveness against mild-moderate disease and up to 100% against severe disease and death. The various clinical trials show a robust safety profile, equal to or better than that of many commonly used vaccines, although they are not free of adverse events. Despite this, there are still significant technical challenges and doubts due to the lack of long-term information. mRNA vaccines represent a new era in vaccination and one of the most important advances in health, science, and technology in recent times. In this review, we will show the basic principles of mRNA vaccines and focus on the vaccines used against COVID-19. Scientific evidence shows that mRNA vaccines are one of the best options not only as a defense against the SARS-CoV-2 pandemic but also as a novel technology against various diseases.

Key words: mRNA vaccines; CVnCoV; mRNA-1273; BNT162.

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RESUMEN

El desarrollo de vacunas basadas en la tecnología de ARNm tiene más de una década de arduo trabajo e importantes avances; varios estudios clínicos se llevan a cabo para probar estas vacunas en el tratamiento y prevención de infecciones y enfermedades como cáncer, citomegalovirus, ébola, virus de hepatitis C, virus de inmunodeficiencia humana, influenza, malaria, rabia y Zika. Sin embargo, no fue hasta la pandemia de COVID-19 en 2020 que tomó un rol protagónico en una importante carrera para desarrollar estrategias terapéuticas contra la enfermedad, principalmente una vacuna. La tecnología de ARNm permite la generación de vacunas de manera rápida y segura, con la posibilidad de escalar la producción a grandes niveles. En la actualidad, ya contamos con vacunas de ARNm contra COVID-19 (Pfizer-BioNtech® y Moderna®) que cuentan con el registro de emergencia de entidades reguladoras, entre ellas la FDA en EUA y la EMA en Europa, y otras tantas en proceso de obtención de datos clínicos que permitirán su disponibilidad en poco tiempo. Por otra parte, los ensayos clínicos de fase 3 siguen su curso. Los análisis preliminares registran niveles de eficacia notablemente altos: en torno a 95% contra la enfermedad leve-moderada y hasta 100% contra la enfermedad grave, incluida la muerte. Los distintos ensayos clínicos muestran un perfil de seguridad sólido, igual o mayor que el de muchas vacunas de uso común, aunque las vacunas no están exentas de eventos adversos. A pesar de lo anterior, existen importantes retos técnicos y dudas debido a la falta de información a largo plazo. Las vacunas de ARNm representan una nueva era en la vacunación y uno de los avances más importantes en salud, ciencia y tecnología en los últimos tiempos. En esta revisión mostraremos los principios básicos de las vacunas de ARNm y nos centraremos en las vacunas utilizadas contra la COVID-19. La evidencia científica demuestra que las vacunas de ARNm son una de las mejores opciones, no solo para combatir la pandemia de SARS-CoV-2 sino como una tecnología novedosa contra diversas enfermedades.

Palabras clave: vacunas ARNm; CVnCoV; mRNA-1273; BNT162.

INTRODUCTION

Vaccines are one of the most important elements in public health, characterized by being able to provide protection against a wide range of diseases, particularly those that are infectious. The basic principle of all vaccines is the generation of immunological memory against a specific microorganism. Vaccines contain molecules of the microorganism against which immunity is to be created. Upon administration, they allow the person to generate the immune response without the need for natural infection. The success of vaccines as a disease prevention and control strategy is extraordinary. Since their use as instruments of public health, they have prevented the infection and death of millions of people for generations around the planet.[1]

The SARS-CoV-2 virus causing COVID-19 (coronavirus disease) was declared a pandemic by the WHO in 2020, and one of the first responses was the urgent need for an effective and safe vaccine against this disease.[2] Although there are strategies that reduce the rate of COVID-19 infections, such as physical distancing, hand washing, and the use of face masks, these measures are not totally effective and, in general terms, are difficult to implement strictly enough as to control the pandemic.[1,2] Currently, there are no widely available therapies to effectively control the disease. In general, we still use treatments focused on symptom control. Large numbers of therapies and drugs are currently being tested in clinical trials; however, they are still far from being widely available.[2]

With public health measures being limited and difficult to implement and without any highly effective treatment against COVID-19, humanity's great bet is on the development and implementation of vaccines. Vaccine development has historically been a challenging journey that takes many years, even decades, and does not always culminate in highly efficient products. Perhaps the biggest effort in terms of knowledge and development in biotechnology during the pandemic has been the development of a new group of vaccines based on synthetic ribonucleic acid (RNA) that contains the genetic information encoding proteins of the SARS-CoV-2 virus.[3] Although not a novel development, these vaccines had never been applied nor produced at a large scale. In this review, we will analyze how RNA-based vaccines work, their application in the fight against COVID-19, and the perspectives of this new generation of vaccines. mRNA vaccine technology is one of the most promising in the healthcare field. We are at the beginning of an exponential growth in their use and applications, which makes them one of the most relevant topics in the scientific community.

General principles of mRNA vaccines

Generally speaking, classic vaccines are based on various strategies that allow our body to be in contact with elements of the virus and mount an immune response. The main strategies are attenuated virus, killed virus, subunits, and mRNA.[1,3,4] There are multiple reviews detailing the main strategies in the creation of vaccines. In this work we will focus on mRNA vaccines, which are based on synthetic mRNA sequences that encode virus proteins. The encoded protein in SARS-CoV-2 is Spike, one of the most important proteins in the functioning of the virus and essential to cell infection. It has also been found that the human body is naturally capable of generating antibodies (Abs) and immune memory against this protein.[4,5,6] Genomic and molecular biology technology have made it possible to sequence SARS-CoV-2 within a few weeks after its isolation, and hundreds of complete genomes of the virus are currently being sequenced every day around the world. This enormous sequencing capacity allows us to rapidly generate new mRNA sequences that could be more effective or even adapt vaccines to new pathogens.[6]

The generation of synthetic mRNA sequences is relatively simple; however, there is still a huge area of development related to stability and scalability in synthesis. Although mRNA must be degraded within a reasonable time after entering the target cell, degradation should also occur after generating sufficient virus protein.[4] On the other hand, the low stability of mRNA poses logistical problems, since these vaccines normally require cold chains that can reach –70°C, which greatly hinders distribution and conservation on a large scale. Scalability is an important issue, as it offers substantial advantages by not relying on living cells or the management of active viruses, as many other vaccines do. Still, few vaccine production facilities currently have the technology to generate the necessary elements for mRNA vaccines.[4,6] A general process of mRNA vaccine development is shown in Figure 1.

There are key elements to ensure the optimum stability of the vaccine. The 5 'and 3' UTRs (untranslated regions) have an influence on both the stability and the translation of mRNA. Basic elements such as cap 5' and a poly (A) tail at the 3' end are basic to ensure an adequate half-life of the molecule. The codons used are also important, mainly in translation. In general, the aim is to use the most common codons in human cells, which allows for a more efficient translation. However, it must also be considered that the change of codons can influence the processivity and precision of the ribosome. Codon usage remains an area of development in mRNA vaccines.[3,4,7]

Chemical modifications are a common practice to increase nucleotide resistance against degradation or translation rate. Some studies show that the change of nucleotides in pseudouridine and 5-methylcysteine increases translation and decreases RNA immunogenicity. Modifying the structure

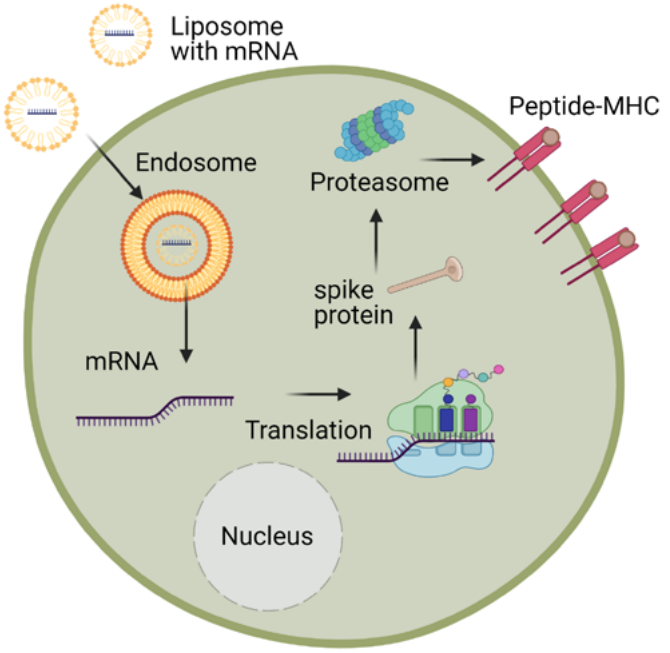

Figure 1. General process of mRNA vaccines.
Lipid particles are endocytosed. Once inside the cell, the mRNA contained in the particles is released into the cytoplasm. The mRNA particles are translated by the cellular ribosomes, expressing the S protein of the SARS-CoV-2 virus. Enzymes of the transcriptional machinery of the virus are also expressed in self-amplification mRNA vaccines. The viral S protein is processed by the proteosome and exposed to immune system cells by the major histocompatibility complex (MHC). Created in BioRender.com

of the 5'cap (usually a cap 1) is another strategy; the anti-reverse cap analogue and phosphorothioate derivatives have been shown to improve transcription by adding the cap only in the forward orientation. Once the features and design of the mRNA are completed, the evaluation of possible structural modifications must be done. The formation of certain secondary structures and double-stranded RNA (dsRNA) can significantly decrease translation rates and increase the immunogenicity of the mRNA at the same time. On the other hand, to avoid contamination with other molecules or even with unwanted secondary structures, purification methods such as fast protein liquid chromatography (FPLC) or high-performance liquid chromatography (HPLC) must be used.[7]

In general, we can distinguish two types of mRNA structures used for vaccines: conventional mRNA and self-amplification mRNA. The first are based on the virus sequence of positive-sense single-stranded RNA. They are composed of the sequence of the antigen of interest flanked by regulatory regions (5´ and 3´ UTR), a 5´cap structure and a poly (A) tail, a structure relatively simple and small. In self-amplifying RNAs the nucleic acid molecule contains the antigen of interest and the information of the transcriptional machinery of the

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virus, particularly the sequence of the RNA-dependent RNA polymerase, usually of some alphavirus. The self-replicating RNAs also contain regulatory sequences which potentiate replication and translation; therefore, they are larger (9–11 kd) and more complex than conventional mRNA. Recent studies have shown advantages and disadvantages of both mRNA structures. Interestingly, it has been noted that self-replicating mRNA needs much lower doses of inoculation and promotes a prolonged and stable expression of the antigen of interest. This is why many current developments are based on self-replicating mRNA technology.[3,4]

A series of extremely important elements are considered for the inoculation and delivery of mRNA molecules. Many formulations that allow the entry of mRNA into cells and their release into the cell cytoplasm have been developed. In general terms, they are vehicles containing genetic material that are capable of fusing with the cell membrane. The most common carriers are lipid nanoparticles (LNPs), cationic lipids, polymers, and protamine sulphate. The cationic peptide protamine has been shown to protect mRNA from degradation by serum RNases. However, protamine-complexed mRNA alone demonstrated limited protein expression and efficacy in a cancer vaccine model, possibly owing to an overly tight association between protamine and mRNA. This issue was resolved by developing the RNActive vaccine platform in which protamine-formulated RNA serves only as an immune activator and not an expression vector.[8,9] In addition to the vehicle used, the inoculation site is essential to obtaining the expected therapeutic results.

Immunogenicity

The use of mRNA vaccines has several beneficial features over subunit, killed, and live attenuated ones, as well as those based on DNA.[3] mRNA vaccines strongly induce both cellular and humoral immune responses. They are relatively safe and effective because they contain only a transient carrier of information that does not interact with the host genome nor needs the whole virus.[4] As it happens, in a natural SARS-CoV-2 infection, the production of specific anti-SARS-CoV-2 Abs induced by mRNA vaccines depends on the activation of the adaptive immune response. It starts when T lymphocytes are presented a relevant peptide derived from the virus by an antigen-presenting cell. This leads to the activation of antigen-specific CD8+ (cellular immunity) and CD4+ T cells, which play a key role in the activation of B lymphocytes. The latter will eventually initiate the production of SARS-CoV-2 specific Abs targeting the SARS-CoV-2 S protein. Studies have shown that these Abs can neutralize the virus in its extracellular stage and thus inhibit the infection by SARS-CoV-2 in vitro and/or in vivo.[5,6]

Karikó et al. found that the inherent immunogenicity of mRNA can be down-modulated to further increase the safety profile.[7] Single-stranded oligoribonucleotides and their degradative products are detected by the endosomal sensors toll-like receptor 7 (TLR7) and 8 (TLR8),[10,11] resulting in type I interferon (IFN-I) production.[12] The authors found that the incorporation of N1-methyl-pseudouridine (m1Ψ) in place of uridine led to a 10-fold increase in translation over unmodified mRNA. Furthermore, they were able to show that this modification in mRNA molecules prevents the activation of TLR7, TLR8 and other innate immune sensors, reducing IFN-I signaling,[13] and undesired vaccine side-effects. For these reasons, many candidates, including the two recently licensed mRNA vaccines mRNA-1273 and BNT162b2, adopted this m1Ψ mRNA modification in their vaccine design. Nucleoside modification also partially suppresses the recognition of dsRNA species.[13]

The immunostimulatory properties of mRNA can conversely be increased by incorporating an adjuvant to increase the potency of some mRNA vaccine formats. These include traditional adjuvants as well as novel approaches that take advantage of the intrinsic immunogenicity of mRNA or its ability to encode immune-modulatory proteins. Self-replicating RNA vaccines have displayed increased immunogenicity and effectiveness after formulating the RNA in a cationic nanoemulsion based on the licensed MF59 (Novartis) adjuvant.[14] Another effective adjuvant strategy is TriMix, a combination of mRNAs encoding three immune activator proteins: CD70, CD40 ligand (CD40L), and constitutively active TLR4. Van Lint et al. found that TriMix mRNA increased the immunogenicity of naked, unmodified, unpurified mRNA in multiple cancer vaccine studies and was particularly associated with increased dendritic cell maturation and CD8+ T cell response.[15] The type of mRNA carrier and the size of the mRNA–carrier complex have also been shown to modulate the cytokine profile induced by mRNA delivery.

The application of mRNA vaccines has been restricted until recently by the instability and inefficient in vivo delivery of mRNA. Recent technological advances have overcome these issues, and multiple mRNA vaccine platforms have been developed in recent years. They have been validated in studies of immunogenicity and efficacy against infectious diseases and several types of cancer in animal models and humans. Recently, mRNA vaccines have elicited potent immunity against infectious disease targets in animal models of influenza, Zika (ZIKV), and rabies viruses, among others, using lipid-encapsulated or naked forms of sequence-optimized mRNA.[16] Highly efficient and non-toxic RNA carriers, like LNPs have been developed to allow prolonged antigen expression in vivo.[17] LNPs have become one of the most appealing and commonly used mRNA delivery tools and often consist of four components: an ionizable cationic lipid, promoting self-assembly into virus-sized particles (~100 nm) and allowing endosomal release of mRNA into the cytoplasm; lipid-linked polyethylene glycol (PEG), which increases the half-life of formulations; cholesterol, a stabilizing agent; and naturally occurring phospholipids that support lipid bilayer

structure. Geall et al. and Pardi et al. demonstrated that LNPs are potent tools for in vivo delivery of self-amplifying RNA and conventional, non-replicating mRNA, respectively.[16,17] mRNA–LNP complexes administered intradermally, intramuscularly, and subcutaneously lead to prolonged protein expression at the site of injection.[17]

Then, to evaluate the efficacy of vaccines, pre-clinical evaluations of SARS-CoV-2 mRNA vaccines have focused on their ability to elicit robust SARS-CoV-2-binding and neutralize Ab responses in mice. Laczkó et al. found that a single 30-µg dose of an mRNA-LNP vaccine, encoding RBD (receptor binding domain) of SARS-CoV-2, promoted high titers of SARS-CoV-2-binding immunoglobulin G (IgG) in mice, just two weeks post-immunization.[18] In every mRNA vaccine candidate (Moderna mRNA-1273, BioNTech/Pfizer BNT162b2, and CureVac CVnCoV) the production of SARS-CoV-2-specific Abs was achieved by a significantly lower dose (0.2–10 µg) than the one used in Laczkó's study. Still, a booster immunization was necessary for NAb (neutralizing antibody) generation at lower doses (1 or 2 µg).[19] These Abs neutralized the virus in vitro, as measured by SARS-CoV-2-based neutralization assays,[18, 19] and their levels were sustained for two months or more post-immunization.[20] All mRNA vaccines induce a potent germinal center response, which produces a more effective and long-lasting antigen-specific Ab response.[21]

As the wild-type SARS-CoV-2 cannot efficiently replicate in common laboratory mouse strains due to the lack of appropriate receptors to initiate viral infection, studies in non-human primates (NHPs) are necessary to determine the efficacy of vaccine candidates. Interestingly, in NHPs, the clinical candidates mRNA-1273 (10 or 100 µg) and BNT162b2 (30 or 100 µg) demonstrated a robust, dose-dependent capacity to elicit SARS-CoV-2 specific Abs after two immunizations. Also, NAb values elicited in NHPs by BNT162b2 were higher than the ones found in SARS-CoV-2 human convalescent sera. The NAb responses elicited by these candidates were combined with in vivo protection against SARS-CoV-2 challenge after the booster immunization. The viral replication found in the upper respiratory tract of the infected animals that received any of these vaccines was transient. Moreover, no viral replication was found in the lower respiratory tract of the vaccinated animals.[22]

A lot has been speculated about the development of Ab disease enhancement (ADE) by SARS-CoV-2 vaccines. This phenomenon is described as the increase in disease severity induced by a vaccine if the subject is later infected by the natural virus. It can be characterized by immunopathology and a T helper 2 cell (Th2) biased response and Ab responses with poor neutralizing activity.[23] Therefore, ADE could occur when a vaccine fails to develop NAbs because of insufficient concentration/affinity or Abs incapable of binding their antigen. Some Middle East respiratory syndrome (MERS) and SARS-CoV-1 vaccines have shown evidence of ADE in animal models, a concern with COVID-19 vaccines. The Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency Vaccines (SPEAC) issued a statement after a scientific working meeting that took place in May 2020, declaring that vaccines inducing strong NAbs and predominant Th1 responses are less likely to induce ADE. The experts also mentioned that the level of NAbs and determination of the relative ratio of binding to NAbs is key to assess the potential risk of ADE in phase 1 clinical trials of COVID-19 vaccines. They suggest to perform a longer follow-up than usual in phase 1 trials to monitor this syndrome in immunized participants.[23]

While no evidence has shown that Ab-dependent enhancement can occur in SARS-CoV-2 infection, the two mRNA vaccines discussed above induce high levels of SARS-CoV-2 binding Abs and NAbs. Therefore, any potential Ab-dependent enhancement could be ruled out.[23,24] Overall, mRNA vaccines seem to activate CD8+ T cell response. A single dose of SARS-CoV-2 mRNA in mice elicits antigen-specific CD8+ T cell responses, characterized by the production of IFN-γ, IL-2, and/or TNF.[18] Preclinical studies of the mRNA clinical candidates have shown that BNT162b2 administration in mice resulted in increased amounts of IFN-γ- and IL-2-secreting CD8+ T cells in the spleen 12 days after immunization.[25] Moderna mRNA-1273 was found to elicit a CD8- T cell response in mice but, in preclinical trials, failed to induce detectable CD8+ T cell responses in macaques, even with high doses (100 µg).[26] Interestingly, in natural SARS-CoV-2 infection in humans, the S protein has been shown to elicit a relatively modest CD8+ T cell response in some COVID-19 cases.[27] Since SARS-CoV Abs levels decrease after two months of natural infection, it has been proposed that the induction of humoral and cellular responses is necessary to generate an optimal long-lasting protective response.[28,29] However, there is no indication that the induction of cytotoxic CD8+ T cells is required for a successful protection against SARS-CoV-2 via vaccination.

Safety

One key advantage of mRNA vaccines is that their production avoids common risks associated with other vaccine platforms (live virus, viral vectors, inactivated virus, and subunit protein vaccines) since they do not require toxic chemicals or cell cultures that could be contaminated with adventitious viruses. Additionally, mRNA is manufactured in a short time, so it is unlikely to get contaminated by microorganisms. This type of vaccines does not contain a live virus, and therefore, does not carry a risk of causing disease in the vaccinated person. The mRNA of the vaccine never enters the nucleus of the cell and does not affect or interact with the person's DNA. Thus, the theoretical risks of infection or integration of the vector into host cell DNA are not a concern in vaccinated people. Probst

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et al. showed that several cell types in mouse dermis take up foreign protein-coding RNA, which can also be demonstrated in human skin. Moreover, the injected mRNA metabolically decays within a few days, making this molecule a merely transient and safe carrier of information.[30] As mRNA is a non-infectious, non-integrating platform, there is no potential risk of insertional mutagenesis. mRNA is degraded by normal cellular processes, and its in vivo half-life can be regulated using various modifications and delivery methods.[31] As mentioned before, studies over the past decade have shown that the immunostimulatory profile of mRNA can be shaped by the incorporation of pseudouridine. This prevents its recognition via PRRs, and therefore, the activation of inflammation and IFN-I production, reducing the unwanted vaccine side-effects.[31,32]

The fact that vaccines are administered to healthy individuals stablishes a strict requirement for safety. Phase 1 to 2b clinical studies are in course to test several mRNA vaccines, and they have shown that these vaccines are safe and reasonably well tolerated. Potential safety concerns that are likely to be evaluated in future preclinical and clinical studies include local and systemic inflammation, the biodistribution and persistence of expressed immunogen, stimulation of auto-reactive Abs, and potential toxic effects of any non-native nucleotides and delivery system components.

Although all the clinical data reviewed indicate that mRNA vaccines are safe to use in humans, this is the first time that a vaccine of this type has been licensed, which raises some potential concerns. There have been rare reports of individuals experiencing anaphylaxis following immunization with COVID-19 mRNA vaccine.[33] As with all vaccines, some people can have allergic reactions to one or more of the components included in the vaccine: an adjuvant, an antibiotic used in the manufacturing process, one of the lipids enveloping the mRNA, or even a salt used as a diluent. As it stands, the offending component of this mRNA vaccine remains unclear, but anaphylaxis represents a major concern for people with a history of severe allergies.

A possible safety concern regarding these novel vaccines is that some mRNA-based vaccine platforms induce a potent IFN-I response[34] associated with inflammation. If it persists, this process becomes chronic, leading to potential autoimmunity.[35] It has been proposed that the identification of individuals at an increased risk of autoimmune reactions before mRNA vaccination could allow for reasonable precautions to be taken. Another potential safety issue is the presence of extracellular RNA during mRNA vaccination. Some studies have addressed this concern and found that extracellular RNA promoted blood coagulation and pathological thrombus formation.[36] In addition, Fischer et al. showed that extracellular naked RNA increases the permeability of tightly packed endothelial cells and may contribute to edema.[37]

The vaccine associated enhanced respiratory disease (VAERD) is a safety issue that requires further investigation regarding SARS-CoV-2 vaccines. Lung immunopathology refers to exaggerated lung inflammation after a viral infection, which may interfere with oxygenation and can lead to a worse disease than what would normally be seen after virus infection in the complete absence of vaccination. Clinical VAERD was first seen in the 1960s among human infants with RSV infection after receiving a formalin-inactivated vaccine against RSV that led to markedly worse respiratory disease as compared to non-vaccinated infants.[38] Some vaccine studies have evaluated the balance of Th1 and Th2 because VAERD has been linked with Th2-biased immune responses in children immunized with whole-inactivated virus vaccines against RSV.[39] Even some inactivated SARS-CoV vaccines have triggered VAERD in some animal models. Studies conducted with mRNA-1273 showed that the Ig subclass and T cell cytokine profile activated after immunization trigger a balanced Th1/Th2 response as compared with SARS-CoV-2 S protein adjuvanted with alum, which clearly skewed the response to Th2 profile. This suggests that mRNA vaccination avoids the Th2-biased immune response linked to VAERD. A major goal of animal studies to support SARS-CoV-2 vaccine candidates through clinical trials is to show that sub-protective responses do not cause VAERD. For the above reasons, mRNA vaccines have been considered relatively safe. Still, they must be evaluated as different mRNA modalities and delivery systems are used in humans and tested in larger patient populations for the first time.

Pfizer-BioNTech vaccine BNT162

Since scientists in China released the genetic sequence of SARS-CoV-2 in January 2020, worldwide research for a potential vaccine was triggered. Having access to global data has been critical to a fast and efficient development. On March 17th, 2020 Pfizer and the German biotech company BioNTech announced their partnership to co-develop a potential mRNA vaccine to prevent the spread of COVID-19. Pfizer stated that both companies would jointly develop BioNTech's mRNA-based vaccine candidate BNT162 to prevent COVID-19 infection, as they had already been working with their German partner to develop an RNA influenza vaccine since 2018.[40]

BioNTech has developed multiple formats and delivery formulations for its mRNA SARS-CoV-2 vaccine platform. Some of them are utilized in *Project Lightspeed*,[41] an initiative to jointly develop and test multiple COVID-19 vaccine candidates as part of a global development program.[42] BioNTech has developed and tested a total of four SARS-CoV-2 vaccine candidates, all of them using the LNP delivery formulation, and three of the mRNA formats (uRNA, modRNA, and saRNA). Two of the four vaccine candidates include a nucleoside modified mRNA (modRNA), one includes a uridine containing

mRNA (uRNA), and the fourth vaccine candidate utilizes self-amplifying mRNA (saRNA). The longer spike sequence is included in two of the vaccine candidates, and the smaller, optimized receptor binding domain (RBD) of the spike protein is included in the other two candidates. The RBD-based candidates contain the piece of the spike that is thought to be the most important for eliciting Abs that can inactivate the virus.[41]

In April, BioNTech and Pfizer received authorization from the German regulatory authority, the Paul-Ehrlich-Institut, to initiate phase 1/2 of the clinical trial for the BNT162 vaccine candidate. The companies concurrently began two phase 1/2 umbrella trials: one with the candidate BNT162b1 in Germany[43, 44] and another one with candidates BNT162b1 and BNT162b2 in the US.[44, 45] BNT162b1 encodes the SARS-CoV-2 RBD trimerized by adding a domain of T4 fibritin (foldon) to increase its immunogenicity through multivalent display. BNT162b2 encodes the SARS-CoV-2 full-length spike, modified by two proline mutations (optimized 2-proline (2P)-mutated SARS-CoV-2 full-length S glycoprotein) to lock it in the prefusion conformation and mimic the intact virus with which the elicited virus-NAbs must interact.[46, 47]

Although different dose schemes were tested in the trials, all doses of BNT162b1 elicited an RBD-specific IgG response in the range of SARS-CoV-2 convalescent plasma within 21 days of the initial vaccination, with a detectable increase after the boost.[44,48] Additionally, both vaccine candidates elicited S-binding IgG Abs at comparable levels after the second immunization[45] and NAb values above the baseline. These NAbs were measured in vitro using a neutralization assay with a modified SARS-CoV-2 reporter virus, and they were detected only after the second immunization.[44,45,48] Although these data indicate that SARS-CoV-2 mRNA vaccines are effective when inducing SARS-CoV-2 IgG responses, a second dose of either mRNA vaccine formulation seems to be required to reach significant levels of NAbs. When evaluated in elderly subjects, both BNT162b1 and BNT162b2 induced antigen-specific IgG titers after the first vaccination, which were enhanced by a second immunization. The elderly population also needed a booster immunization to induce NAb production. Notably, NAb titers were overall lower in the elderly when compared to younger subjects.[45] It was observed that BNT162b2 elicited robust and durable CD4+ and CD8+ T cell responses in most of the trial subjects. The SARS-CoV-2-specific total CD4+ T cells promoted by BNT162b1 were polarized toward a Th1 functional profile, as measured by the frequency of SARS-CoV-2-specific CD4+ T cells producing IFN-γ and IL-2 but not IL-4, upon stimulation with SARS-CoV-2 peptides.[44,48]

BNT162b1 and BNT162b2 also showed to be safe when injected to adults. In the safety evaluation of these two candidates, pain and tenderness were reported as the most common adverse events. Fever, fatigue, and chills were the most frequently reported systemic adverse events. Reactogenicity was dose-dependent and more pronounced after the boost dose. It is important to mention that BNT162b2 induced less adverse events than BNT162b1, particularly in participants aged 65–85 years. After analyses of data from their phase 1/2 trials in Germany and the U.S., BioNTech and its collaborators selected BNT162b2 for use in their subsequent phase 3 studies.[44] The D614G mutation was included in BNT162b2 in phase 1/2 trials since this is the most commonly observed SARS-CoV-2 S variant in mutational analyses reported in the literature.[49] As of December 30, 2020, BNT162b2 had been authorized for emergency use in the United States. As such, mRNA vaccine administration to the public has commenced. Along with this decision also comes the issue of confounding the continuation of phase 3 studies. The individuals originally in the placebo control groups will also be vaccinated, rendering the long-term double-blinded study of SARS-CoV-2 vaccine-induced immunity impossible.

BioNTech/Pfizer have recently published data of the ongoing clinical phase 3 of BNT162b2.[50] In about 44,000 participants enrolled in the study, the two-dose immunization regimen (30-µg doses, 21 days apart) conferred a remarkable 95% protection against COVID-19. Only eight individuals developed COVID-19 while 162 cases were reported in the placebo group. BNT162b2 also proved to be safe: only 27% of the vaccinated individuals and 12% of the placebo group reported adverse events, mostly short-term mild-to-moderate local reactions.

Moderna Therapeutics vaccine mRNA-1273

It has been found that S proteins from members of the coronavirus family undergo a dramatic structural rearrangement to fuse virus and host cell membranes, promoting the delivery of the viral genome into the target cells. It was previously shown that prefusion-stabilized protein immunogens that preserve neutralization-sensitive epitopes are an effective vaccine strategy for enveloped viruses. Two proline substitutions (2P) at the apex of the central helix and heptad repeat 1 were identified and effectively stabilized MERS-CoV and SARS-CoV in the prefusion conformation. Therefore, just 24 hours after the release of the SARS-CoV-2 isolate sequence, 2P mutations were substituted into S positions aa986 and 987 to produce prefusion-stabilized SARS-CoV-2 S (S-2P) protein. Shortly after, Moderna and the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID), within the National Institutes of Health (NIH), initiated the production of mRNA-1273, an LNP–encapsulated mRNA vaccine expressing SARS-CoV-2 S-2P as a transmembrane-anchored protein with the native furin cleavage site (mRNA-1273),[51, 52] In March 2020, the FDA authorized the study of this vaccine candidate to proceed to clinical trials.[53]

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In a phase 1 study of mRNA-1273 conducted at Emory University in Atlanta, 45 participants aged 18–55 years received two immunizations (prime and boost) with 25, 100, or 250 µg of the SARS-CoV-2 mRNA vaccine, 28 days apart. The vaccine achieved NAb induction in all the participants. Two weeks after the second dose, the title of these Abs, detected by enzyme-linked immunosorbent assay (ELISA) for full S- and RBD-specific IgG, was superior to that observed in recovered COVID-19 patients. Antigen-specific IgG titers further increased after the boost viral neutralization was measured by in vitro neutralization assays with a pseudotyped lentivirus and wild-type SARS-CoV-2. NAbs reached levels in the range of convalescent serum only after the second immunization in all vaccine groups. mRNA-1273 potentially induces a durable Ab response, since participants of the 100-µg dosage group were followed up for 119 days after the initial vaccination (90 post-boost), and, despite a slight decrease, NAb levels remained significantly elevated in all participants.[54]

The NIH-led phase 1 study of mRNA-1273 completed the enrollment of the three-dose cohorts mentioned above and expanded to an additional six cohorts: three of older adults (ages 56–70) and three of elderly adults (ages 71 and above). The participants of this study were immunized twice with either 25 or 100 µg mRNA-1273, and a robust binding and neutralizing Ab response was detected after two doses.[55] It was also observed that individuals over 56 years of age developed a higher NAb response after a second immunization in response to the higher dose (100 µg).

In phase 1 trials, mRNA-1273 elicited a measurable SARS-CoV-2-specific total CD4+ T cell response that was strongly biased towards the production of Th1 cytokines, with minimal Th2 cytokine production.[55] By contrast, the SARS-CoV-2-specific CD8+ T cell response was almost undetectable in most vaccinated individuals, even after the boost dose. These results are in line with those obtained after immunizing rhesus macaques with mRNA-1273 during preclinical trials, where no antigen-specific CD8+ T cell response was found, even with doses as high as 100 µg. It is unclear why mRNA-1273 is unable to promote effective CD8+ T cell response in larger animal models and humans, and it is a concern that needs further investigation.[56]

There were no serious adverse events reported during the phase 1 trial of mRNA-1273 that met the criteria to halt the trial. Local pain at the injection site was the most common while systemic events including fever, chills, and headache were registered with increased incidence and severity after the booster immunization and with the higher vaccine doses.[57] The same safety profile was also observed in the elderly.[55] As mentioned before, the mRNA-1273 vaccine did not show evidence of enhanced respiratory disease after infection in the short term.[58]

In October 2020, Moderna completed the enrollment of the phase 3 COVE Study, and in November. mRNA-1273 met its primary efficacy endpoint in the first interim analysis of the phase 3 COVE study. Interestingly, Moderna announced a longer shelf life for mRNA-1273 at refrigerated temperatures in that same month. In December 2020, The FDA authorized the emergency use of mRNA-1273 in individuals aged 18 years or older in the United States. The phase 3 clinical trial for Moderna vaccine mRNA-1273 is currently undergoing.[59] This trial consists of around 30,000 participants whose ages range from 18 to 85 years. All participants received a two-dose injection series of either 100 µg of mRNA-1273 or a saline placebo, separated by 28 days. The results show a 94.1% efficacy rate of mRNA-1273; only 11 COVID-19 cases were reported in the vaccine group versus 185 cases in the placebo group. The safety profile of this vaccine is also very favorable, as there are no safety issues reported.

CureVac Vaccine CVnCoV

CureVac NV (Nasdaq: CVAC) is a company of German origin with experience in the design of mRNA technology. In 2020 it began the process to create a vaccine against COVID-19 based on mRNA. The vaccine candidate called CVnCoV is a complete stabilized pre-fusion S protein-based mRNA, using LNP as a vehicle. Unlike other vaccines, CVnCoV is made exclusively of nucleotides without chemical changes based on its proprietary RNActive ® technology.[19] In October 2020, the company published its results in mice and hamsters;[19] the vaccine presented a strong humoral immune response of IgG1 and IgG2a SARS-COV-2 virus neutralizing Abs. There were IFN-γ +/TNF + CD4 + and CD8 + responses. In December 2020, the data of his study in Rhesus monkeys were released, proving the vaccine induced a robust cellular and humoral immune response, as the animals were protected against infection by SARS-CoV-2. Histological and pathological analysis as well as general evaluations show that the vaccine is safe to use in non-human primates.[19, 60]

In December 2020, the phase 2 b/3 clinical study of the CVnCoV vaccine against COVID-19 in adults began (ClinicalTrials.gov Identifier NCT04652102). This randomized, double-blind clinical trial studies 36,500 participants from various centers around the world, including Mexico. The study is designed to evaluate disease prevention in adults (18 years and older) after the application of the vaccine (12 ug) or placebo in the deltoid area on days 1 and 29 (two inoculations). The clinical trial is in progress and the first results were expected in May 2021. One of the benefits of the CVnCoV vaccine is that it can be stored for at least 3 months at temperatures between 2 and 8°C. CureVac has generated various collaborations with technology companies as Tesla and renowned pharmaceutical companies such as Bayer, Celonic, GSK, and Novartis. It aims to

ensure the large-scale production of the vaccine and generate new vaccines against various variants of the coronavirus that may be detected.

Other mRNA vaccines

There are several developments of mRNA-based vaccines from various institutions around the world; among the projects with the greatest advances are: i) self-replicating mRNA vaccine by Arcturus Therapeutics in association with the Duke-NUS School of Medicine in Singapore, and ii) the Chinese ARCoV vaccine from the Academy of Military Medical Sciences, Suzhou Abogen Biosciences and Walvax Biotechnology. Assays in mice have shown the first induces immune response and protection against SARS-CoV-2 infection. Preliminary results obtained in the clinical trial phase 1/2 showed efficacy and safety, so that a clinical trial phase 2 began in Singapore and US.[2] ARCoV presented protective effects and safety in phase 1, and phase 2 of this vaccine is currently ongoing.[2,66] There are various developments of new vaccines against COVID-19 in countries such as Japan, India, South Korea, Italy, France, and several others. Still, most of these mRNA vaccine projects are in phase 1 or 1/2 of study and, although promising, they will likely have solid results until 2022 or later (Table 1).[2,66]

DISCUSSION AND CONCLUSION

mRNA vaccines have proven their effectiveness and safety in real life. In the months after their emergency authorization by the different regulatory entities, millions of doses of Pfizer-BioNTech and Moderna vaccines have been administered in 83 and 35 countries, respectively.[61] In all studies and statistics, the protection and safety levels shown in clinical trials are favorable. Other mRNA vaccines against COVID-19 are expected to enter the market and be used massively around the world in the coming months.[2, 61] Among the concerns that still exist regarding the use of this type of vaccine, perhaps the most important is its long-term effects. Although everything indicates that it is capable of generating a protective and lasting immune response, we have yet to learn how this immune response behaves over long periods of time.

The pandemic has forced us to increase our knowledge of mRNA vaccines at an extraordinary high rate as technical, development, production, and distribution capabilities are expanded. All these advances ensure that mRNA vaccines will be one of the most widely used strategies in the control of infectious diseases in the coming years. mRNA is a safe and effective method of vaccination and offers a solution to counter the threat of emerging infectious diseases. mRNA vaccines can direct the expression of virtually any membrane-bound, soluble, or polyprotein antigens, mimicking antigen expression during natural infection. Since their effect is only transient, they are highly useful in the development of prophylactic and therapeutic alternatives. In addition, this technology potentially improves morbidity and mortality rates.[62]

mRNA vaccines have been rigorously assessed for safety, and clinical trials have shown that they provide a long-lasting immune response. They are safer for the patient since they are not produced using infectious elements as pathogen particles or inactivated pathogens. Moreover, mRNA does not integrate itself into the host genome and the RNA strand in the vaccine is degraded once the protein is made.[3] Early clinical trial results indicate that these vaccines generate a reliable immune response and are well tolerated by the vast majority of the population, with few side effects.[3]

In recent years, multiple mRNA vaccine platforms have been developed and validated in studies of immunogenicity and efficacy against infectious diseases and several types of cancer, in animal models and humans. mRNA vaccines have elicited a potent immunity against infectious diseases like influenza virus and ZIKV. In a phase 1 randomized clinical trial for mRNA vaccines against H10N8 and H7N9 influenza viruses, favorable safety and reactogenicity profiles were observed, and no serious adverse events related to the vaccine were reported.[63] Additionally, a modified mRNA vaccine protected against ZIKV and diminished the production of Abs enhancing DENV infection in cells or mice.[64] These advances have demonstrated the potential of mRNA-based vaccines. To date, clinical trials of COVID-19 mRNA-based vaccines have shown that the safety profile and efficacy rate are very favorable.[45]

mRNA vaccines can be created quickly and produced at a large scale, which reduces their cost in the long run. In addition, they are produced in a laboratory and have the potential for rapid, high-volume manufacturing with the precision and flexibility of antigen design necessary to provide both timely and effective responses to large outbreaks and epidemics. They also offer a more flexible stockpiling approach. Low-volume libraries of frozen plasmid and/or unformulated mRNA can be potentially stored for decades and then rapidly formulated and distributed as threat levels rise.[65]

Despite all the advantages of mRNA-based vaccines, there are technical challenges to overcome, the most important being storage as they need to be frozen or refrigerated due to the thermolability of the RNA. Work is ongoing to reliably produce vaccines that can be stored outside the cold chain, which will be much more suitable for use in countries with limited or no refrigeration facilities.

The global effort to create mRNA-based vaccines against COVID-19 has greatly advanced mRNA technology and increased the speed of mRNA vaccine development. This will

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Table 1. mRNA vaccines against SARS-CoV-2.

Vaccine	Manufacturer	Efficacy	Dose	Administration	Storage	Phase
Comirnaty (tozinameran or BNT162b2)	Pfizer-BioNtech and Fosun Pharma	91.30%	2 doses, 3 weeks apart	Intramuscular	Freezer storage only from –25°C to –15°C	Emergency use/Phase 4
mRNA-1273	Moderna and National Institute of Allergy and Infectious Diseases (NIAID)	90%	2 doses, 4 weeks apart	Intramuscular	30 days under refrigeration, 6 months at –20°C	Emergency use/Phase 4
CVnCoV	CureVac	Unknown	2 doses, 4 weeks apart	Intramuscular	Stable at least 3 months at 2–8°C	Phase 3
ARCT-021	Arcturus Therapeutics and the Duke-NUS School of Medicine in Singapore	Unknown	Unknown	Intramuscular	Unknown	Phase 2
ARCoV	Academy of Military Science (AMS), Suzhou Abogen Biosciences and Walvax Biotechnology	Unknown	2 doses, 2 or 4 weeks apart	Intramuscular	Unknown	Phase 3
LNP-nCoVsaRNA	Imperial College London	Unknown	2 doses	Intramuscular	Unknown	Phase 1
ChulaCov19	Chulalongkorn University	Unknown	2 doses	Intramuscular	Unknown	Phase 1
PTX-COVID19-B	Providence Therapeutics	Unknown	2 doses, 4 weeks apart	Intramuscular	Unknown	Phase 1
CoV2 SAM (LNP)	GlaxoSmithKline	Unknown	2 doses, 30 days apart	Intramuscular	Unknown	Phase 1
mRNA-1273.351	Moderna and National Institute of Allergy and Infectious Diseases (NIAID)	Unknown	3 doses, 28 or 56 days apart	Intramuscular	Unknown	Phase 2
MRT5500	Sanofi Pasteur and Translate Bio	Unknown	2 doses, 3 weeks apart	Intramuscular	Unknown	Phase 1/2
DS-5670a	Daiichi Sankyo	Unknown	2 doses	Intramuscular	Unknown	Phase 1/2
HDT-301	SENAI CIMATEC	Unknown	2 doses, 4 weeks apart	Intramuscular	Unknown	Phase 1
mRNA-1283	Moderna	Unknown	2 doses, 4 weeks apart	Intramuscular	Unknown	Phase 1
EXG-5003	Elixirgen Therapeutics	Unknown	1 dose	Intradermal	Unknown	Phase 1/2
mRNA COVID-19 vaccine	Shanghai East Hospital and Stemirna Therapeutics	Unknown	Unknown	Intramuscular	Unknown	Phase 1

likely benefit other health care areas, particularly oncology. There have been major efforts for several years to use mRNA technology to fight cancer, with promising results. In this way, mRNA vaccines become a new paradigm not only in vaccination but in health in general.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Review Article

Musculoskeletal pain in college students: a systematic review

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ABSTRACT

Introduction: To date, there is no systematic study focused on posture-related musculoskeletal pain in college students. **Objective:** Summarize the evidence of musculoskeletal pain in college students. **Eligible criteria:** Year of publication between 2015 and 2020, observational studies published in English, full text available, and analysis of the presence of musculoskeletal pain in college students. MESH terms and Boolean operators used for the search were pain AND musculoskeletal AND college students. **Data sources:** Databases used were PubMed, ClinicalKey, ProQuest, ResearchGate, and ScienceDirect. **Results:** A total of 318 records were identified out of which 296 were excluded, and only 22 were included for analysis. The majority of the records involved college students form the general population, while five involved dental students and five, health science students. The majority of the records involved a setting in which the student was in a constant sitting position, only two involved a dental setting, and one involved a laboratory setting. **Conclusions:** The most reported sites of pain were neck, shoulders, and upper and lower back.

Key words: musculoskeletal pain; college students.

RESUMEN

Introducción: A la fecha no hay una revisión sistemática cuyo enfoque sea dolor musculoesquelético en estudiantes de universidad. **Objetivo:** Resumir evidencia de dolor musculoesquelético en estudiantes de universidad. **Criterios de selección:** Año de publicación entre 2015 y 2020, estudios observacionales en idioma Inglés, texto completo disponible, análisis de la presencia de dolor musculoesquelético en estudiantes de universidad. Se usaron los términos MeSH y operadores booleanos dolor Y musculoesquelético Y estudiantes de universidad. Fuentes de información: PubMed, ClinicalKey, ProQuest ScienceDirect y ResearchGate. **Resultados:** Un total de 318 artículos fueron identificados, de los cuales 296 fueron excluidos; solo 22 fueron incluidos en el análisis. La mayoría de los estudios involucra a estudiantes de la población universitaria en general, cinco artículos involucran a estudiantes de odontología y cinco más, a estudiantes de ciencias de la salud. La mayoría de los artículos incluyen espacios en los que el estudiante debe permanecer en una posición sentada constantemente; solo dos artículos involucran un espacio de consultorio dental y uno, el espacio de laboratorio médico. **Conclusiones:** Los sitios de dolor más reportados fueron cuello, espalda alta y baja y hombros.

Palabras clave: dolor musculoesquelético; estudiantes de universidad.

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INTRODUCTION

College students often use notebooks and electronic devices for notetaking during class time. They tend to adopt inadequate postures while using these electronic devices, which predisposes them to develop musculoskeletal dysfunction.[1,2] The overuse of the upper extremities due to repetitive activities, maintaining postures while writing, brief periods of rest, and academic/life stress can cause the muscles to be in constant activation. Maintaining postures can lead to conditions as tendinitis and carpal tunnel syndrome, among others.[1] The fact that a laptop keyboard and screen are linked and do not move independently will force a user to choose a convenient hand/wrist or head/neck posture.[2-4] In the 2015–2016 school year, there were approximately 3.6 million, 945 higher-education students in Mexico; 364,894.5 of them at the National Autonomous University of Mexico (UNAM) and 11,527 at Universidad Anahuac México Norte, as well as 386,219 higher-education teachers. Of these 3.6 million students, 51% use computers and/or electronic tablets for notetaking and schoolwork.[5] It is essential to identify the sites and prevalence of musculoskeletal pain among college students as the previous history of pain is a risk factor for a new episode of pain,[6] pain intensity, and disability.[7] If untreated, pain can cause a person to go into disability leave.[8-10] Musculoskeletal pain in multiple sites and depressive symptoms are associated with predicting long-term disability leave.[11] Among healthcare workers, musculoskeletal problems and mental health problems account for the most working days lost. The most reported pain sites are lower back and lower and upper limbs; pain in the latter resulted in the most prolonged absence from work. Furthermore, women accounted for 87.9% of absences and had longer return to work time than men.[12] In a study involving a one-year follow-up of disability leave due to musculoskeletal pain, those workers who had sick days due to pain and decreased over the year and people who had sick days and increased over the year all had modifiable lifestyle factors at the time they were in disability leave, such as BMI, smoking status and vigorous leisure-time physical activity.[8-10] There are previous systematic reviews on musculoskeletal pain in different populations like young workers,[13] dental professionals,[14] office workers,[15] waste collection workers,[16] and farmers.[17] To the author's knowledge, there is no systematic review that covers musculoskeletal pain in college students. The aim of this systematic review is to summarize the existing literature regarding musculoskeletal pain among college students.

METHODS

Literature search. This review was done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Registration of this review was not possible since the preliminary search of the literature had already started by the time the registration was intended to be done, and PROSPERO only allows registration when the review is in its initial stages. The research question was formulated using Condition, Context, Population (CoCoPop) guidelines.[18] Data extracted was as follows: authors, year of publication, country of origin, condition studied, context studied, population studied, and measurement instrument.

Study selection. The databases used to search for records were PubMed, ClinicalKey, ProQuest, ResearchGate, and ScienceDirect. Records were considered eligible if they had been published between 2015 and 2020, they were observational studies published in English, the full text was available, they analyzed the presence of musculoskeletal pain, and the study population were college students. MESH terms and Boolean operators used for the search were pain AND musculoskeletal AND college students. Two researchers individually identified records, and if necessary, decisions to include or exclude a record were made through consensus.

Quality assessment. The appraisal of the records was done by one researcher using the AXIS tool.[18] Records were not excluded based on the appraisal. Both researchers had full access to all records, appraisal, and data extraction spreadsheets through a shared Google Drive folder. A meta-analysis was not possible due to the heterogeneity of the sample size, population, outcomes, and measurement instruments.

RESULTS

Figure 1 shows the record selection process. A total of 318 records were identified. After duplicates were removed, 315 records remained while 275 records were excluded after the title and/or abstracts review. The full texts of the remaining records (38) were then assessed. After the full text assessment, 16 records were excluded: three on the studied population, ten on the relationship between furniture and pain, and three on cell phone usage and pain. Only 22 records were included in the analysis. The data extraction is summarized in Table 1.

Study Characteristics

Publication dates ranged from 2015 to 2020. Four records were from Saudi Arabia, three from India, two from USA, two from Brazil, one from South Africa, one from Australia, one from Ethiopia, one from China, one from the United Arab Emirates, two from Pakistan, one from Turkey, one from Ireland, and one from Spain.

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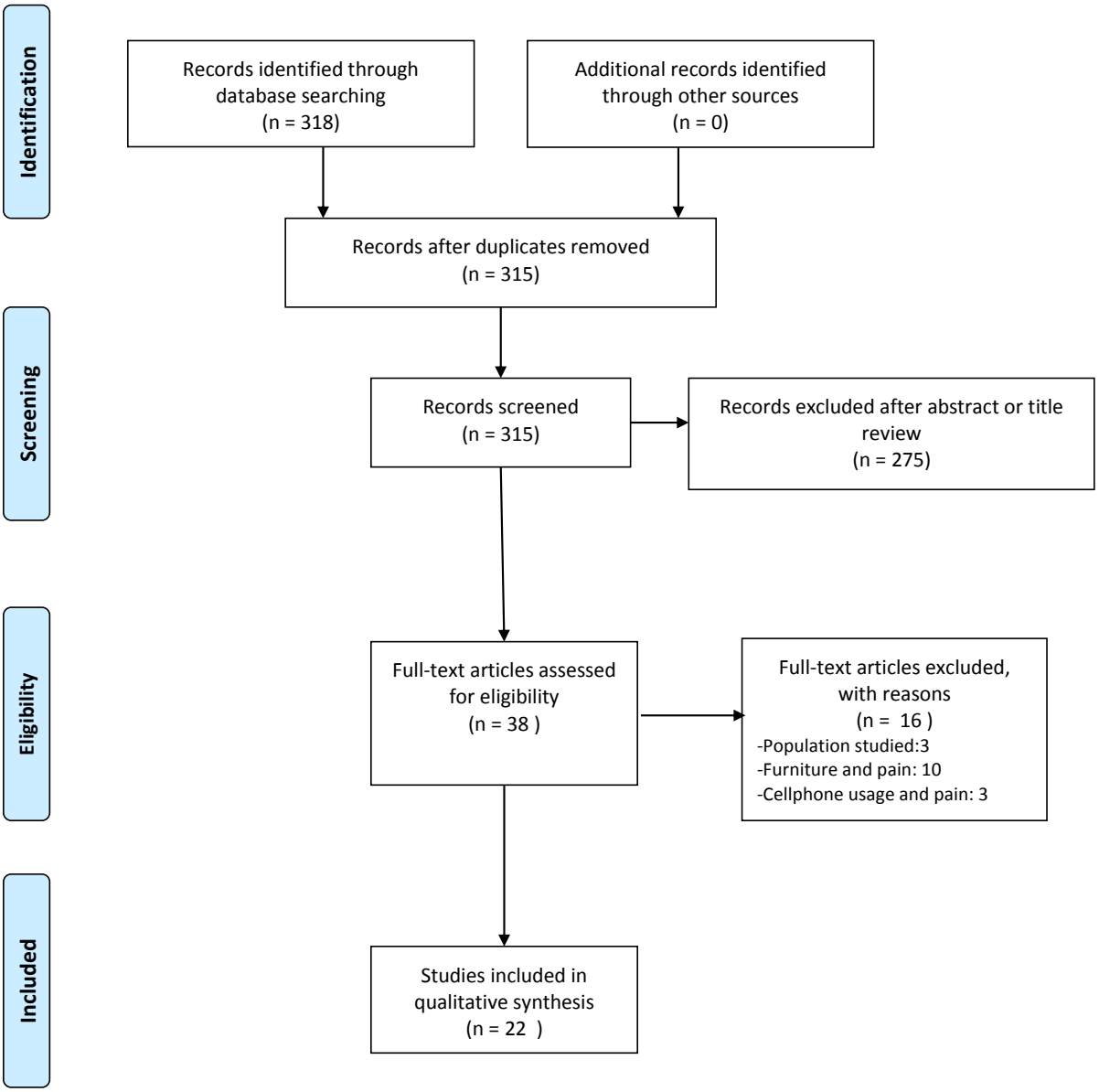

FIGURE 1. Diagram of search strategy.

Musculoskeletal pain sites and prevalence

One record studied the presence of musculoskeletal symptoms in neck (59.5%), lower back (46.8), and shoulder (40.0%)[30]; another reported the prevalence of musculoskeletal symptoms: 91% in at least one body part, 69.7% in the neck, 61.8% in the upper back, and 55.1% in the lower back.[19] One record studied the presence and site of musculoskeletal pain caused by computer use (14.2% neck, 13.8% upper back) and aggravated by computer use (28.9% neck, 25.9% lower back, 24.3% upper back).[20] One record studied the prevalence of lower-back pain (56.6% overall, 12-month prevalence 48.8%).[21] One studied the prevalence of musculoskeletal pain in the past 12 months (65.4% lower back, 53.9% upper back, 48.6% neck pain).[22] One studied the prevalence of neck pain (49.2% in the previous 12 months).[20] Three records studied the prevalence of musculoskeletal pain among females and males. One studied the prevalence of musculoskeletal discomfort (53.8% of total students) and its difference between males and females (neck, 28.9% males and 42.7% females; wrist, 24.4% males and 29.2% females; lower back, 21.1% males and 27.1% females; upper back, 16.7% males and 17.7% females). Females reported a significantly higher prevalence of neck ($p = 0.05$) and shoulder ($p = 0.006$) discomfort.[23] Others studied general musculoskeletal disorders and reported their prevalence in males (65%) and females (89.1%) without a statistically significant difference ($p = 0.083$).[19] Some studies focused on the prevalence of work-related

Table 1. Study characteristics.

	Authors	Year	Country	Condition	Context	Population (N=6,500)	Instrument of measure
1	AlShayhan and Saadeddin [39]	2017	Saudi Arabia	"Low back pain (56.6% overall, 12-month prevalence 48.8%)"	No specific setting	"Health science students (n=1,052,746 females and 306 males)"	Nordic musculoskeletal Questionnare
2	Bubric and Hedge [21]	2016	USA	"Musculoskeletal discomfort amongst males and females (neck 28.9% males and 42.7% females; wrist 24.4% males and 29.2% females; lower back 21.1% males and 27.1%; upper back 16.7% males and 17.7%)"	Laptop use	"Undergraduate and graduate students (n=186, 148 undergraduate and 38 graduate students;90 males and 96 females)"	Online questionnare
3	Bueno et al. [35]	2019	Brazil	"Musculoskeletal symtpoms (61.49% neck,22.03% elbow/forearm and 49.62% lumbar region in the last 12 months)"	Smartphone use	"University students (n=522, 329 females and 193 males)"	Nordic Musculoskeletal Questionnare for the neck and shoulder region
4	Hosteng et al. [26]	2019	USA	"Physical discomfort (68.5% reported pain in at least one area over the past 12 months; positive association between sitting time and discomfort(r=.28,p<0.01))"	Classroom setting	"Undergraduate college students (n=54, 36 females and 18 males)"	General Comfort Scale
5	Hough and Nel [32]	2017	South Africa	"Musculoskeletal discomfort (62.5% regards to pain or numbness(neck 63.9%), stiffness(neck 47.2%) and spasms(44.4%))"	Laptop use	"Third year students residing on campus who owned a laptop (n=72, 55 females and 17 males)"	Self administered questionnare
6	Kamal et al. [21]	2020	Saudi Arabia	"Musculoskeletal disorders (89.1% female and 65% male)"	Clinical settings	"Preclinical dental students (n=86, 40 females and 36 males)"	Short survey questionnare
7	Nahar and Sayed [36]	2018	India	"Musculoskeletal pain (neck pain 65%,lower back pain 61%, 35% upper back pain and 30% shoulder and wrists pain)"	Workstation use in university	"Students in computer science bachelor (n=100, 42 females and 58 males)"	Cornell Musculoskeletal Dysfunction Questionnare
8	Osama, Ali and Malik [31]	2018	Islamabad	"Musculoskeletal discomfort (Neck 75.7%, lower back 62.5%, upper back 58.8%, right shoulder 62.5%, left shoulder 44.1%, buttocks/hips 36%)"	Computer use	"Undergraduate university students (n=136)"	Student spcecific Cornel Musculoskeletal Discomfort Questionnare
9	Penkala, El-Debal and Coxon [37]	2018	Australia	"Musculoskeletal problems (34.5% in the last 12 months (lower back 27.3%, neck 23.6%, upper back 20.0% and soulders (15.5%); 21.8% in the last 7 days (lower back15.5%, neck 10.0%, upper back 13.6% and shoulders (6.4%))"	Laboratory setting	"Medical science students (n=110, 76 female 32 males and 2 others)"	Modified Standarised Nordic musculoskeletal Questionnare
10	Rambhad, Pande and Radke [27]	2020	India	"Musculoskeletal disorders (51% reported having pain, 41% reported sometimes having pain)"	Preclinical setting	"Second year undergraduate dental students (n=100)"	General questionnare
11	Weleslassie et al. [40]	2020	Ethiopia	"Neck pain (49.2% in the previous 12 months)"	No specific setting	"Undergraduate students (n=419, 144 females and 275 males)"	Adapted Nordic Questionnare
12	Woo, White and Lai [33]	2016	China	"Musculoskeletal complaints (49.9% experiencie musculoskeletal symptoms (78.1% shoulder, neck 72.9%, wrist/hand 43.4%, lower back 39.4%, upper back 38.6%, elbow 18.7%)"	Use of electronic devices	"University students (n=503, 299 males and 204 females)"	Multiple choice questionnare

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Table 1. Study characteristics (*continuation*).

	Authors	Year	Country	Condition	Context	Population (N=6,500)	Instrument of measure
13	Nadeem et al. [22]	2019	Pakistan	"Musculoskeletal pain lower back pain (last 12 months 65.4%) upper back (last 12 months 53.9%),neck pain (last 12 months 48.6%)"	No specific setting	"Undergraduate physical therapy students (n= 321, 50 males and 271 females)"	Nordic Musculoskeleal Questionnare
14	Zafar and Almosa [28]	2019	Saudi Arabia	"Work related musculoskeletal disorders Females (shoulder 47%, neck 43%, lower back 42%, upper back 33%) Males (lower back 33%, upper back 26% neck 26% shoulder 24%)"	No specific setting	"Undergraduate dental students (n=142, 88 females and 54 males)"	Self made questionnare
15	Caromano et al. [24]	2015	Brazil	"Discomfort by body region cervical (42.40%) shoulder (left 38.75%, right 34.57%) lumbar area (36.22%) head (31.37%) sacral (32.91%)"	Sitting posture in different activities	"University students (n=47, 42 females and 5 males)"	Self reported diary
16	Kim et al. [25]	2016	India	"Musculoskeletal disorders postgraduates (neck 53%, lower back 44%, shoulder 35%, upper back 33%, wrist and ankle 13%) undergraduates(upper back 44%, lower back 45%, neck 40%, shoulder 48%, wrist 25%)"	No specific setting	"Postgraduates and undergraduate dental students (n=320, 160 postgraduates and 160 undergraduates)"	Standard Nordic Questionnare
17	Hashim et al. [38]	2021	United Arab Emirates	"Musculoskeletal disorders one body site (past week 48.5%, previous year 68.3%), low back (38.6% previous week, 61.4% previous year) neck (28.7% previous week, 52.5% previous year) shoulder (23.3% previous week, 44.1% previous year)"	No specific setting	"Undegraduate dental students (n=202,152 female and 50 males)"	Modified Standarised Nordic musculoskeletal Questionnare
18	Haroon et al. [4]	2018	Pakistan	"Musculoskeletal pain at least in one body site (74.4% past 12 months, 38.9% past seven days) low back (38.6% past 12 months, 16.1% past seven days)neck(33.1% past 12 months, 13.6% past seven days) shoulders(27.8% past 12 months, 119% past seven days) knees(24.2% past 12 months, 11.1% past seven months)"	No specific setting	"Medical students (n= 360, 256 females and 104 males)"	Standard Nordic Questionnare
19	Ekşioğlu [19]	2015	Turkey	"Musculoskeletal symptoms 91% at least in one body part, neck (69.7%), upper back (61.8%), lower back (55.1%)"	No specific setting	"Undergraduate university students (n=89, 35 females and 54 males)"	Improved version of the Student Specific Cornell Musculoskeletal Discomofort Questionnare
20	Dockrell, Bennet and Culleton-Quinn [20]	2015	Ireland	"Musculoskeletal symptoms caused by computer use (14.2% neck, 13.8% upper back) aggravated by computer use (28.9% neck, 25.9%low back, 24.3%upper back)"	Computer use	"Undergraduate university students (n= 239, 199 females and 40 males)"	Modified Standarised Nordic musculoskeletal Questionnare
21	Rodríguez et al. [29]	2020	Spain	"Musculoskeletal pain previous 12 months(68.8% neck, 61.9% lumbar, 39.1% dorsal area) during lockdown (69.9% neck, 63.4% lumbar area, 41.2% dorsal area)"	Prior and during COVID-19 lockdown	"University students (n= 1,198, 846 females and 352 males)"	Spanish Standardized Kuorinka Modified Nordic Questionnare
22	Alsalameh et al. 30	2019	Saudi Arabia	"Musculoskeletal symptoms Neck (59.5%) lower back (46.8) shoulder (40.0%)"	Smartphone use	"Medical students (n= 242, 85 females and 157 males)"	Nordic Musculoskeletal Questionnare

musculoskeletal disorders in females (shoulder 47%, neck 43%, lower back 42%, upper back 33%) and males (lower back 33%, upper back 26% neck 26%, shoulder 24%).[28] One record studied discomfort by body region: cervical region (42.40%), shoulder region (left 38.75%, right 34.57%), lumbar region (36.22%), head region (31.37%), and sacral region (32.91%).[24] One record studied the prevalence of musculoskeletal disorders in postgraduate (neck 53%, lower back 44%, shoulder 35%, upper back 33%, wrist and ankle 13%) and undergraduate (upper back 44%, lower back 45%, neck 40%, shoulder 48%, wrist 25%) dental students.[25] One record studied the prevalence of musculoskeletal disorders in the past week and the previous year, in regards to only one body site they found a prevalence of 48.5% in the past week and 68.3% in the previous year and per body site they found a prevalence of low back 38.6% previous week and 61.4% previous year in low back, 28.7% previous week and 52.5% previous year in the neck, 23.3% previous week and 44.1% previous year in the shoulder.[38] One record studied the prevalence of musculoskeletal pain in one body site during the last 12 months (74.4%) and the last seven days (38.9%). The prevalence in the lower back was 38.6% in the past 12 months and 16.1% in the past seven days; in neck-shoulders, 33.1% in the past 12 months and 13.6% in the past seven days; in shoulders, 27.8% in the past 12 months and 11.9% in the past seven days; and in the knee, 24.2% in the past 12 months and 11.1% in the past seven days.[4]

One record studied physical discomfort in undergraduate college students during class; the authors found a positive association between sitting time and discomfort ($r = .28$, $p < 0.01$). Furthermore, 68.5% of the students reported pain in at least one area over the past 12 months.[26] One record reported the prevalence of musculoskeletal pain: 51% of dental students reported having pain while 41% often suffered pain during preclinical work.[27] The most reported pain sites were neck, shoulders, and lower and upper back.[19, 20, 22, 24, 25, 28-30] Additionally, other studies reported pain in buttocks/hip[31] and the knees.[4] Only two records reported differences in sex besides the previously mentioned most reported sites of pain, and one reported a difference in sex regarding lower back pain. One record reported a higher prevalence of pain or wrist numbness in females (7 females, 12.7%; and 6 males, 35.3%; 95% CI –46.8%, –1.3%),[32] and another found females had a higher prevalence of upper limb musculoskeletal disorders (59.3% vs 43.5% in males, $p < 0.001$) and shoulder discomfort (84.3% vs 72.3% in males, $p < 0.050$).[33] An additional record reported that males had a higher lifetime prevalence of lower back pain (65% vs 53.1% in females, $p < 0.001$)[9]. Only one record studied the prevalence of musculoskeletal pain in the last 12 months and during the COVID-19 lockdown. It reports a prevalence of 68.8% in neck, 61.9% in lumbar area, and 39.1% in dorsal area in the last 12 months against 69.9% in neck, 63.4% in lumbar area, and 41.2% in dorsal area during the COVID-19 lockdown. [29]

Context studied

Two records involved laptop use,[7,26] two involved smartphones,[30,35] one involved classroom setting,[26] one involved clinical setting (dental students),[21] one involved workstation in university (computer science students),[36] and two involved computer use.[20,31] Only one involved laboratory setting (medical science students),[37] one involved preclinical setting (dental students),[23] one involved use of electronic devices,[10,] eight involved no specific setting, [4,19,22,25,28,38-40] one involved different sitting postures,[24] and one involved the COVID-19 lockdown.[29]

Population studied

In regards to population (Total N = 6,500), four records involved medical (n = 1,052, 746 females and 306 males[39]; n = 360, 256 females and 104 males[4]; and n = 242, 85 females and 157 males[30]) and health science students (n = 110, 76 females, 32 males, and 2 others).[37] Two records involved dental students (n = 86 total, 40 females, 36 males,[21] and n=100 total[27]), two involved undergraduate dental students (n=202 total,152 female and 50 males[27] and n=142 total, 88 females and 54 males[28]) and one involved postgraduates and undergraduate dental students (n = 320 total, 160 postgraduates and 160 undergraduates[25]). One record included undergraduate physical therapy students (n = 321, 50 males and 271 females[22]) and another one, computer science students (n = 100, 42 females and 58 males).[36] The rest involved undergraduate and graduate students (n = 186 total, 148 undergraduate and 38 graduate students[23]) university students (n = 522 total, 329 females and 193 males[35]; n = 1,198, 846 females and 352 males[29]; and n = 47 total, 42 females and 5 males[24]), undergraduate college students (n = 54 total, 36 females and 18 males,[26]) third-year students residing on campus (n = 72 total, 55 females and 17 males[32]) undergraduate university students (n = 239 total, 199 females and 40 males[20]; and n = 89 total, 35 females and 54 males[19]), undergraduate students (n = 419 total, 144 females and 275 males[40]) and university students (n = 503 total, 299 males and 204 females). [33]

Instruments used to measure musculoskeletal pain

Four records used the Nordic Musculoskeletal Questionnaire (NMQ),[22, 25, 30, 39] one used the NMQ for neck and shoulder region,[35] one used the Spanish Standardized Kuorinka Modified NMQ,[29] one used the General Comfort Scale,[26] one used the Cornell Musculoskeletal Dysfunction Questionnaire (CMDQ),[40] one used the Student Specific CMDQ (SS-CMDQ),[31] and another one used an improved version of the SS-CMDQ.[19] Three studies used a modified version of the standardized NMQ,[20, 37, 38] one used an adapted form of the NMQ,[40] one was an online questionnaire,[23] one used a self-reported diary,[24] and five used a self-made questionnaire.[21, 27, 32, 33,28]

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Record appraisal

The summary of the record appraisal is shown in Table 2. All records but one (not explicitly stated[28]) had clear aims/objectives while the study design was appropriate for those aims.[4, 19, 20, 22, 25, 28, 29, 32, 35, 36, 38] Only five records justified their sample by sample size calculations.[4, 29-31, 40] Only one record did not explicitly state the target population.[28] Four records stated the sample used might not be representative of the target population.[26,32,33,4] Two used a convenience sample,[26, 33] one used a non-randomized sample,[32] and one found the sample used was too small.[4] In all but two records the selection process was likely to represent the target population.[24, 30] Although only eight records had non-responders, none of the records provided details as to what had been done to address this.[4, 20, 28, 33, 37-40] All the risk factors and outcome variables were properly measured according to the aims of the studies.[4, 19-26, 28-31, 33, 37, 38, 41] All but one record defined a clear statistical significance[25] while only three failed to specify their statistical methods sufficiently.[25, 27, 36] In all records except one, basic data were described.[27] In all records, the response rate was described using either a numerical or percentage value, as in the three non-responder records,[33, 37, 40] or the rest of the records with a response rate of 100%.[21-27, 29-32, 35, 36, 39] Six of the eight non-responder records provided information about non-responders[4, 20, 28, 33, 38, 39] and two provided no information.[37, 40] All records showed internally consistent results; the results of the analysis were followed by discussions and justified conclusions.[4, 19-26, 28-33, 35-39] Eight records did not discuss limitations of the study.[22, 24, 25, 27, 30, 33, 36, 38] None of the records reported funding or conflict of interests that could affect results. Only three records did not get approval by local ethics committees or showed signed informed consent.[19, 27, 36] One of those three records explained the procedures to participants prior to the commencement of the study but did not get the participants' signed informed consent.[36]

DISCUSSION

According to Szczygieł E. et al. it was until 1980 when Majeske and Buchanan were able to demonstrate the favorable changes in the body when sitting with good lumbar support, preventing lower back pain. Lengsfeld and De Carvalho noted the importance of having a lower backrest, as it maintains the correct angulation between the L-5 and S-1 vertebrae, thus preventing the overload of L-4 to L-5 intervertebral discs1.[13] The pelvis position is essential to maintaining the curvature of the spine, as it decreases the intervertebral pressure of the discs, preventing spine overload and reducing tension in soft tissues. Furthermore, it is also essential to have an accurate head and neck placement. To decrease the additional tension at the neck and shoulder muscles, the eyes should be at the same height as the computer screen/monitor (30º of cervical flexion according to Burgess-Limerick), as it has been shown to decrease neck hyperextension (between the joints of C0-C1 and C1-C2)[42].Over the past decade, there has been an increase in sedentary lifestyles. People spend more time sitting (at school, in the car, or in front of a computer) than performing a physical activity that demands a higher energy consumption.[43] In 1997, on average, a person spent around 5.9 hours a week sitting while, in 2003, it was 14.6 hours, approximately. However, since 2012, people sit more than 7 hours a day (above all, young adults between ages 18 and 25), and most of the time, it is with an incorrect posture.[42] From 2006 to 2015, the sedentary lifestyle increased on average by 18 minutes a day, 8% more than in previous years.[42]

Several studies have shown that in older adults sitting for too long in a single position is associated with cardiometabolic problems, cancer, type 2 diabetes, and early death, while reducing the time of sitting (or interrupting the periods with moderate physical activity) lowers LDL cholesterol levels, reduces joint pain, and may improve a person's mental health.[44] A short-term sedentary lifestyle contributes to hepatic insulin resistance and characteristic dyslipidemia, resulting in liver triglyceride aggregation from a metabolic point of view. Sedentary time can represent 60% of our day, increasing the risk of cardiovascular mortality, type 2 diabetes, hypertension, and can be associated with visceral fat buildup (adipose tissue).[45] In 2017, there were 38 million deaths due to non-communicable diseases, of which 82% were in developing countries. These deaths were caused by three main factors: 1) physical inactivity, 2) unbalanced diet, and 3) time spent in a sitting position (e.g., in front of a computer, commuting, schools, and office).[46] People who sit for 8 to 11 hours a day increase their chance of mortality by 15% every three years and increase the risk of developing obesity, type 2 diabetes, cancer, and cardiovascular disease.[47] It is necessary to identify the population groups that are more likely to have a sedentary life: students (mostly college students), women, and people who use computer/electronics devices.[31] In recent years, laptop use among college students has increased by 77%. Unlike a desktop computer, the laptop allows for different body postures when using it, benefiting the person from maintaining the same position for a long time.[33] A study was conducted at Shifa Tameer-e-Millat University in Pakistan to identify postural conditions associated with computer use and the factors that may contribute to the accumulation of this discomfort. Participants were students from 17 to 25 years of age at the same university who had to take the SS-CMDQ. It which has two versions, one for male and one for female students, participants are asked whether they have presented pain or discomfort in any area of the body in the last week. If so, they have to state how many times a week/day and show in a diagram where the discomfort is. At the end of the study, students indicated that the musculoskeletal areas with the most significant ailments and discomfort were neck (75.5%), lumbar area (62.5%), and cervical area (58.8%). They were also asked how many hours a day they spent in front of a computer, and the average result was between 2.5 and 3 hours (approximately 5 hours a week).[31]

Table 2. Risk of bias assessment.

	Authors	Aims/ objectives clear	Study design appropiate for the aims	Sample size justified	Target population defined	Sample appropiate to represent the target population	Selection process of participants likely to represent the target population	Measures undertaken to adress non responders	Risk factors and outcome variables measured appropiatly to the aims	Clear statistical significance defined
1	AlShayhan and Saadeddin [39]	YES	YES	NO	YES	YES	YES	NO	YES	YES
2	Bubric and Hedge [21]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
3	Bueno et al. [35]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
4	Hosteng et al. [26]	YES	YES	NO	YES	NO	YES	No non responders	YES	YES
5	Hough and Nel [32]	YES	YES	NO	YES	NO	YES	No non responders	YES	YES
6	Kamal et al. [21]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
7	Nahar and Sayed [36]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
8	Osama, Ali and Malik [31]	YES	YES	YES	YES	YES	YES	No non responders	YES	YES
9	Penkala, El-Debal and Coxon [37]	YES	YES	NO	YES	YES	YES	NO	YES	YES
10	Rambhad, Pande and Radke [27]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
11	Weleslassie et al. [40]	YES	YES	YES	YES	YES	YES	NO	YES	YES
12	Woo, White and Lai [33]	YES	YES	NO	YES	NO	YES	NO	YES	YES
13	Nadeem et al. [22]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
14	Zafar and Almosa [28]	YES	YES	NO	YES	YES	YES	NO	YES	YES
15	Caromano et al. [24]	YES	YES	NO	YES	YES	NO	No non responders	YES	YES
16	Kim et al. [25]	YES	YES	NO	YES	YES	YES	No non responders	YES	NO
17	Hashim et al. [38]	YES	YES	NO	YES	YES	YES	NO	YES	YES
18	Haroon et al. [4]	YES	YES	YES	YES	NO	YES	NO	YES	YES
19	Ekşioğlu [19]	YES	YES	NO	YES	YES	YES	No non responders	YES	YES
20	Dockrell, Bennet and Culleton-Quinn [20]	YES	YES	NO	YES	YES	YES	NO	YES	YES
21	Rodríguez et al [29]	YES	YES	YES	YES	YES	YES	No non responders	YES	YES
22	Alsalameh et al [30]	NO	YES	YES	NO	YES	NO	No non responders	YES	YES

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Table 2. Risk of bias assessment (*continuation*).

	Methods described sufficiently to be repeated	Basic data described	Response rate described	If appropiate, information about non responders described	Results internally consistent	Presence of results for the analyses described	Discussion and conclusions justified	Limitations discussed	Funding or conflict of interests that could affect results	Ethical approval or informed consent attained
1	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES
2	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
3	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
4	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
5	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
6	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
7	NO	YES	YES	No non responders	YES	YES	YES	NO	NO	NO
8	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
9	YES	YES	YES	NO	YES	YES	YES	YES	NO	YES
10	NO	NO	YES	No non responders	YES	YES	YES	NO	NO	NO
11	YES	YES	YES	NO	YES	YES	YES	YES	NO	YES
12	YES	YES	YES	YES	YES	YES	YES	NO	NO	YES
13	YES	YES	YES	No non responders	YES	YES	YES	NO	NO	YES
14	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES
15	YES	YES	YES	No non responders	YES	YES	YES	NO	NO	YES
16	NO	YES	YES	No non responders	YES	YES	YES	NO	NO	YES
17	YES	YES	YES	YES	YES	YES	YES	NO	NO	YES
18	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES
19	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	NO
20	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES
21	YES	YES	YES	No non responders	YES	YES	YES	YES	NO	YES
22	YES	YES	YES	No non responders	YES	YES	YES	NO	NO	YES

The World Health Organization (WHO) indicates that a sedentary lifestyle affects at least 60% of the world's population. This negative impact increases the likelihood of chronic illness, muscle pain, poor posture, and ergonomic problems. Less physical activity, as sitting in one position or having no activity at all, can lead to a loss in nitrogen and calcium in the musculoskeletal system as well as a decrease in heart muscle (up to 11%) and oxygen intake (28%), which can mean that the muscles will not have the required oxygen consumption, making them more prone to injury.[34, 48]

In this review, the most reported pain sites are neck, lower and upper back, and shoulders; some participants have also reported pain in wrists, hands, and buttocks or hips. The time spent continuously sitting, the number of breaks, head position while working at a desk, and physical activity are all musculoskeletal pain determinant.[49] Forward neck posture is not associated with pain in adolescents[50] nor is neck flexion while using a smartphone device (text neck).[51] Casas et al. analyzed different sittings categories and how each type affects posture. They determined that, in chairs with armrests, sitting with a rounded back with the feet supported on another chair and sitting with a rounded back with the legs crossed were identified as significant and associated with both acute and chronic neck pain.[52] There is a direct link between lower back pain and sitting on a chair. Lumbar lordosis with chest kyphosis must be maintained to reduce the risk of muscle tensions and allow the upper limbs to have a free range of motion but simultaneously support the elbow joints-forearm on the armrest.[53, 54] A sitting position is defined by a variety of factors, like workplace setting, characteristics of chair and desk, job specifications, environmental factors (room temperature and lighting), human anatomical characteristics, and sitting time during school/study hours.[42,53] When using computers (or electronic devices) to study, 31.5% of students sit with posterior pelvic tilt and neck flexion, 18% slump (collapsed trunk), and 10% are in a prone position or lateral position over a flat surface. However, there has been an increase in laptop use (77% of students), which could benefit the student from maintaining the same position for extended times.[33] Maintaining a low position for a long time can lead to changes in posture that may develop into musculoskeletal disorders. Being seated for long periods and in an inadequate position generates alterations in postural balance and can trigger long-term muscle overloads on anatomical structures.[42]

Some authors report differences in pain intensity and prevalence between females and males, with males having a higher prevalence of lifetime lower back pain[39] and females having a higher prevalence of neck and shoulder discomfort,[23] upper body musculoskeletal disorders, shoulder discomfort,[33] and pain or numbness in wrists[32] This is in line with what has been previously reported. Women have a higher prevalence of upper extremity musculoskeletal disorders with factors ranging from job-related (work stress, architecture), psychological and psychosocial (social support, conflicting schedules), and cultural (housework, pain complaints), to biological (hormones, muscle fiber type) as potential candidates as to why there is such difference.[55] In a nationwide prospective cohort study covering five years in the Dutch population, females had higher rates of disability pension due to musculoskeletal problems like back and shoulder problems and neck symptoms.[56] A study done in 2015 by Vafadar, Coté, and Archambault shows that when asked to reproduce a shoulder angle, women immediately tend to overestimate the angle leading to an increased shoulder flexion, while men tend to both overestimate and underestimate the angle.[57] It could be argued that female college students overestimate the angle needed to do everyday tasks, and as a result, they produce a greater shoulder flexion that leads to increased muscle activation and eventual discomfort.

In our review, participants in several studies are dental students in a preclinical setting, and in one case, students in a laboratory setting. The analysis of pain and posture must be different as the settings in these populations are not those of the rest of the population (sitting in front of a desk vs. dental or laboratory workstations). Among dental professionals, the most reported pain sites are neck, back, and shoulders. Awkward postures and the number of working hours without breaks are risk factors for developing musculoskeletal pain[14] and pain in more than one site was associated with decreased work quality and quantity.[8-10] Among laboratory technicians, the most affected pain sites are trunk and knees, with awkward posture identified in neck, trunk, wrists, forearms, and shoulders.[58] Taken all together, it seems that posture per se does not cause musculoskeletal pain, but rather the time spent in that posture.

Several strategies to treat musculoskeletal problems due to posture have been proposed. Ergonomic interventions have not been effective in reducing the intensity and frequency of neck and lower back pain in the short and long term.[59] Among dental workers, ergonomic chairs, magnification lenses, broader and lighter instruments, ergonomics training courses, and prismatic glasses reduce musculoskeletal pain frequency and severity.[54, 60] Having 5-minute work breaks consisting of standing and stretching every 30–45 minutes effectively reduces musculoskeletal discomfort and muscle tension.[56] The most effective strategy to prevent and treat musculoskeletal pain appears to be physical activity. People with chronic musculoskeletal pain already exhibit low physical activity levels.[61] Physical activity is effective in reducing pain in the wrists, shoulder, cervical and lumbar spine,[1] and preventing episodes of neck pain[62]; it is also a good treatment for chronic neck pain.[63] Ten minutes of daily resistance exercise effectively reduce shoulder and neck pain.[64] Exercise treats and prevents upper extremity musculoskeletal pain.[65] Exercise plus education prevents[66] and treats episodes of lower back pain and work absence.[67] Between workplace modifications, the

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use of technologies or devices, and educational or behavioral interventions, only exercise and its combination with education effectively treat lower back pain in a workplace environment.[68] Exercise combined with cognitive intervention aimed at pain and movement effectively reduces chronic musculoskeletal pain in laboratory technicians.[69] Furthermore, physical activity levels can predict a return to work and subjective employability at one year of disability leave.[70] The promotion of physical activity and an active lifestyle can be especially beneficial to college students as it can carry over to the professional field as healthy lifestyle habits and behaviors. However, all these interventions must be taken with caution as their effectiveness has been measured in a professional setting (workplace, laboratory, or dental clinic) with professionals (office workers, laboratory workers, or dental professionals) and not college students as the study population.

Strengths

To the authors' knowledge, this is the first systematic review of musculoskeletal pain with college students as the study population. Second, the studies included in the analysis were done in different countries with different types of sub-populations (medical students, dental students, general students) and in different settings (classroom, dental clinic, laboratory), meaning that this is a widespread phenomenon not exclusive to one population or setting.

Limitations

An important limitation of this review is the lack of a meta-analysis. Mixed subpopulations and different settings made a meta-analysis not suitable for this review. Another limitation noted by the authors was the different types of instruments used to measure musculoskeletal pain, ranging from validated instruments to self-made questionnaires and simple yes or no questions. The majority of the studies did not have a justified sample, which may have biased their results or limited the extent to which their results can be generalized.

CONCLUSIONS

The most-reported pain sites were neck, lower and upper back, and shoulders. A particular interest must be taken in college students as previous pain episodes can be a risk factor for recurrent pain, so they may start to develop musculoskeletal pain at a young age. It is crucial to see posture from a global and holistic point of view to make recommendations that can be applied in universities to promote a healthy lifestyle, reduce the risk of musculoskeletal problems, and prevent the early onset of chronic diseases.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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Review Article

Probiotic foods as functional foods for modulating obesity

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ABSTRACT

Introduction: Obesity is a multifactorial chronic disease that involves many internal and external factors, causing the death of at least 2.8 million people each year, according to the World Health Organization. Therefore, there is a need for nobler treatments to lose weight. Probiotic foods are classified as functional foods due to their nutritional contribution. **Objective**: To describe the impact of probiotic foods in the treatment of obesity. **Methods**: A review of 61 studies from different databases was carried out. **Results:** It is known that probiotics have several action mechanisms that beneficially affect the gut microbiota to maintain homoeostasis in the whole organism. The relationship between these microorganisms and the control and modulation of a person's body weight has been observed. **Conclusion:** The beneficial effects of probiotics are strain-specific and may impact obesity through different action mechanisms, such as abdominal fat decrease, changes in inflammatory biomarkers, microbiota restoration, and reduction in triglycerides serum levels.

Key words: obesity; functional foods; probiotic foods; gut microbiota; gut brain axis.

RESUMEN

Introducción: La obesidad es una enfermedad crónica multifactorial que involucra muchos factores internos y externos que causan la muerte de al menos 2.8 millones de personas cada año de acuerdo con la Oganización Mundial de la Salud. Por tanto, surge la necesidad de encontrar tratamientos más nobles. Los alimentos probióticos se clasifican como alimentos funcionales por su aporte nutricional. **Objetivo:** Describir el impacto de los alimentos probióticos en el tratamiento de la obesidad. **Metodología**: Se llevó a cabo una revisión de 61 estudios de diferentes bases de datos. **Resultados:** Se sabe que los probióticos tienen varios mecanismos de acción que afectan de manera beneficiosa a la microbiota intestinal para mantener la homoeostasis en todo el organismo. Se conoce la relación entre estos microorganismos y el control y modulación del peso corporal de una persona. **Conclusión:** Los efectos benéficos de los probióticos son específicos de la cepa y pueden impactar a la obesidad a través de diferentes mecanismos de acción como disminución de la grasa abdominal, cambios en biomarcadores inflamatorios, restauración de la microbiota y reducción de los niveles de triglicéridos séricos .

Palabras clave: obesidad; alimentos funcionales; alimentos probióticos; microbiota intestinal; eje intestino-cerebro.

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INTRODUCTION

Obesity is characterized by being a multifactorial chronic disease that involves many environmental, genetic, and metabolic factors. It refers to an abnormal increase in body fat. The body mass index (BMI) is usually used as a diagnostic measure; this value is derived from the relationship between weight and height (kg/m^2), classifying an individual as underweight < 18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25–29.9 kg/m^2, and obese > 30 kg/m^2.[1,2] In early 2020, the World Health Organization (WHO) warned that obesity and overweight had reached global epidemic figures, affecting approximately 40% of the population (children, teenagers, and adults), and caused the death of at least 2.8 million people each year. It also pointed out that those who show greater susceptibility are men under 50 years old.[2] The pathophysiology of obesity may seem very simple: Calorie consumption exceeds energy expenditure. However, it is not only the result of bad habits, such as a diet high in sugar, alcohol, and fat, or a sedentary lifestyle. There is evidence that the pathogenesis involves processes much more complex than just calorie accumulation.[3] There are multiple etiological factors that have been identified, among them are: absence of leptin (an adipokine that increases satiety),[4] genetic mutations,[5] and hypothyroidism, characterized by a low concentration of thyroid hormones triiodothyronine (T3) and thyroxine (T4), associated with a decrease in metabolic activity. Obesity is also a risk factor associated with other chronic ailments as diabetes, cardiovascular diseases, cancer, arterial hypertension, coronary heart disease, cerebrovascular accidents, lung diseases, liver diseases, and osteoarthritis.[6]

If dietary approaches and physical activity are not sufficient to achieve goals, health professionals may consider implementing drug and supplement therapies (like appetite suppressants). In severe cases, options like bariatric surgery (stomach portion removal) may be considered. These strategies can have unfavorable side effects;[7] therefore, new approaches are necessary for weight loss improvement. Consumers nowadays believe that food may directly impact their health. There is a general perception on the benefits of different food therapies that may be more effective than medical treatments. So, many food products not only satisfy hunger and provide different necessary nutrients but also contribute with essential components for the prevention of different diseases and improve physical and mental health. They are known as functional foods.[8]

The Science of Functional Foods in Europe (FUFOSE) defines functional foods as those *"that satisfactorily demonstrate in adequate concentrations beneficially affect one or more functions in the human body beyond nourishing it in such a way as to improve its state of health and/or prevent diseases. They are still food and part of a normal diet."*[9] They can be used as weight management tools to fight obesity and enhance weight loss by regulating appetite, satiety, energy output, thermogenesis, and adipogenesis.[10] Carbohydrate-based food (brown rice), protein-based food (legumes), fruits and vegetables, and specific beverages (tea) are selected foods due to the potential effects on weight loss.[10]

Probiotic foods are well known and widely used because they possess various nutritional and therapeutic properties to the host when administered in adequate amounts. They are considered functional foods that may be used strategically as weight management tools against obesity, specifically restoring balance by acting directly upon the gut microbiota. The gut microbiota are all the living microorganisms mainly in the colon. The microbiota has digestive properties and is also involved in nutrition and immunity as well as other homeostatic aspects.[11] In recent years, much attention has been drawn to the contribution of gut microbiota to the development of obesity. Research has shown that the microbiota of obese individuals is structurally and functionally different from healthier individuals. Most obesity studies evaluating overweight and obese patients show an imbalance characterized by a lower diversity and alterations in action mechanisms (immune dysregulation, energy, and gut hormone regulation, and proinflammatory mechanisms), which can lead to uncontrolled weight.[12,13] The objective of this review is to describe the impact of probiotic foods in the treatment of obesity, the microorganisms involved, and their action mechanisms.

METHODS

A literature search was conducted using Google Scholar as the main search engine. On the other hand, the scientific databases used were National Center of Biotechnology (NCBI), PubMed Central (PMC), ResearchGate, MDPI SciELO, Wiley Online Library, Frontiers, Science Direct, and MDPI Karger. The following keywords were used: obesity AND probiotics, obesity AND functional foods, functional food AND probiotic foods, gut microbiota AND obesity, gut microbiota AND probiotics, gut microbiota AND gut-brain axis. The articles were selected based on the following criteria: open accessibility, publication year (2015–2021; but some older articles were selected due to their methodology and results), studies in English and Spanish, and clinical trials and animal obesity model studies using probiotic foods.

RESULTS

Gut microbiota and obesity

Gut microbiota refers to the all the microorganisms found in an environment whereas microbiome refers to all the microorganisms and their genes, a collection of their genomes.

Sometimes these terms are used as synonyms but differ from one another.[14] Gut microbiota consists of three main phyla: Bacteroidetes (Porphyromonas, Prevotella, Bacteroides), Firmicutes (Ruminococcus, Clostridium, Lactobacillus and Eubacteria) and Actinobacteria (Bifidobacteria), along with Proteobacteria, Fusobacteria, and Verrumicrobia.[15] The gut microbiota comprises all commensal and pathogenic bacteria residing in the gastrointestinal tract (GIT). It plays a key role in the maintenance of health, metabolism modulation, and disease pathogenesis.[16] To do so, in the host's organism, it executes various action mechanisms of nutritional and immune nature.[17,18] It enhances nutrient and mineral absorption, enzyme synthesis, water-soluble B and K vitamins, amino acids, folic acid, and biotin. It also favors the production of short-chain fatty acids (SCFAs) through the fermentation of non-digestible substrates of dietary fibers. In addition, the gut microbiota and the innate immune system are in extensive communication. This means gut microbiota stimulates the development of nonspecific or innate (physical and chemical barriers, epithelial surfaces) and specific or adaptive (lymphocytes and their antibodies) immune system components as well as the maturation of immune cells, just after birth and during the host's entire life. The gut represents a stable ecological niche for its inhabiting bacteria that rely on the host's physiology to maintain their basic biological processes. Then, it establishes an interaction, called symbiosis, with the host.[19] It contributes to all the physiology from digestion to fertility, and it affects the brain function for the regulation of the host's appetite.[20]

Appetite regulation and host metabolism

The hypothalamus is the main center of homeostatic control of energy balance through the neural and humoral pathways.[21] This center involves a homeostatic regulation of food intake by energy inflow and expenditure or outflow, and it communicates directly with the gut; this circuit is called gut-brain-axis.[22] It is an enteroendocrine system and is the largest endocrine organ in the human body. Its enteroendocrine cells (EEC) are distributed throughout the intestinal tract and secrete several peptides that act like hormones (hunger hormones) and neurotransmitters: gastrin, ghrelin, somatostatin, serotonin, cholecystokinin (CCK), glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY). They act as a response to the presence of food (nutrient and mechanical stimuli). These hormones and neurotransmitters mediate effects on the secretion of other gastrointestinal substances (insulin, bile acids, and gastric acids), gut motility, and food intake by the activation of the vagus nerve (VN).[23,24] The latter innervates all the digestive system, and its fibers are all distributed across all the layers of the digestive system. VN sends a signal to the hypothalamus to control satiation and the host energy balance and metabolism. Evidence indicates that the gut microbiota plays an important role in a bidirectional communication between the brain and the gut. The bacteria greatly influence the host energy metabolism, produce several proteins that activate satiety pathways, and subsequently, affect food intake and energy homoeostasis.[24]

Gut microbiota in gut-brain axis

The VN does not cross the epithelial layer, so it is not in direct contact with the microbiota. The nerve fiber only senses microbiota signals through the diffusion of bacterial compounds, metabolites, and enterochromaffin cells. These cells detect signals coming from the gut microbiota through toll-like receptors (TLR), which recognize bacterial lipopolysaccharides (LPS) and others in order to express peptide hormones for modulating appetite.[25] Gut microbiota-derived metabolites can modify the release of these hormones and neurotransmitters. Some dietary nutrients are converted into plasma metabolites by the gut microbiota, including SCFAs (lactate, butyrate, and acetate), dopamine, and serotonin. SCFAs play a special role in satiety and inflammation and can suppress VN activity during food intake and in the blood brain barrier. They also mediate the G protein-coupled receptors (GPR41 and GPR43) that inhibit fat accumulation in adipose tissue and promote glucose metabolism in the liver and muscle.[26]

Dysbiosis in obesity

Several studies have found an association between microbial dysbiosis in obesity and VN signaling. In obese subjects, there is an imbalance of the hunger hormones and neurotransmitters as well as changes in gut microbiota composition and proportions and derived metabolites. Such imbalance causes diverse pathophysiologies related to weight imbalance and improper energy homoeostasis.[27] The gut microbiota of healthy individuals changes in time due to aging and environmental factors such as dietary habits, type of food, lifestyle, and antibiotics, among others. Between individuals there are large differences in microbiome attributed to age, ethnicity, lifestyle, and diet.[28] These factors could modify its composition, leading to a dysbiosis in which opportunistic microorganisms take advantage to cause diseases. Dysbiosis is defined as a reduction in microbial diversity, a combination of the loss of beneficial bacteria and an increase in pathogenic ones. This imbalance likely promotes diet-induced obesity and metabolic complications. It is also associated with diarrhea, irritable bowel syndrome, allergies, multiple sclerosis, type 1 and type 2 diabetes, rheumatoid arthritis, Alzheimer's and Parkinson's diseases, autism, and atherosclerosis.[29] Accordingly to the scientific literature Firmicutes and Bacteroidetes ratio (F/B) has been associated with host homoeostasis and is frequently cited as a hallmark of obesity.[30]

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Firmicutes/Bacteroidetes ratio (F/B)

The Firmicutes/Bacteroidetes ratio is an eventual biomarker or hallmark of obesity. Alterations affecting these phyla were first described in obese animals and subjects that exhibited increased abundance of Firmicutes at the expense of Bacteroidetes. When the subjects were submitted to a calorie-restricted diet for one year, Bacteroidetes increased as compared to Firmicutes. In fact, higher proportions of Bacteroidetes are found in people whose diet is rich in fiber. On the other hand, Firmicutes are found in people who consume large amounts of protein, sugar, starch, and calories. Firmicutes are more capable of extracting energy from foods than Bacteroidetes, promoting a more efficient calorie absorption and weight gain.[31] So, restoring the balance of the gut microbiota through the use of probiotics is clinically an important target to treat obesity and other diseases related to the imbalance in gut microbiota. One method is reversing microbial dysbiosis by the consumption of probiotics.[31]

Probiotics

The term probiotic means *for life* in Greek. Through the years, different definitions have been proposed, and in 2001 the United Nations Food and Agriculture Organization (FAO) adopted the current formal definition: *"Live microorganisms that, when administered in adequate doses, confer benefits for the health of the organism."*[32] Probiotics can be naturally found within or added to food products (probiotic foods), as fermented milk or dairy products (yogurt, cheese, and kefir) and fruit juice. It has been demonstrated that these products transport the bacteria to the intestine. Once in the gut, they colonize the colon, avoiding the adhesion of pathogens. They act directly on the microbiota and affect its composition and function.[33] Probiotics that potentially reduce the F/B ratio, and subsequently obesity, are mostly bacteria from the genera *Lactobacillus* (*L. rhamnosus, L. paracasei*, and *L. salivarius)* and *Bacillus* (*B. amyloliquefaciens*) and yeasts from the genus *Saccharomyces* (*S. boulardii*).[34] It is well known that probiotics have multiple effects on the host. These mechanisms affect the development of gut microbiota and inhabit the host to ensure a proper balance between the microorganisms necessary for the optimal functions of the organism.[35]

Action mechanisms of probiotics

Major probiotic action mechanisms of the gut microbiota include enhancement of epithelial barrier, which is not clearly understood yet. Several studies indicate that some bacteria, specifically the genera Lactobacillus, modulate the regulation and transcription of several genes encoding adherence junction proteins, as E-cadherin and B-catenin, their phosphorylation, and protein kinase C (PKC).[36] Probiotics execute other types of action mechanisms:[37, 38]

Increased adhesion to intestinal mucosa and concomitant inhibition of pathogen adhesion (manipulation of intestinal microbial communities). Several *Lactobacillus* proteins have been shown to promote mucous adhesion and interaction with intestinal epithelial cells, as surface adhesins that mediate the attachments. Probiotics also cause alterations in intestinal mucins that prevent pathogen binding; and induce the release of small peptides/proteins from epithelial cells against pathogens.[37]

Production of antimicroorganism substances. Probiotics are able to produce low-molecular-weight (LMW) peptides, such as organic acids (acetic acid and lactic acid) and antibacterial peptides bacteriocins that inhibit pathogenic bacteria.[37]

Modulation of immune system. Probiotic bacteria are well known for interacting with epithelial, dendritic cells (DCs), monocytes, macrophages, and lymphocytes that can exert an immunomodulatory effect.[38]

Effects on gut microbiota

The possibility that the diet affects the gut microbiota has been discussed within the scientific community for a long time. Indeed, human diets may have direct effects on the microbiota, which results in changes in its composition. Experiments using animal models and subjects have demonstrated that some foods may contribute to the restoration of the F/B ratio and gut balance in general (vitamins, minerals, amino acids and dietary fiber) while others might affect the intestinal microbiota (fat and sugar-enriched diet).[39] Due to their potential action mechanisms in the host, probiotics are considered a functional food for weight management.[40] In this review, several clinical trials were found to describe the potentially beneficial effect of probiotic foods in the gut microbiota of obese experimental models (Table 1).

DISCUSSION

Probiotics confer health benefits to the host when administered in adequate amounts. They are commonly used as food supplements that improve the host's intestinal balance. It is known that the metabolic activity of gut microorganisms affects host homoeostasis, and variations in its composition are associated with obesity pathogenesis and some other complications.[41] Among the probiotics included in many functional foods and dietary supplements for human and animal consumption are Bifidobacterium and Lactobacillus spp., these are the predominant and subdominant groups of

Table 1. Clinical trials using probiotics for weight management in animal and human obesity models.

PROBIOTIC (BACTERIA STRAIN)	PROBIOTIC FOOD	CHARACTERISTICS	DOSE/ DURATION	RESULTS
Clostridium butyricum[53]	Cottage cheese and Greek-style yogurt made from pasteurized cow's, goat's or camel's milk The final products were inoculated with *Clostridium butyricum* (CFUs not specified) after manufacture	Model: 30 female C57BL/6 mice, 6–8 weeks old, weighing 14–16 g Induced rodent diet 5001 (mix of sugars, fat, proteins, vitamins, and minerals)	1 mL/day/ 5 weeks	Significant gut microbiota enrichment was observed in mice supplemented with cow milk cheese and camel milk, manifesting an increase in *Clostridiales, Ruminococcaceae, Lachnospiraceae,* and *Anaerostipes spp*), producing butyrate (one of the main short-strain fatty acids, SCFAs, linked to protective effects against metabolic syndrome)
Bifidobacterium spp. and *Lactobacillus spp.*[54]	Cheese The final product was inoculated with *Bifidobacterium spp.* and *Lactobacillus spp.* (CFUs not specified) after manufacture	Model: SD albino rats and C57BL/6J mice Induced High-fat diet (HD), mix of sugars, proteins, vitamins and minerals, to increase weight	Not specified	Lactobacillus strains were more effective in reducing weight gain, fatty acid synthesis, and live intake due to the enhanced adiponectin production and AMPK activation related with the expression of lipid oxidative genes in adipose tissue, liver, and skeletal muscle
Lactobacillus gasseri SBT2055[55]	Yogurt The final product was inoculated with *Lactobacillus gasseri* SBT2055 (5×10^{10} CFU/100g) after manufacture	53 obese people (29 men and 14 women) aged 33–63; BMI 24.2–30.7 kg/m^2	200 g/ day/12 weeks	Decrease in abdominal and subcutaneous fat (4.6%), BMI, and waist and hip circumference.
Lactobacillus plantarum[56]	Cheese The final product was inoculated with *Lactobacillus plantarum* (5×10^{11} CFU/100 g) after manufacture	40 obese people (20 men and 20 women) aged 30–69 Diagnosis of metabolic syndrome characterized by obesity	50 g/day/ 3 weeks	Reduced serum triglycerides, arterial blood pressure, and BMI
Lactobacillus fermentum and *Lactobacillus amylovorus*[57]	Yogurt Two yogurt treatments: Yogurts inoculated with 1.08×10^9 CFUs of *Lactobacillus fermentum* (LB) or 1.30×10^9 CFUs of *Lactobacillus amylovorus* (LA)	28 obese people (10 men and 18 women) aged 18–60 years BMI 25–32 kg/m^2; induced HD (mix of sugars, proteins, vitamins, and minerals) to increase weight	Not specified	Participants lost 4% (LA group), 3% (LB group), and 1% (control group) of total fat mass , respectively, but body weight and composition did not differ significantly between treatments LA treatment resulted in the largest fat reduction and the largest decrease in prevalence of some bacteria clusters in gut, as *Clep*, related to a greater loss of body adiposity
Lactobacillus fermentum TSI2 and *Lactobacillus fermentum* S2[59]	Yogurt The final product was inoculated with 8 log CFU/mL of *Lactobacillus fermentum* TSI2 and *Lactobacillus fermentum* S2 after manufacture	Model: Male SD rats, 6 weeks old, weighing 200 g Induced HD (mix of sugars, proteins, vitamins, and minerals) to increase weight	1 mL/200 g body weight/ 8 weeks	TSI and MIX groups presented significantly smaller adipocytes than HF group in abdominal fat tissue Adiponectin increased in TSI and epididymal fat was lower in S2 than in HF

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Table 1. Clinical trials using probiotics for weight management in animal and human obesity models (*continuation*).

PROBIOTIC (BACTERIA STRAIN)	PROBIOTIC FOOD	CHARACTERISTICS	DOSE/ DURATION	RESULTS
Lactobacillus plantarum DK211[60]	FWB The product was manufactured with *Lactobacillus plantarum* DK211 (10^9 CFU/mL)	Model: Male SD rats, 4 weeks old, weighing 156.04 ± 11.74 g Induced HD (mix of sugars, proteins, vitamins, and minerals) to increase weight	3000 mg/ day Over 4 weeks	HDFWB showed significantly lower organ weights, abdominal and epididymal fat pads, LDL-cholesterol levels, blood glucose, serum insulin, and serum appetite-related hormones (leptin and ghrelin) vs HD
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, and *Bifidobacterium animalis*[61]	Yogurt Conventional yogurt containing starter cultures *Lactobacillus delbrueckii subsp. bulgaricus* (CFU: 10^8/mL) and Streptococcus thermophilus (CFU: 10^8/mL) Probiotic yogurt containing starter cultures and additional *Bifidobacterium animalis* (CFU: 10^8/mL)	Model: 30 male mice, 5 weeks old Induced HD (mix of sugars, proteins, vitamins, and minerals) to increase weight	2–3 mL/ day/60 days	Intestinal microbiota improved in conventional yogurt group with increased *Lactobacillus spp*. population Both yogurts improved histology of small intestine; normalized glucose, LDL, HDL, and cholesterol in serum; and promoted cytokine production (IL-6, IL-10 and INF-γ), that reinforce epithelial barrier to prevent inflammatory response
Streptococcus thermophilus, Lactobacillus acidophilus, Enterococcus faecium, and *Lactobacillus rhamnosus*[58]	Yogurt 3 yogurt treatments: Test yogurt StLa fermented with two strains of *Streptococcus thermophilus* (CFU: 10x10^7/mL each) and two strains of L*actobacillus acidophilus* (CFU: 2x10^7/mL each) Test yogurt StLr: was fermented with two strains of *Streptococcus thermophilus* (CFU: 8x10^8/mL each) and one strain of *Lactobacillus rhamnosus* (CFU: 2x10^8/mL each) Test yogurt GAIO® (G): fermented using the Ukrainian bacterial culture CAUSIDO® This culture contained one strain of *Enterococcus faecium* (CFU: 6 x 10^7/mL each) and two strains of *Streptococcus thermophilus* (CFU: 1 x 10^9/ mL each) GAIO® (G): fermented milk product	70 obese adults (20 men and 50 women) aged 18–55, BMI 25–37 kg/m^2	450 mL/ day/8 weeks	There was a decrease in LDL-cholesterol after the consumption of GAIO® due to a possible cholesterol reabsorption in the small intestine

Abbreviations: CFU= colony forming units/mL, SCFA= short chain fatty acids, SD= Sprague Dawley rats, HD: induced high fat diet, BMI= body mass index, GAIO® (G): fermented milk product, LA= *Lactobacillus amylovorus* group, LB= *Lactobacillus fermentum* group, *Clep*= Clostridium cluster, TSI= HD-fed rats orally administered yogurt fermented by *L. fermentum* TSI (n=8), HF= High-fat diet, MIX= HD-fed rats orally administered yogurt fermented by a mixture of *L. fermentum* TSI and S2 (n=8) , FWB= fermented whey beverage, HDFWB= high fat diet plus fermented whey beverage, LDL= low density lipoprotein, HDL= high density lipoprotein.

the gastrointestinal microbiota.[42] Both strains are lactic acid bacteria (LAB) and have been reported to improve the inhibition of pathogenic microorganisms and protection against gastrointestinal diseases through the maintenance of the intestinal barrier and the enhancement of immune response.[43]

Those LAB considered commercial probiotics belong mostly to the Lactobacillus genus, with over one hundred species recognized. Among them are those shown in Table 1: *L. acidophilus, L. casei, L. gasseri, L. fermentum, L. plantarum,* and *L. rhamnosus.* They are generally recognized as safe (GRAS). The Bifidobacterium genus (*B. infantis, B. animalis, B. longum,* and *B. Breve B3*) is also found in probiotic products but not as frequently.[44] For decades, Lactobacilli have been used as an effective therapy to treat gastrointestinal conditions and other pathologies, displaying an overall positive safety profile.[45] Lactobacillus strains are the most widely reported in the articles here mentioned (see Table 1). The changes observed after probiotic intake in experimental groups compared to control include SCFA production and an increase in gut bacteria count. There is also a reduction in abdominal fat, hip circumference, and proinflammatory cytokines (IL-1, IL-6, IL-10, and INF-γ) as well as lower levels of sugar, cholesterol, and triglycerides. It is worth mentioning that these effects are considered biomarkers associated with weight loss control and could influence weight management strategies. Nevertheless, the efficacy and action mechanisms are strain-dependent or strain-specific. Then, they cannot be ascribed indistinctly or extended to all probiotics of the same genus or species. Some strains may equally play a significant role against obesity in their own way or have a greater positive impact.[46] For example, a meta-analysis conducted by Million, Angelakis, Paul, Armougom, Leibovici and Raoult (2012) reported that *L. casei strain Shirota (LAB 13), L. gasseri, L. rhamnosus,* and *L. plantarum* have a more positive effect on weight loss.[47] In fact, they appear to be protective against obesity. Results reported in table 1 show some antiobesity effects, such as a decrease in abdominal and subcutaneous fat (*L. gasseri SBT2055*) and a reduction in serum triglycerides and LDL cholesterol (*L. rhamnosus*). Clinical and experimental studies suggest that *L. plantarum* shows the most promising effects against several pathological conditions, including obesity. This strain is able to inhibit weight gain and fat accumulation.[48] Furthermore, lowers serum cholesterol, glucose and triglycerides levels in blood.[49] Changes observed in Table 1, after *L. plantarum* intake also include the reduction of serum appetite related hormones (leptin and ghrelin) On the other hand, *L. fermentum* and *L. acidophilus* are associated with weight gain. These bacteria are widely present in many products intended for increasing energy efficiency, as freeze-dried foods (fruits, vegetables, cereals, meat, and poultry). Finally, *Clostridium butyricum, Lactobacillus amylovorus, Enterococcus faecium,* and *Lactobacillus delbrueckii subsp. bulgaricus* are not commonly consumed by humans. In fact, they are still under experimentation. Still, the results after intake (Table 1) demonstrate that these bacteria strains could be applied as future probiotics for weight control purposes.

Probiotics are classified as modified functional ingredients added to the food that owe their special properties to bioactive compounds. Those a considered as primary and secondary metabolites of nutritious and non-nutritive natural components found in small proportions. Despite this, they can trigger mechanisms that improve human health as they have antioxidant, anti-inflammatory, antifungal, and antibacterial properties.[50] In Table 1, all authors mention that probiotics were added to the food, and that all the food matrixes were fermented dairy products. They are commonly found in this type of food; products such as yogurt and cheese are excellent probiotic vectors due to their nutritional composition, acidity, and shelf life. Cheese is also a suitable transport for bacteria given its high buffering capacity, a result of its high fat content and dense structure, that may protect them during gastric transit.[51]

CONCLUSION

Several works suggest that some probiotic strains may have a greater positive impact in the treatment of obesity while others exert a beneficial effect on weight loss. Some of the changes observed in Table 1 after probiotic intake include reduction in abdominal fat, but also reduction of triglycerides serum levels and proinflammatory cytokines (IL-1 IL-6, IL-10, INF-γ). Available data suggest significant therapeutic effects of probiotics in weight management, and some strains have been approved for consumption. Still, some sanitary regulators, as the Food and Drug Administration, have not approved any probiotics related to overweight/obesity treatment.[52] Studies and clinical trials support the therapeutic effects of probiotics. However, older studies (before 2010) fail to understand or associate the action mechanisms involved, and little or no continuity has been reported. Further work is necessary to meet all quality standards for medical applications. Probiotics may provide an advanced understanding of obesity etiology and metabolic consequences. Even these markers can be a direct target for future obesity perspectives and new and innovative techniques for weight management.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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Review Article

Clinical advances in multiple sclerosis and amyotrophic lateral sclerosis treatment: A review

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ABSTRACT

Neurodegenerative diseases are clinical manifestations that depend on the anatomy and function of the affected areas. Amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) are some of these diseases, but they are also autoimmune and their etiology makes treatments limited and of little therapeutic efficacy. Currently, some clinical research advances can be pillars for the development of new treatments for these diseases. Therefore, the objective of this review is to describe the latest clinical advances in ALS and MS as well as their results in clinical recovery in randomized clinical trials, meta-analyzes, and full-text systematic reviews conducted in humans and rats, published in English and Spanish in the last 5 years, using PubMed, SciELO, and Cochrane. For clinical trials to be included, they had to provide a detailed breakdown of randomization methods, diagnostic criteria, intervention details, and efficacy evaluation. The results show that, so far, available medications, like riluzole and edaravone for ALS and fingolimod, dimethyl fumarate, and IFN β-1b for MS, only prolong the life of the patient. Among these drugs are also glutamate neurotransmitter antagonists, immunomodulators and even antioxidants; each of them showed significant improvement in the reviewed trials. Similarly, other non-pharmaceutical treatments, as the 600-mg dose of curcumin in the diet for ALS, showed improvement of the patients' conditions. Regarding MS, more studies should be carried out on autotransplantation with adipose-derived mesenchymal stem cells (AdMSCs) to investigate the potential therapeutic benefit of this technique in phases prior to secondary-progressive (SPMS).

Key words: clinical advances; treatment and multiple/amyotrophic lateral sclerosis; sclerosis.

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RESUMEN

Las enfermedades neurodegenerativas son manifestaciones clínicas que dependen de la anatomía y función de las zonas afectadas. La esclerosis lateral amiotrófica (ELA) y la esclerosis múltiple (EM) son algunas de estas enfermedades, además, son autoinmunes y su etiología hace que los tratamientos sean limitados y de escasa eficacia terapéutica. Actualmente, algunos avances en la investigación clínica de estas enfermedades pueden ser pilares para el desarrollo de nuevos tratamientos. Por tanto, el objetivo de esta revisión es describir los últimos avances clínicos en ELA y EM, así como los resultados de recuperación clínica en ensayos clínicos aleatorios, metanálisis y revisiones sistemáticas de textos completos realizados en humanos y ratas, publicados en inglés y español en los últimos 5 años mediante PubMed, SciELO y Cochrane. Para ser incluidos, los ensayos clínicos debieron proporcionar un desglose detallado de los métodos de aleatorización, los criterios de diagnóstico, los detalles de las intervenciones y la evaluación de la eficacia. Los resultados muestran que hasta ahora solo existen medicamentos que prolongan la vida del paciente, riluzol y edaravona para ELA y fingolimod, dimetilfumarato e IFN β-1b para EM, principalmente. Entre estos medicamentos se encontraron también antagonistas del neurotransmisor glutamato, inmonumoduladores e incluso antioxidantes; cada uno de ellos mostró una mejoría significativa en los ensayos revisados. De igual forma, otros tratamientos no farmaceúticos demostraron mejoría de los pacientes con dichas enfermedades, como la dosis de 600 mg de cúrcuma en la dieta de los pacientes con ELA. En cuanto a EM, se deben realizar más estudios sobre el autotrasplante con células madre derivadas de tejido adiposo mesenquimatoso (AdMSC) para investigar el beneficio terapéutico potencial de esta técnica en fases previas a la esclerosis múltiple secundaria progresiva (SPMS por sus siglas en inglés).

Palabras clave: avances clínicos; tratamiento y esclerosis múltiple/esclerosis lateral amiotrófica; esclerosis.

INTRODUCTION

Neurodegenerative diseases are those clinical features that depend on the anatomy and function of the affected areas. As they progress, they produce atrophy in the damaged areas of the cortex, which can be detected in imaging studies or macroscopic examinations of the brain. Histologically, they are characterized by gliosis and neuronal loss by apoptosis. In some cases, the activation of the microglia is observed, suggesting that a low-grade inflammatory mechanism contributes to the diseases. Some of these diseases have well-defined genetic alterations, but others are sporadic. The latter currently constitute a primary field in biomedical research due to the lack of effective treatments to stop their progress and the failure of treatments to allow the patients' recovery.[1,2]

There are several subclassifications of neurodegenerative diseases, one of which is autoimmune in nature. Autoimmunity refers to the presence of autoantibodies or T lymphocytes that react against autoantigens, and it does not necessarily imply that the appearance of self-reactivity has pathological consequences. Autoimmunity is found in all people and increases with age; however, autoimmune diseases arise only when the transgression of one or more of the basic mechanisms regulating immune tolerance leads to self-reactivity and, therefore, to tissue damage.[2] Amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) are two autoimmune neurodegenerative diseases. But compared to other neurodegenerative diseases, just a few therapeutic strategies have been developed to treat them since their etiology makes treatments limited and with little therapeutic efficacy. Some advances in clinical research can be pillars for the development of new treatments for these diseases. The objective of this study was to describe the latest clinical advances in ALS and MS as well as the results of clinical recovery in randomized clinical trials, meta-analyzes, and full-text systematic reviews conducted in humans and rats published in English and Spanish in the last 5 years using PubMed, SciELO, and Cochrane.

AMYOTROPHIC LATERAL SCLEROSIS

ALS is a neurodegenerative disease characterized by a primary degeneration of motor neurons that causes weakness and atrophy of the affected muscles. It is the most common motor neuron disease and is characterized by the degeneration of both upper and lower motor neurons.[3] From a macroscopic point of view, the ALS brain appears normal, although atrophy of the precentral gyrus is sometimes observed; the spinal cord is thinned, and the anterior motor roots are atrophic and gray in color, as compared to the posterior ones, which are sensitive. Histologically, the main alteration in ALS is the loss of motor neurons with gliosis in the anterior horn of the spinal cord, stem, and motor cortex. Seen in the surviving motor neurons, Bunina bodies are eosinophilic inclusions, 2–5 µ in diameter in the cell body, reactive for ubiquitin. The white matter of the spinal cord shows pallor of the corticospinal tracts.[2] The clinical picture is characterized by the onset of muscle twitching and weakness of one limb (usually upper) that then spreads to other parts of the body. As the disease

progresses and nerve cells are destroyed, muscles weaken. This eventually affects chewing, swallowing, speaking, and breathing. Eventually, ALS affects the control of the muscles needed to move, speak, eat, and breathe. There is no cure for this disease and there is no involvement of the sensory part or bladder dysfunction as in myelopathies, which is the first differential diagnosis. Risk factors for developing ALS are smoking; hereditary factor (5–10% patients inherit Familial Lateral Amyotrophic Sclerosis), exposure to environmental toxins, sex (it has been shown that male sex present higher prevalence), and age (40–65 years). [4]

Pathophysiology

The disease process is virtually limited to the motor neurons of the cerebral cortex, brainstem, and spinal cord, and certain forms of progressive ataxia affecting only the Purkinje cells of the cerebellum. It is based on neuron exhaustion and loss: Not only do the cell bodies disappear but also their dendrites, axons, and myelin sheaths, a phenomenon that is not accompanied by an intense tissue or cellular response. The cerebrospinal fluid (CSF) shows little if any change in protein content.[5] Several processes at cellular level characterize the death of individual cells. The pathological neuronal degeneration process is very different because it refers to a series of changes in mature neurons that occurs over an extended period, leads to cell death, and usually leaves a discrete glial scar but not regional tissue necrosis. In many models of degenerative disease, cell loss encompasses the activation of specialized genes, although their chronological evolution and cell morphology are not apoptotic. It has become clearer that mechanisms other than programmed cell death are essential for understanding degenerative diseases and that the clinical manifestations of such disorders occur even before cell destruction. For example, interference with synapse signaling and supporting glial cell dysfunction is as important as morphological neuronal death.[5]

Conventional clinical treatments

ALS is the most common motor neuron disease in adults, and no effective treatment is currently available beyond supportive care with some moderately life-prolonging supportive medications. Among these drugs is riluzole; it is absorbed orally and metabolized in the liver by cytochrome P450 (CYP)-mediated hydroxylation and glucuronidation. It has presynaptic and postsynaptic effects as it not only inhibits glutamate release but also blocks postsynaptic glutamate receptors like NMDA (N-methyl-d-aspartate) and kainate and inhibits voltage-gated Na^+ channels. The recommended dose is 50 mg twice a day, taken one hour before or two hours after a meal. It is generally well tolerated but can eventually cause nausea or diarrhea. Although the magnitude of its effect in ALS is small, riluzole represents a significant therapeutic milestone in the treatment of a disease refractory to all previous treatments.[3,7] Another drug used in the treatment of ALS is edaravone, a small molecule with free radical-scavenging properties that can reduce oxidative stress. It has been used in Japan for acute strokes since 2001 and was approved by the FDA for ALS under an orphan drug designation. The treatment is given in cycles, the first one is given intravenously daily for 14 days, followed by a 14-day break, then in subsequent cycles, 10 out of 14 days followed by a 14-day break. The drug is metabolized to glucuronide and sulfate and is excreted mainly in the urine as glucuronide, producing a terminal t1/2 of 4.5–6 h.[3]

MULTIPLE SCLEROSIS

MS or plaque sclerosis is a chronic neurological inflammatory and autoimmune disease characterized by demyelinating lesions and axonal damage in the central nervous system (CNS) that cause disability. It affects 1 in 1000 people, especially women (2:1 ratio compared to men). It generally affects people from 16–55 years old. The race most affected is white, of northern European descent. It begins with the appearance of focal inflammatory lesions in the cerebral white matter and a demyelination of neurons. Axons are preserved in the early phase of the disease, but the disease can cause deterioration or permanent damage to the nerves over time. Myelin is a lipoprotein structure that isolates axons, facilitating smooth and high-speed transmission of nerve impulses in the CNS. MS is the most common autoimmune neurodegenerative disease in subjects between 20 and 50 years of age, [9] and the patient may have one or more of the following types of cells involved of the injury:

- *Pattern 1:* T cells and macrophages are around the capillaries in the lesion, but the oligodendrocytes remain mostly intact and there are no antibodies. It occurs in 15% of patients.[10]
- *Pattern 2:* The lesion presents T cells and macrophages around the capillaries with intact oligodendrocytes but complement activation. It occurs in 58% of patients.[10]
- *Pattern 3:* There are diffuse lesions with inflammation and damaged oligodendrocytes, along with microglial activation and loss of MAG (myelin-associated glycoprotein). There is also partial oligodendrocyte remyelination and apoptosis. It occurs in 26% of patients.[10]
- *Pattern 4:* Lesions show abrupt edges, degenerated oligodendrocytes, and myelin ring. There is no complement activation or MAG loss. It only occurs in 1% of the patients.[10]

MS is classified into several subtypes based on how it develops clinically:[11]

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- *Relapsing-remitting (RRMS):* It is characterized by acute pictures of signs and symptoms that last weeks or months, alternated with periods of complete or partial resolution or the appearance of new signs and symptoms with recoveries. About 85% of people are diagnosed at this point. People aged 20–40 are diagnosed.[11]

- *Secondary-progressive (SPMS):* Appears 10–20 years after the installation of the relapsing-remitting form. Remissions become infrequent and are usually replaced by a gradual worsening of neurological symptoms over months to years. If neurological sequelae presents, the progression of early lesions is considered. Between 30 and 50% of patients with RRMS eventually develop SPMS.[11]

- *Primary-progressive (PPMS):* It is a disabling form of the disease marked by a constant worsening of symptoms, usually without definite recurrences or remission periods. About 15% of people with MS have this condition, which is diagnosed in between ages 40 and 60.[11]

- *Progressive-relapsing (PRMS):* It is a subtype of the primary-progressive form that can have rare relapses superimposed on a slow progression. It is the most aggressive subtype of the disease and affects between 3 and 5% of the cases. Unlike the relapsing-remitting form, PRMS shows a scarcity of brain and spinal lesions on MRI that differs from the patient's pathological, immunological, and clinical manifestations. It returns continuously from the beginning of the disease, presenting periodic severe exacerbations.[11]

Pathophysiology

In MS, tissue damage and neurological symptoms are the result of an immune mechanism directed against myelin sheath antigens made up of oligodendrocytes lining axons (neuronal extensions). The tissues of the nervous system and the spinal cord are protected by the blood-brain barrier (BBB), which is dysfunctional in these patients and allows macrophages and lymphocytes (T and B) to enter and carry out their autoimmune attack. There is inflammation, demyelination, reactive gliosis, and axonal damage. The most widely accepted hypothesis regarding its origin postulates that MS is the result of a genetic predisposition, an unknown environmental factor and a local or systemic factor that activates the autoimmune T cells. It is believed that this local factor could be certain infections (mostly by Epstein-Barr virus) or low vitamin D levels and little sun exposure. This would cause an autoimmune reaction, triggering inflammation and demyelination reaction. Activated CD4+ appear to adhere to the surface of endothelial cells in CNS vessels and migrate to the CNS, crossing the BBB. This leads to increased expression of cell adhesion molecules, matrix metalloproteinases, and pro-inflammatory cytokines. Together, they attract additional immune cells, break down the extracellular matrix to aid their migration, and activate autoimmune responses against antigens, such as basic protein, myelin, myelin-associated glycoprotein, myelin oligodendrocyte glycoprotein, proteolipid protein, αB-crystallin, phosphodiesterases, and S-100 protein. The binding of these target antigens by antigen-presenting cells triggers an immune response that can involve cytokines, macrophages, and complement. Antibodies against antigens found in white matter and oligodendrocytes can cause direct demyelination by cellular immunity by complement activation inducing cytolysis (fragments of antibodies against the basic protein of myelin have been found in patients affected by EM). Or indirectly demyelination by humoral immunity inducing the activation of macrophages and microglial cells that, through the trimolecular complex (formed by T cell receptors, antigens and receptors of the Histocompatibility class II molecule) would produce cytokines, such as factor a of tumor necrosis and interferon g that would generate nitro-oxygenation reactions producing amino acids, complement components, or proteolytic and lipolytic enzymes. The immune attack on the myelin strips the axons and thus reduces nerve conduction as it triggers neurological symptoms. Each patient will develop different symptoms depending on the neurons affected, but the disease can be very disabling.[10,12,13]

Conventional Treatment

The density and opening of internodal Na^+ channels leads to inflammation and edema that release cytokines, adhesion products, and others, as nitrous oxide (NO). These cause demyelination and slow the conduction of nerve impulse through the axons, which eventually triggers the symptoms of the disease. The short-term treatment is based on recover brain functions with the resolution of edema, changes in pH and the reduction of inflammation, while in the long term will be done by the recovery of Na^+ channels, although this recovery will always result in sequelae of the pathology.[10]

Treatment of the acute phase. Treatment should not be delayed more than 48–72 hours, and high doses of corticosteroids should be started. Treatment is based on the administration of 6-methylprednisolone 1 g IV for about 5 days, followed by a descending oral regimen of prednisolone 1 mg/kg/day that can range between 15 and 30 days.[10]

Treatment to prevent the progression of the disease. There is a wide variety of drugs that cause a nonspecific suppression of the immune system.[10,14]

- Immunosuppressants as azathioprine, cyclophosphamide, cyclosporine, FK506, methotrexate, mitoxantrone, deoxyspergualin, monoclonal antibodies, sulfasalazine, and total lymphoid irradiation.[10]

- Immunomodulators as linomide, immunoglobulins IV, or plasmapheresis in cases of resistance.[10]

- Administration of autologous T-cell clones by irradiation, inducing activated T cell withdrawal.[10]

- Modification of the cytokine system: Among the current treatments for the disease, there is the subcutaneous or intramuscular injection of Interferon B; which has diverse immunomodulatory effects and therefore multiple adverse effects, including influenza, depression, leukopenia, and hepatotoxicity. Two types of interferons are available on the spanish market; 1b which is administered subcutaneously and 1a, the latter for intramuscular administration, with a new variant with a higher dose recently introduced 1a subcutaneously.[10,14] Another subcutaneous injection that is usually given is glatiramer acetate (GA), which has effects on T cells and much fewer adverse effects than interferon B. However, it has not yet reached Mexico.[10]

- Inactivation of inflammatory mediators through inhibition of metalloproteases: In oral treatment there is dimethyl fumarate, which prevents the activation of the nuclear factor 2 transcription pathway and thereby reduces the inflammation of the disease. Another oral treatment is reriflunomide; a pyrimidine blocker that at the molecular level reduces the division of inflammatory cells.[10,14]

- Inhibition of migration through the blood-brain barrier: by blocking adhesion molecules by ICAM-1, VCAM-1, VLA-4 antibodies or by blocking cytokines: natalizumab, a monoclonal antibody that is applied intravenously, attacks α4-integrin and thus reduces the entry of lymphocytes to the Central Nervous System to maintain myelin integrity.[10]

- By promoting remyelination: fingolimod; sphingosine receptor modulator that is taken by mouth. Sphingosine is a complex aminoalcohol of great importance, since it is a precursor component of sphingolipids in general. The phospholipid or sphingolipid complex of interest to rescue with the receptor, is sphingomyelin; because it would keep the axons protected.[10,14]

It should be noted that all these oral medications must be given daily and thus are usually annoying for the patient. That is why a drug administered monthly is also mentioned.[14]

METHODS

Inclusion and exclusion criteria. This review contains randomized clinical trials, meta-analyses, and full-text systematic reviews conducted in humans and rats, published in English and Spanish in the last five years. It excludes non-randomized trials, quasi-randomized trials, case reports, trials written in languages other than English or Spanish, and trials conducted in species other than humans or rats. Articles that do not have concise and well-supported conclusions or that do not talk about the treatment of MS or ALS are also excluded. For clinical trials to be included, they must provide an in-depth breakdown of randomization methods, diagnostic criteria, details of interventions, and efficacy evaluation. The duration of treatment and patient follow-up is unlimited.

Types of intervention: Any type of intervention is included as long as it is randomized.

*Data sources and search strategy***.** The following databases were searched from February 2015 to February 2020 for studies relevant to this review: PubMed, SciELO and Cochrane. Since each database has its own limits, there was a little variation between the limits in each one, but they all have the same year limits and MeSH keywords. The articles without enough limits for species, language, or studies were analyzed by the researchers. They were discarded if they were not randomized clinical trials, meta-analyzes, or full-text systematic reviews conducted in humans and rats, published in English and Spanish in the last 5 years. Articles without concise and well-supported conclusions or not dealing with MS or ALS treatment were excluded. Here we present the limits stablished on each database. PubMed: The research was made with the MeSH keywords clinical advances, treatment and multiple sclerosis and clinical advances, treatment and amyotrophic lateral sclerosis. The limits were full text, meta-analysis, randomized controlled trial, and systematic review from 2015 to 2020 in humans and other animals in English and Spanish. SciELO: The research was made with the MeSH keywords clinical advances, treatment and multiple sclerosis and clinical advances, treatment and amyotrophic lateral sclerosis. The limits were articles from 2015 to 2020 in English and Spanish. Cochrane: The research was made with the MeSH keywords clinical advances, treatment and multiple sclerosis and clinical advances, treatment and amyotrophic lateral sclerosis. The limits were essays originally published between 2015 and 2020.

Type of participants: Neither the age nor the nationality of the subjects involved was taken into account.

Type of result measurement: The validity of the results of the studies was determined by detecting systematic errors or biases (selection, performance, performance bias, attrition/loss, attrition bias, or detection biases). The trials correctly designed and carried out were included in the review.

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RESULTS

- PubMed: It gave 64 results for Multiple Sclerosis and 9 results for Amyotrophic Lateral Sclerosis.
- Scielo: It gave 1 result for Multiple Sclerosis and 0 results for Amyotrophic Lateral Sclerosis.
- Cochare: It gave 18 results for Multiple Sclerosis and 0 results for Amyotrophic Lateral Sclerosis.

From the 3 databases PubMed, Scielo y Cochrane; there were selected just 12 articles for MS, and 3 articles for AML. So many of the discarded articles, weren't concise, well-supported or did not talk about the treatment of Multiple Sclerosis or Amniotrophic Lateral Sclerosis. Here is the most relevant information of these articles.

NEW CLINICAL THERAPEUTIC STRATEGIES

Amyotrophic Lateral Sclerosis

To find a treatment for this deadly disease, several therapeutic targets were actively sought, including kinases (phosphorylase enzymes whose function is to transfer phosphate groups from ATP to a substrate in order to activate or deactivate it) that did not phosphorylate the proteins involved in the pathological pathology as expected and nonsteroidal anti-inflammatory drugs (NSAIDs) that did not reduced the inflammation cascade caused by the active antibodies of the disease, the silencing of key genes, and the modulation or replacement of specific cell populations that cause autoimmunity. However, none have shown significant improvement as clinical trials have yet to define the safety and tolerability profiles of pharmacological, gene, and cellular therapies.[4] On the other hand, a randomized clinical trial investigated the efficacy of oral curcumin supplementation (600 mg/day). It is a natural antioxidant compound that due to its inhibitory power of the activity of histone acetyltransferases (HAT) and its beneficial anticancer and anti-inflammatory effects, was planned to inhibit the HAT activity of anti-inflammatory cells in a double-blind study.[6] The study used two groups: group A received placebo for 3 months and then curcumin for the next 3 months, and group B took the experimental substance for the entire 6 months. The study performed clinical evaluations and measured oxidative stress biomarkers, including oxidative protein products (AOPP), iron reducing capacity (FRAP), and lactate. Group B showed a reduction in AOPPs ($p < 0.01$) not detected in group A. Additionally, the FRAP assay values remained stable in group B, while group A showed a reduction without treatment and a later increase with therapy. In an assay, lactate was lower in group B *vs* group A ($p < 0.01$). It was concluded that curcumin treatment shows encouraging results that indicate a slight slowdown in the progression of the disease. It improves aerobic metabolism and oxidative damage, which also contributes to a deeper understanding of the pathogenic mechanisms of ALS.[6]

The nutritional status is an important prognostic factor in ALS. Then, a randomized study analyzed the safety, tolerability, and efficacy of nutritional counseling with or without an application of mHealth to maintain or increase body weight in ALS. In this randomized, open-label, standard-of-care, single-center controlled clinical trial, 78 participants were enrolled. Adults with ALS were randomized to one of three nutritional interventions: advice from their doctor or nurse (standard care), advice from a registered dietitian (RD, in person), or advice supported by the mHealth app. Although nutritional advice from a dietitian has been shown to be safe, it does not help maintain weight significantly better than standard care in patients with ALS.[8] No effective treatment is currently available for acute phase of ALS beyond supportive care with some moderately life-prolonging supportive medications. All the new and significant clinical advances on the treatment to prevent the progression of ALS are shown in Table 1.

Multiple Sclerosis

Among the most recent works on MS, there is a mouse model that suggests the transplantation of neuron cell precursors can be a good therapy. These immature cells can become neuronal cells and replace the lost myelin sheath. They also have a protective effect against leukemia since they secrete the cytokine leukemia inhibitory factor.[13] There are also several studies that show the effectiveness of drugs such as ocrelizumab, daclizumab, laquinimod, and masitinib. Ocrelizumab is a humanized anti-CD20 monoclonal antibody in phase 3 trials for progressive forms of MS; it selectively targets CD20-positive B cells, key contributors to myelin and axonal damage. In two global multicenter, double-blind, randomized phase-3 trials in 1656 patients with RRMS, OPERA I, and OPERA II, ocrelizumab significantly reduced the number of lesions detected by MRI compared to IFN β-1a. Daclizumab, on the other hand, is a human monoclonal immunoglobulin G1 antibody directed against the CD25 receptor, expressed on active T cells that could prevent the formation of interleukin-2 receptors in MS. It has even been associated with the activation of regulatory CD56 natural killer cells, which potentially destroy activated autologous T cells. This would likely give daclizumab the ability to reduce T cell proliferation. Laquinimod, another possible treatment, is an oral quinolone-3-carboxamide derived from linomide. Although its action mechanism is unclear, it is presumed to reduce leukocyte migration to the CNS, which would prevent neurological deterioration. Last, masitinib is a selective tyrosine kinase inhibitor that controls mast cell survival, migration, and degranulation by inhibiting certain growth and activation signaling pathways.[15]

Table 1. Conventional treatment *vs* clinical advances in amyotrophic lateral sclerosis.

Amyotrophic Lateral Sclerosis Treatment to prevent the progression of the disease	
• **Riluzole:** The recommended dose is 50 mg twice a day, taken one hour before or two hours after a meal.[3, 7] • **Edaravone:** It is given intravenously, with the first round daily for 14 days, followed by a 14-day break, then in subsequent cycles, 10 out of 14 days followed by a 14-day break.[3]	• **Diet:** oral curcumin supplementation (600 mg/day). In a double-blind study it was concluded that curcumin treatment shows encouraging results that indicate a slight slowdown in the progression of the disease, improving aerobic metabolism and oxidative damage.[6] • **Nutritional counseling:** In a randomized, open-label, standard-of-care, single-center controlled clinical trial; adults with ALS were randomized to one of three nutritional interventions: advice from their doctor or nurse ("standard care"), advice from a registered dietitian (RD) ("in person"), or advice supported by a mHealth app. Although nutritional advice from a dietitian has been shown to be safe, it does not maintain weight significantly better than standard care in patients with ALS.[8]

Several trials seeking to provide treatments related to non-genetic factors as diet. Such is the case of a meta-analysis of twelve studies and 950 patients that found vitamin D supplementation plays a therapeutic role in the treatment of MS. Although more trials are required to confirm the appropriate dose, it paints a promising picture.[16] Another systematic review from 2017 suggests amantadine provides a small improvement in fatigue for some people with MS. Still, beneficial effects in advanced stages of the disease have not yet been proven.[17] An additional review found that depression and anxiety are the most common comorbidities in MS, each of which affect more than 20% of the population. The prevalence of psychiatric comorbidities is high even at the time of MS diagnosis and increases throughout the course of the disease. This review revealed that symptomatic therapies used to manage MS can cause many mental diseases to patients, like depression and anxiety. They proved that corticosteroids cause transient depression, mania, and psychosis. And the mental effects of interferon are right now under investigation since the concerns about its probability to cause depression.[18]

It has been found that motor and cognitive rehabilitation can improve functional and structural brain plasticity in MS patients. Since by continuously evaluating the brain of patients in rehabilitation programs based on computer-assisted exercises/video games performed in an outpatient setting or at home, and continuously evaluation of brain structure by imaging techniques such as MRIs; changes in white matter microarchitecture, task-related activation and/or functional connectivity were presented after selective and task-oriented training. A relevant correlation was found between improved motor function and brain changes shown in MRI, supporting the hypothesis that training-induced brain plasticity can lead recovery on patients with MS.[19] There wasn't any advances on the acute effective treatment. Since then, the already known treatment will be the one used; 1 gram of 6-methylprednisolone I.V. for 5 days followed by 15–30 days of prednisolone, at a rate of 1 mg/kg/day.[10] All significant advances on new clinical therapies about treatment to prevent the progression of MS are shown in Table 2.

Advancements in licensed therapies

Dimethyl fumarate (DMF) was licensed in 2013 as a first-line oral therapy for patients with RRMS. It is highly effective, neuroprotective, and immunomodulatory and shows a favorable benefit-risk profile. However, the effects of DMF on the immune system of MS patients were unclear prior to marketing. Therefore, a systematic review in 2018 clarified the pharmacokinetics of DMF and its effect on the molecular pathways related to immunity. The evidence from the collected studies pointed to a multifactorial working mechanism of DMF treatment in MS that leads to a restored immune balance favoring a more tolerogenic or anti-inflammatory immune profile. DMF reduces the relapse rate, the number of brain injuries, and protects MS patients from further deterioration of motor and cognitive function; in addition, it has an immunomodulatory effect that modifies the altered immune balance of MS. Treatment with DMF selectively reduces CD8+ T cells and inflammatory memory subtypes of T and B cells in MS patients. This is in part due to the restored cytolytic function of $CD56^{bright}$ NK cells in DMF-treated MS patients. Furthermore, the expression or production of pro-inflammatory cytokines by T cells and B cells is reduced by shifting the Th1/Th17 response and pro-inflammatory B cells to an anti-inflammatory response. Therefore, treatment with DMF inhibits the activation and proliferation of T cells as well as the expression of antigen presentation and costimulatory markers in B cells.[20]

Cognitive impairment affects 40–65% of patients with MS. Therefore, another study evaluated the effects of fingolimod and IFN β-1b on the progression of cognitive impairment using MRI and clinical outcomes in patients with RRMS for 18 months.

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Table 2. Conventional treatment vs clinical advances in multiple sclerosis.

Multiple Sclerosis Treatment to prevent the progression of the disease	
• **Immunosuppressants:** azathioprine, cyclophosphamide, cyclosporine, FK506, methotrexate, mitoxantoin, deoxyspergualine, monoclonal antibodies, sulfasalazine or even total lymphoid irradiation. • **Immunomodulators:** linomide, i.v. immunoglobulins or plasmapheresis in cases of resistance. • **Administration of autologous T cell clones by irradiation**: ○ Modification of the cytokine system: Among the current treatments for the disease, there is the subcutaneous or intramuscular injection of Interferon B. Two types of interferon are available on the Spain market; 1b which is administered subcutaneously and 1a, the latter for intramuscular administration, with a new variant with a higher dose recently introduced 1a subcutaneously. ○ Another subcutaneous injection that is usually given is GA, which has effects on T cells and much fewer adverse effects than interferon B. However, it has not yet reached Mexico. • **Inactivation of inflammatory mediators through inhibition of metalloproteases:** ○ In oral treatment there is dimethyl fumarate and teriflunomide. • **Inhibition of migration through the blood-brain barrier, by blocking adhesion molecules by ICAM-1, VCAM-1, VLA-4 antibodies or by blocking cytokines:** ○ Natalizumab, a monoclonal antibody that is applied intravenously. • **Remyelination promoters:** ○ Fingolimod; sphingosine receptor modulator that is taken by mouth. [10, 14]	• **Neuron cell precursors:** these immature cells can become neuronal cells that replace the lost myelin sheath.[13] • **Monoclonal antibodies:** ○ Ocrelizumab: humanized anti-CD20 monoclonal antibody in phase 3 clinical development of progressive forms of MS that selectively targets CD20-positive B cells, key contributors to myelin and axonal damage. It has significantly reduced the number of lesions detected by MRI compared to IFN beta-1a in phase 3 studies.[15] ○ Daclizumab: It has even been associated with the activation of regulatory CD56 natural killer cells, which potentially destroy activated autologous T cells: which would give it the ability to reduce T cell proliferation. However, this is not yet confirmed.[15] • **Immunomodulators:** ○ Laquinimod: Although its mechanism of action is not clear, it is presumed that it reduces the migration of leukocytes to the CNS, which would prevent neurological deterioration in the patien.t.[15] ○ Masitinib: selective tyrosine kinase inhibitor that controls mast cell survival, migration, and degranulation by inhibiting certain growth and activation signaling pathways.[15] • **Diet:** vitamin D supplementation has a therapeutic role in the treatment of MS. Although more trials are required to confirm the appropriate dose, it paints a promising picture.[16] • **Symptomatically treatment:** ○ Amantadine may provide a small improvement in fatigue for some people with multiple sclerosis, although beneficial effects in the advanced stages of the disease have not yet been proven.[17] ○ Motor and cognitive rehabilitation can improve functional and structural brain plasticity in patients with MS. Since by continuously evaluating the brain of patients in rehabilitation programs based on computer-assisted exercises / video games performed in an outpatient setting or at home, by means of imaging techniques such as MRIs; changes in white matter microarchitecture, task-related activation and / or functional connectivity were described after selective and task-oriented training. [19]

The study included RRMS patients with cognitive impairment randomized (2:1) to fingolimod (0.5 mg daily)/IFN β-1b (250 µg every other day). Impairment was assessed using Rao's Brief Repeatable Battery test and the Delis-Kaplan executive function system. MRI parameters, Expanded Disability Status Scale (EDSS) scores, and relapses were measured. Patients (157) were randomized, of which 30 discontinued the study. At month (M) 18, both treatment groups showed improvement in all cognitive parameters. The relapse rate, the total number, and the volume of the lesions enhanced with gadolinium T2/T1 were higher with IFN β-1b, as well as the percentage of change in brain volume during the study. The safety and tolerability of both treatments were similar to previous studies. Both treatments showed improvement in cognitive parameters. Fingolimod demonstrated significantly better effects on MRI parameters and relapse rate. A trial of longer duration and lower dropout rate is necessary to observe the full expression of the differential effects on the cognitive impairment scales that reflect the differences between the groups on MRI. But still, the study confirms the favorable benefit-risk profile of fingolimod.[21]

Another triple-blind, placebo-controlled study evaluated autologous transplantation with adipose-derived mesenchymal stem cells (AdMSCs). The patients were randomized to receive a single infusion of placebo, low-dose (1 x 106 cells/kg), or high-dose (4 x 106 cells/kg) autologous AdMSC product and were followed for 12 months. Autologous AdMSC infusion is safe and feasible in patients with SPMS. Larger studies and probably an earlier stage treatment would be needed to investigate the potential therapeutic benefit of this technique. However, the study demonstrated that AdMSC infusion is a safe and feasible procedure in patients with SPMS.[22]

An attempt was also made in 2016 to find out whether erythropoietin (EPO), part of an endogenous neuroprotective system in the brain, could be a viable treatment in progressive MS. A phase 2, single-center, randomized, double-blind, placebo-controlled trial was conducted in which 52 patients with SPMS or PPMS were assigned to treatment with recombinant EPO (48,000 IU) or placebo IV 17 times over 24 weeks. No difference was found in the primary outcome between the EPO group and the placebo group since none of the secondary outcomes, neither clinical nor MRI measures showed significant differences.[23] Many studies also proved that active derivatives of vitamin A suppress the formation of pathogenic T cells in patients with MS. Therefore, a 1-year randomized placebo-controlled clinical trial examined 101 patients with RRMS to determine the degree of impact of vitamin A on disease progression in MS. The treated group received 25,000 IU/d of retinyl palmitate for six months followed by 10,000 IU/d of retinyl palmitate for another six months. Results of EDSS and Multiple Sclerosis Functional Composite (MSFC) were recorded at the beginning and end of the study. The results showed that the mean ± SD of the changes in clinically probable multiple sclerosis (CPEM) in the treated group was –0.14 ± 0.20 and in the placebo group, –0.31 ± 0.19. The CPEM improved significantly ($P < 0.001$) in the treatment group. There were no significant differences between the mean ± SD of the EDSS changes in the treated (0.07 ± 0.23) and placebo (0.08 ± 0.23) groups ($P = 0.73$). Similarly no significant differences were observed between the mean ± SD of the annualized relapse rate in the treated group (–0.36 ± 0.56) and the placebo groups (–0.53 ± 0.55) ($P = 0.20$). Vitamin A then improved the total MSFC score in RRMS patients, but did not change EDSS, relapse rate, or active brain lesions. The study then provides class II evidence that treatment with high-dose EPO is not effective in patients with moderately advanced progressive MS.[24]

GA is an immune modulating medication currently used to treat RRMS and clinically isolated syndrome (CIS). It is a random sized 40–100 amino acid polymer composed of primarily L-alanine, L-lysine, L-glutamic acid, and L-tyrosine. The premise behind its mechanism of action lies in the construct that patients with MS have antibodies directed against myelin basic protein (MBP), a component of the myelin sheaths of neurons within the CNS. Its chemical construct allows GA to mimic MBP and thus be a decoy for the antibodies in these patients, reducing the inflammatory response. The postulates from multiple clinical studies are that GA shifts the immune response from a pro-inflammatory state comprised of Th1 T-Cells to regulatory, non-inflammatory Th2 T-Cells. While GA cannot penetrate the BBB, its ability to induce peripheral Th2 cells and their subsequent crossing of the BBB allows the reduction of further inflammation within the CNS. This action mechanism has been called *bystander suppression*. Thus far, in preclinical levels it has been noted that GA induces a neuroprotective and/or neuro-regenerative effect. The drug increases neurotrophic factors like brain-derived neurotrophic factor (BDNF), which has been discovered to be vital to neuronal and glial cell survival.[25]

It has been shown to have a clear beneficial effect on spasticity in patients with RRMS previously treated with IFN-β. In treatment-naïve patients, its effect is less pronounced but without evidence of worsening spasticity. Only two studies have been conducted to evaluate this effect; the first, published in 2010 by Meca-Lallana et al., was a prospective observational pilot study conducted in two cohorts of patients with RRMS and spasticity who were going to be treated with GA: a cohort previously treated with IFN-β who switched treatment for reasons of safety or lack of efficacy (n = 13) and a cohort of naïve patients (n = 15). After 18 months of follow-up and compared to baseline, all patients who switched from IFN-β to GA showed a significant reduction in mean scores on the MAS for the right and left hemibodies and in scores of the PSFS and Global Pain Scale. In patients who started GA as the first disease-modifying drug, no change in their degree of spasticity was seen on any of the clinical scales used, probably

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because the baseline mean degree of spasticity was very mild and lower than those of patients treated with IFN-β. However, they did show a significant reduction in H reflex latency in the left hemibody and H/M ratio in the right hemibody (these are two objective electrophysiological indicators of improvement in spasticity, whose sensitivity, validity and reproducibility have been previously confirmed).[26]

DISCUSSION

Some vital data for the management of autoimmune neurodegenerative pathologies presented here is important. It must be noted that, once a diagnosis has been made, the patients and their relatives must be informed about the nature of the disease with total clarity and, above all, the recent diagnosis does not imply an unfavorable prognosis in all cases. Secondly, the precautions that people must have, as avoiding exposure to viral diseases, is relevant. The treatment must be multidisciplinary since several areas are involved in diseases. Thirdly, the ideal treatment is to be chosen taking into account the new scientific discoveries regarding the disease in question.

In ALS, no effective treatment was found beyond supportive care with some moderately life-prolonging supportive medications such as riluzole (which inhibits glutamate release, blocking postsynaptic glutamate NMDA and kainate receptors, and inactivating voltage-gated Na^+ channels) and edaravone (small molecule that reduces oxidative stress). Meanwhile, in recent lines of research regarding pathology, a randomized clinical trial investigated the efficacy of oral curcumin supplementation (600 mg / day) and concluded that treatment with this substance shown a slight slowdown in disease progression. But it is need to know the molecular mechanism of the substance in new investigations in order to know how it improves the aerobic metabolism and reduce oxidative damage of the disease. Knowing this we may have more therapeutic ideas that enhance the effects of the curcumin. Other studies did not reveal new therapeutic targets; since the randomized study that looked at the safety, tolerability, and efficacy of nutritional counseling for maintaining or increasing body weight in ALS, showed that nutritional counseling didn't make a change on the weight of the ALS patients who take the counseling nutritional course compared with the standard care ALS patients.[8] Last, the study that analyzed the treatment with kinases, NSAIDs, key gene silencing, and modulation or substitution of specific cell populations promoting autoimmunity did not demonstrate any important therapeutic benefits.[4] Then, the ALS patient still has not found a treatment to cure their disease. It is needed to keep searching new therapeutic dianas in order to prolong lifes of ALS patients more than the few months gained with riluzole and edaravon. In addition, due to the discoveries demonstrated in curcumin; the effect of curcumin needs to be enhanced and empowered with new medicines.

The conventional treatment for MS in the acute phase should not be delayed more than 48–72 hours and must start with high-dose corticosteroids, 6-methylprednisolone 1 g IV, and prednisolone for 5 days. It prevents disease progression and nonspecifically suppresses the immune system. The actual treatments between the ones a doctor who treats a MS patients can choose are immunosuppressants, immunomodulators, the administration of autologous T cell clones by irradiation, the modification of the cytokine system with Interferon B or GA, the inactivation of inflammation mediators by means of the inhibition of metalloproteases with dimethyl fumarate, the inhibition of migration through the blood-brain barrier and the promotion of remyelination with fingolimod. While new clinical therapeutic strategies suggest a transplantation of neuron cell precursors (that replace the lost myelin covering) and drugs that improve the panorama of the disease with monoclonal antibodies, such as ocrelizumab, daclizumab in order to prolong patients life's; other laboratories need to keep searching ways to cure the disease with new therapeutic dianas. Other trials use treatments related to non-genetic factors. A study using vitamin D is still in process because the appropriate dose is yet to be found. Symptomatic therapies used to control MS can cause depression or anxiety. Several reviews have found that corticosteroids can cause transient depression, mania, or psychosis. Pivotal trials of IFN-β for MS raised concerns about this therapy, which evidences the need for new therapies that do not cause this type of comorbidities. Similarly, it was concluded that motor and cognitive rehabilitation can improve plasticity in MS patients, as a relevant correlation was found between improved function and brain changes detected by MRI. This supports the hypothesis that training-induced brain plasticity is specifically related to the trained domain.[19]

All this, in conjunction with the advances in therapies already authorized; such as the recent understanding of the pharmacokinetics of dimethyl fumarate or the recent discovery of the positive effects of fingolimod and interferon beta-1b (IFN β-1b) in the progression of cognitive impairment by magnetic resonance imaging; show that MS, although it still has no cure, does have multiple ways of allowing the patient to have an improvement in their prognosis and quality of life compared to previous therapeutic lines.

Scientific advances are so accelerated that even a triple-blind, placebo-controlled study evaluated autologous transplantation with AdMSCs. Autologous AdMSC infusion is safe and feasible in SPMS patients, but larger and probably earlier studies are still necessary to investigate the potential therapeutic benefit of this technique. Other treatments have also been tried and discarded; EPO and vitamin A (in a randomized controlled clinical trial) provided class II evidence that high doses of EPO are not effective in patients with moderately advanced progressive MS.[24]

CONCLUSIONS

It is necessary to continue searching for more information on the pathophysiology of amyotrophic lateral sclerosis and multiple sclerosis in order to find their main causes and thus the way to reverse them. To date, there are only medications that prolong the patient's life, as riluzole and edaravone for amyotrophic lateral sclerosis and fingolimod, dimethyl fumarate and IFN β-1b for multiple sclerosis. Importantly, a 600-mg dose of curcumin added to the diet in amyotrophic lateral sclerosis has shown to inhibit the activity of histone acetyltransferases in anti-inflammatory cells and help the prognosis. In addition, more studies should be carried out on autologous transplantation with adipose-derived mesenchymal stem cells to investigate their potential therapeutic benefit in phases prior to secondary-progressive multiple sclerosis.

CONFLICT OF INTEREST

There was no affiliation with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), and non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed and showed in this manuscript.

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Review Article

Association between gut microbiota and obesity. A review of the evidence

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ABSTRACT

Obesity is a worldwide health problem that is causally associated with genetic and nutritional factors. Nevertheless, other factors, as gut microbiota, have been proposed to play a role in its development. The living microorganisms that inhabit the organism are known as microbiota, and in humans, the microbiota consists of four main groups: *Firmicutes, Bacteroidetes, Proteobacteria,* and *Actinobacteria*. Although it was traditionally believed the human body was in a bacteria-free environment during embryonic development and colonization occurred at birth, there is evidence that colonization begins in the uterus. Naturally, the intestinal microbiota exhibits several metabolic functions, but numerous factors can modify the proportion of families of bacteria that compose it. Such modifications can lead to a state of dysbiosis that can cause the development of secondary overweight, obesity, and in the future, chronic metabolic diseases. However, not all the results are consistent with this hypothesis. There is evidence that fecal transplantation by modifying the composition of the intestinal microbiota significantly reduces the patient's body weight and food consumption. Bariatric surgery is less favored and the appearance of species that reduce low-grade inflammation is observed. Therefore, this review aims to analyze the available literature on the ability of the gut microbiota to alter homeostasis and increase the probability of developing overweight and obesity.

Key words: obesity; gut microbiota; dysbiosis; overweight; metabolism.

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RESUMEN

La obesidad es un problema de salud a nivel mundial que se asocia en forma causal con factores genéticos y nutricionales. Sin embargo, se propone que otros factores, como la microbiota intestinal, participan en su desarrollo. Los microorganismos vivos en el organismo son conocidos como microbiota y en el ser humano esta se divide en cuatro grupos principales: *Firmicutes, Bacteroidetes, Proteobacteria y Actinobacteria*. Tradicionalmente se pensaba que el organismo se encontraba en un entorno libre de bacterias durante el desarrollo embrionario y su colonización ocurría al nacer, pero la evidencia apunta al inicio de la colonización en el útero. De forma natural, la microbiota intestinal exhibe varias funciones metabólicas y varios factores pueden modificar la proporción de sus familias de bacterias. Tales modificaciones pueden conducir a un estado de disbiosis que puede provocar el desarrollo de sobrepeso y obesidad de forma secundaria y, en el futuro, enfermedades metabólicas. Sin embargo, no toda la evidencia es consistente con esta hipótesis. Aunque el trasplante fecal produce una disminución significativa en el peso corporal del paciente y el consumo de alimentos, además de la cirugía bariátrica, al modificar la composición de la microbiota, también favorece la aparición de especies que disminuyen la inflamación de bajo grado característica de la obesidad. Por lo tanto, esta revisión tiene como objetivo analizar la literatura disponible sobre la capacidad de la microbiota intestinal para alterar la homeostasis y aumentar la probabilidad de desarrollar sobrepeso y obesidad.

Palabras clave: obesidad; microbiota intestinal; disbiosis; sobrepeso; metabolismo.

INTRODUCTION

Obesity, defined as the abnormal or excessive accumulation of fat that poses a health risk, has become a public health problem worldwide due to the increase in its gradual prevalence observed for the past 50 years. Therefore, in the late 90s, the World Health Organization (WHO) declared it a global epidemic. The number of infants and young children (0–5 years) who are overweight or obese increased from 32 million in 1990 to 38 million in 2019.[1] Mexico ranks first in childhood obesity and has one of the highest rates of overweight among Organization for Economic Cooperation and Development (OECD) countries.[2] Unfortunately, most of the strategies against obesity undertaken in Mexico have failed to reduce the prevalence of the epidemic. Between 2016 and 2018, obesity increased by 1% in combined prevalence with overweight in children under five years of age, 1.1% in children from 5 to 11 years old (34.4% vs. 35.5% respectively) and 3.5% in the group of 12 to 19 years. Finally, in the same period, the combined prevalence of overweight and obesity in adults increased by 3.6% in men (69.4 vs. 73%) and 3.8% in women (73% vs 76.8%).[3]

The relevance of obesity lies in its association with the appearance of metabolic alterations and pathological conditions, such as insulin resistance, type 2 diabetes, hypertension, fatty liver, asthma, sleep apnea, cardiovascular diseases, and at least eleven types of cancer.[4-7] Although genetic factors, lifestyle, diet, and the imbalance between food intake and physical activity are closely related to obesity, others are related to this pathology.[6-7] Such is the case of the mother's nutritional status, birth weight, the microbiota in early stages of life, sedentary life, obesity in parents and the composition and metabolic expression of the intestinal microbiota.[5,6,8,9] In this study, we present evidence supporting the ability of the intestinal microbiota to alter the homeostasis of the human body, which increases the probability of becoming overweight or obese.

THE INTESTINAL MICROBIOTA

The living microorganisms residing in an ecological niche of the organism are known as microbiota. It is traditionally estimated that the human body contains about 10^{14} cells, of which 90% are prokaryotic and 10% eukaryotic. Then, in essence, our organism is mainly composed of non-human cells, most of them bacteria, that are predominantly lodged in it.[10] There are about 500 bacterial species, and 99% of them belong to the four prominent families: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%), and *Actinobacteria* (3%).[11] Figure 1 shows the variety, quantity, and main species of bacteria that colonize the human digestive tract. Traditionally, the body of the human fetus has been considered free of bacteria while colonization is to begin at the moment of its birth. However, there is growing evidence of bacteria in the placenta, umbilical cord, and amniotic fluid in healthy term pregnancies. So, colonization could begin in the uterus.[12-14]

Regardless of the moment of the first colonization occurs, small concentrations of *Staphylococcus, Streptococcus, Propionibacterium, Corynebacterium, E. coli, and Streptococcus* are found immediately after birth. They are responsible for the preparation of a favorable environment for colonization by the genera *Bifidobacterium*, *Bacteroides,* and *Clostridium*, between days 4 and 7 of extrauterine life.[10] However, in children born by cesarean section, initial colonization is more frequent

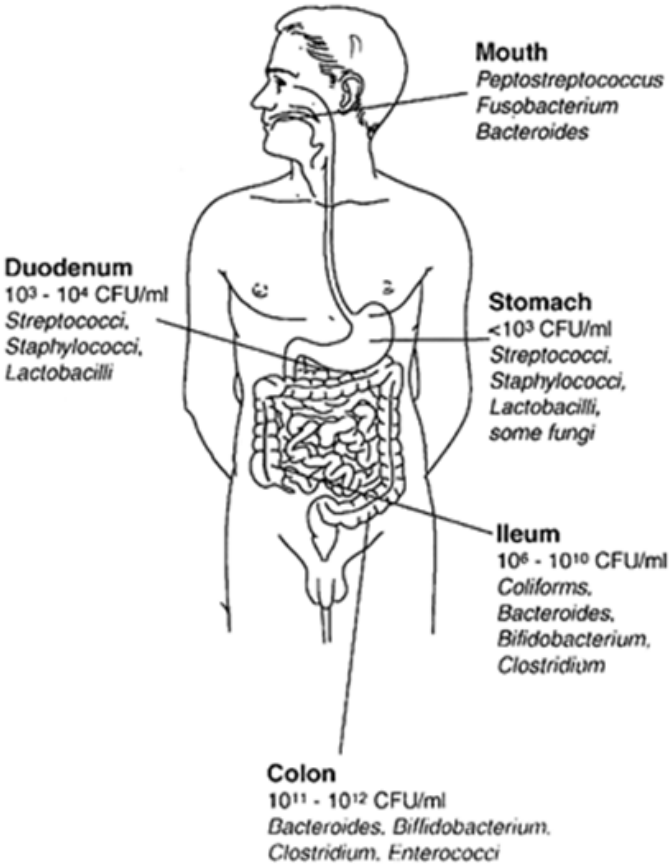

Figure 1. Microorganisms of the gastrointestinal system.

In the upper gastrointestinal tract (from the stomach to the small intestine), the number of colonies is low due to the characteristics prevailing in the lumen that hinder significant colonization. Conditions for growth and permanence in the colon are optimal, mainly due to motor activity and the number of nutrients. Consequently, the highest concentration of intestinal microbiota is located in the large intestine. CFU: colony-forming unit; ml: milliliter. Taken from Thompson-Chagoyán et al. (10).

with environmental microorganisms. After birth, anaerobic colonization is delayed, and the microbiota is composed of microaerophilic bacteria, facultative anaerobes, and sporulated bacteria found in the hospitable environment. Between days 4 and 6, anaerobic bacteria appear in children born vaginally but only in 9% of those delivered by cesarean section. The microorganisms introduced during the first seven days of life have a low colonization capacity, and there is a delay in acquiring anaerobic *Bacteroides*, *Bifidobacteria, E. coli*, and other Gram (-) bacteria. However, the exposure and acquisition of these bacteria among children in developing countries occur earlier.[10] From the first year of life, bacterial colonization gradually changes. At two years, it is very similar to the adult intestinal microbiota, although it shows a more significant number of facultative anaerobes. Finally, it is considered identical to that of the adult in late childhood (9–11 years).[10]

Intestinal microbiota functions

There are three large functions of the intestinal microbiota. *Metabolic*: The microbiota generates energy through the metabolic substrates present in the lumen of the intestine (dietary fiber, lipids, proteins, starches, mucins, epithelial desquamation cells, bacterial wastes, bile acids, and cholesterol). It is in charge of the production of short-chain fatty acids (SCFAs).[15,16] *Trophic:* The intestinal microbiota affects the differentiation of epithelial cells by the interaction between resident microorganisms and their metabolic products, mainly by SCFAs. This stimulates the proliferation and differentiation of the gut cells directly.[10] *Protective:* The gut microbiota has a profound influence on gene expression in the intestinal mucosa involved in defense of the organism by regulating the intestinal barrier function. The intestinal mucosa acts as a natural barrier against the adhesion of pathogenic bacteria, antigens, and toxic substances of the intestinal lumen.

The modulation of antigen transport can be attributed to Lactobacilli since the intestinal antigen translocation decreases significantly in rats treated with these microorganisms.[10] In addition, resident microorganisms in the intestine produce bacteriocins, which lowers the pH and depletes the nutrients required by the pathogenic bacteria.

Through all these mechanisms, the intestinal epithelium acts as a barrier against the growth of pathogenic bacteria, antigens, and toxic substances of the intestinal lumen.[16] Intestinal bacteria also protect the host through promoting the secretory immunoglobulin A (IgA) against some pathogens, the restoration of some commensal organisms, and the reduction in enterotoxins without increasing the number of aggressor microorganisms.[10] Simultaneously, the multiplication and complexity of the intestinal microbiota are essential for expanding the immune system and its specialization. The relationship between the gut-associated lymphoid tissue (GALT) and the intestinal microbiota in the early stages of life is crucial for developing interactions between the mucosa and systemic immunoregulation. Further maturation of GALT requires constant stimuli of bacteria from the intestinal microbiota. Homeostasis of the digestive ecosystem is determined by the balance between the microbiota, intestinal permeability, and local immunity so that any event that affects any of these components impacts others and negatively impacts the entire organism.[10]

ASSOCIATION BETWEEN INTESTINAL MICROBIOTA AND OBESITY

Even though the relationship between obesity and the intestinal microbiota has been undoubtedly proven, several studies show a significantly lower accumulation of body fat in microbiota-

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free mice than in those with intestinal microbiota.[16] Significant differences in the gut microbiome (group of microorganisms, their genes, and metabolites) have been found between genetically modified obese mice (ob/ob) and mice born with ob/+ and +/+ non-obese genotypes to the same litter.[17] Mechanisms that link the gut microbiota with obesity include the abnormal composition of the microbiota (dysbiosis); increased energy extraction from food; effect on the intestinal barrier integrity; modulation of the immune system and inflammation, and the production of specific metabolites that have an impact on different organs and tissues (GALT, the intestinal barrier, the brain, the liver, and adipose tissue, among others).[10]

Early contact with microorganisms and obesity risk

For many years, scientists held the idea that the human gut was free of microorganisms and that the fetus developed in a sterile environment because colonization began at the time of delivery. However, recent studies have shown that the intrauterine environment is not sterile even in physiological pregnancies by showing that there are microorganisms in the amniotic fluid, umbilical cord, fetal membranes, and the placenta, both in children born vaginally and by cesarean section.[10,12,14,18-20] The microbiota found in the amniotic fluid and the placenta are remarkably similar because the microorganisms pass from the intestine into the mother's bloodstream and reside in the placenta or pass through it into the amniotic fluid.[14,20] Colonization continues after birth, and in the initial microbiota of the newborn, the phyla *Proteobacteria* and *Actinobacteria* have a predominance.[20] However, several factors such as the route of delivery, gestational age, the type of food, and the use of antimicrobials during pregnancy, among others, make *Firmicutes* and *Bacteroidetes* the predominant phyla at the expense of lower *Proteobacteria* and *Actinobacteria.*[21,22] All of these modify the exchange of microbiota between the mother and her child in various degrees. This causes an aberrant colonization that several studies have shown predisposes the child to obesity in later stages of life.[23]

The mother's weight modifies the child's intestinal microbiota composition since children of obese mothers have greater amounts of *Faecalibacterium* spp., *Oscillibacter* spp., *Blautia* spp., and Eubacterium spp.[24] They produce SCFAs, which favors a significant energy production from intestinal waste and an increased risk of obesity at later ages.[25] Additionally, obese pregnant women have a more significant amount of *Bacteroides* in the vaginal tract during the third trimester of pregnancy.[26] If their children are born this way, they come into early contact with this type of bacteria. Therefore, their microbiota will contain a greater quantity of these microorganisms, which predisposes them to suffer obesity in later stages of life. Finally, some authors have proposed that the presence of high concentrations of *Staphylococcus aureus* and low levels of *Bifidobacterium* spp. in the intestinal microbiota in early childhood could predict the future appearance of overweight or obesity.[9]

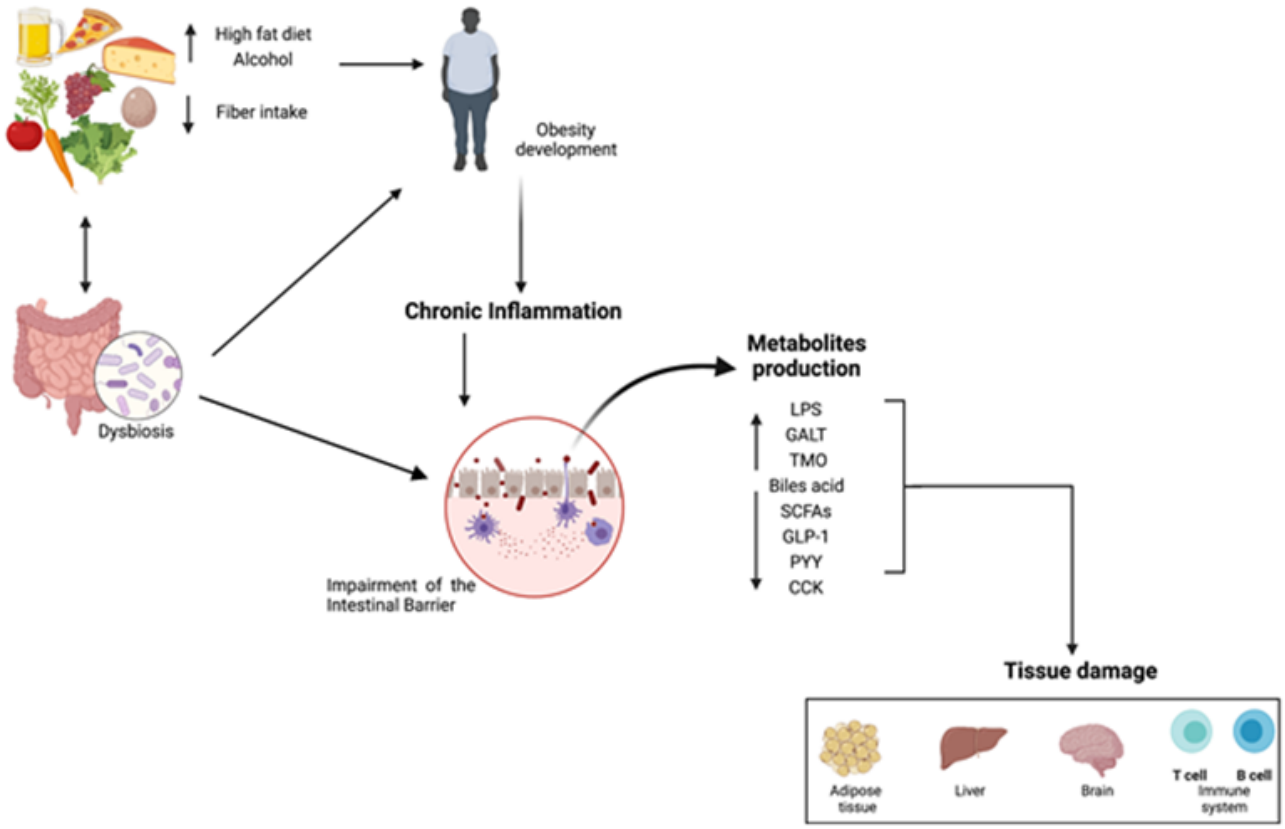
The type of feeding during lactation is equally crucial for developing obesity due to the microorganisms that colonize the child's intestine. In formula-fed infants, the number of facultative aerobic bacteria (Staphylococcus, Streptococcus, Enterobacteriaceae) in the stool is markedly higher, and colonization by the genus Bifidobacterium generally begins some days later than in breastfed infants. *Bacteroides* predominate among anaerobes and are present in a large number of *Enterobacteriaceae.*[20] After the first feeding, the microbiota changes rapidly. In children exclusively breastfed within a few weeks, bifidobacteria are the dominant microorganisms, possibly due to factors in breast milk stimulating the growth of these bacteria and not found in infant formulas.[10]

Altered intestinal microbiota (dysbiosis)

The taxonomy of the gut microbiota comprises phyla, classes, families, genera, and species. In the first taxonomic class, the dominant microorganisms are *Actinobacteria, Proteobacteria, Fusobacteria*, *Verrucomicrobia, Firmicutes*, and *Bacteroidetes*. The last two represent 90% of the intestinal microbiota. In contrast, *Proteobacteria, Fusobacteria, Actinobacteria, Tenericutes, Verrucomicrobia, Synergistetes,* and *Cyanobacteria* account for the remaining 10%.[27] Studies indicate that obese patients have a different gut microbiota with increased Firmicutes and a decrease in Bacteroidetes.[28,29] This imbalance is known as dysbiosis[15] and causes loss of integrity of the mucous barrier, degradation of the intestinal mucous layer, pro-inflammatory cytokines increase, and increased oxidative stress (Figure 2). It is also found in duodenal fluid samples, where the predominant phyla are Firmicutes and Actinobacteria, accompanied by a considerable decrease in Bacteroidetes.[21,28,30] This finding is secondary to the limited availability of mucin in the duodenal mucus compared to that found in the stomach and colon.[30]

Several studies link the increase or decrease in other specific intestinal microorganisms to obesity and overweight. They report a reduction in the amount of one or more of the following: *Methanobrevibacter smithii; Clostridium perfringens, Lactobacillus,* and *Bifidobacteria; Staphylococcus aureus*, *Escherichia coli,* and *Faecalibacterium prausnitzii;* and *Clostridium, Ruminococcus, Blautia, Veillonella,* and *Bacteroides fragiles.*[9,31-35]

Some authors describe an increase in certain microorganisms accompanied by a decrease in *Lactobacillus reuteri*, *Lactobacillus casei/paracasei*, and *Lactobacillus planctarum*.[26,32]



LPS: Lipopolysaccharide; GALT: Gut-associated lymphoid tissue; TMO: Trimethylamine N-oxide; SCFAs: Short-chain fatty acids; GLP-1: Glucagon-like peptide 1; PYY: Peptide YY; CCK: Cholecystokinin.

Figure 2. There is a close relationship between gut microbiota and obesity.

Dysbiosis and chronic inflammation modify the intestinal epithelium, causing damage to the intestinal barrier and translocation of LPS. Similarly, there is an increase in TMO concentrations and a decrease in SCFA concentrations, and in response to oxidative stress, pro-inflammatory cytokines increase. On the other hand, in response to LPS translocation and inflammation, the different cells of the GALT rise in the immune system activity. All the alterations derived from dysbiosis and mild inflammation, characteristic of obesity, in the long term cause damage to different organs and systems, such as adipose tissue, the liver, the brain, and the immune system. At the nutritional level, the hormones GLP-1, PYY, CCK play a prominent role in satiety in various metabolic functions, such as glucose homeostasis, nutrient absorption, and several more.

These alterations in the intestinal microbiota disappear when the individual loses weight. However, the amount of *Bacteroidetes* is a more precise indicator of an individual's body mass index (BMI) than that of *Firmicutes*, likely due to the more significant variability of *Firmicutes.*[32,34,35] Finally, the decrease in the bacterial family *Christensenellaceae spp.* has been proposed as a reasonably sensitive biomarker of obesity because it modulates the intestinal microbiota composition and reduces weight gain in humans.[36]

Production and fermentation of short chain fatty acids and energy production

The gut microbiota obtains a significant amount of energy through the production and fermentation of SCFAs (acetate, propionate, and butyrate) from unabsorbed food, intestinal cells, and carbohydrates [fructans, starches, and polysaccharides (cellulose, galactomannans, arabinogalactan, xylan, and pectins)], waste products of microorganisms, intestinal secretions and other metabolic substances (such as mucin).[10]

When they ferment the indigestible polysaccharides from the diet into monosaccharides and SCFAs, gut microorganisms produce a significant amount of calories, which contribute with up to 10% of an individual's basal caloric expenditure or are stored as adipose tissue.[15,16] Obese subjects show reduced taxonomic diversity but a higher fermentation capacity than lean individuals, which increases the amount of energy produced from carbohydrates and lipids.[5]

In several studies with intestinal germ-free animals, lower body fat was found than animals with intestinal microbiota, although

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the former consumed a more significant amount of food.[15] When they performed a fecal transplant from other animals or humans in gnotobiotic, the percentage of fat increased significantly as food consumption considerably decreased.[36] Similarly, when receiving microorganisms from obese subjects, animals showed a more significant accumulation of body fat than those that were implanted lean animals' microbiota.[36] This increased ability to obtain energy has also been found in humans, as the intestinal production of SCFAs in the feces of obese children and adults is significantly higher than that observed in lean individuals.[37] Several types of bacteria are associated with a remarkable ability to obtain energy from non-metabolized products located in the gut lumen, resulting in increased liver lipogenesis when the energy is absorbed. At the same time, these bacteria inhibit the fasting-induced adipocyte factor (FIAF), an LPL inhibitor, which favors triglyceride storage in adipocytes.[16]

Modification of peripheral metabolism by the microbiota and its products

In addition to the effects on energy extraction from food, colonization of germ-free mice increases lipid storage in adipocytes and regulates it in the body by inducing lipogenesis in the liver.[16] The increased synthesis of triglycerides produces excess energy that reduces the protein kinase activity initiated by adenosine monophosphate (AMPK) in skeletal muscle and in the liver. This leads to the inhibition of ATP generation metabolic pathways.[36,38] Furthermore, SCFAs induce apoptosis of intestinal epithelial cells and interact with G-protein-coupled receptors 41 and 43 (GPR41 and GPR43), which favors the production of peptide YY (PYY) and glucagon-like peptide 1 (GLP-1). When they interact with SCFAs, these hormones promote an increase in leptin production, which inhibits neuropeptide Y and melanocortin, both with catabolic effect, under normal conditions. Thus, the adipose tissue acts on the sympathetic nervous system (SNS), causing anorexia and reducing energy intake. When there is dysbiosis despite the elevated leptin serum levels, there is no anorexic and catabolic effect as a result of the simultaneous resistance to the action of leptin. This seems to be due to the lack of leptin supply to the CNS.[39-41] These changes in metabolism induce a more significant food intake and an increase in glucose-induced insulin production.[42] They also trigger an inflammatory response responsible for obesity-related disorders, such as insulin resistance, lipogenesis, and increased triglyceride storage.[43] Similarly, in germ-free animals, the activity of lipoprotein lipase (LPL), an enzyme that increases the cellular uptake of fatty acids and favors the accumulation of triglycerides, is reduced.[16] Still, if these animals have FIAF, also known as angiopoietin-like protein 4 (Angptl4), there is an increase in LPL activity and a consequent accumulation of body fat.[36,38]

Modification of microbiota composition by diet and its impact on metabolic functions

Diet is one of the essential factors in determining the conformation of the intestinal microbiota. It has been described in experimental animals that the consumption of a diet that provides more than 30% of calories in fat promotes the development of obesity. The typical western diet contributes with 32.8% of calories in fat and high levels of refined sugars, salt, saturated fats, trans-fatty acids and low fiber.[5,44] One way to explain the association between the change in the intestinal microbiota produced by the diet consumed and the subsequent appearance of obesity is that the enterotypes do not undergo modifications by the place where the host lives but by the ingested diet, which causes the prevalence of Prevotella, Bacteroides or Ruminococcus.[44] The diet is rich in fats and animal proteins, the Bacteroides enterotype appears. When the diet is rich in carbohydrates, Prevotella predominates, while there is a significant growth of Ruminococcus in diets containing abundant non-digestible fiber and inulin.[45] However, some authors reject this explanation because the enterotype of an individual can be highly variable. Additionally, putative discrete clusters are less useful as disease biomarkers than a predictive model built from the relative abundance of the raw taxon.[46]

Association between host genotype, intestinal microbiota, and obesity

The existence of a close relationship between the host genotype, the modification of the intestinal microbiota composition, and the development of obesity is widely accepted Several studies describe a close relationship between the host genotype, the change in the gut microbiota composition, and the subsequent development of obesity.[47] In experimental deficient in leptin mice (ob/ob), the microbiome is rich in genes associated with recovering energy from food and a significant decrease in Bacteroidetes and Bifidobacterium sp.[48] The ApoA1 gene, associated to the modification of the intestinal microbiota, codes for Apolipoprotein A1, whose mutation is linked to metabolic diseases. The polymorphism of apoA1 is related to modifications in the intestinal microbiota characterized by increased *Desulfovibrionaceae* and depletion of the members of the *Bifidobacteriaceae* family.[49] The gene phospholipase D1 (PLD1), by hydrolyzing glycerol phospholipids, the phospholipase D1 (PLD1) gene produces phosphatidic acid, an intracellular messenger associated with the onset of obesity.[50] The microbiota in obese subjects produced by PLD1 shows a significant growth of the genus *Akkermansia muciniphila*, an inducer of obesity in mice, according to several reports.[5,51]

Fecal transplant and weight reduction

The introduction of a fecal suspension from a healthy person into a patient's gastrointestinal tract to restore the altered microbial ecosystem is known as a fecal microbiota transplant.[52] Although this procedure is not new, it was initially used to treat *Clostridium difficile* infection, and it is currently considered highly effective in treating the recurrent disease caused by this microorganism. At present, multiple studies use fecal transplantation to treat other conditions in which there is an alteration of the intestinal microbiota (Figure 3). The results obtained are promising; however, there are other studies with inconclusive or contrary results.[52–54] It has been demonstrated, in animal models, that the intestinal microbiota of obese and thin subjects can induce similar phenotypes and that the microbiota of lean mice, transplanted into obese mice, reduces the adipose tissue gain in the recipient.[55] The opposite occurs when patients who received intestinal microbiota transplants as part of the treatment for repeated infections by *Clostridium difficile* or anorexia nervosa significantly increased their weight.[56,57] Thus, most of the centers where fecal transplantation is carried out in obese patients, discarded donors with pathogens in cultures, intestinal diseases, or communicable diseases. They also reject all those with a BMI > 30 kg/m^2, malnutrition or metabolic syndrome.[58] However, the characteristics a donor must have to obtain optimal results (super donor), as in other conditions, are not yet known.

Another fundamental aspect that has promoted the microbiota transplantation for treating obesity is that 12 months after the procedure, the microbial composition in the feces of the transplanted individual; it is very similar to that of the donor, probably because the transplanted microbiota produces the appearance of phenotypes identical to those of the donor in the recipient[59] The intestinal microbiota transplantation exerts its effect likely through manipulating the intestinal microbiota composition, strengthening the intestinal barrier, suppressing pathogens, and modulating the immune system.[54]

Bariatric surgery and obesity treatment

Bariatric surgery produces reduction in weight and fat mass of 65–75%. This effect is due to the decrease in caloric intake and a significant change in the composition of the intestinal microbiota.[59] This fact that has been proven in several studies, although only in Roux-en-Y gastric bypass because this surgery causes alterations of pH and oxygen content, increases the concentration of bile acids, and decreases the number of nutrients that reach the colon.[60-64]

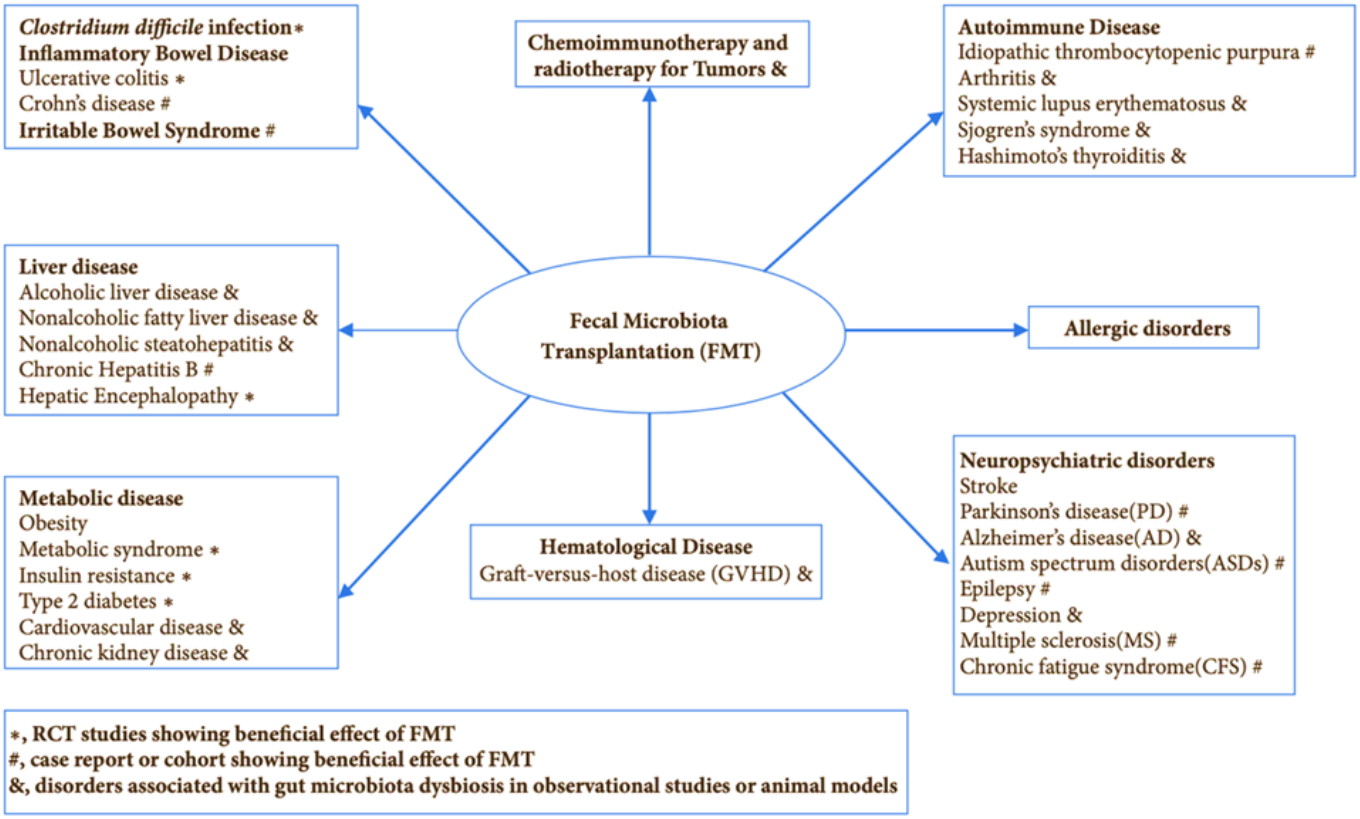

FIGURE 3. Current and potential uses of intestinal microbiota transplantation, types of studies carried out, and results obtained in both animal and human models. (Taken from Zhou et al.[59]).

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The Roux-en-Y gastric bypass also induces *Bacteroidetes* and *Proteobacteria* growth and a reduction in *Firmicutes*.[65] It increases *Faecalibacterium prausnitzii* species, to which the decrease in low-grade inflammation, characteristic of obesity, is attributed. The prolonged weight reduction after surgery is due to the modification of cholesterol metabolism by Sarcina. This effect occurs regardless of the energy ingested and a decrease in gastric leptin concentration, elevated in obese patients immediately after surgery and preceding changes in serum hormone concentration.[66] Finally, the following new species have even been described after bariatric surgery: *Anaerotruncus massiliensis, Bariatricus massiliensis, Eisenbergiella massiliensis,* and *Ruminococcus phoceensis.*[67-70]

CONCLUSION

The evidence that associates the intestinal microbiota with obesity relies on the presence of dysbiosis, where there is an increase in certain microorganisms and a reduction in others. Another consistent finding is that the obese patient's microbiota contains less taxonomic diversity but can produce a more significant amount of energy from food waste, desquamation cells, and secretions of the gastrointestinal tract. Similarly, the microbiota can carry out metabolic changes that favor food intake. In turn, the intake promotes intestinal permeability and low-grade inflammation due to lipopolysaccharides and reduces leptin and metabolic changes. These two increase the amount of energy consumed and produce resistance to insulin, lipogenesis, and triglyceride storage. Many of these changes are attributed to diet, the host's genotype, or early contact with certain types of microorganisms. Despite all this evidence, research is yet to demonstrate whether the changes found are the cause of obesity or just a manifestation of this pathology. The results obtained with fecal transplantation and bariatric surgery are encouraging, since both modify the body composition of obese subjects. The isolated bacteria that appear as body weight is reduced are directly associated with the loss of fat tissue and can be used to modify the intestinal microbiota selectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest. No financial support was required.

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