VNIVERSITAT DE VALÈNCIA

Programa de Doctorado en Enfermería Clínica y Comunitaria

IL-6 y ejercicio físico: efectos del ejercicio físico sobre la IL-6 y sus consecuencias en la salud

Tesis doctoral por compendio de artículos

Pablo Gómez Rubio

Directoras:

Dra. Isabel Trapero Gimeno

Dra. Cristina Buigues González

VNIVERSITAT DE VALÈNCIA

Isabel Trapero Gimeno, Doctora en Medicina, profesora titular de Universidad del área de Enfermería, Universitat de València y Cristina Buigues González, Doctora en Enfermería, profesora contratada doctora del área de Enfermería, Universitat de València,

CERTIFICAN

Que Pablo Gómez Rubio, Licenciado en Ciencias de la actividad física y deporte por la Universitat de Valencia, ha realizado su tesis doctoral bajo nuestra dirección con el título de "IL-6 y ejercicio físico: efectos del ejercicio físico sobre la IL-6 y sus consecuencias en la salud".
Una vez revisado el presente trabajo, consideramos que reúne las condiciones para ser presentado y defendido como TESIS DOCTORAL por compendio de publicaciones.

Y para que conste a los efectos oportunos firmamos el presente certificado.

En Valencia, a 23 de enero de 2023

Fdo: Isabel Trapero

Fdo: Cristina Buigues

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A todos aquellos que me han animado y me han acompañado. Gracias.

En particular a ti Isabel, muchísimas gracias.

RESUMEN

Introducción: la Interleukina 6 (IL-6) se han convertido en uno de los principales focos de investigación en el estudio de las causas e interacciones que subyacen al desarrollo de multitud de síndromes y enfermedades así como de los procesos relacionados con el envejecimiento, todos ellos teniendo como característica común un mayor estado de inflamación. El ejercicio ha evidenciado ser una potente herramienta para la mejora de la salud de las personas tanto sanas como afectadas por determinadas enfermedades. **Objetivos:** el propósito de este compendio de artículos es estudiar las interrelaciones que se producen entre el ejercicio y la IL-6 en personas con un estado de inflamación alterado, profundizando en el análisis de las caraterísticas del ejercicio y sus consecuencias sobre loos niveles de IL-6. **Metodología:** Hemos realizado dos revisiones con dos poblaciones diferentes que se caracterizan por un estado alterado de inflamación sistémica y un experimento de campo en el que corroborar los datos extraidos de las revisiones y observar si los resultados de los tests de saliva se pueden extrapolar a los resultados obtenidos con los tests sanguíneos. **Resultados:** El ejercicio es capaz de modular los niveles de IL-6 en personas con un estado de inflamación alterado como es en la Esquizofrenia y en el deterioro que se produce en el proceso de envejecimiento. La variación de los parámetros del ejercicio utilizado para estimular a las personas provoca efectos diferentes en los niveles plasmáticos de IL-6 dando lugar a efectos agudos o crónicos que producen adaptaciones diferentes. Los resultados de los tests de saliva muestran una correlación significativa y positiva entre el número de criterios de fragilidad cumplidos con lo niveles de IL-6 ($r=0,26$; $p=0,025$, Spearman) y una correlación negativa entre los criterios específicos de actividad física ($r=-0,31$; $p=0,007$, Spearman). También se observan diferencias significativas entre los individuos robustos y frágiles en la concentración de IL-6 salival ($p< 0,043$, prueba de Kruskal-Wallis). **Conclusión:** El ejercicio es un potencial tratamiento para la prevención y desaceleración del desarrollo de ciertas enfermedades y síndromes caracterizados por un estado de inflamación alterado. Los tests de saliva es una potencial herramienta diagnóstica para el control de los niveles de IL-6 por su valor ecológico, por su accesibilidad económica y por su sencilla utilización.

ABSTRACT

Introduction: Interleukin-6 (IL-6) has become one of the main focuses of research in the study of the causes and interactions underlying the development of a multitude of syndromes and diseases as well as processes related to aging, all of them having as a common characteristic an increased state of inflammation. Exercise has proven to be a powerful tool for improving the health of both healthy people and those affected by certain diseases. **Objectives:** the purpose of this compendium of articles is to study the interrelationships that occur between exercise and IL-6 in people with an altered state of inflammation, deepening the analysis of the characteristics of exercise and its consequences on IL-6 levels. **Methodology:** We have carried out two reviews with two different populations characterized by an altered state of systemic inflammation and a field experiment to corroborate the data extracted from the reviews and to observe whether the results of the saliva tests can be extrapolated to the results obtained with the blood tests. **Results:** Exercise is able to modulate IL-6 levels in people with an altered inflammatory state such as in schizophrenia and in the deterioration that occurs in the aging process. The variation of the exercise parameters used to stimulate people causes different effects on the plasma levels of IL-6 giving rise to acute or chronic effects that produce different adaptations. The results of the saliva tests show a significant positive correlation between the number of frailty criteria met and IL-6 levels ($r=0.26$; $p=0.025$, Spearman) and a negative correlation between specific physical activity criteria ($r=-0.31$; $p=0.007$, Spearman). Significant differences are also observed between robust and frail individuals in salivary IL-6 concentration ($p<0.043$, Kruskal-Wallis test). **Conclusion:** Exercise is a potential treatment for the prevention and deceleration of the development of certain diseases and syndromes characterized by an altered inflammatory state. Saliva testing is a potential diagnostic tool for the control of IL-6 levels because of its ecological value, economic accessibility and ease of use.

RESUM

Introducció: la Interleukina 6 (IL-6) s'han convertit en un dels principals focus d'investigació en l'estudi de les causes i interaccions que subjauen al desenvolupament de multitud de síndromes i malalties així com dels processos relacionats amb l'envelliment, tots ells tenint com a característica comuna un major estat d'inflamació. L'exercici ha evidenciat ser una potent eina per a la millora de la salut de les persones tant sanes com afectades per determinades malalties. **Objectius:** el propòsit d'aquest compendi d'articles és estudiar les interrelacions que es produeixen entre l'exercici i la IL-6 en persones amb un estat d'inflamació alterat, aprofundint en l'anàlisi de les característiques de l'exercici i les seues conseqüències sobre loos nivells de IL-6. **Metodologia:** Hem realitzat dues revisions amb dues poblacions diferents que es caracteritzen per un estat alterat d'inflamació sistèmica i un experiment de camp en el qual corroborar les dades extretes de les revisions i observar si els resultats dels tests de saliva es poden extrapolar als resultats obtinguts amb els tests sanguinis. **Resultats:** L'exercici és capaç de modular els nivells de IL-6 en persones amb un estat d'inflamació alterat com és en l'Esquizofrènia i en la deterioració que es produeix en el procés d'envelliment. La variació dels paràmetres de l'exercici utilitzat per a estimular a les persones provoca efectes diferents en els nivells plasmàtics de IL-6 donant lloc a efectes aguts o crònics que produeixen adaptacions diferents. Els resultats dels tests de saliva mostren una correlació significativa i positiva entre el nombre de criteris de fragilitat complits amb l'anivelles de IL-6 ($r=0,26$; $p=0,025$, Spearman) i una correlació negativa entre els criteris específics d'activitat física ($r=-0,31$; $p=0,007$, Spearman). També s'observen diferències significatives entre els individus robustos i fràgils en la concentració de IL-6 salival ($p< 0,043$, prova de Kruskal-Wallis). **Conclusió:** L'exercici és un potencial tractament per a la prevenció i desacceleració del desenvolupament d'unes certes malalties i síndromes caracteritzades per un estat d'inflamació alterat. Els tests de saliva és una potencial eina diagnòstica per al control dels nivells de *IL-6 pel seu valor ecològic, per la seua accessibilitat econòmica i per la seua senzilla utilització.

ÍNDICE DE CONTENIDOS

ÍNDICE DE FIGURAS Y TABLAS

Figuras

Tablas

INTRODUCCIÓN

0. INTRODUCCIÓN

La tesis que vamos a exponer se presenta como compendio de tres artículos, dos de ellos son revisiones bibliográficas y el tercero es un trabajo de campo. Atendiendo a la normativa especificada por la Escola de Doctorat de la Universitat de València para este tipo de tesis doctoral, procederemos a presentar un resumen global de la temática enmarcado en el marco teórico del documento, de los principales resultados obtenidos y de las conclusiones.

Esta tesis profundiza en las interacciones existententes entre la IL-6 y el ejercicio físico, centrándose en los posibles efectos que el ejercicio tiene sobre los niveles de IL-6 y las consecuencias sobre la salud de las personas.

Actualmente el sistema inmune y, concretamente, la Interleukina 6 (IL-6) se han convertido en uno de los principales focos de investigación en el estudio de las causas e interacciones que subyacen al desarrollo de multitud de síndromes y enfermedades, así como de los procesos relacionados con el envejecimiento.

Estando interesados en estas investigaciones y siendo conocedores de la multitud de beneficios que la literatura científica otorga a la páctica de ejercicio físico tanto en población adulta como en poblaciones con desarrollo de diferentes enfermedades, quisimos profundizar en el estudio de las posibles interacciones y relaciones entre la IL-6 y el ejercicio físico con el objetivo de investigar si la práctica de ejercicio físico podría influir sobre los niveles sistémicos de IL-6 y, en consecuencia, sobre los procesos inflamatorios desadaptativos que se dan en ciertas poblaciones.

En un primer momento, teniendo en cuenta que nos surgió la posibilidad de llevar a cabo un proyecto en común con un centro de referencia especializado en la atención de personas con Esquizofrenia, y siendo conocedores de que uno de los mecanismos que podría subyacer al desarrollo de la Esquizofrenia son las complejas interacciones entre el sistema inmune y el sistema nervioso, realizamos una revisión para conocer los efectos que el ejercicio tiene tanto en los niveles de IL-6 como en el rendimiento cognitivo de esta población. Las evidencias actuales, apoyadas por los estudios genéticos, relacionan este desorden con un desequilibrio de las citokinas y, en consecuencia, una desregulación a la alta de los procesos inflamatorios, estando la IL-6 en el foco de muchas de estas investigaciones. También quisimos introducir la variable rendimiento cognitivo por la importancia que los déficits cognitivos tienen en esta enfermedad y porque existe evidencia de que niveles de IL-6 se relacionan con el rendimiento en diferentes funciones cognitivas.

Una vez realizada la revisión y dispuestos a ejecutar el estudio de campo, no se pudo llevar a cabo por las dificultades con el acceso a la muestra y principalmente con la recogida de medidas en

los diferentes momentos propuestos por el diseño del estudio. Así que decidimos realizar una nueva revisión en personas mayores ya que disponíamos de facilidades para acceder a una muestra de esta población. El envejecimiento está asociado a una considerable pérdida de las funciones motoras y a un progresivo descenso de las capacidades de aprendizaje, de memoria y de las funciones cognitivas en general. Actualmente se conoce que los valores basales de IL-6 en personas mayores sedentarias son más altos que en personas que realizan actividad física habitual. De este modo, en esta segunda revisión profundizamos en las relaciones que el ejercicio físico tiene sobre el sistema inmune, y sobre la IL-6 en particular, a través de los diferentes protocolos de ejercicio físico que se están utilizando y los efectos que provocan en personas mayores.

Finalmente, realizamos el estudio de campo en personas mayores a las que realizamos medidas tanto de los niveles de IL-6 como de los criterios de fagilidad de Fried. La fragilidad es un síndrome clínico relacionado con la edad en el que la inflamación tiene un papel fundamental. Existen numerosas investigaciones que, mediante el análisis de muestras sanguíneas, indican que el incremento de los niveles de ciertos marcadores inflamatorios como la IL-6 están asociados a la fragilidad. Teniendo en cuenta estos resultados/asociación, en este estudio de campo quisimos observar, mediante la recogida y análisis de muestras salivares, la relación entre los niveles de IL-6 y la fragilidad, y más concetamente la relación entre los niveles de IL-6 y el criterio de actividad física de Fried. Los tests de saliva ya se habían utilizado en otros síndromes inflamatorios pero no en personas con fragilidad y nos parecía interesante tanto la utilización de este tipo de tests por su valor ecológico y por no ser invasivo, como comprobar si los resultados eran similares a los encontrados en investigaciones que analizaban muestras sanguíneas.

MARCO TEÓRICO

1. MARCO TEÓRICO

1.1. IL-6

La interleukina-6 (IL-6) pertenece a la familia de las citokinas neuropoyéticas cuyos miembros tienen un peso molecular de 21 kDa en su forma no glicosilada [1]. El gen IL6 contiene cuatro intrones y cinco exones y está ubicado en el cromosoma 7p21 en el genoma humano. La citoquina es sintetizada por una variedad de células que incluyen monocitos, fibroblastos, células endoteliales y varios tipos de células del sistema nervioso central (SNC), y su concentración es modulada por numerosos factores que incluyen agentes proinflamatorios, patógenos virales y bacterianos, neurotransmisores, y segundos mensajeros [2]. La regulación compleja de la expresión del gen IL-6 es proporcionada por diferentes sitios de unión para factores de transcripción, incluidos los del factor nuclear KB (NF-KB), el factor nuclear-IL-6 (NF-IL-6) y la proteína activadora i, y dos elementos sensibles a glucocorticoides (GRE 1 y GRE2) [3]. Bajo condiciones fisiológicas, los niveles de IL-6 en el SNC son bajos, mientras que sus niveles aumentan durante la lesión cerebral, la inflamación y la enfermedad. Otra causa que conduce a un aumento en la concentración de IL-6 en el cerebro es la entrada neta desde la sangre al parénquima cerebral que conduce a un eventual aumento en la producción central de citoquinas inflamatorias [4].

La IL-6, además, está implicada en la regulación del metabolismo energético [5]. Los niveles circulantes elevados de IL-6 influyen tanto en la homeostasis de la glucosa [6] como en el metabolismo de los lípidos [7]. Si bien los efectos de la IL-6 en la homeostasis de la glucosa son contradictorios [8], existe un mayor consenso con respecto a su papel en la regulación del metabolismo de los lípidos. La infusión de IL-6 en individuos sanos estimula la lipólisis y la oxidación de ácidos grasos [9]. Los estudios in vitro de adipocitos y miotubos han confirmado los efectos de la IL-6 sobre la lipólisis y la oxidación de ácidos grasos, y la evidencia muestra que la IL-6 induce estos efectos al aumentar la proteína quinasa activada por AMP (AMPK) [10]. Estudios en roedores apoyan un efecto regulador de la IL-6 sobre la masa de tejido adiposo. Los ratones knockout para IL-6 de cuerpo entero desarrollan obesidad de inicio maduro y un aumento general de la masa grasa, un fenotipo parcialmente revertido por la inyección de IL-6 [5].

Se ha demostrado que los niveles de citokinas varían a lo largo de la vida así como el balance entre citokinas antiinflamatorias y proinflamatorias [11], sugiriendo que la elevada producción de citokinas inflamatorias y el descenso de los niveles de citokinas antiinflamatorias incrementan la respuesta inflamatoria central perjudicando la plasticidad sináptica [12] y, en consecuencia, empeorando el rendimiento cognitivo que se asocia al envejecimiento y a determinadas enfermedades. De esta forma, ya se asocian algunos biomarcadores inflamatorios como predictores del declive cognitivo [11].

En el adecuado balance entre citokinas antiinflamatorias y proinflamatorias, parece que tiene un papel relevante la IL-6, citokina que da lugar a respuestas tanto proinflamatorias como antiinflamatorias en función de sus niveles [13], y que es capaz de atravesar la barrera sanguínea cerebral y modular los procesos inflamatorios centrales dando lugar, si los niveles son elevados, a un incremento de la neurodegeneracion e influenciando negativamente en las funciones cognitivas [14].

Actualmente son numerosos los estudios en los que existe evidencia a cerca del protagonismo que tiene la IL-6 en los procesos inflamatorios a través de los cuales el sistema inmune interrelaciona con el sistema nervios central [5].Se ha observado que los niveles de IL-6 incrementan con la edad y que unos niveles altos se asocian con el declive cognitivo que se produce en la vejez [15-17]. Se ha observado/analizado/contemplado que la IL-6 correlaciona de forma inversa con el volumen de sustancia gris del hipocampo [18] y con el nivel de integridad de la sustancia blanca del cerebro [14]. Estas interacciones de la IL-6 con el SNC sería uno de los mecanismos mediante el cual el sistema inmune influye tanto en el rendimiento de las funciones cognitivas como en la neurodegeneración y funcionalidad cognitiva.

Existen evidencias de que altos niveles de IL-6 periférico afectan negativamente a la memoria y al aprendizaje [18], asociándose a un pobre rendimiento cognitivo en general [19] y que el rendimiento en tareas cognitivas desciende en procesos agudos inflamatorios en los que se aumentan los niveles de IL-6 [20]. Por otro lado, se ha constatado que mediadores inflamatorios periféricos como la IL-6 son capaces de atravesar la barrera sanguínea cerebral y modular los procesos inflamatorios centrales dando lugar a un incremento de la neurodegeneración e influenciando negativamente en las funciones cognitivas [14]. Concretamente se ha observado que mayores niveles basales de la citokina IL-6 aumentan el riesgo de futuro descenso cognitivo relacionado con la edad [21,22].

También se ha observado un incremento de IL-6 en enfermedades caracterizadas por una disminución cognitiva o demencia así como en desórdenes depresivos [23], de forma que se considera que tener unos bajos niveles de IL-6 podría reducir el riesgo de desarrollar una enfermedad

neurodegenerativa [24]. Las últimas revisiones indican que los altos niveles de inflamación y de IL-6 se relacionan con los síntomas negativos de diferentes enfermedades como la Esquizofrenia [25].

Respecto a los efectos que los niveles de IL-6 provocan, a pesar de que la mayoría de estudios hablan de los efectos negativos que tiene su incremento [23] y de sus propiedades proinflamatorias, varios estudios sugieren que el aumento moderado de los niveles provocados por el ejercicio podría dar lugar a una respuesta antiinflamatoria inhibiendo la producción de otras citokinas proinflamatorias como la IL-1b y TNFa, reduciendo los niveles de inflamación y desarrollando un efecto neuroprotector [26].

1.2. EJERCICIO FÍSICO

Teniendo en cuenta las evidencias de la literatura científica, está ampliamente corroborado que el ejercicio tiene numerosos efectos beneficiosos sobre el sistema nervioso central, de forma que ejerce/realiza/desempeña tanto un efecto neuroprotector retrasando y decelerando el descenso del rendimiento cognitivo que se produce por la edad y en determinadas enfermedades así como mejorando y optimizando diferentes funciones cognitivas como el aprendizaje, la memoria o las funciones ejecutivas [27].

Siguen habiendo muchas cuestiones sin resolver sobre los mecanismos concretos a través de los cuales el ejercicio da lugar a estos efectos beneficios sobre el sistema cognitivo [25], pero existe cada vez mayor evidencia acerca de que el sistema inmune participa de forma predominante. Firth et al, en 2017 [28], concluyeron que los beneficios que el ejercicio tiene sobre la Esquizofrenia se deben a cambios estructurales cerebrales mediados por el sistema inmune. Para que estos efectos beneficiosos que provoca el sistema inmune se produzcan es fundamental un buen balance entre citokinas proinflamatorias y antiinflamatorias consiguiendo modular y consolidar la reorganización de redes neuronales [29]. Y en este adecuado balance entre citokinas antiinflamatorias y proinflamatorias, tiene un papel relevante la IL-6 [13].

Ya son numerosos los estudios que han revelado un efecto antiinflamatorio consecuente al ejercicio que potencia efectos positivos sobre la neuroplasticidad [12]. En consecuencia, empieza a considerarse al ejercicio como un desacelerador de la edad biológica. El ejercicio físico, como estímulo estresor, induce diferentes respuestas inmunes asociadas a la producción de interleukinas elicitando una respuesta antiinflamatoria a través de la inflamación. También existe evidencia que demuestra que el ejercicio físico está asociado con una reducción sistémica de la inflamación [24]. El buen balance entre citokinas antiinflamatorias y proinflamatorias podría ser uno de los mecanismos

a través del cual el ejercicio ejercería sus efectos inmunoprotectores e inmunoregulatorios. Los nuevos estudios genéticos sugieren que estos efectos del ejercicio estarían relacionados con una regulación a la alta de los genes involucrados en la producción de leucocitos y en la regulación a la baja de la inflamación [30].

Estudiar el tipo de ejercicio realizado y profundizar en las variables de dicho ejercicio puede ayudarnos a descubrir biomarcadores y moléculas terapéuticas que podrían ser la base de numerosos trastornos relacionados con la inactividad física. Sin embargo, es difícil diseccionar los mecanismos subyacentes a los cambios inducidos por el ejercicio, ya que el ejercicio es un proceso muy complejo que involucra simultáneamente respuestas integradoras y adaptativas en múltiples tejidos y órganos a nivel celular y sistémico.

Se han realizado muchos estudios utilizando diferentes tipos de ejercicios y diferentes métodos en sus protocolos de actuación. Algunos estudios utilizan protocolos de ejercicio aeróbico que están diseñados para mejorar la resistencia cardiorrespiratoria al aumentar el esfuerzo metabólico, lo que requiere un esfuerzo de respiración, frecuencia cardíaca y flujo sanguíneo para igualar la intensidad del esfuerzo. Estos protocolos de ejercicio aeróbico se desarrollan a través de una actividad que involucra la integración de grandes grupos musculares, como en la propulsión rítmica de la masa corporal durante movimientos de diferentes intensidades (por ejemplo, caminar, trotar o correr) o actividades de menor impacto mecánico (p. ej., natación o ciclismo) [31]. Por otro lado, hay otros estudios que utilizan protocolos de Entrenamiento de resistencia, que se realiza para mejorar la fuerza muscular y la resistencia muscular y se refiere a un método especializado de acondicionamiento muscular que normalmente requiere una variedad de equipos, como pesas libres, máquinas de pesas, elásticos. bandas y/o ejercicios de peso corporal. El ejercicio de resistencia se diferencia del ejercicio aeróbico en que se centra en sobrecargar repetidamente los músculos durante las contracciones estáticas, isométricas o dinámicas, lo que provoca una conexión más estrecha y fuerte entre la musculatura y el sistema nervioso [32].

Pese al gran esfuerzo de las últimas décadas por dilucidar los mecanismos celulares y moleculares del ejercicio agudo y crónico, la mayor parte de la biología del ejercicio sigue siendo poco conocida.

Actualmente comienzan a aparecer estudios que indican que parametros del ejercicio como la intensidad y duración son importantes para que la regulación de citokinas sea la adecuada para provocar una respuesta antiinflamatoria que origine el efecto protector, tanto en personas sanas como en personas con diferentes patologías [30].La intensidad y la duración del ejercicio están asociados a diferentes vías de utilización de la energía para realizar el ejercicio propuesto. Si el ejercicio se realiza a una intensidad baja o moderada, la glucosa derivada del hígado o de la ingestión oral, y ácidos

grasos libres de tejido adiposo proporciona principalmente el combustible necesario para el músculo esquelético. Si la intensidad del ejercicio es aumentada, la contribución de los ácidos grasos libres de tejido adiposo circulantes es modestamente disminuido mientras que el uso de la glucosa circulante es ampliamente regulado al alza. Si el ejercicio es continuado durante más de una hora a una intensidad fija, el uso de la energía de la oxidación de lípidos se inclina. De esta forma, la variación en la intensidad y duración del ejercicio, pueden afectar a los niveles de glucosa intramuscular y sanguínea [26].

A pesar de los estudios realizados con diferentes protocolos tanto aeróbicos como de entrenamiento de resistencia y de la profunda investigación entorno a los parámetros del ejercicio de intensidad y duración, las respuestas siguen siendo complejas. Anatómicamente, el músculo esquelético es el órgano más grande que constituye alrededor del 40% de la masa corporal total y, por lo tanto, desempeña un papel importante en la regulación del metabolismo. Junto con los efectos locales del músculo esquelético sobre el metabolismo, recientemente se ha descubierto que, al igual que los adipocitos, el músculo esquelético es un órgano secretor responsable de la producción de varios cientos de péptidos clasificados como 'miokinas' [33].

El descubrimiento de las miokinas ha abierto una nueva puerta para entender la biología del ejercicio, aportando evidencia de que los músculos son capaces de comunicarse con otros órganos, como el hueso, el hígado, el tejido adiposo, el cerebro, etc. [26].

1.3. FRAGILIDAD

El envejecimiento, además de estar asociado a una considerable pérdida de las funciones motoras, también cursa con un progresivo descenso de las capacidades de aprendizaje, de memoria y de las funciones cognitivas en general [34,35]. Existen numerosos modelos que tratan de analizar todos los factores que llevan a este declive congnitivo y motor mostrando la importancia tanto de variables extrínsecas (estres psicológico, polución, nivel de sedentarismo, etc.) como de variables intrínsecas (genética, neurotrasmisores, etc.) [36], dando explicación a la neurodegeneración y al declive de las funciones cognitivas que se produce en la vejez [37]. Uno de los factores que actualmente más se están estudiando es la interacción entre el sistema inmune y los procesos inflamatorios con el desarrollo de la vejez, generalmente conocido como "inflamm-aging" [38]. Relacionado con este proceso de inflamación y deterioro, actualmente se están realizando numerosas investigaciones sobre el síndrome de fragilidad [39-41].

La fragilidad es un síndrome clínico crucial relacionado con la edad que se define comúnmente como un estado de mayor vulnerabilidad a los factores estresantes externos y se produce como consecuencia del deterioro acumulativo de muchos sistemas fisiológicos a lo largo de la vida [42]. Este síndrome describe el deterioro físico y funcional que ocurre como consecuencia de ciertas enfermedades (p. ej., cáncer, infección crónica, etc.) pero que también puede ocurrir en ausencia de enfermedad [43]. En los últimos años, la fragilidad ha sido reconocida como una prioridad de salud pública emergente y la necesidad de un reconocimiento e intervención tempranos es apremiante [44]. Esto se debe a que es ampliamente aceptado como un tema crítico para el envejecimiento de la población en todo el mundo, ya que es relevante para múltiples resultados adversos, como la hospitalización, la institucionalización y la mortalidad prematura [45]. Aún no se ha alcanzado una definición de fragilidad aceptada internacionalmente a pesar de la importancia de este síndrome clínico. La definición más utilizada considera la fragilidad como un fenotipo que comprende cinco indicadores (Fried, 2001) [46]:

1) Pérdida de peso. La pérdida de peso se definió como la pérdida no intencional de 4,5 kg o más en el último año.

2) Agotamiento autoinformado. Se indicó agotamiento si los sujetos respondían positivamente a alguna de las afirmaciones de la Escala de Depresión del Centro de Estudios Epidemiológicos [47]: "Sentí que todo lo que hacía me costaba mucho esfuerzo al menos tres o cuatro días a la semana" y "Sentí que no podía seguir haciendo cosas por lo menos tres o cuatro días a la semana", al referirse a la semana anterior, o si el sujeto respondía "A menudo" o "La mayor parte del tiempo" a la pregunta "¿Con qué frecuencia en la última semana ¿Sientes que todo lo que hiciste fue un esfuerzo?", que también está incluida en la escala de depresión del Centro de Estudios Epidemiológicos [48]. Se estimó que todos los criterios de agotamiento estaban presentes.

3) Actividad física (AF). La baja AF se cuantificó mediante la adaptación española del Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ) readaptado para mujeres [49,50]. El MLTPAQ fue administrado por un entrevistador capacitado a quien se le proporcionaron instrucciones precisas y una lista detallada de PA. Los participantes recibieron una lista de actividades y se les pidió que marcaran las que habían realizado durante el último año. Para evitar en lo posible el sesgo de memoria, se recopilaron primero las actividades realizadas en la última semana, seguidas de las realizadas en el último mes, el último trimestre y finalmente el último año, incluyendo siempre los primeros periodos. Para fines de validación se utilizó únicamente la información referente al último año. A partir de este cuestionario se calculó el gasto total de energía en PA en el tiempo libre (EEPA) y se utilizó para cuantificar la PA [51].

4) Rendimiento motor lento. La lentitud se evaluó mediante la prueba de velocidad de marcha de 4-6 m, ajustada por sexo y altura según los estándares de la Batería Breve de Rendimiento Físico [52]. Se estimó que una velocidad de marcha baja corresponde al peor quintil para sexo y talla del grupo.

5) Debilidad. Para determinar la debilidad, se midió la fuerza con un dinamómetro hidráulico Jaymar, según los estándares de las Poblaciones Hispanas Establecidas para los Estudios Epidemiológicos del Adulto Mayor [53].

La fragilidad se diagnostica clínicamente por la presencia de tres o más de estos indicadores y está precedida por una etapa prodrómica conocida como prefragilidad, definida por tener uno o dos indicadores [46].

Los cambios fisiopatológicos que subyacen y preceden a la fragilidad no se conocen claramente. La etiología de la fragilidad se ha asociado con cambios en varios sistemas fisiológicos, incluidos los sistemas de inflamación, coagulación, hematológico y endocrino [54]. La inflamación es uno de esos posibles cambios fisiopatológicos que puede estar estrechamente relacionado con la fragilidad [55]. Las citoquinas proinflamatorias pueden influir en la fragilidad ya sea directamente, al promover la degradación de proteínas, o indirectamente al afectar importantes vías metabólicas [56]. Los investigadores han informado que los niveles elevados de marcadores inflamatorios, como las interleucinas-6 (IL-6), el factor de necrosis tumoral (TNF-) y la proteína C reactiva (PCR) están asociados con con una fuerza muscular reducida, mala actividad física, funcional, fragilidad y mortalidad [57-59]. Está bien establecido que la IL-6 es un biomarcador potencial de fragilidad, que podría evaluarse como un objetivo para la intervención [60], y se considera un marcador predictivo de incidentes de fragilidad [61].

Por su fácil aplicación en la práctica clínica y su valor pronóstico en las decisiones terapéuticas de los médicos especialistas, la identificación precoz de un estado de fragilidad es fundamental en los adultos mayores. Los biomarcadores pueden ayudar a identificar a los pacientes que corren el riesgo de volverse frágiles [62]. Los biomarcadores proporcionan índices objetivos y medibles para estados normales y/o patogénicos, así como respuestas a intervenciones terapéuticas [63]. Son útiles para detectar cambios subclínicos que conducen a indicadores observables de fragilidad y probar protocolos de intervención para la fragilidad, ya que reflejan cambios en los procesos fisiológicos que subyacen y/o se relacionan con la fragilidad [64].

Para comprender mejor la etiología, el curso temporal y las consecuencias de los procesos inflamatorios y desarrollar mejores intervenciones, se necesitan nuevos métodos para evaluar la inflamación que se puedan realizar en contextos ecológicos, a lo largo del tiempo y en múltiples momentos a lo largo del día. [65]. La sangre ha sido el fluido biológico habitual en el que se miden

muchos analitos con fines clínicos y de investigación. Esto es razonable, porque la sangre es un fluido sistémico que llega a todas las partes del cuerpo y puede influir en el comportamiento biológico de los tejidos o, por otro lado, recibir y transportar componentes derivados de los tejidos que reflejan lo que está sucediendo en los tejidos corporales [66]. Aunque la detección de biomarcadores en suero y sangre es una práctica estándar, es deseable el uso de muestras no invasivas. Al estudiar otros métodos, algunos autores informaron que la saliva es una herramienta de diagnóstico potencial debido a su facilidad de recolección y no invasividad [67]. La saliva humana contiene proteínas, péptidos, hormonas y enzimas, cada uno de los cuales puede usarse fácilmente para evaluar diferentes enfermedades [68]. Se han identificado aproximadamente 1400-2000 proteínas en la saliva [69]. El número de proteínas en la saliva muestra su diversidad, y cada una de estas proteínas se puede utilizar como una herramienta sencilla para evaluar la toxicidad, la infección y los niveles inmunológicos y hormonales. La saliva humana representa imágenes de todo el cuerpo y también se conoce como el "espejo del cuerpo". Un biomarcador es una nanoproteína medible que se utiliza para predecir un estado biológico [70]. Algunos estudios han analizado los niveles de IL-6 salival en pacientes no solo con patologías orales comunes [71], sino también en enfermedades sistémicas como el síndrome de ovario poliquístico [72], síndrome de dolor vesical, trastornos del sueño [73] y enfermedades inflamatorias, pero se desconoce la diferencia entre los niveles salivales de IL-6 en pacientes con síndrome de fragilidad.

Existe consenso sobre el papel de los niveles de IL-6 en sangre y su correlación con la vejez, con determinadas enfermedades degenerativas y con el síndrome de fragilidad en personas mayores. También existe consenso sobre los numerosos efectos beneficiosos que el ejercicio tiene sobre el sistema nervioso central y el sistema inmune, y más concretamente sobre la IL-6. En la presente tesis, se ha tratado de profundizar en las relaciones entre la IL-6, el ejercicio y los procesos inflamatorios que se producen tanto en el curso del envejecimiento como en enfermedades degenerativas. Propondremos estrategias de ejercicio concretas para afianzar los beneficios que éste tiene sobre los niveles de IL-6 en función de los resultados obtenidos en las dos primeras revisiones y veremos la utilidad del test de saliva como herramienta diagnóstica del síndrome de Fragilidad además de la relación de éste con los niveles de IL-6 y con el ejercicio.

OBJETIVOS

El objetivo general planteado en la presente tesis fue estudiar las interrelaciones existentes entre el ejercicio físico y el sistema inmune y, más concretamente, entre el ejercicio físico y la IL-6 para ver de qué forma el ejercicio físico puede modular los niveles de esta interleukina, alterada en determinadas circunstancias de desregulación del sistema inmunitario.

Tratamos de dar respuesta a este objetivo general a través de los siguientes objetivos específicos estudiados en los tres artículos publicados que comprenden la tesis:

- Conocer los efectos agudos y crónicos que el ejercicio físico tiene sobre la IL-6 y profundizar en las consecuencias que sobre esta última provoca la variación de dicho ejercicio a través de sus parámetros de carga (intensidad y volumen).

- Revisar las implicaciones del sistema inmune y la IL-6 en el desarrollo y curso de determinadas enfermedades o síndromes, así como en poblaciones especiales.

- Conocer las consecuencias de la realización de ejercicio físico en poblaciones que han desarrollado alguna enfermedad o síndrome en el que el sistema inmune y la IL-6 están implicados.

- Analizar la viabilidad del test de saliva para conocer los niveles de IL-6 y comparar sus resultados con los tests sanguíneos.

RESULTADOS

A continuación presentamos los resultados acerca de las relaciones entre la IL-6, el ejercicio y los procesos inflamatorios producidas en el envejecimiento y en las enfermedades degenerativas.

2. RESULTADOS

2.1. Resultados del primer artículo: The effects of exercise on IL-6 levels and cognitive performance in patiens with schizophrenia.

En este artículo se realizaron búsquedas en las bases de datos electrónicas CINAHL, Scopus, Web of Science, Embase, Cochrane Library y PubMed sin límite temporal inicial hasta noviembre de 2018 con los siguientes términos de búsqueda de palabras clave: "esquizofrenia" y "ejercicio" o "actividad física" y “IL-6” y “cognición”. Esta primera búsqueda no nos mostró nigún resultado, así que llevamos a cabo a continuación dos búsquedas independientes. Una primera búsqueda introduciendo las palabras claves "esquizofrenia" y "ejercicio" o "actividad física" y “IL-6”, y una segunda búsquda con las palabras claves "esquizofrenia" y "ejercicio" o "actividad física" y “cognición”. Finalmente, y debido a los pocos resultados obtenidos, realizamos otras dos búsquedas cuyas palabras clave fueron, por un lado "esquizofrenia" y "ejercicio" o "actividad física", y por otro lado "esquizofrenia" y "IL-6". También se revisaron Google Scholar y las listas de referencias de los artículos recuperados utilizando la estrategia de búsqueda descrita anteriormente para identificar cualquier publicación adicional potencialmente relevante.

Esquizofrenia y ejercicio

Ejercicio en relación con la cognición

Ya hay mucha evidencia que sugiere que el ejercicio afecta la plasticidad cerebral al inducir la neurogénesis y causar cambios estructurales [27]. El ejercicio juega un papel importante en la alteración metabólica, estructural y funcional de las conexiones cerebrales, y es un factor importante en la plasticidad cerebral que puede ser protector contra el deterioro cognitivo resultante del envejecimiento [12]. Estos efectos se reflejan en el funcionamiento cognitivo a nivel cerebral, tanto en la mejora de diferentes funciones cognitivas como en la prevención del deterioro cognitivo que se produce a causa del envejecimiento (Figura 1).

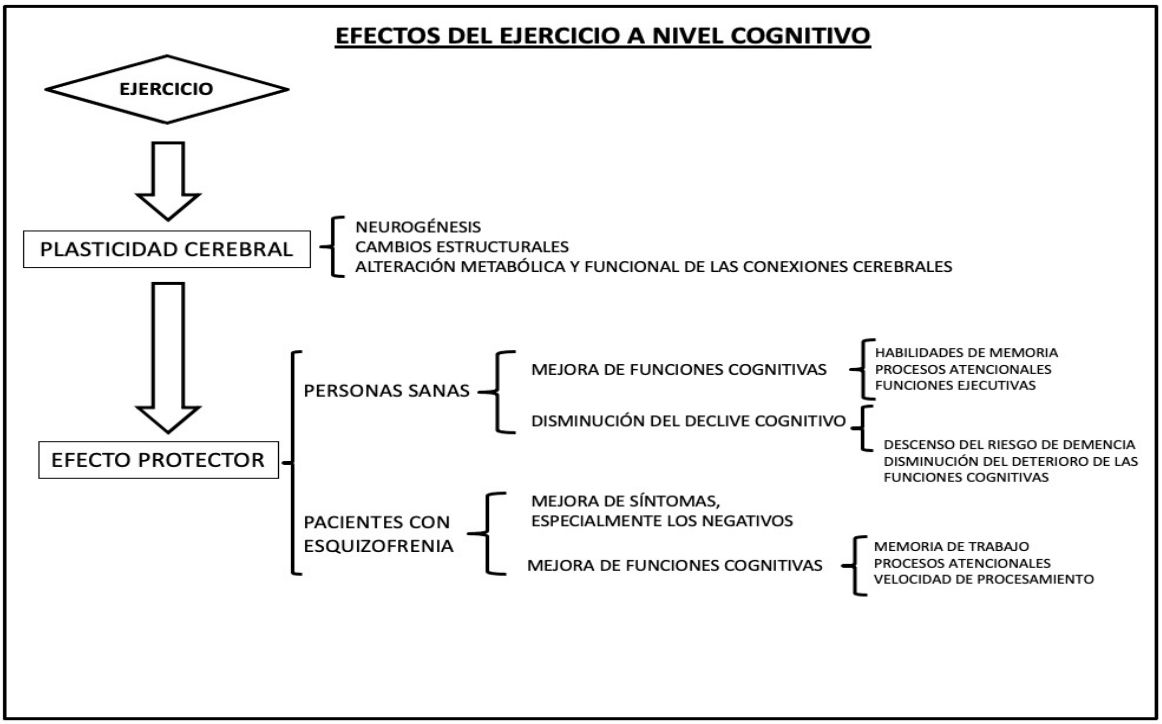

Figura 1. Efectos del ejercicio a nivel cognitivo

El ejercicio tiene un papel potencial en la plasticidad cerebral que conduce a un efecto neuroprotector en personas sanas y en pacientes con esquizofrenia. Este efecto neuroprotector tiene consecuencias positivas en la mejora de las funciones cognitivas, en el retraso del deterioro cognitivo y en la mejora de la sintomatología de la esquizofrenia.

Se han observado mejoras en las funciones cognitivas principalmente en términos de memoria (tareas de memoria implícita, memoria inmediata y reconocimiento), procesos de atención y en funciones ejecutivas [24]. También se ha demostrado un mejor rendimiento en tareas verbales, perceptivas y aritméticas en niños que realizan ejercicio regularmente en comparación con niños sedentarios [74].

Como ya se mencionó, numerosos estudios han demostrado que el ejercicio previene el deterioro cognitivo relacionado con la edad [75], reduce el riesgo de desarrollar demencia y ralentiza la disminución del deterioro de la función ejecutiva [76]. El efecto neuroprotector inducido por el ejercicio también se ha observado en personas con demencia y discapacidad cognitiva [77], reduciendo sus síntomas y mejorando las funciones cognitivas tanto en jóvenes como en adultos [78].

Esquizofrenia y ejercicio

En relación con la esquizofrenia, los estudios actuales indican que el ejercicio puede reducir los síntomas psiquiátricos y mejorar las funciones cognitivas en diferentes subdominios, como se informa en la guía de la Asociación Europea de Psiquiatría (EPA) [79]. Los beneficios del ejercicio para tratar este trastorno están relacionados con sus efectos a nivel del cerebro, y el efecto protector producido en el hipocampo por el ejercicio es similar tanto en pacientes con esquizofrenia como en adultos sanos [80] (Figura 1). Varios estudios diferentes han observado que estos cambios causados por el ejercicio a nivel del cerebro mejoran tanto los síntomas positivos como negativos de la esquizofrenia. La reducción en la incidencia de síntomas negativos es especialmente relevante porque la medicación estándar es menos efectiva para tratarlos [78].

Además, hay evidencia de que el ejercicio también mejora el rendimiento en tareas que involucran memoria de trabajo, velocidad de procesamiento y procesos atencionales [81]. Específicamente, la realización de actividad física ligera se correlacionó positivamente con el rendimiento cognitivo, mientras que la actividad física moderada y vigorosa se asoció con una mejoría en los síntomas de la esquizofrenia [82]. El mecanismo o mecanismos exactos que subyacen a esta mejora cognitiva mediada por el ejercicio son actualmente desconocidos, pero varios estudios han indicado que el sistema inmunológico como es probable que esté involucrado.

Esquizofrenia, ejercicio y sistema inmunológico

El sistema inmune en relación con la cognición

Varios estudios han encontrado que el sistema inmune está involucrado en la modulación homeostática de la neurogénesis [83],observando que, entre otros mecanismos, la estimulación de citoquinas de nivel periférico altera varios neurotransmisores clave que optimizan la neurogénesis [84].. El efecto positivo que tiene el sistema inmune en este contexto está condicionado por complejas interacciones entre las células cerebrales que realizan funciones inmunes y las neuronas a través de neurotransmisores y citoquinas pro y antiinflamatorias [29].

Por lo tanto, estas mejoras resultaron en ganancias en los procesos de aprendizaje y memoria [85] y produjeron una asociación inversa entre los procesos inflamatorios y el rendimiento en el razonamiento espacial, la memoria a corto plazo, el aprendizaje verbal y las pruebas de función ejecutiva [14]. Muchos estudios señalan que un equilibrio adecuado entre las citoquinas proinflamatorias y antiinflamatorias subyace a estas mejoras en los procesos cognitivos al modular y consolidar la reorganización de las redes neuronales [29].

Sin embargo, también se ha demostrado que los niveles de citoquinas y el equilibrio entre las citoquinas proinflamatorias y antiinflamatorias varían a lo largo de la vida de un individuo [11], *lo* que sugiere que la producción de altas cantidades de citoquinas inflamatorias y una disminución en los niveles antiinflamatorios, aumenta la respuesta inflamatoria central, dañando la plasticidad sináptica [12] y, en consecuencia, también aumentar el empeoramiento del rendimiento cognitivo asociado a la edad. Por lo tanto, ciertos biomarcadores inflamatorios ya se utilizan para predecir el deterioro cognitivo [11].

El sistema inmunológico y la esquizofrenia

La inflamación se considera actualmente un mecanismo potencial en el desarrollo y progresión de la esquizofrenia [25]. Los pacientes con esquizofrenia tienen niveles aumentados de marcadores de citoquinas inflamatorias y, más específicamente, este aumento puede estar relacionado con los síntomas negativos de la enfermedad [86]. Por lo tanto, un equilibrio inadecuado entre las citoquinas proinflamatorias y antiinflamatorias causaría disfunción del sistema inmunológico, deteriorando el neurodesarrollo y desregulando la neurotransmisión cerebral en pacientes con esquizofrenia [87].

El sistema inmunológico y el ejercicio

Mantener un buen equilibrio entre las citoquinas proinflamatorias y antiinflamatorias podría ser uno de los mecanismos a través de los cuales el ejercicio ejerce sus efectos inmunoprotectores e inmunorreguladores [88]. El ejercicio, como factor estresante, induce diferentes respuestas inmunes asociadas con la producción de interleucinas, provocando así una respuesta antiinflamatoria a través de la inflamación [89]. Existe amplia evidencia de que el ejercicio se asocia con una reducción sistémica de la inflamación [24].

Además, nuevos estudios genéticos sugieren que el ejercicio puede activar genes involucrados en la producción de leucocitos, así como aquellos que regulan a la baja la inflamación. Aunque este mecanismo no se entiende del todo, comienzan a aparecer estudios que indican que parámetros del ejercicio como la intensidad y la duración son factores importantes en la regulación suficiente de las citoquinas para inducir esta respuesta antiinflamatoria protectora tanto en individuos sanos como en aquellos con diferentes patologías [30]. Sin embargo, la interacción de múltiples factores en el sistema inmune hace que queden dudas sobre los mecanismos exactos que lo interrelacionan con la esquizofrenia y el ejercicio, aunque recientemente han surgido trabajos que destacan un papel importante para la IL-6 en este contexto.

Esquizofrenia, ejercicio e IL-6

IL-6 y la cognición

Numerosos estudios han publicado evidencia sobre el papel de la IL-6 en los procesos inflamatorios que interconectan el sistema inmune con el sistema nervioso central (SNC). Los niveles de IL-6 se correlacionan inversamente con el volumen de materia gris del hipocampo [18], así como con la integridad de la materia blanca en el cerebro [14]. Uno de los mecanismos por los cuales el sistema inmune influye en el rendimiento de la función cognitiva, la neurodegeneración y la funcionalidad cognitiva, involucra las interacciones entre la IL-6 y el SNC (Figura 2).

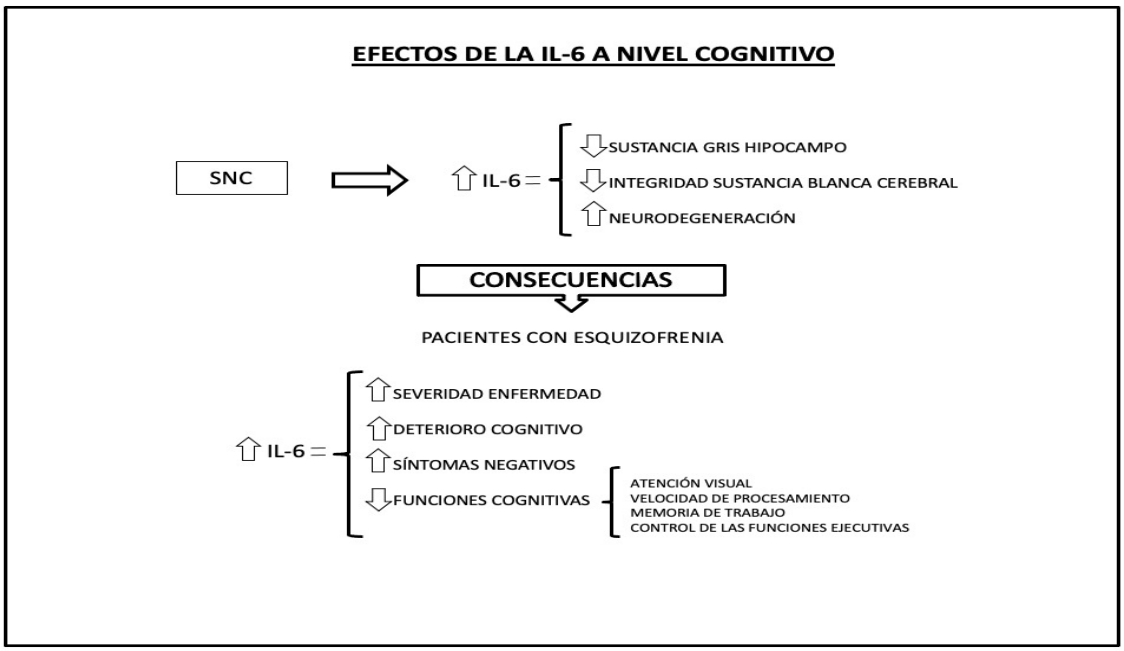

Figura 2. Efectos de la IL-6 a nivel cognitivo.

La IL-6 tiene numerosos efectos en el sistema nervioso central (SNC). Estos efectos tienen diferentes consecuencias en los pacientes con esquizofrenia. Con respecto a la función cognitiva, existe evidencia de que los altos niveles de IL-6 periférica afectan negativamente la memoria y el aprendizaje [18], se asocia con un rendimiento cognitivo generalmente deficiente [19], y que el rendimiento cognitivo-tarea es menor en los procesos inflamatorios agudos que implican mayores niveles de IL-6 [20]. También es importante que los mediadores inflamatorios periféricos como la IL-6 puedan cruzar la barrera hematoencefálica y modular los procesos inflamatorios centrales, lo que lleva a un aumento de la neurodegeneración e influye negativamente en la función cognitiva [14]. Específicamente, la presencia de altos niveles basales de IL-6 aumenta el riesgo de deterioro cognitivo

futuro relacionado con la edad [21,22]. El aumento de IL-6 también se ha observado en la demencia y otras enfermedades caracterizadas por el deterioro cognitivo, así como en los trastornos depresivos [23]. Por lo tanto, la presencia de niveles bajos de IL-6 puede reducir el riesgo de desarrollar enfermedades neurodegenerativas [24].

Sin embargo, a pesar de que la mayoría de los estudios discuten los efectos negativos de los niveles elevados de IL-6, hay evidencia de que los niveles moderados de IL-6 también pueden tener un efecto antiinflamatorio y protector [13].

IL-6 y esquizofrenia

Hay mucha evidencia en la literatura con respecto a la desregulación del sistema inmunológico y la presencia de altos niveles de citoquinas proinflamatorias como la IL-6 en pacientes con esquizofrenia [90]. Además, los altos niveles de IL-6 se asocian con la morfología cerebral alterada y la gravedad de la enfermedad [22], así como con el deterioro cognitivo continuo [91] y la disminución de la atención visual, la velocidad de procesamiento, la memoria de trabajo y la función de control ejecutivo [92] observada a medida que progresa la esquizofrenia (Figura 2). Mirando más específicamente los diferentes síntomas de la esquizofrenia, parece que los altos niveles de inflamación e IL-6 están asociados con los síntomas negativos de la enfermedad [25].

Il-6 y ejercicio

El ejercicio induce un aumento en los niveles plasmáticos de diferentes citoquinas, incluyendo IL-6 [93]. En el año 2000, Steenberg et al. publicaron el primer estudio que muestra que la actividad músculo-esquelética relacionada con el ejercicio aumenta los niveles plasmáticos de IL-6 [94]. Los siguientes estudios mostraron que la magnitud de esta elevación plasmática de IL-6 derivada de la fibra muscular causada por el ejercicio se correlaciona con diferentes parámetros como la intensidad y la duración de la actividad física [26].

Además del efecto agudo que el ejercicio tiene sobre el aumento de los niveles plasmáticos de IL-6, diferentes estudios también han demostrado que con el tiempo, después de un protocol de ejercicio prolongado, hace que los niveles basales de IL-6 disminuyan [90]. Esta reducción también está relacionada con la intensidad y la duración del ejercicio físico, así como con otras variables como los niveles de condición física de los participantes del estudio [95]. Las personas que realizan ejercicio

regular en su vida diaria muestran niveles basales más bajos de IL-6 en comparación con aquellos que son sedentarios [94].

Aunque, como ya hemos mencionado, la mayoría de los estudios asocian la IL-6 con respuestas proinflamatorias, numerosos estudios también han observado que el aumento agudo de los niveles de IL-6 causado por el ejercicio da lugar a una respuesta antiinflamatoria al inhibir, por ejemplo, la activación de TNFα (factor de necrosis tumoral alfa) cuya actividad es altamente proinflamatoria [13] y estimulando la producción de otras moléculas predominantemente antiinflamatorias del sistema inmune [96].

2.2. Resultados del segundo artículo: The beneficial effect of physical exercise on inflamatory markers in older individuals.

Para el trabajo de este artículo se realizaron búsquedas en las bases de datos electrónicas CINAHL, Scopus, Web of Science, Embase, Cochrane Library y PubMed desde diciembre de 2009 hasta el 10 de diciembre de 2019 con los siguientes términos de búsqueda de palabras clave: "envejecimiento" o "personas mayores" y "ejercicio" o "actividad física". ' y 'marcador inflamatorio'. Esto produjo información insuficiente y, por lo tanto, también realizamos cuatro búsquedas más utilizando el marcador inflamatorio específico (TNF-α, PCR, IL-6 e IL-10) como términos de búsqueda de palabras clave. También se revisaron Google Scholar y las listas de referencias de los artículos recuperados utilizando la estrategia de búsqueda descrita anteriormente para identificar cualquier publicación adicional potencialmente relevante.

Se identificaron 253 registros a través de la búsqueda en la base de datos descrita anteriormente. Sin embargo, con el objetivo de analizar artículos en los que se hayan descrito protocolos de ejercicio aeróbico así como protocolos de ejercicio de fuerza en humanos, excluyendo artículos en animales, muestra de pacientes con patologías y artículos sin descripción del protocolo de ejercicio o en los que se realice una combinación de protocolos de ejercicio, finalmente analizamos 24 estudios. De ellos obtuvimos lo descrito a continuación:

El ejercicio de resistencia se distingue del ejercicio aeróbico en que se centra en sobrecargar repetidamente los músculos durante las contracciones estáticas, isométricas o dinámicas, lo que hace que las conexiones entre la musculatura y el sistema nervioso se vuelvan más cercanas y fuertes [97].

Se encontró una amplia variedad de estudios que utilizaron diferentes tipos de programas de ejercicio y protocolos de actividad. Algunos estudios utilizaron programas de ejercicio aeróbico diseñados para mejorar la aptitud cardiorrespiratoria al aumentar el esfuerzo metabólico requerido

para completar el ejercicio, lo que requiere que los participantes aumenten su frecuencia respiratoria, frecuencia cardíaca y flujo sanguíneo para que coincida con la intensidad del ejercicio. Estos protocolos de ejercicio aeróbico implican actividades que requieren el uso de grandes grupos musculares, como en la propulsión rítmica de masas corporales durante movimientos a diferentes intensidades (por ejemplo, caminar, trotar o correr) o a través de actividades con un menor impacto mecánico (por ejemplo, natación o ciclismo). Otros estudios utilizaron protocolos de entrenamiento de resistencia diseñados para mejorar la fuerza muscular y la resistencia a través de métodos especializados de acondicionamiento muscular; estos normalmente involucraban algunos equipos de gimnasio como pesas libres, máquinas de pesas, bandas elásticas y / o ejercicios de peso corporal.

La mayoría de los estudios analizaron los efectos del ejercicio físico sobre la IL-6 en personas mayores que utilizaron protocolos de ejercicio aeróbico [98-105,31,32]. Sin embargo, estos estudios fueron heterogéneos e incluyeron poblaciones de entre 40 y 95 años, utilizaron diferentes protocolos de ejercicio aeróbico, duraron entre 2 y 12 meses, y se preformaron entre 2 y 5 veces por semana a una intensidad de 45% a 80%. No obstante, los resultados obtenidos en estos diferentes estudios generalmente mostraron que el ejercicio aeróbico regular disminuyó la presencia de marcadores inflamatorios como IL-6, TNF-α y PCR, y aumentó la IL-10. Sin embargo, los resultados fueron muy variables debido a la variación en sus protocolos y criterios de inclusión, como también describieron otros autores [106].

Curiosamente, actualmente se están llevando a cabo más estudios de ejercicio físico con un enfoque en el entrenamiento de fuerza en poblaciones de personas mayores de [52 a 65 años]. En cuanto a la IL-6, los resultados de los estudios que implementaron protocolos de entrenamiento de fuerza fueron más contradictorios que los de los estudios de ejercicio aeróbico. El entrenamiento de fuerza tendió a producir un menor efecto crónico sobre IL-6, TNF-α, CRP e IL-10, mientras que también resultó en un aumento agudo en los niveles de IL-6 [107,108]. Sin embargo, los estudios de entrenamiento de fuerza se vieron limitados por el hecho de que incluyeron menos participantes (8 a 17 individuos) que los estudios de ejercicio aeróbico y porque sus características también fueron muy heterogéneas, tanto en términos de los ejercicios implementados como de su intensidad, los grupos musculares involucrados, la duración del protocolo (1-8 meses) y la frecuencia del ejercicio (2-3 veces por semana).

La Tabla 1 y la Tabla 2 incluyen información sobre el/los autor/es y el año de publicación de los estudios analizados; la muestra investigada, con detalles de edad, tipo de ejercicio, ejercicio de protocolo, tipo de respuesta; y finalmente, sus resultados con respecto a los niveles de marcadores inflamatorios.

Tabla 1. Resumen de los estudios incluidos en esta revisión (Respuesta crónica)

Referencia	Edad	Aer (ejercicio aeróbico)/RT (entrenamiento de resistencia)	Protocolo	Resultados
[98] Irwin y Olmstead, 2012	59-86	Aer: Tai Chi Chih	40 min/sesión, 3 sesiones/semana durante 16 semanas; Intensidad moderada	IL-6 ¯ CRP Û
[99] Tartibian et al., 2015	50-65	Aer: Caminando	25–30 min/día,3–4 días/semana con 45–55% de HRmax durante las primeras 8 semanas; 40–45 min/día,4–6 días/semana con 55–65% hr durante las últimas 8 semanas	IL-6 ¯ CRP ¯ TNF- α ¯
[100] Nishida et al., 2015	65-85	Aer: ejercicio de escalón de banco	10–20 min/sesión, 3 veces/día, y un objetivo de 140 min/semana durante 12 semanas; umbral de lactato	IL-6 Û TNF- αÛ
[31] Abdollahpour et al., 2016	50-74	Aer: ejercicio aeróbico	50 min/día, 3 días/semana durante 6 meses; 70–80% HRmax	IL-6 ¯ TNF- αÛ
[101] Nascimento et al., 2015	60-70	Aer: ejercicio aeróbico	16 semanas	IL-6 ¯
[32] El-Kader y Al-Jiffri, 2019	60-68	Aer: cinta de correr	40 min/sesión, 3 sesiones/semana durante 6 meses. 60–80% HRmax	IL-6 ¯ IL-10 - TNF- α ¯
[102] Lee et al., 2015	65-70	Aer: caminar en cinta de correr	40 min/sesión, 3 sesiones/semana durante 8 semanas. 40–70% HRmax	IL-6 ¯ CRP ¯ TNF- α ¯
[103] Lima et al., 2015	63-72	Aer: cinta de correr	20-30 min/sesión, 3 sesiones/semana durante 30 sesiones. 50–80% HRmax	IL-6 ¯ TNF- αÛ
[104] Muscari et al., 2010	65-71	Aer: cinta de correr	1h/sesión, 3 sesiones/semana durante 12 meses. 70% HRmax	CRP ¯
[109] El-Kader et al., 2019	60-67	RT: 9 máquinas de resistencia	9 ex; 3x8-12 rep. 60" res/set. 60-80% RM	IL-6 ¯ IL-10 - TNF- α ¯
[110] Ihalainen et al., 2019	65-75	RT: entrenamiento de fuerza de cuerpo entero	7-9 ex, 2-5 x 4-12 repeticiones, 6 meses, 70-90% RM	IL-6 ¯ CRP ¯
[111] Cao et al., 2019	65	RT: 6 máquinas de resistencia	6 ex, 2-3 x 10-30 repeticiones, 6 semanas, 40-80% RM	CRP Û
[112] Cunha et al., 2019	65-74	RT: 9 pesas libres de ejercicio	9 ex, 1x15 repetición, 20min/sesión, 3 sesiones/semana 12 semanas	CRP ¯
[113] Alikhani y Sheikholeslami-Vatani, 2019	60-64	RT: 6 máquinas de resistencia	6 ex, 4x10 rep, 1h/sesión, 3 sesiones/semana, 12 semanas, 75% RM	TNF- αÛ
[114] Rodríguez-Miguelez et al., 2014	68-70	RT: prensa de piernas; banco de curl de bíceps; cubierta de picoteo sentada	3 ex; miembro inferior y pecho; vigoroso; 3 × 08–12 Rep; 2×/semana, 8 semanas.	IL-6 ¯ CRP ¯
[115] Strandberg et al., 2015	66-70	RT: extensión de la pierna	5 ex; cuerpo entero; vigoroso; 3 × 15 a 08 Rep; 2×/semana, 24 semanas.	IL-6 ¯ CRP ¯
[116] Hagstrom et al., 2016	46-60	RT: prensa de piernas	8 ex; cuerpo entero; vigoroso; 3 × 08–10 Rep; 3×/semana; 32 semanas.	IL-6 ¯ CRP ¯ TNF- αÛ
[117] Nunes et al., 2016	57-65	RT: extensión de la pierna	8 ex; cuerpo entero; vigoroso; 6 × 08–12 Rep; 3×/semana, 16 semanas.	IL-6 ¯ TNF- αÛ

(Continuación Tabla 1)

Referencia	Edad	Aer (ejercicio aeróbico)/RT (entrenamiento de resistencia)	Protocolo	Resultados
[118] Tomeleri et al., 2018	65-75	RT: prensa de pecho; fila sentada; empuje del tríceps; rizo predicador; prensa horizontal de piernas; extensión de rodilla; rizo de rodilla; pantorrilla sentada levantada	8 ex; cuerpo entero; moderado; 3 × 10-15RM, 3×/semana, 12 semanas.	IL-6 ↓ CRP ↓ TNF- α↓
[119] Ogawa et al., 2010	80-89	RT: bandas	4 ex,2x10 rep, 1 sesión/semana, 12 semanas	CRP ↓ IL-6 ⇔ TNF- α⇔
[120] Marques et al., 2013	62-73	RT: máquinas de resistencia	60 min/sesión, 3x8 repetición, 3 sesiones/semana, 32 semanas, 75-80% RM	CRP ↓ IL-6 ↓ TNF- α⇔

Tabla 2. Resumen de los estudios incluidos en esta revisión (Respuesta aguda).

Referencia	Edad	Aer (ejercicio aeróbico)/RT (entrenamiento de resistencia)	Protocolo	Resultados
[107] Nascimento et al., 2016	60-74	RT: Ejercicio de resistencia excéntrica	7 series de 10 repeticiones al 110% de 10 RM con un descanso de 3 min/set	IL-6 48h después↓
[108] Gmiat et al., 2017	40-45	RT: Entrenamiento en Circuito de Alta Intensidad	10 ejercicios, 30 segundos/ejercicio con 10 segundos de descanso. 3 veces con un descanso de 2 min/tiempo	IL-6 48h después↓
[105] Windsor et al., 2018	67-77	Aer: ciclismo	24 min al 40%	IL-6 48h después↓ IL-10 ⇔ TNF- α⇔

2.3. Resultados del tercer artículo: Salivary Il-6 concentration is associated with frailty syndrome in older individuals.

Finalmente, tras realizar dos artículos de revisión de la literatura existente centrados en nuestro objeto de estudio, se llevó a cabo el trabajo de campo con la recogida y análisis de muestras obtenidas en población anciana.

Para el estudio se reclutaron un total de 72 sujetos (84,7% mujeres). Se realizó un estudio transversal con perfil residencial en 2019, entre personas mayores institucionalizadas residentes en centros de larga estancia (GeroResidencias La Saleta) en la provincia de Valencia (España). Los criterios de exclusión fueron: demencia, enfermedad psiquiátrica grave (esquizofrenia, trastorno bipolar, etc.) o ceguera; infecciones agudas o cáncer conocido. Esta investigación se recopiló de acuerdo con los requisitos de la Declaración de Helsinki, y todo el protocolo del estudio fue aprobado

por el comité de ética local. Se obtuvo el consentimiento informado por escrito de todos los participantes. Medimos las características sociodemográficas y los cinco criterios de fragilidad (pérdida de peso involuntaria, poca energía o agotamiento, movilidad lenta, debilidad muscular y poca actividad física) según los criterios de fragilidad de Fried [5], y realizamos evaluaciones geriátricas utilizando el Tinetti índice, prueba MMSE, escala de Yesavage, índice de Barthel y escala de insomnio de Atenas (AIS) para estimar la gravedad del insomnio.

Se recogieron muestras de saliva de los pacientes descritos anteriormente en horario de mañana, con al menos 2 horas de ayuno (entre 9-11 horas de la mañana) utilizando el sistema Salivette ® Cortisol (Sarstedt, Alemania). Se centrifugaron 100 µl de cada muestra y el sobrenadante se alicuotó en tubos eppendorf y se congeló (-80ºC) hasta su posterior análisis. Las muestras se llevaron a temperatura ambiente y se realizó un análisis ELISA de inmunoensayo utilizando el "High Sensitivity Human Elisa Kit for IL-6 (AbCam ab46042) de acuerdo con las instrucciones del fabricante. Los cambios en la intensidad del color y la absorbancia a 450 nm y 490 nm fueron se leyó usando el lector de microplacas ELISA, y se preparó una curva estándar trazando las lecturas de absorbancia de los estándares contra sus concentraciones usando el programa Graphpad.

Vimos que el 5% de los pacientes eran robustos, el 54,9% prefrágiles y el 36,6% frágiles. El *criterio* de Fried más común en la muestra fue la mala actividad física (75%) seguida de la velocidad de caminata lenta (68.1%), agotamiento (29.2%), debilidad (22.5%) y pérdida de peso (8.3%). Las evaluaciones psicogeriátricas se muestran en la Tabla 3.

Tabla 3. Características de la muestra de estudio

Categoría	Valor medio	Gama
Edad (años)	83,3 ± 6,9	66-99
Escala de Yesavage (síntomas depresivos)	3,5 ± 3,2	0-11
Escala de insomnio de Atenas	4.6 ± 4	0-16
Índice de Barthel (actividades básicas de la vida cotidiana)	74,1 ± 22	25-100
Índice de balance de Tinetti	10,5 ± 3,6	1-16
Índice de marcha de Tinetti	8,0 ± 2,8	0-12
Criterios de fragilidad	0 criterios=6 individuos 1 criterio=18 individuos 2 criterios=22 individuos 3 criterios=19 individuos 4 criterios=7 individuos	

. La media ± desviación estándar y el rango para cada valor/escala. La evaluación geriátrica se evaluó mediante escalas validadas: tinetti de marcha e índice de equilibrio para determinar el riesgo de caídas, valoración del deterioro cognitivo mediante el mini-mental (MMSE), escala de Yessavage para la depresión geriátrica, índice de Barthel para medir las actividades de la vida diaria y la movilidad, Escala de insomnio de Atenas (AIS) para estimar la gravedad del insomnio.

También evaluamos la relación entre la concentración salival de IL-6 y las escalas geriátricas y los criterios de fragilidad. Se observó que la IL-6 no se asoció significativamente con ninguna de las escalas de evaluación geriátrica (índice de marcha de Tinetti: r = 0,18; p = 0,12; Índice de equilibrio de Tinetti: r = 0,22; p = 0,064, Spearman); Índice de Barthel para medir las actividades de la vida diaria y la movilidad (r = 0,91; p = 0,44, Spearman); mini- mental (MMSE) para el deterioro cognitivo (r = -0,15; p = 0,20); Escala de Yesavage para la depresión geriátrica (r =-0,23; p = 0,08, Spearman); o la Escala de Insomnio de Atenas para la gravedad del insomnio (r = 0,17; p = 0,14, Spearman). Y tampoco se observaron correlaciones significativas entre el recuento de IL-6 y el agotamiento autoinformado (r = 0,23; p = 0,848, Spearman), la fuerza de agarre de la mano (r = 0,13; p = 0,280, Spearman) o el rendimiento motor lento (r = 0,13; p = 0,267, Spearman).

Sin embargo, hubo una correlación significativa y positiva entre el número de criterios de fragilidad cumplidos con lo niveles de IL-6 (r=0,26; p=0,025, Spearman) (Fig. 3A) y una correlación negativa entre los criterios específicos de actividad física (r=-0,31; p=0,007, Spearman)(Fig.3B)

No hubo diferencias significativas (p=0,07, Kruskal-Wallis) para el recuento de IL-6 entre los diferentes niveles de fragilidad, pero sí se establecieron diferencias significativas entre los valores de IL-6 y el sexo (p=0,496, U-Mann Whitney) y la edad (r= -0,053; p=0,658).

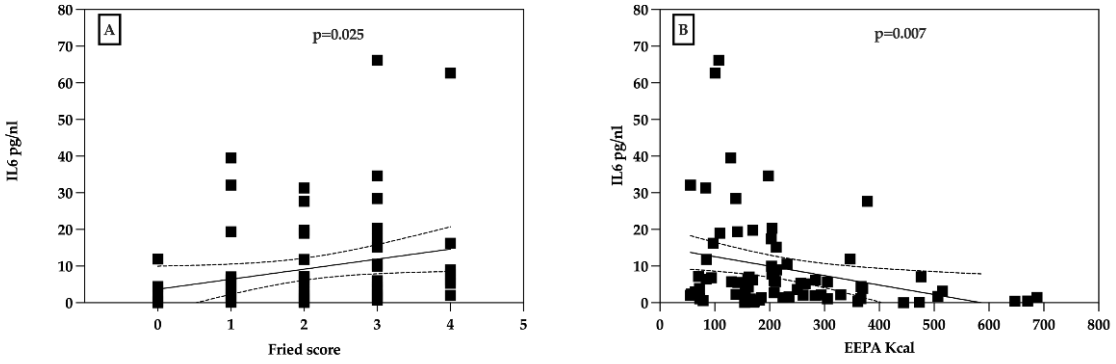

Figura 3. Correlación entre la IL-6 y la puntuación de Fried y la actividad física. (A) Correlación entre la concentración de IL-6 en la saliva y la puntuación de fragilidad. (B) Correlación entre la concentración de IL-6 en la saliva y el gasto energético total de PA en tiempo libre (EEPA).

Hubo diferencias significativas entre los individuos robustos y frágiles en la concentración de IL-6 salival (p< 0,043, prueba de Kruskal-Wallis) (Figura 4).

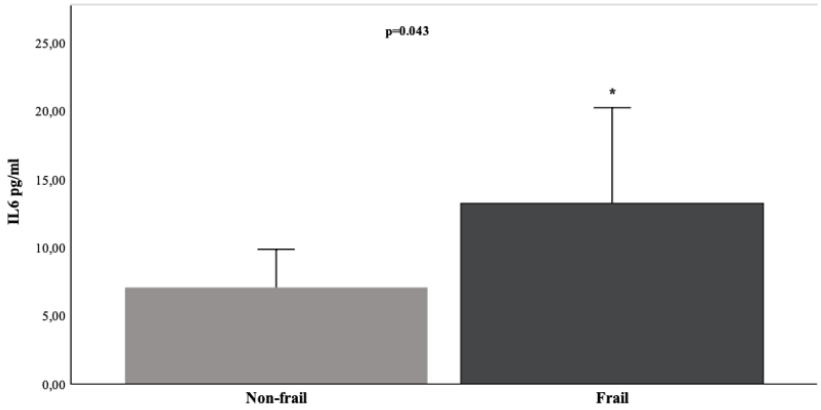

Figura 4. Diferencia entre el síndrome de fragilidad y la IL-6 (pg/mL). La fragilidad se midió de acuerdo con los 5 criterios propuestos por Fried et al. [66]. Los pacientes Robustos y pre-frágiles se agruparon en el grupo no frágil. Los pacientes frágiles fueron aquellos que cumplieron con 3 o más de los criterios de fragilidad.

Para cada uno de los criterios de fragilidad, hubo diferencias significativas en la IL-6 entre los sujetos que cumplieron con el criterio de fragilidad relacionado con la reducción de la actividad física y los que no lo hicieron (p = 0,007,U-Mann Whitney), como se muestra en la figura 5A. Con respecto al criterio de fragilidad "Pérdida involuntaria de peso" hubo una diferencia significativa en la concentración salival de IL-6 entre los individuos que cumplieron este criterio en comparación con los que no lo hicieron (p = 0,002, U-Mann Whitney) (Figura. 5B).

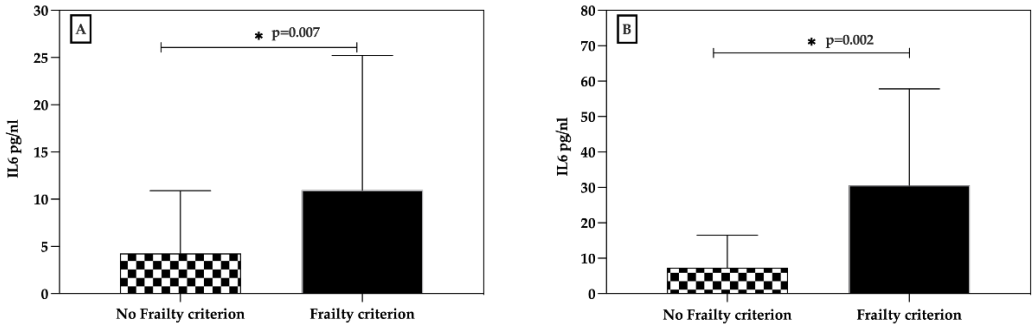

Figura 5. (A) Diferencia entre los criterios de fragilidad de la actividad física y (B) IL-6 (pg/mL) y los criterios de fragilidad de la pérdida de peso e IL-6 (pg/mL). La fragilidad se midió de acuerdo con los 5 criterios propuestos por Fried et al. [66].

Finalmente, con el objetivo de analizar la sensibilidad y la especificidad de la concentración salival de IL-6 para discriminar a los individuos frágiles versus no frágiles (robustos), se realizó el análisis de la curva de operación receptora (ROC). El área bajo el valor de la curva fue de 0,697 con IC del 95%: 0,566–0,827 (Figura 6), y el valor de corte de 3,74 pg/ml tuvo una sensibilidad del 69,0% y una especificidad del 52,8%.

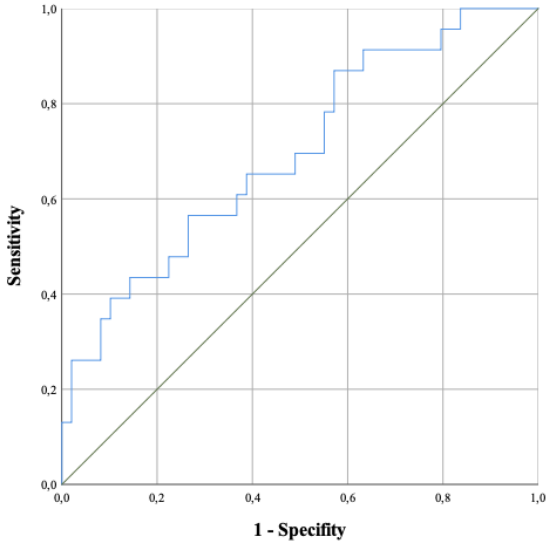

Figura 6. Curva de características operativas del receptor (ROC) para la concentración de IL-6 y la capacidad de discriminar entre individuos frágiles versus individuos robustos + prefrágiles.

DISCUSIÓN

3. **DISCUSIÓN**

Nuestro objetivo principal fue estudiar las interrelaciones existentes entre el ejercicio físico y el sistema inmune y, más concretamente, entre el ejercicio físico y la IL-6 para ver de qué forma el ejercicio físico puede modular los niveles de esta interleukina alterada en determinadas circunstancias de desregulación del sistema inmunitario.

De las revisiones realizadas y de los resultados obtenidos en los artículos podemos enfatizar la importancia que tienen la IL-6, el ejercicio y las interrelaciones entre ellos en el desarrollo, curso y control de ciertas enfermedades y síndromes degenerativos así como en el proceso normal de envejecimiento. Numerosos estudios evidencian que tanto la IL-6 tiene un rol principal en su desarrollo y avance [25], como que las respuestas de los niveles de IL-6 al ejercicio podrían estar detrás de los efectos beneficiosos que dicho ejercicio tiene sobre estas poblaciones mostrando, por ejemplo, una mejoría en tareas que implican memoria de trabajo, velocidad de procesamiento y procesos atencionales [28], y un efecto protector en la plasticidad cerebral que sería la responsable de la mejoría de la sintomatología [80].

Respondiendo a nuestro primer objetivo especifico planteado, el ejercicio físico, como estímulo estresor, provoca al inicio un respuesta aguda que daría lugar a un aumento de los niveles plasmáticos de IL-6 [96], que aunque en un primer momento pueda resultar inadecuado para el estado habitual de inflamación y de valores elevados de IL-6 que tienen las personas de las poblaciones estudiadas, con la continuidad del ejercicio y la adaptación a su ejecución de forma habitual se conseguiría dar lugar a una respuesta crónica en la que, por un lado, descenderían los niveles basales de IL-6 y, por otro, disminuirían los niveles de IL-6 que se producen como respuesta aguda.

Con esta doble repuesta crónica se favorecería, por un lado, la respuesta antiinflamatoria y, por otro lado, el descenso de los niveles basales de IL-6, reduciendo de forma sistémica el estado de inflamación característica de estas poblaciones y favoreciendo el efecto neuroprotector del cerebro que está detrás de la mejora que el ejercicio tiene sobre las funciones cognitivas [80].

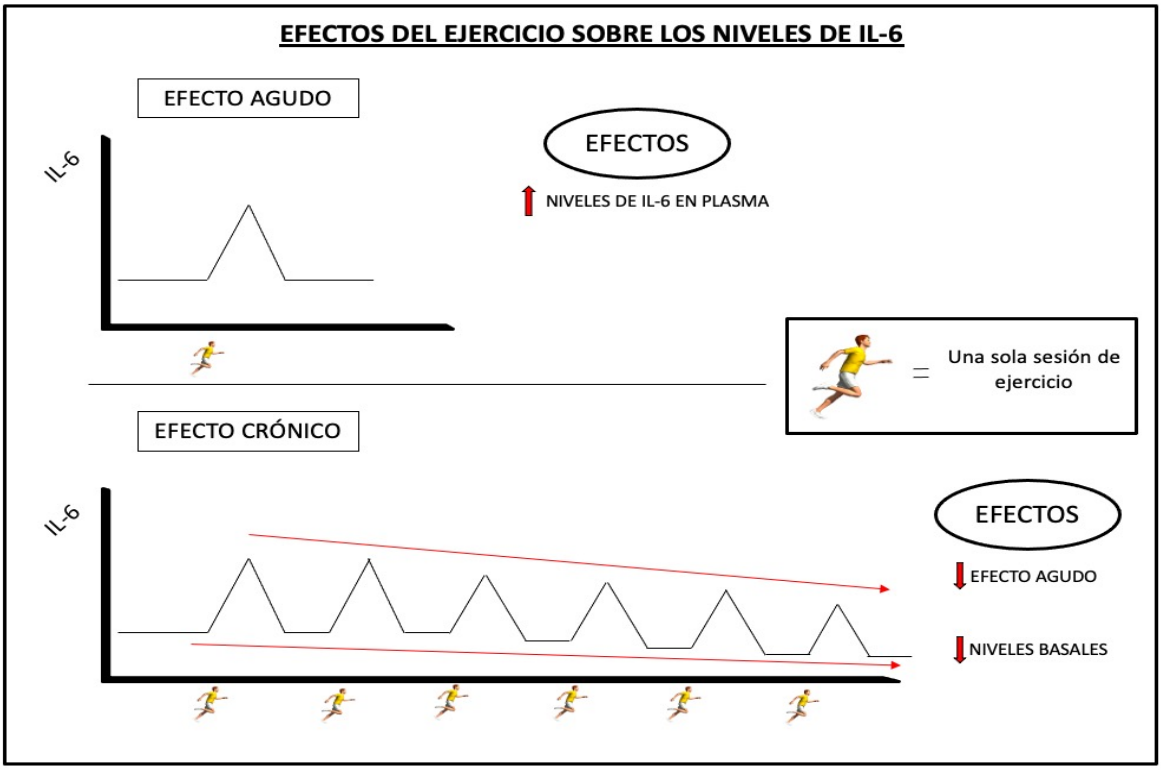

Figura 7. Efectos del ejercicio y niveles de IL-6.

Por lo tanto, teniendo en cuenta los datos aportados, un determinado estímulo de ejercicio repetido en el tiempo y con los parámetros de entrenamiento adecuados podrían modular los niveles de IL-6 dando lugar a efectos positivos en las poblaciones estudiadas. Sin embargo, aunque se necesitan más estudios para conocer cómo afecta la variacion de los diferentes parámetros del ejercicio (intensidad, duración) [26] así como el tipo de ejercicio a los niveles plasmáticos de IL-6, tanto en su efecto agudo como en su efecto crónico, comienzan a verse evidencias acerca de la interrelación entre la IL-6 y el ejercicio, aunque no conozcamos los mecanismos exactos de la regulación de la IL-6 através del ejercicio.

Los niveles plasmáticos de IL-6 podrían estar regulados durante el ejercicio tanto por señales centrales como por señales periféricas. A nivel periférico, numerosos estudios han revelado que tanto las fibras musculares Tipo I como Tipo II dan lugar a la producción de IL-6 en respuesta a las contracciones musculares, provocando un aumento de los niveles plasmáticos como efecto agudo [77]. Este aumento de la liberación de IL-6 está mediado por el glucógeno muscular de forma que

conforme va descendiendo éste se produce un mayor incremento de la producción de IL-6 intramuscular, dando como consecuencia un aumento de los niveles plasmáticos. Sin embargo, con la continuidad del entrenamiento, y como efecto crónico, se produce una mayor expresión intramuscular de receptores de IL-6 (IL-6R) que sugiere una mayor sensibilidad del músculo a los niveles de IL-6 intramusculares provocando que los niveles plasmáticos de IL-6 no aumenten tanto [103]. Por lo tanto, a nivel muscular, la baja concentración de glucógeno muscular provocada por la ejecución del ejercicio físico estimula la producción de IL-6 que se ve reflejada en un incremento de sus niveles plasmáticos. Sin embargo, como adaptación a dicho ejercicio físico, se produce una mayor sensibilidad a la IL-6 intramuscular gracias a la regulación al alta de sus receptores (IL-6R), que da lugar a que ante un mismo ejercicio físico que provoque un descenso del glucógeno muscular, los niveles de IL-6 plasmáticos no se incrementen tanto.

Por otro lado, a nivel central (SNC) se ha observado que los niveles de IL-6 plasmáticos también dependen de señales cerebrales. Sin embargo, mientras que la liberación de IL-6 a nivel muscular aumenta con el descenso de los niveles de glucosa, el cerebro muestra una señal inversa de forma que cuando los niveles de glucosa sanguínea son altos, el cerebro contribuye a que la circulación de IL-6 en sangre sea mayor, aboliendo su liberación cuando los valores de glucosa en sangre son bajos. [121], cumpliendo ambas señales (la liberación muscular y la liberación cerebral) una función de equilibrio homeostático de los niveles de IL-6 plasmáticos. Durante el ejercicio, se ha observado que se produce un aumento de la liberación de IL-6 a nivel central aunque es mucho menor que la liberación de IL-6 que se produce a nivel muscular.

Aunque este mecanismo no está comprendido de forma completa, comienzan a aparecer estudios que indican que parametros del ejercicio como la intensidad y duración son importantes para que la regulación de citokinas sea la adecuada para provocar una respuesta antiinflamatoria que origine el efecto protector, tanto en personas sanas como en personas con diferentes patologías [30]. La intensidad y la duración del ejercicio puede ser uno de los mecanismos que inciden en las señales centrales y periféricas que regulan los niveles plasmáticos de IL-6. La intensidad y la duración del ejercicio están asociados a diferentes vías de utilización de la energía para realizar el ejercicio propuesto. Si el ejercicio se realiza a una intensidad baja o moderada, la glucosa derivada del hígado o de la ingestión oral, y ácidos grasos libres de tejido adiposo proporciona principalmente el combustible necesario para el músculo esquelético. Si la intensidad del ejercicio es aumentada, la contribución de los ácidos grasos libres de tejido adiposo circulantes es modestamente disminuido

mientras que el uso de la glucosa circulante es ampliamente regulado al alza. Si el ejercicio es continuado durante más de una hora a una intensidad fija, el uso de la energía de la oxidación de lípidos se inclina [26]. De esta forma, la variación en la intensidad y duración del ejercicio, pueden afectar a los niveles de glucosa intramuscular y sanguínea y, en consecuencia, a los niveles plasmáticos de IL-6.

Por lo tanto, los niveles plasmáticos de IL-6 parecen descender a medio y largo plazo [90] (efecto crónico) cuando se utilizan protocolos de ejercicio aeróbico, siendo la duración del ejercicio el parámetro que resultaría más determinante. Respecto al efecto agudo, parece que los mayores incrementos de IL-6 se producen a nivel periférico (intramuscular) [93]. En este incremento de los niveles de IL-6 y su consecuente adaptación, con la continuidad del ejercicio, en la regulación al alza de los receptores intramusculares de IL-6 que provoca un descenso de la salida de IL-6 al plasma sanguíneo, parece que es determinante el parámetro de la intensidad del ejercicio ya que necesitamos provocar una deplección del glucógeno muscular para provocar esta adaptación.

Nuestros siguientes objetivo fueron encaminados a detrminar las implicaciones del sistema inmune y la IL-6 en el desarrollo y curso de determinadas enfermedades o síndromes, así como en poblaciones especiales y conocer los beneficios que podría tener la realización de ejercicio físico sobre ellas, actuando sobre los niveles de IL-6.

Actualmente, ya son numerosas las evidencias de los efectos positivos que el ejercicio tiene sobre la Esquizofrenia [25]. También se conoce el importante rol que la IL-6 juega en el desarrollo y sintomatología de la Esquizofrenia, y cómo el ejercicio es capaz de modular la respuesta de la IL-6.

Poder incidir a través del ejercicio como tratamiento en la modulación de los niveles de IL-6 de las personas con Esquizofrenia, tendría una especial relevancia por lo económico y accesible de su utilización, mas si cabe teniendo en cuenta que los tratamientos estandares de medicacion no han conseguido unos efectos importantes sobre los sintomas negativos de la enfermedad, pero que se han visto disminuidos gracias al ejercicio físico.

El hecho de que los protocolos de Resistance Training focalice sus efectos a nivel muscular, nos hace intuir que sería recomendable realizar este tipo de ejercicio (con la intensidad adecuada), ya que conseguiríamos una mayor deplección de los depósitos de glucógeno con la consiguiente

proliferación de receptores intramusculares de IL-6. De esta forma, aunque en un principio la liberación de IL-6 provocada por dichos estímulos diera lugar a un aumento de los niveles de IL-6 plasmáticos, con la continuidad del ejercicio y el aumento de IL-6R bajarían los niveles de IL-6 que llegan al torrente sanguíneo, consiguiendo una adaptación crónica que descendería los niveles plasmáticos y los incrementos agudos de Il-6 fueran cada vez menores.

Por otro lado, conforme se vayan produciendo estas adaptaciones, se debería combinar este tipo de ejercicio con otros predominantemente aeróbicos para potenciar el descenso de los niveles basales que se han observado en los estudios longitudinales, centrándonos en la duración como parámetro más relevante de este tipo de ejercicio. Independientemente del tipo de ejercicio realizado (aeróbico o Resistance Training), parece que los parámetros del ejercicio realizado, intensidad y duración, son factores determinantes para conseguir los efectos deseados sobre los niveles de IL-6 [95].

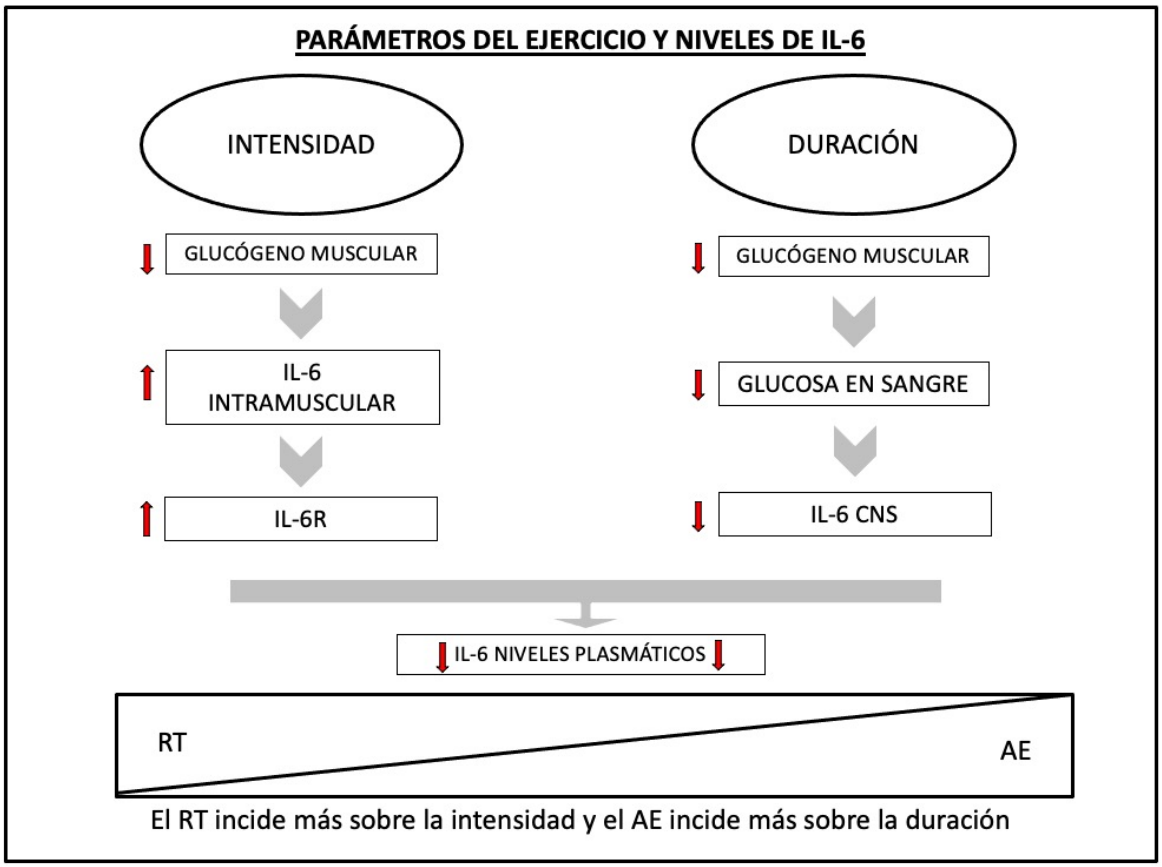

Figura 8. Parámetros del ejercicio y niveles de IL-6.

Además de la influencia que tienen la intensidad y duración del ejercicio realizado sobre los niveles de IL-6 y sus adaptaciones, también son importantes tanto el nivel de forma del sujeto como su estado de inflamación. Se ha observado que, ya sea utilizando protocolos de ejercicio aeróbico como de Resistance Training, los sujetos que muestran un mayor efecto tanto agudo (incremento de los niveles de IL-6) como crónico (descenso de los niveles basales de IL-6) son aquellos que tienen un bajo nivel de forma y aquellos que tienen un estado de inflamación inicial alto [110].

Respecto al nivel de forma de los sujetos, hay que constatar que la intensidad y duración del ejercicio utilizado en los protocolos de los estudios puede ser la suficiente para provocar adaptaciones en los sujetos que no están habituados a realizar ejercicio físico, pero no estimular lo suficiente a aquellos sujetos que mantienen un hábito diario de práctica de ejercicio, por lo que el protocolo diseñado en el estudio no les provocaría el estrés suficiente que dé lugar a las adaptaciones comentadas. En cuanto al estado inicial de inflamación del sujeto, los resultados de los estudios muestran que ante estímulos estresores menores provocados por el ejercicio se consigue recuperar el equilibrio homeostático inflamatorio [25].

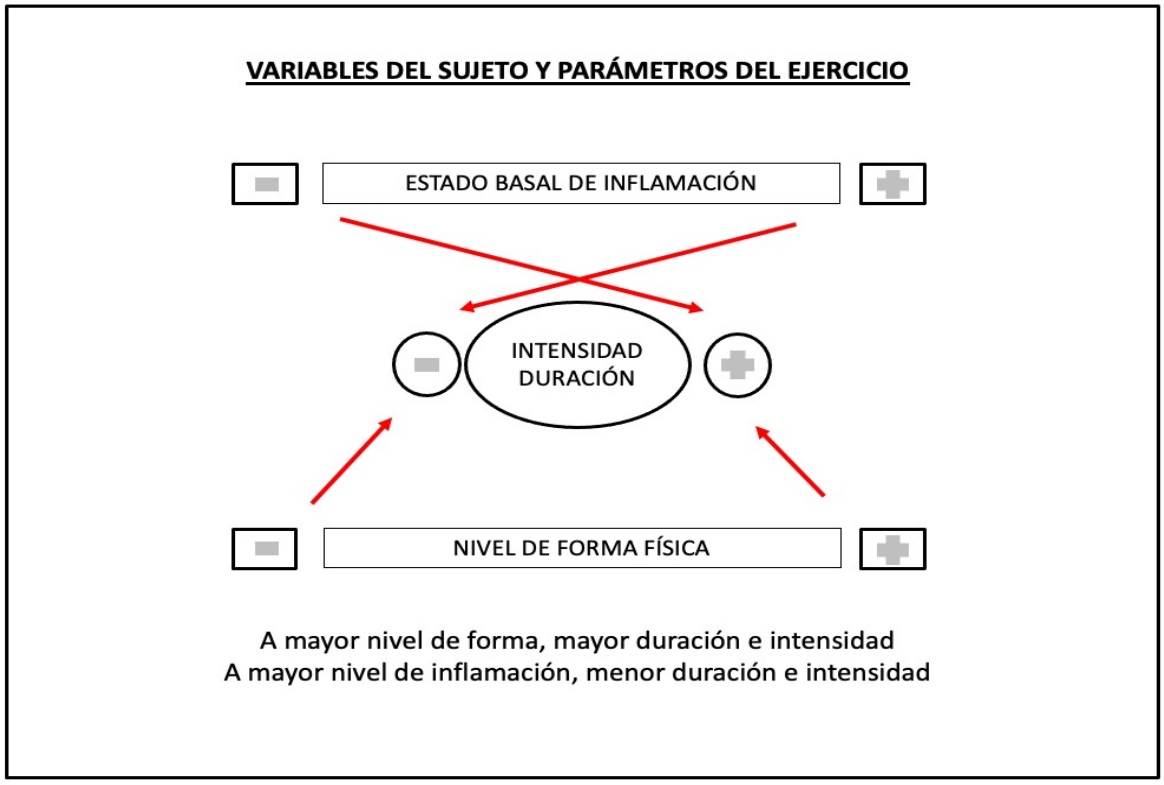

Figura 9. Parámetros del ejercicio y variables del sujeto.

Por lo tanto, teniendo en cuenta que son determinantes en los efectos que el ejercicio provoca en los niveles de IL-6 tanto los parámetros de dicho ejercicio como el nivel del sujeto, se hace imprescindible que los protocolos de actuación con ejercicio físico se adapten de forma individualizada a las características de cada sujeto.

Finalmente, concluyendo con los objetivos planteados, quisimos estudiar si el análisis de los niveles de IL_6 en muestras de saliva, se podían extrapolar a los resultados obtenidos en muestras sanguíneas. En este sentido, aunque los resultados obtenidos en este estudio que relacionan la IL-6 con la fragilidad y específicamente con los niveles de actividad física están bien establecidos en la literatura, el hecho de que estos resultados se obtengan a través de la saliva sugieren que esta técnica puede ser utilizada como una herramienta diagnóstica potencial. Resultados similares se han obtenido en valores salivales de IL-6 en pacientes diagnosticados con enfermedades inflamatorias como diabetes [122], sepsis [67] y enfermedad de Huntington [123]. Más recientemente, un número creciente de estudios han sugerido que los biomarcadores salivales pueden ser útiles para el diagnóstico y seguimiento de algunas enfermedades o afecciones, independientemente de sus concentraciones en sangre [66]. Algunos estudios han obtenido resultados que indican que la IL-6 salival está altamente correlacionada con los niveles sanguíneos [124]. De esta forma, la prueba de Saliva puede ser una herramienta no invasiva para el diagnóstico de fragilidad, sin que la técnica de muestreo implique un estrés adicional para el paciente que implique cambios en los niveles que podrían ocurrir como resultado del estrés asociado con la extracción de sangre [73], y es barato, se puede llevar a cabo en contextos ecológicos a lo largo del tiempo y en múltiples momentos a lo largo del día [65].

Por lo tanto, las pruebas de Saliva podrían utilizarse para el diagnóstico y para indicar la existencia o el riesgo de desarrollar una enfermedad, así como la respuesta a una terapia en particular. El diagnóstico salival es un field que surge rápidamente y que depende del desarrollo de biomarcadores sensibles y específicos que pueden emplearse en entornos clínicos a gran escala. Es razonable suponer que futuros estudios clínicos demostrarán progresivamente la utilidad de los biomarcadores salivales relacionados con moléculas inflamatorias como la IL-6 para el diagnóstico y seguimiento de algunas enfermedades, y específicamente para el diagnóstico de la fragilidad.

CONCLUSIONES

Actualmente, ya son numerosas las evidencias de los efectos positivos que el ejercicio tiene sobre la salud de las personas. También se conoce el importante rol que el sistema inmune y, en particular, la IL-6 juega en el desarrollo y sintomatología de de diferentes enfermedades y síndromes.

En este compendio de artículos nos hemos centrado en las interacciones que se producen entre el ejercicio y la IL-6 en personas que sufren enfermedades y síndromes caracterizados por un estado de inflamación sistémico. Hemos observado que el ejercicio es capaz de modular la respuesta de IL-6 y hemos profundizado en cómo conseguir que dicha respuesta sea la adecuada para descender los niveles plasmáticos de IL-6 de las personas que tienen un estado de inflamación alterado.

Teniendo en cuenta los datos aportados, un estimulo de ejercicio repetido en el tiempo y con los parámetros de entrenamiento adecuados (intensidad, duración, tipo de entrenamiento...) podrían modular los niveles de IL-6 dando lugar a efectos positivos como tratamiento tanto en la mejoría de las funciones cognitivas como en la sintomatología de enfermedades y procesos inflamatorios.

También hemos utilizado una potencial herramienta diagnostica como son los tests de saliva por su valor ecológico, por su accesibilidad económica y por su sencilla utilización. El hecho de que los resultados obtenidos puedan extrapolarse a los resultados obtenidos en pruebas sanguíneas, hacen de los tests de saliva una herramienta de testeo preventivo como de control del estado de inflamación.

Poder incidir a través del ejercicio como tratamiento en la modulación de los niveles de IL-6, tendría una especial relevancia por lo económico y accesible de su práctica, mas si cabe teniendo en cuenta los múltiples beneficios conocidos que su práctica tiene tanto a nivel intrapersonal como social.

BIBLIOGRAFÍA

5. BIBLIOGRAFÍA

1. May, L.T.; Santhanam, U.; Sehgal, P.B. On the multimeric nature of natural human interleukin-6. *J Biol Chem*. **1991**, May 25;266(15):9950-5. PMID: 2033081

2. Gruol, D.L. ; Nelson, T.E. Physiological and pathological roles of interleukin-6 in the central nervous system. *Mol Neurobiol.* **1997** Dec;15(3):307-39. doi: 10.1007/BF02740665. PMID: 9457704

3. Dendorfer, U.; Oettgen, P.; Libermann, T.A. Multiple regulatory elements in the interleukin-6 gene mediate induction by prostaglandins, cyclic AMP, and lipopolysaccharide. *Mol Cell Biol.* **1994** Jul;14(7):4443-54. doi: 10.1128/mcb.14.7.4443-4454.1994. PMID: 8007951; PMCID: PMC358816.

4. Trapero, I. ; Cauli, O. Interleukin 6 and cognitive dysfunction. *Metab Brain Dis*. **2014** Sep;29(3):593-608. doi: 10.1007/s11011-014-9551-2. Epub 2014 Apr 30. PMID: 24782046.

5. Wallenius, V.; Wallenius, K.; Ahre´ n, B.; Rudling, M.; Carlsten, H.; Dickson, S.L.; Ohlsson, C.; and Jansson, J.O. Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med*. **2002** *8*, 75–79. doi: 10.1038/nm0102-75. PMID: 11786910.

6. Febbraio, M.A.; Hiscock, N.; Sacchetti, M.; Fischer, C.P.; and Pedersen, B.K. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes*. **2004**. *53*, 1643–1648. doi: 10.2337/diabetes.53.7.1643. PMID: 15220185.

7. Petersen, E.W.; Carey, A.L.; Sacchetti, M.; Steinberg, G.R.; Macaulay, S.L.; Febbraio, M.A.; and Pedersen, B.K. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. *Am. J. Physiol. Endocrinol. Metab*. **2005**. *288*, E155–E162. doi: 10.1152/ajpendo.00257.2004.

8. Pedersen, B.K.; and Febbraio, M.A. Point: Interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. *J. Appl. Physiol*. **2007**. *102*, 814–816. doi: 10.1152/japplphysiol.01208.2006.

9. Van Hall, G.; Steensberg, A.; Sacchetti, M.; Fischer, C.; Keller, C.; Schjerling, P.; Hiscock, N.; Møller, K.; Saltin, B.; Febbraio, M.A.; and Pedersen, B.K. Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J. Clin. Endocrinol. Metab*. **2003**. *88*, 3005–3010. doi: 10.1210/jc.2002-021687.

10. Carey, A.L.; Steinberg, G.R.; Macaulay, S.L.; Thomas, W.G.; Holmes, A.G.; Ramm, G.; Prelovsek, O.; Hohnen-Behrens, C.; Watt, M.J.; James, D.E.; Kemp, B.E.; Pedersen, B.K.; Febbraio, M.A. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes.* **2006**. *55*, 2688–2697. doi: 10.2337/db05-1404.

11. Scheinert, R.B.; Asokan, A.; Rani, A.; Kumar, A.; Foster, T.C.; Ormerod, B.K. Some hormone, cytokine and chemokine levels that change across lifespan vary by cognitive status in male. *Comportamiento cerebral. Inmune.* **2015**, págs. 49, 216–232. doi: 10.1016/j.bbi.2015.06.005.

12. Di Benedetto, S.; Müller, L.; Wenger, E.; Düzel, S.; Pawelec, G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neuroscience and Biobehavioral Reviews* **2017**, 75, 114–128. http://doi.org/10.1016/j.neubiorev.2017.01.044

13. Philippe, M.; Krüsmann, P.J.; Mersa, L.; Eder, E.M.; Gatterer, H.; Melmer, A.; Ebenbichler, C.; Burtscher, M. Acute effects of concentric and eccentric exercise on glucose metabolism and interleukin-6 concentration in healthy males. *Biol. Deporte.* **2016**, 33, 153-158. doi: 10.5604/20831862.1198634

14. Marsland, A.L.; Gianaros, P.J.; Kuan, D.C-H.; Sheu, Reino Unido; Krajina, K.; Manuck, S.B. Brain morphology links systemic inflammation to cognitive function in midlife adults. *Cerebro Behav Immun*. **2015**, 48, 195–204. http://doi.org/10.1016/j.bbi.2015.03.015

15. Yaffe, K.; Kanaya, A.; Lindquist, K.; Simonsick, E. M.; Harris, T.; Shorr, R. I. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA*. **2014**. 292, 2237–2242. doi: 10.1001/jama.292.18.2237.

16. Dik, M. G.; Jonker, C.; Hack, C. E.; Smit, J. H.; Comijs, H. C.; and Eikelenboom, P. Serum inflammatory proteins and cognitive decline in older persons. *Neurology.* **2015**. 64, 1371–1377. doi: 10.1212/01.WNL.0000158281.08946.68.

17. Mooijaart, S. P.; Sattar, N.; Trompet, S.; Lucke, J.; Stott, D. J.; Ford, I. Circulating interleukin-6 concentration and cognitive decline in old age: the PROSPER study. *J. Intern. Med.* **2013**. 274, 77–85. doi: 10.1111/joim.12052.

18. Kline, R.; Wong, E.; Haile, M.; Didehvar, S.; Farber, S.; Sacks, A.; Pirraglia, E.; de Leon, M.J.; Bekker, A. Peri-Operative Inflammatory Cytokines in Plasma of the Elderly Correlate in Prospective Study with Postoperative Changes in Cognitive Test Scores. *Int J Anesthesiol Res*. **2016**, 4(8), 313–321. http://doi.org/10.19070/2332-2780-1600065

19. Puzianowska-Kuźnicka, M.; Owczarz, M.; Wieczorowska-Tobis, K.; Nadrowski, P.; Chudek, J.; Slusarczyk, P.; Skalska, A.; Jonas, M.; Franek, E.; Mossakowska, M. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immunity & Ageing*. **2016**, 13–21. http://doi.org/10.1186/s12979-016-0076-x

20. Paine, N.J.; Bosch, J.A.; Ring, C.; Drayson, M.T.; van Zasten, J.J.C.S.V. Induced mild systemic inflammation is associated with impaired ability to improve cognitive task performance by practice. *Psychophysiology*. **2015**, 52, 333-341. http://doi.org/10.1111//psyp..12360

21. Bradburn, S.; Sarginson, J.; Murgatroyd, C.A. Association of Peripheral Interleukin-6 with Global Cognitive Decline in Non-demented Adults: A Meta-Analysis of Prospective Studies. *Frente. Envejecimiento Neurosci.* **2018**, 9:438. doi: 10.3389/fnagi.2017.00438

22. Shivakumar, V.; Debnath, M.; Venugopal, D.; Rajasekaran, A.; Kalmady, S. V.; Subbanna, M.; Narayanaswamy, J.C.; Amaresha, A.C.; Venkatasubramanian, G. Influence of correlation between HLA-G polymorphism and Interleukin-6 (IL6) gene expression on the risk of schizophrenia. *Cytokine*. **2018**, *107*, 59–64. http://doi.org/10.1016/j.cyto.2017.11.016

23. Gruol, D.L. IL-6 regulation of synaptic function in the CNS. *Neuropharmacology*. **2015**, 96(0 0), 42–54. http://doi.org/10.1016/j.neuropharm.2014.10.023

24. Thielen, J-W.; Kärgel, C.; Müller, B.W.; Rasche, I.; Genius, J.; Bus, B.; Maderwald, S.; Norris, D.G.; Wiltfang, J.; Tendolkar, I. Aerobic Activity in the Healthy Elderly Is Associated with Larger Plasticity in Memory Related Brain Structures and Lower Systemic Inflammation. *Front. Aging Neurosci.* **2016**, 8:319. doi: 10.3389/fnagi.2016.00319

25. Goldsmith, D. R.; Haroon, E.; Miller, A. H.; Strauss, G. P.; Buckley, P. F.; Miller, B. J. TNF-α and IL-6 are associated with the deficit syndrome and negative symptoms in

patients with chronic schizophrenia. *Schizophrenia Research*. **2018**. 199(C), 281–284. http://doi.org/10.1016/j.schres.2018.02.048

26. Huh, J. Y. The role of exercise-induced myokines in regulating metabolism. *Archives of Pharmacal Research*. **2018**. *41*(1), 14–29. http://doi.org/10.1007/s12272-017-0994-y

27. Mandolesi, L.; Polverino A.; Montuori S.; Foti F.; Ferraioli G.; Sorrentino P.; Sorrentino G. Effects of Physical Exercise on Cognitive Functioning and Wellbeing: Biological and Psychological Benefits. *Front. Psychol* **2018**, 9:509, 1–11, http://doi.org/10.3389/fpsyg.2018.00509

28. Firth, J.; Cotter, J.; Carney, R.; Yung, A.R. The pro-cognitive mechanisms of physical exercise in people with schizophrenia. *British Journal of Pharmacology.* **2017**, 174, 3161–3172. http://doi.org/10.1111/bph.13772

29. Levin, S.G.; Godukhin, O.V. Modulating Effect of Cytokines on Mechanisms of Synaptic Plasticity in the Brain. *Biochemistry (Moscow).* **2017**, 82-3, 264-274. http://doi.org/10.1134/S000629791703004X

30. Liu, D.; Wang, R.; Grant, A. R.; Zhang, J.; Gordon, P. M.; Wei, Y.; Chen, P. (2017). Immune adaptation to chronic intense exercise training: new microarray evidence. *BMC Genomics*. **2017**, 18–29. http://doi.org/10.1186/s12864-016-3388-5

31. Abdollahpour, A.; Khosravi, N.; Eskandari, Z.; Haghighat, S. Effect of six months of aerobic exercise on plasma interleukin-6 and tumor necrosis factor-alpha as breast cancer risk factors in postmenopausal women: a randomized controlled trial. *Iran Red Crescent Med. J.,* **2016**, 19:e27842.

32. Abd El-Kader, S.M.; Al-Shreef, F.M.; Al-Jiffri, O.H. Impact of aerobic exercise versus resisted exercise on endothelial activation markers and inflammatory cytokines among elderly. *Afri Salud Sci*. **2019**. 19(4), 2874-2880. doi: 10.4314/ahs.v19i4.9.

33. Henningsen, J.; Rigbolt, K.T.; Blagoev, B.; Pedersen, B.K.; Kratchmarova, I. Dynamics of the skeletal muscle secretome during myoblast differentiation. *Mol Cell Proteom.* **2010**. 9:2482–2496. doi: 10.1074/mcp.M110.002113.

34. M.T. Heneka, M.T.; O'Banion, M.K.; Terwel, D.; Kummer, M.P. Neuroinflammatory processes in Alzheimer's disease, *J. Neural Transm*. **2010**. 117 (8), 919–947. doi: 10.1007/s00702-010-0438-z.

35. Forster, M.J.; Dubey, A.; Dawson, K.M.; Stutts, W.A.; Lal, H.; Sohal, R.S. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc. Natl. Acad. Sci.* **1996**. 93 (10), 4765–4769. doi: 10.1073/pnas.93.10.4765.

36. Bott, N.T.; Bettcher, B.M.; Yokoyama, J.S.; Frazier, D.T.; Mtthew, W.; Karydas, A.; Yaffe, K.; Kramer, J.H. Youthful Processing speed in older adults: genetic, biological and behavioral predictors of cognitive processing speed trajectories in aging. *Front. Aging Neurosci.* **2017**. 9:55. doi: 10.3389/fnagi.2017.00055.

37. DeCarlo, C. A.; Tuokko, H. A.; Williams, D.; Dixon, R. A.; MacDonald, S. W. BioAge: toward a multi-determined, mechanistic account of cognitive aging. *Ageing Res. Rev.* **2014**. 18, 95–105. doi: 10.1016/j.arr.2014.09.003.

38. Franceschi, C.; Bonafe, M.; Valensin, S.; Olivieri, F.; De Luca, M.; Ottaviani, E.; De Benedictis, G. Inflamm-aging. An evolutionary perspective on im- munosenescence. *Ann. N. Y. Acad. Sci*. **2000**. 908, 244–254. doi: 10.1111/j.1749-6632.2000.tb06651.x.

39. Sieber, C.C. Frailty - From concept to clinical practice. *Exp Gerontol*. **2017**. Jan;87(Pt B):160-167. doi: 10.1016/j.exger.2016.05.004.

40. Leng, S.X. ; Xue, Q.L. ; Tian, J. ; Walston, J.D. ; Fried, L.P. Inflammation and frailty in older women. *J Am Geriatr Soc*. **2007**. Jun;55(6):864-71. doi: 10.1111/j.1532-5415.2007.01186.x.

41. Li, H. ; Manwani, B. ; Leng, S.X. Frailty, inflammation, and immunity. *Aging Dis*. **2011**. Dec;2(6):466-73.

42. Hubbard, R.E.; Woodhouse, K.W. Frailty, inflammation and the elderly. *Biogerontology.* **2010**. *11*, 635–641. https://doi.org/10.1007/s10522-010-9292-5

43. Fernández-Garrido, J.; Navarro-Martínez, R.; Buigues-González, C.; Martínez-Martínez, M.; Ruiz-Ros, V.; Cauli, O. The value of neutrophil and lymphocyte count in frail older women. *Experimental Gerontology*. **2014**. 54(1-7), 35-41. http://doi.org/10.1016/j.exger.2013.11.019

44. Wang, J.; Maxwell, C.A.; Yu, F. Biological Processes and Biomarkers Related to Frailty in Older Adults: A State-of-the-Science Literature Review. *Biological Research for Nursing*. **2018**. 21(1), 80–106. http://doi.org/10.1177/1099800418798047

45. Fried, L.P.; Ferrucci, L.; Darer, J.; Williamson, J.D.; Anderson, G. Untangling the concepts of disability, frailty, and comorbidity: Implications for improved targeting and care. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2004**. 59(3), M255–M263. https://doi.org/10.1093/gerona/59.3.M255

46. Fried, L.P.; Tangen, C.M.; Waltson, J.; Newman, A.B.; Hirsch, C.; Gottdiener, J.; Seeman, T.; Tracy, R.; Kop, W.J.; Burke, G. Frailty in Older Adults: Evidence for a Phenotype. *Journals Gerontol. Ser. A Biol. Sci. Med. Sci.* **2001**. 56 (3), M146-M157. https://doi.org/10.1093/gerona/56.3.m146

47. Orme, J. G.; Reis, J.; Herz, E. J. Factorial and Discriminant Validity of the Center for Epidemiological Studies Depression (CES-D) Scale. *J. Clin. Psychol.* **1986**. *42* (1), 28–33. https://doi.org/10.1002/1097-4679(198601)42:1<28::aid-jclp2270420104>3.0.co;2-t.

48. Radloff, L. S. The CES-D Scale : A Self-Report Depression Scale for Research in the General Population. *Appl. Psychol. Meas.* **1977**. *1* (3), 385–401.

49. Elosua, R.; Garcia, M.; Aguilar, A.; Molina, L.; Covas, M. I.; Marrugat, J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. *Med. Sci. Sports Exerc.* **2000**. *32* (8), 1431–1437. https://doi.org/10.1097/00005768-200008000-00011.

50. Ruiz Comellas, A.; Pera, G.; Baena Díez, J. M.; Mundet Tudurí, X.; Alzamora Sas, T.; Elosua, R.; Torán Monserrat, P.; Heras, A.; Forés Raurell, R.; Fusté Gamisans, M.; et al. Validación de Una Versión Reducida En Español Del Cuestionario de Actividad Física En El Tiempo Libre de Minnesota (VREM). *Rev. Esp. Salud Publica* **2012**. *86* (5), 495–508.

51. Jacobs, D. R. J.; Ainsworth, B. E.; Hartman, T. J.; Leon, A. S. A Simultaneous Evaluation of 10 Commonly Used Physical Activity Questionnaires. *Med. Sci. Sports Exerc.* **1993**. *25* (1), 81–91. https://doi.org/10.1249/00005768-199301000-00012.

52. Guralnik, J.; Simonsick, E.; Ferrucci, L.; Glynn, R.; Berkman, L.; Blazer, D.; Scherr, P.; Wallace, R. A Short Physical Performance Battery Assessing Lower Extremity Function: Association with Self-Reported Disability and Prediction of Mortality and Nursing Home Admission. *J Gerontol.* **1994**. *49* (2), M85-94.

53. Ottenbacher, K. J.; Branch, L. G.; Ray, L.; Gonzales, V. A.; Peek, M. K.; Hinman, M. R. The Reliability of Upper- and Lower-Extremity Strength Testing in a Community Survey of Older Adults. **2002**. 1423–1427. https://doi.org/10.1053/apmr.2002.34619.

54. Walston, J.J.; McBurnie, M.A.M.; Newman, A.A.; Tracy, R.P.R.; Kop, W.J.W.; Hirsch, C.H.C.; Gottdiener, J.J.; Fried, L.P.L. 2002. Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. *Arch. Intern. Med.* **2002.** 162, 2333–2341. doi:10.1001/archinte.162.20.2333

55. Chen, X.; Mao, G.; Leng, S.X. Frailty syndrome: an overview. *Clin. Interv. Aging*. **2014**. 9, 433-441. http://dx.doi.org/10.2147/cia.s45300.

56. Lang, P.O.; Michel, J.P.; Zekry, D. Frailty syndrome: a transitional state in a dynamic process. *Gerontology*. **2009**. 55(5):539-49. http://dx.doi.org/10.1159/000211949.

57. Schaap, L.A.; Pluijm, S.M.; Deeg, D.J.; Visser, M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med* **2006**. 119:526, e9–e17.

58. Roubenoff, R.; Parise, H.; Payette, H.A. Cytokines, insulin-like growth factor1, sarcopenia, and mortality in very old community-dwelling men and women: the Framingham heart study. *Am J Med.* **2003**.115:429–35.

59. Schaap, L.A.; Pluijm, S.M.; Deeg, D.J..Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. *J Gerontol A Biol Sci Med Sci.* **2009**. 64:1183–9.

60. Tembo, M.C.; Holloway-Kew, K.L.; Bortolasci, C.C.; Brennan-Olsen, S.L.; Williams, L.J.; Mark A. Kotowicz, M.A.; Pasco, J.A. Association between serum interleukin-6 and frailty in older men: cross-sectional data. *European Geriatric Medicine***. 2021**. 12, 887–892. https://doi.org/10.1007/s41999-021-00490-8.

61. Semmarath, W.; Seesen, M.; Yodkeeree, S.; Sapbamrer, R.; Ayood, P.; Malasao, R.; Penprapa Siviroj, P.; Limtrakul, P. The Association between Frailty Indicators and Blood-Based Biomarkers in Early-Old Community Dwellers of Thailand. *Int. J. Environ. Res. Public Health.* **2019**. *16*, 3457; doi:10.3390/ijerph16183457.

62. Ma, L.; Sha, G.; Zhang, Y.; Li, Y. Elevated serum Il-6 and adiponectin levels are associated with frailty and physical function in Chinese older adults. *Clinical Interventions in Aging*. **2018**. 13, 2013-2020. https://doi.org/10.2147/CIA.S180934.

63. Colburn, W.; DeGruttola, V.; DeMets, D.; Downing, G.; Hoth, D.; Oates, J. ..Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics*. **2001**. 69, 89–95. doi:10.1067/mcp.2001.113989.

64. Corwin, E. J.; Ferranti, E. Integration of biomarkers to advance precision nursing interventions for family research across the lifespan. *Nursing Outlook*. **2016**. 64, 292–298. doi:10.1021/acssen- sors.7b00425.

65. Slavish, D.C.; Graham-Engeland, J.E.; Smyth, J.M.; Engeland, C.G. Salivary Markers of Inflammation in Response to Acute Stress. *Brain Behav Immun*. **2015**. 0, 253–269. doi:10.1016/j.bbi.2014.08.008.

66. Chávez-Alderete, J.; Gochicoa-Rangel, L.; Del-Río-Hidalgo, R.; Guerrero-Zúñiga, S.; Mora-Romero, U.; Benítez-Pérez, R.; Rodríguez-Moreno, L.; Torre-Bouscoulet, L.; Vargas, M.H. Salivary concentrations of cytokines and other analytes in healthy children. *Cytokine*. **2021**. 138, 155379. https://doi.org/10.1016/j.cyto.2020.155379.

67. Galhardo, L. F.; Ruivo, G. F.; De Oliveira, L. D.; Parize, G.; Dos Santos, S. S. F.; Pallos, D.; Leao, M. V. P. Inflammatory markers in saliva for diagnosis of sepsis of hospitalizes patients. *Eur J Clin Invest.* **2020**. 50 (5), 13219. https://doi.org/10.1111/eci.13219.

68. Khurshid, Z.; Naseem, M.; Sheikh, Z.; Najeeb, S.; Shahab, S.; Zafar, M.S. Oral antimicrobial peptides: Types and role in the oral cavity. *Saudi Pharm. J.* **2016**. 24, 515–524. https://doi.org/10.1016/j.jsps.2015.02.015.

69. Xiao, H.; Wong, D.T. Proteomics and its applications for biomarker discovery in human saliva. *Bioinformation.* **2011**. 5 (7), 294–296. doi:10.6026/97320630005294.

70. Gröschl, M.; Köhler, H.; Topf, H.-G.; Rupprecht, T.; Rauh, M. Evaluation of saliva collection devices for the analysis of steroids, peptides and therapeutic drugs. *J. Pharm. Biomed. Anal.* **2008**. 47 (3), 478–486. https://doi.org/10.1016/j.jpba.2008.01.033

71. Melguizo-Rodríguez, L.; Costela-Ruiz, V.J.; Manzano-Moreno, F.J.; Ruiz, C.; Illescas-Montes, R. Salivary Biomarkers and Their Application in the Diagnosis and Monitoring of the Most Common Oral Pathologies. *Int J Mol Sci*. **2020**. Jul 21;21(14):5173.

72. Ozcaka, O.; Ceyhan, B.O.; Akcali, A.; Bicakci, N.; Lappin, D.F.; Buduneli, N. Is there an interaction between polycystic ovary syndrome and gingival inflammation?. *J Periodontol*. **2012**. 83: 1529–37.

73. Ibáñez-Del Valle, V.; Navarro-Martínez, R.; Ballestar-Tarin, M.L.; Cauli, O. Salivary Inflammatory Molecules as Biomarkers of Sleep Alterations: A Scoping Review. *Diagnostics* (Basel). **2021**. Feb 10;11(2):278.

74. Ostermann, S.; Herbsleb, M.; Schulz, S.; Donath, L.; Berger, S.; Eisenträger, D.; Siebert, T.; Müller, H-J.; Puta, C.; Voss, A.; Gabriel, H.W.; Koch, K.; Bär, K-J. Exercise Reveals the Interrelation of Physical Fitness, In ammatory Response, Psychopathology, and Autonomic Function in Patients With Schizophrenia. *Schizophrenia Bulletin***, 2018,** 39- 5, 1139–1149. http://doi.org/10.1093/schbul/sbs085

75. Niemann, C.; Godde, B.; Staudinger, U. M.; Voelcker-Rehage, C. Exercise-induced changes in basal ganglia volume and cognition in older adults. *Neuroscience*. **2014**, 281, 147–163. doi: 10.1016/j.neuroscience.2014.09.033

76. Hollamby, A.; Davelaar, E. J.; Cadar, D. Increased physical fitness is associated with higher executive functioning in people with dementia. *Front. Public Heal.* **2017**, 5:346. doi: 10.3389/fpubh.2017.00346

77. Pedersen, B. K.; Febbraio, M. Muscle-derived interleukin-6—A possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain, Behavior, and Immunity*. **2005**, *19*(5), 371–376. http://doi.org/10.1016/j.bbi.2005.04.008

78. Dauwan, M.; Begemann, M.J.H.; Heringa, S.M.; Sommer, I.E. Exercise Improves Clinical Symptoms, Quality of Life, Global Functioning, and Depression in Schizophrenia: A Systematic Review and Meta-analysis, *Schizophrenia Bulletin*. **2016**, vol. 42-3, 588–599. http://doi.org/10.1093/schbul/sbv164

79. Stubbs, B.; Vancampfort, D.; Hallgren, M.; Firth, J.; Veronese, N.; Solmi, M.; Brand, S.; Cordes, J.; Malchow, B.; Gerber, M.; Schmitt, A.; Correll, C.U.; De Hert, M.; Gaughran, F.; Schneider, F.; Kinnafick, F.; Falkai, P.; Möller, H-J.; Kahl, K.G. EPA guidance on physical activity as a treatment for severe mental illness: a meta-review of the evidence and Position Statement from the European Psychiatric Association (EPA), supported by the

International Organization of Physical Therapists in Mental Health (IOPTMH). *European Psychiatry*. **2018**, *54*, 124–144. http://doi.org/10.1016/j.eurpsy.2018.07.004

80. Van der Stouwe, E. C. D.; Van Busschbach, J. T.; de Vries, B.; Cahn, W.; Aleman, A.; Pijnenborg, G. H. M. Neural correlates of exercise training in individuals with schizophrenia and in healthy individuals_ A systematic review. *NeuroImage: Clinical* **2018**, *19*, 287–301, http://doi.org/10.1016/j.nicl.2018.04.018

81. Firth, J.; Cotter, J.; Elliott, R.; French, P.; Yung, A.R. A systematic review and meta-analysis of exercise interventions in schizophrenia patients. *Psychological Medicine*. **2015**, 45, 1343–1361. http://doi.org/10.1017/S0033291714003110.

82. Hidese, S.; Matsuo, J.; Ishida, I.; Hiraishi, M.; Teraishi, T.; Ota, M.; Hattori, K.; Kunugi, H. Relationship of Handgrip Strength and Body Mass Index With Cognitive Function in Patients With Schizophrenia. *Front. Psychiatry*. **2018**, 9:156. doi: 10.3389/fpsyt.2018.00156

83. Leiter, O.; Kempermann, G.; Walker, T.L. A common language: how neuroimmunological cross talk regulates adult hippocampal neurogenesis. *Stem Cells Int.* **2016**, 1681590.

84. Hasegawa-Ishii, S.; Inaba, M.; Umegaki, H.; Unno, K.; Wakabayashi, K.; Shimada, A. Endotoxemia-induced cytokine-mediated responses of hippocampal astrocytes transmitted by cells of the brain-immune interface. *Sci. Rep*. **2016**, 6, 25457.

85. McAfoose, J.; and Baune, B. T. Evidence for a cytokine model of cognitive function, *Neurosci. Biobehav. Rev*. **2009**, 33, 355- 366.

86. Noto, C.; Maes, M.; Ota, V.K.; Teixeira, A.L.; Bressan, R.A.; Gadelha, A.; Brietzke, E. High predictive value of immune-inflammatory biomarkers for schizophrenia diagnosis and association with treatment resistance. *World J. Biol. Psychiatry*. **2015**, 1–8.

87. Rodrigues-Amorim, D.; Rivera-Baltanás, T.; Spuch, C., Caruncho, H. J.; González-Fernandez, Á.; Olivares, J. M.; Agís-Balboa, R. C. Cytokines dysregulation in schizophrenia: A systematic review of psychoneuroimmune relationship. *Schizophrenia Research* **2018**, *197*(C), 19–33, http://doi.org/10.1016/j.schres.2017.11.023

88. van der Geest, K. S. M.; Wang, Q.; Eijsvogels, T. M. H.; Koenen, H. J. P.; Joosten, I.; Brouwer, E.; Hopman, M.T.E.; Jacobs, J.F.M.; Boots, A.M.H. Changes in peripheral immune cell numbers and functions in octogenarian walkers – an acute exercise study. *Immunity & Ageing*. **2017**, 14:5. http://doi.org/10.1186/s12979-017-0087-2

89. Gjevestad, G.O.; Holven, K.B.; Ulven, S.M. Effects of Exercise on Gene Expression of Inflammatory Markers in Human Peripheral Blood Cells: A Systematic Review. *Informes de riesgo cardiovascular actuales*. **2015**. 9, (7):34. doi: 10.1007/s12170-015-0463-4 PMID: 26005511

90. Lavratti, C.; Dorneles, G.; Pochmann, D.; Peres, A.; Bard, A.; de Lima Schipper, L.; Lago, P.D.; Wagner, L.C.; Elsner, V.R. Exercise-induced modulation of histone H4 acetylation status and cytokines levels in patients with schizophrenia. *Physiology & Behavior*. **2017**, *168*(C), 84–90. http://doi.org/10.1016/j.physbeh.2016.10.021

91. Borovcanin, M.M.; Jovanovic, I.; Radosavljevic, G.; Pantic, J.; Minic Janicijevic, S.; Arsenijevic, N.; Lukic, M.L. Interleukin-6 in Schizophrenia—Is There a Therapeutic Relevance?. *Front. Psychiatry*. **2017**, 8:221. doi: 10.3389/fpsyt.2017.00221

92. Misiak, B. A.; Czykiewicz, B. O. S.; Kotowicz, K.; Rybakowski, J. K.; Samochowiec, J.; Frydecka, D. Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. *Investigación de la esquizofrenia*. **2018**. *192*(C), 16–29. http://doi.org/10.1016/j.schres.2017.04.015

93. Monteiro-Junior, R.S.; de Tarso Maciel-Pinheiro, P.; da Matta Mello, E.; da Silva, L.F.; Terra, R.; Carneiro, L.S.F.; Rodrigues, V.D.; Nascimento, O.J.M.; Deslandes, A.C.; Laks, J. Effect of Exercise on Inflammatory Profile of Older Persons: Systematic Review and Meta-Analyses. *Journal of Physical Activity and Health*. **2018**, 15, 64-71. http://doi.org/10.1123/jpah.2016-0735

94. Kapilevich, L.V.; Zakharova, A.N.; Kabachkova, A.V.; Kironenko, T.A.; Orlov, S.N. Dynamic and Static Exercises Differentially Affect Plasma Cytokine Content in Elite Endurance- and Strength-Trained Athletes and Untrained Volunteers. *Front. Physiol.* **2017**, 8:35. doi: 10.3389/fphys.2017.00035

95. Cronin, O.; Keohane, D.M.; Molloy, M.G.; Shanahan, F. The effect of exercise interventions on inflammatory biomarkers in healthy, physically inactive subjects: a systematic review. *An International Journal of Medicine*. **2017**, 110: 10, 629-637. http://doi.org/10.1093/qjmed/hcx091

96. Pruimboom, L.; Raison, C.L.; Muskiet, F.A.J. Physical Activity Protects the Human Brain against Metabolic Stress Induced by a Postprandial and Chronic Inflammation. *Behavioural Neurology.* **2015**, http://doi.org/10.1155/2015/569869

97. Miller, K.J.; Gonçalves-Bradley, D.C.; Areerob, P.; Hennessy D.; Mesagno, C.; Grace, F. Comparative effectiveness of three types to treat clinical depression in older adults: a systematic review and network meta-analysis of randomised controlled trials. *Ageing Research Reviews*, **2020**, 58, 100999.

98. Irwin, M. R.; Olmstead, R. Mitigating cellular inflammation in older adults: a randomized controlled trial of Tai Chi Chih. *Am. J. Geriatr. Psychiatry,* **2012**, 20, 764–772.

99. Tartibian, B.; FitzGerald, L. Z.; Azadpour, N.; Maleki, B. H. A randomized controlled study examining the effect of exercise on inflammatory cytokine levels in post-menopausal women. *Post Reprod. Health,* **2015**, 21, 9–15.

100. Nishida, Y.; Tanaka, K.; Hara, M.; Hirao, N.; Tanaka, H.; Tobina, T. Effects of home-based bench step exercise on inflammatory cytokines and lipid profiles in elderly Japanese females: a randomized controlled trial. *Arch. Gerontol. Geriatr*., **2015**, 61, 443–451.

101. Nascimento, C.M.C.;Cominetti, M.R.; Pereira, J.R.; Andrade, L.P.; Garuffi, M.; Kerr, D.S.; Forlenza, O.V.; Stella, F. Regular multimodal aerobic exercise reduces pro-inflamatory cytokines and improves BNDF peripheal levels and executive functions in elderly MCI individuals with different BDNF VAL66MET genotypes. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, **2015**, 11 (7), 323.

102. Lee, J.S.; Chang, G.K.; Tae, B.S.; Hyo, G.K.; Sung, J.Y. Effects of 8-week combined training on body composition, isokinetic strength, and cardiovascular disease risk factors in older women. *Aging Clin Exp Res*, **2015**, 27, 179–186

103. Lima, L.G.; Bonardi, J.M.T.; Campos, G.O.; Bertani, R.F.; Scher, L.M.L.; Louzada-Junior, P.; Moriguti, J.C.; Ferriolli, E.; Lima, N.K.C. Effect of aerobic training and aerobic and resistance training on the inflammatory status of hypertensive older adults. *Aging Clin Exp Res*, **2015**, 27, 483-489.

104. Muscari, A.; Giannoni, C.; Pierpaoli, L.; Berzigotti, A.; Maietta, P.; Foschi, E.; Ravaioli, C.; Poggiopollini, G.; Bianchi, G.; Magalotti, D.; Tentoni, C.; Zoli, M. Chronic endurance exercise training prevents aging-related cognitive decline in healthy older adults: a randomized controlled trial. *Int J Geriatr Psychiatry*, **2010**, 25, 1055–1064.

105. Windsor, M.T.; Bailey, T.G.; Perissiou, M.; Greaves, K.; Jha, P.; Leicht, A.S.; Russell, F.D.; Golledge, J.; Askew, C.D. Acute inflammatory responses to exercise in patients with abdominal aortic aneurysm. *Med Sci Sports Exerc.,* **2018,** 50(4), 649-658.

106. Zheng, G.; Qiu, P.; Xia, R.; Lin, H.; Ye, B.; Tao, J.; Chen, L.; Effect of Aerobic Exercise on Inflammatory Markers in Healthy Middle-Aged and Older Adults: A Systematic Review and Meta-Analysis *of Randomized* Controlled Trials. *Front. Aging Neurosci.,* **2019**, 11, 98.

107. Nascimento, D.C.; Navalta, J.W.; Durigan, J.L.Q.; Marqueti, R.C.; Tibana, R.A.; Franco, O.L.; Almeida, J.A.; Camarço, N.F.; Neto, I.V.S.; Prestes, J. Acute eccentric resistance exercise decreases matrix metalloproteinase activity in obese ederly women. *Clin Physiol Funct Imaging*, **2016**, 36, 139-145

108. Gmiat, A.; Micielska, K.; Kozlowska, M.; Flis, D.J.; Smaruj, M.; Kujach, S.; Jaworska, J.; Lipinska, P.; Ziemann, E. The impact of a single bout of high intensity circuit training on myokines cencentrations and cognitive functions in women of different age. *Physiology & Behavior,* **2017**, 179, 290-297.

109. Abd El-Kader, S.M.; Al-Jiffri, O.H.; Aerobic exercise modulates cytokine pro le and sleep quality in elderly.. *Afri Health Sci*, **2019**, 19(2), 2198-2207.

110. Ihalainen, J.K.; Inglis, A.; Mäkinen, T.; Newton, R.U.; Kainulainen, H.; Kyröläinen, H.; Walker, S. Strength Training Improves Metabolic Health Markers in Older Individual Regardless of Training Frequency. *Front. Physiol.,* **2019**, 10:32.

111. Cao Dinh, H.; Njemini, R.; Onyema, O.O.; Beyer, I.; Liberman, K.; De Ddobbeleer, L.; Renmans, W.; Vander Meeren, S.; Jochmans, K.; Delaere, A.; Knoop, V.; Bautmans; I. Strength Endurance Training but Not Intensive Strength Training Reduces Senescence-Prone T Cells in Peripheral Blood in Community-Dwelling Elderly Women**.** *J Gerontol A Biol Sci Med Sci.,* **2019**, 13, 74(12), 1870-1878.

112. Cunha, P.M.; Ribeiro, A.S.; Nunes, J.P.; Tomeleri, C.M.; Nascimento, M.A.; Moraes, G.K.; Junior, P.S.; Barbosa, D.S.; Venturini, D.; Cyrino, E.S. Resistance training performed with single-set is sufficient to reduce cardiovascular risk factors in untrained older women: The randomized clinical trial. Active Aging Longitudinal Study. *Archives of Gerontology and Geriatrics*, **2019**, 81, 171-175.

113. Alikhani, S.; Sheikholeslami-Vatani, D. Oxidative stress and anti-oxidant responses to regular resistance training in young and older adult women. *Geriatr Gerontol Int,* **2019**, 19(5), 419-422.

114. Rodriguez-Miguelez, P.; Fernandez-Gonzalo, R.; Almar, M.; Mejias, Y.; Rivas, A.; de Paz, J.A.; Cuevas, M.J.; Gonzalez-Gallego, J. Role of Toll-like receptor 2 and 4 signaling pathways on the inflammatory response to resistance training in elderly subjects. *Age (Dordr.),* **2014**, 36, 9734.

115. Strandberg, E.; Edholm, P.; Ponsot, E.; Wahlin-Larsson, B.; Hellmen, E.; Nilsson, A.; Engfeldt, P.; Cederholm, T.; Riserus, U.; Kadi, F. Influence of combined resistance

training and healthy diet on muscle mass in healthy elderly women: a randomized controlled trial. *J. Appl. Physiol*., **2015**, 119, 918–925.

116. Hagstrom, A.D.; Marshall, P.W.; Lonsdale, C.; Papalia, S.; Cheema, B.S.; Toben, C.; Baune, B.T.; Fiatarone Singh, M.A.; Green, S. 2016. The effect of resistance training on markers of immune function and inflammation in previously sedentary women re- covering from breast cancer: a randomized controlled trial. *Breast Cancer Res. Treat.*, **2016**,155, 471–482.

117. Nunes, P.R.; Barcelos, L.C.; Oliveira, A.A.; Furlanetto Junior, R.; Martins, F.M.; Orsatti, C.L.; Resende, E.A.; Orsatti, F.L. Effect of resistance training on muscular strength and indicators of abdominal adiposity, metabolic risk, and inflammation in postmenopausal women: controlled and randomized clinical trial of efficacy of training volume. *Age (Dordr.),* **2016**, 38, 40.

118. Tomeleri, C.M.; Souza, M.F.; Burini, R.C.; Cavaglieri, C.R.; Ribeiro, A.S.; Antunes, M.; Nunes, J.P.; Venturini, D.; Barbosa, D.S.; Sardinha, L.B.; Cyrino, E.S. Resistance training reduces metabolic syndrome and inflammatory markers in older women: a randomized controlled trial. *J. Diabetes*, **2018**, 10, 328–337.

119. Ogawa, K.; Sanada, K.; Machida, S.; Okutsu, M.; Suzuki, K. Resistance Exercise Training-Induced. Muscle Hypertrophy Was Associated with Reduction of Inflammatory Markers in Elderly Women**.** *Mediators of Inflammation***, 2010**, ID 171023.

120. Marques, E.A.; Mota, J.; Viana, J.L.; Tuna, D.; Figuereido, P.; Guimaraes, J.T.; Carvalho, J. Response of bone mineral density, inflammatory cytokines, and biochemical bone markers to a 32-week combined loading exercise programme in older men and women. *Archives of Gerontology and Geriatrics*, **2013**, 57, 226–233.

121. Nybo, L.; Moller, K.; Pedersen, B.K.; Nielsen, B.; Secher, N.H. Association between fatigue and failure to preserve cerebral energy turnover during prolonged exercise. *Acta Physiol. Scand*. **2003**. 179, 67–74.

122. Agho, E.T.; Owotade, F.J.; Kolawole, B.A.; Elijah Olufemi Oyetola, E.O.; Adedeji, T.A. Salivary in ammatory biomarkers and glycated haemoglobin among patients with type 2 diabetic mellitus. *BMC Oral Health.* **2021**. 21:101. https://doi.org/10.1186/s12903-021-01453-y.

123. Corey-Bloom, J.; Fischer, R. S.; Kim, A.; Snell, C.; Georgia M. Parkin, G. M.; Granger, D. A.; Granger, S. W.; Thomas, E. A. Levels of Interleukin-6 in Saliva, but Not Plasma, Correlate with Clinical Metrics in Huntington's Disease Patients and Healthy Control Subjects. *Int. J. Mol. Sci.* **2020**. *21*, 6363; doi:10.3390/ijms21176363.

124. Byrne, M.L.; O'Brien-Simpson, N.M.; Reynolds, E.C.; Walsh, K.A.; Laughton, K.; Waloszek, J.M.; Woods, M.J.; Trinder, J.; Allen, N.B. Acute phase protein and cytokine levels in serum and saliva: A comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain Behav. Immun.* **2013**. *34*, 164–175. https://doi.org/10.1016/j.bbi.2013.08.010.

ANEXOS

6. ANEXOS

6.1. Artículo 1

diseases

MDPI

Review

The Effects of Exercise on IL-6 Levels and Cognitive Performance in Patients with Schizophrenia

Pablo Gómez-Rubio * and Isabel Trapero

Departamento de Enfermeria, Facultad de Enfermería y Podología, Univerdidad de Valencia, 46010 Valencia, Spain; Isabel.Trapero@uv.es

* Correspondence: pagorubio1@hotmail.com; Tel.: +34-606-784-540

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Abstract: Exercise plays an important role in brain plasticity, leading to improvements in cognitive function and delaying the cognitive deterioration of healthy people. These effects can be observed in individuals with schizophrenia through improvements in their performance in cognitive tasks and a decrease in the symptomology of the disease. In this review we examine the current evidence for the roles that exercise and the immune system play in patients with schizophrenia, and specifically analyze the interleukin-6 (IL-6) pathway as a potential mechanism resulting in these positive effects. Inflammation and high levels of IL-6 are associated with both the severity of schizophrenia and the cognitive impairment suffered throughout the disease. Performing regular exercise can modulate IL-6 by lowering its basal levels and by causing lower acute increases in the plasma levels of this cytokine in response to exercise (an anti-inflammatory response to physical exertion). Although there is evidence for the positive effects of physical exercise on schizophrenia, more studies will be required to better understand how variation in different exercise parameters affects both the acute and chronic plasma levels of IL-6.

Keywords: interleukin-6; schizophrenia; physical exercise; immune system

1. Introduction

Schizophrenia affects approximately 24 million people around the world and is considered a severe psychiatric disorder characterized by 'positive symptoms' (hallucinations and delusions), 'negative symptoms' (including avolition and anhedonia), and cognitive deficits (such as deficiencies in perception, memory, and attention) [1]. Despite extensive research on this disorder, standard treatments including medication and cognitive behavioral therapy have not been shown to be effective, mainly in relation to negative symptoms and cognitive deficits [2].

One of the reasons why current treatments are ineffective is that schizophrenia is still sometimes misunderstood because of the complexity of its pathogenesis [3]. The manifold interactions between the immune system and the nervous system could underlie the development of schizophrenia as part of a complex mechanism. Evidence suggests that an increasing level of a stressing hormone may activate the inflammatory arm of the immune system and, hence, trigger the expression of genes responsible to elicit a chronic and low-grade inflammation state [4]. The interest in immune/inflammatory changes and their associated oxidative consequences as a potential determinant/key in the pathophysiology of schizophrenia has been recently restored. The latest evidence supports a model which describes that the onset of oxidative stress stimuli and a consequent immune dysfunction may alter cellular homeostasis which, in turn, may determine an aberrant growth of interneurons and, consequently, a psychotic symptomatology [5]. Within this context, the possible relationship between a psychotic breakdown evolving in schizophrenia and inflammation proteins, such C-reactive protein (CRP), has been investigated [6]. CRP is thought to assist in complement binding to foreign and damaged

cells and to affect the humoral response to disease [7]. It is also believed to play an important role in innate immunity, as an early defence system against infections [8], but future research is needed to investigate the relationships between CRP levels and cytokines [9]. The current evidence, supported by genetic studies, relates this disorder to an imbalance in cytokines and, consequently, to dysregulated triggering of inflammatory processes, with IL-6 being at the center of many of these studies [10].

This new evidence regarding the possible relationships between the immune system and schizophrenia, together with the lack of efficacy of standard treatments, means that exercise is now being considered as a possible treatment for this disease. This is because, when combined with the adequate prescription of other treatments, it has beneficial effects on the immune system. In fact, some studies have already been published that show a reduction in the symptomology of patients with schizophrenia thanks to exercise interventions [11].

In this article we examine the relationships between schizophrenia, exercise, and the immune system, and specifically look at the current evidence regarding IL-6 as one of the potential mechanisms through which exercise could produce improvements in the symptoms of schizophrenia.

Our search strategy conducted an electronic database search of CINAHL, Scopus, Web of Science, MEDLINE, Embase, the Cochrane Library, and PubMed from inception on 10 May 2018. The keyword search terms used were as follows: 'schizophrenia' and 'exercise' or 'physical activity' and 'cognitive' or 'cognition' and 'IL-6′. As we did not find enough information, we carried out two more searches. First of all we used the following keyword search terms: 'schizophrenia' and 'exercise' or 'physical activity' and 'IL-6′. Secondly, we used the following keyword search terms: 'schizophrenia' and 'cognitive' or 'cognition' and 'IL-6′. Google Scholar and the reference lists of retrieved articles were also searched in order to identify any additional relevant publications.

2. Schizophrenia and Exercise

2.1. Exercise in Relation to Cognition

There is already a lot of evidence to suggest that exercise affects brain plasticity by inducing neurogenesis and causing structural changes [12]. Exercise plays an important role in the metabolic, structural, and functional alteration of brain connections, and is an important factor in brain plasticity that can be protective against the cognitive decline consequent to aging [13]. These effects are reflected in cognitive functioning at the cerebral level, both in the improvement of different cognitive functions and in the prevention of the cognitive deterioration that occurs because of aging (Figure 1).

Improvements in cognitive functions have mainly been observed in terms of memory (implicit memory tasks, immediate memory, and recognition), attention processes, and in executive functions [14]. Better performance in verbal, perceptual, and arithmetic tasks has also been shown in children who regularly perform exercise compared with sedentary children [15].

As already mentioned, numerous studies have shown that exercise prevents the cognitive decline related to age [16], reduces the risk of developing dementia, and slows the decline in executive-function impairment [17]. The neuroprotective effect induced by exercise has also been observed in people with dementia and cognitive disability [18], reducing their symptoms and improving cognitive functions in both young people and adults [19].

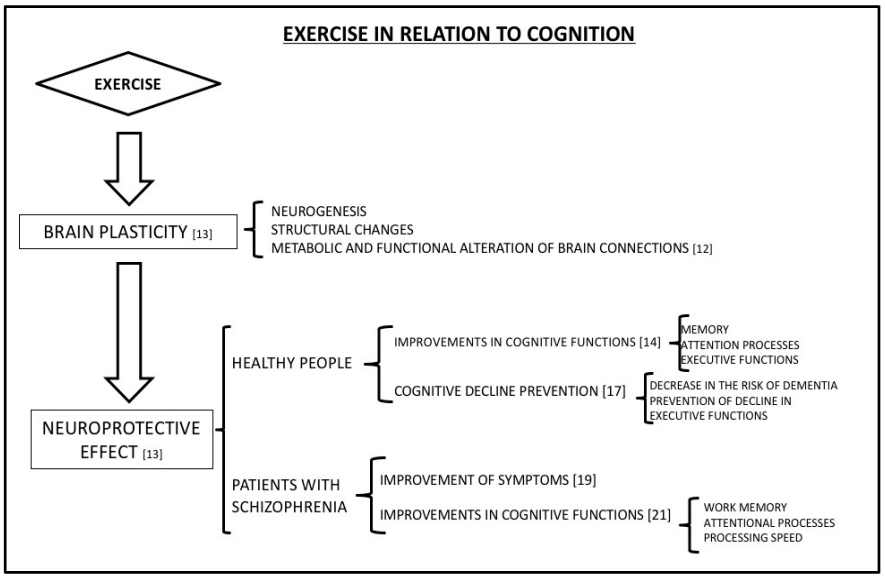

Figure 1. Exercise has a potential role in brain plasticity that leads to a neuroprotective effect in healthy people and in patients with schizophrenia. This neuroprotective effect has positive consequences in the improvement of cognitive functions, in the delay of cognitive deterioration, and in the improvement of the symptomatology of schizophrenia.

2.2. Schizophrenia and Exercise

In relation to schizophrenia, current studies indicate that exercise can reduce psychiatric symptoms and improve cognitive functions in different subdomains, as reported in the European Psychiatric Association (EPA) guidance [20]. The benefits of exercise for treating this disorder are related to its effects at the level of the brain, and the protective effect produced in the hippocampus by exercise are similar both in patients with schizophrenia and in healthy adults [11] (Figure 1). Several different studies have observed that these changes caused by exercise at the level of the brain improve both the positive and negative symptoms of schizophrenia. The reduction in the incidence of negative symptoms is especially relevant because standard medication is less effective at treating them [19].

Furthermore, there is evidence that exercise also improves performance in tasks involving working memory, processing speed and attentional processes [21]. Specifically, undertaking light physical activity positively correlated with cognitive performance, while moderate and vigorous physical activity was associated with an improvement in the symptoms of schizophrenia [22]. The exact mechanism or mechanisms that underlie this exercise-mediated cognitive improvement are currently unknown, but several studies have indicated that the immune system as is likely involved.

3. Schizophrenia, Exercise and the Immune System

3.1. The Immune System in Relation to Cognition

Several studies have found that the immune system is involved in the homeostatic modulation of neurogenesis [23], observing that, among other mechanisms, stimulation of peripheral-level cytokines alters several key neurotransmitters that optimize neurogenesis [24]. The positive effect that the immune system has in this context is conditioned by complex interactions between the brain cells that perform immune functions and neurons via neurotransmitters and pro- and anti-inflammatory cytokines [25].

Thus, these improvements resulted in gains in learning and memory processes [26] and produced an inverse association between inflammatory processes and performance in spatial reasoning, short-term memory, verbal learning, and executive-function tests [27]. Many studies point out that

an adequate balance between pro- and anti-inflammatory cytokines underlies these improvements in cognitive processes by modulating and consolidating the reorganization of neural networks [25].

Nevertheless, it has also been shown that cytokine levels and the balance between pro- and anti-inflammatory cytokines vary over an individual's lifetime [28], suggesting that production of high amounts of inflammatory cytokines and a decrease in anti-inflammatory levels increase the central inflammatory response, damaging synaptic plasticity [13] and consequently also augmenting age-associated worsening of cognitive performance. Thus, certain inflammatory biomarkers are already used to predict cognitive decline [28].

3.2. The Immune System and Schizophrenia

Inflammation is currently considered a potential mechanism in the development and progression of schizophrenia [29]. Patients with schizophrenia have increased levels of inflammatory cytokine markers and, more specifically, this increase may be related to the negative symptoms of the disease [30]. Therefore, an inadequate balance between pro- and anti-inflammatory cytokines would cause immune system dysfunction, impairing neurodevelopment and deregulating brain neurotransmission in patients with schizophrenia [10].

3.3. The Immune System and Exercise

Maintaining a good balance between pro- and anti-inflammatory cytokines could be one of the mechanisms through which exercise exerts its immunoprotective and immunoregulatory effects [31]. Exercise, as a stressor, induces different immune responses associated with the production of interleukins, thus eliciting an anti-inflammatory response through inflammation [32]. There is ample evidence that exercise is associated with a systemic reduction in inflammation [14].

Additionally, new genetic studies suggest that exercise may activate genes involved in the production of leukocytes as well as those that downregulate inflammation. Although this mechanism is not fully understood, studies are beginning to appear that indicate that exercise parameters such as intensity and duration are important factors in sufficiently regulating cytokines to induce this protective anti-inflammatory response both in healthy individuals and in those with different pathologies [33]. However, the interaction of multiple factors in the immune system mean that doubts remain about the exact mechanisms which interrelate it with schizophrenia and exercise, although recent work has emerged highlighting an important role for IL-6 in this context.

4. Schizophrenia, Exercise and IL-6

4.1. IL-6 and Cognition

Numerous studies have published evidence regarding the role of IL-6 in the inflammatory processes which interconnect the immune system with the central nervous system (CNS). IL-6 levels inversely correlate with hippocampal gray matter volume [34] as well as with the integrity of the white matter in the brain [27]. One of the mechanisms by which the immune system influences cognitive function performance, neurodegeneration, and cognitive functionality, involves interactions between IL-6 and the CNS (Figure 2).

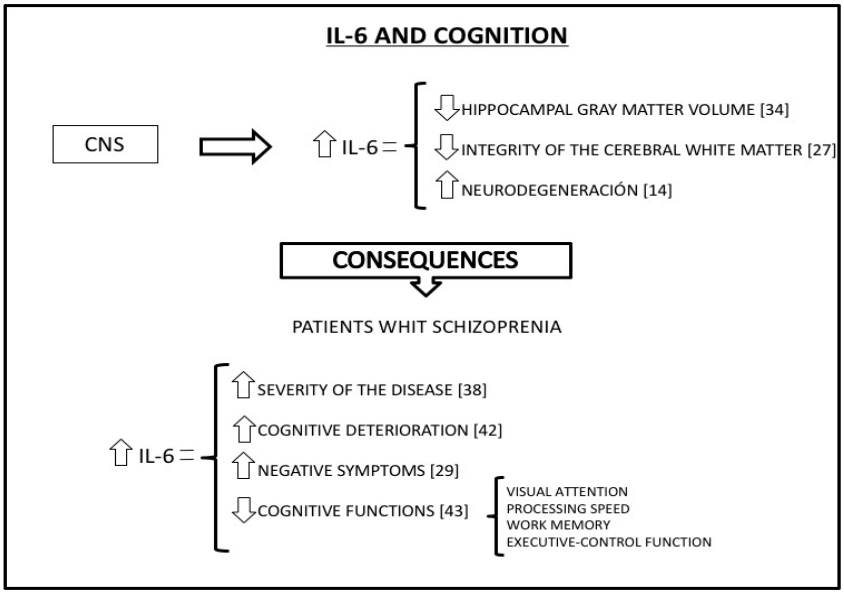

Figure 2. IL-6 has numerous effects in the central nervous system (CNS). These effects have different consequences in the patients with schizophrenia.

Regarding cognitive function, there is evidence that high levels of peripheral IL-6 negatively affects memory and learning [34], is associated with generally poor cognitive performance [35], and that cognitive-task performance is lower in acute inflammatory processes involving increased levels of IL-6 [36]. It is also important that the peripheral inflammatory mediators such as IL-6 can cross the blood–brain barrier and modulate central inflammatory processes, leading to increased neurodegeneration and negatively influencing cognitive function [27]. Specifically, the presence of high basal IL-6 levels increases the risk of future cognitive age-related decline [37,38]. Increased IL-6 has also been observed in dementia and other diseases characterized by cognitive decline, as well as in depressive disorders [39]. Thus, presence of low levels of IL-6 may reduce the risk of developing neurodegenerative diseases [14].

However, even though most studies discuss the negative effects of elevated IL-6 levels, there is evidence that moderate levels of IL-6 may also have an anti-inflammatory and protective effect [40].

4.2. Il-6 and Schizophrenia

There is a lot of evidence in the literature regarding immune system deregulation and the presence of high levels of pro-inflammatory cytokines such as IL-6 in patients with schizophrenia [41]. Moreover, high levels of IL-6 are associated with the altered brain morphology and the severity of the disease [38] as well as with the continuing cognitive deterioration [42] and decline in visual attention, processing speed, working memory, and executive-control function [43] seen as schizophrenia progresses (Figure 2). Looking more specifically at the different symptoms of schizophrenia, it appears that high levels of inflammation and IL-6 are associated with the disease's negative symptoms [29].

4.3. Il-6 and Exercise

Exercise induces an increase in the plasma levels of different cytokines, including IL-6 [44]. In the year 2000, Steenberg et al. published the first study to show that the skeletal-muscle activity connected with exercise increases plasmatic IL-6 levels [45]. Following studies showed that the magnitude of this muscle-fiber derived plasma IL-6 elevation caused by exercise correlates with different parameters such as the intensity and duration of the physical activity [46].

In addition to the acute effect that exercise has on the increase of plasma IL-6 levels, different studies have also shown that over time, following a protocol of prolonged exercise, causes baseline

IL-6 levels to decrease [41]. This reduction is also related to the intensity and duration of physical exercise as well as other variables such as the fitness levels of the study participants [47]. People who perform regular exercise in their daily lives show lower baseline IL-6 levels compared to those who are sedentary [45].

Although, as we have already mentioned, most studies associate IL-6 with proinflammatory responses, numerous studies have also observed that the acute increase in IL-6 levels caused by exercise gives rise to an anti-inflammatory response by inhibiting, for example, the activation of tumor necrosis factor alpha (TNFα) whose activity is highly proinflammatory [40] and by stimulating the production of other predominantly anti-inflammatory immune system molecules [48].

5. Discussion

The evidence available in the scientific literature strongly corroborates that exercise has numerous beneficial effects on the CNS; it exerts a neuroprotective effect, delays and slows the decline in cognitive performance produced by age, and improves and optimizes different cognitive functions such as learning, memory, and executive functions [12]. As set out by the EPA guide [20], the benefits related to exercise seen in healthy people are also observed in patients with schizophrenia, both in terms of symptomatology and in improvements in cognitive function.

Nonetheless, many unresolved questions remain about the specific mechanisms through which exercise mediates these beneficial effects on the cognitive system, both generally in healthy people [29] and especially, in patients with schizophrenia [49]. However, the evidence suggesting that the immune system plays a predominant role in these effects is mounting. Numerous studies have revealed that exercise has an anti-inflammatory effect that positively affects neuroplasticity [13]. Related to exercise, several studies have revealed that pro- and anti-inflammatory cytokines must be properly balanced so that the immune system can exert positive or negative effects on brain neuroplasticity, to modulate and consolidate the reorganization of neural networks, and the benefits that exercise has on schizophrenia are because it mediates cerebral structural changes via the immune system [25].

When pro- and anti-inflammatory cytokines are adequately balanced, the cytokine IL-6 appears to play a significant role in both pro- and anti-inflammatory responses, depending on the levels of its expression [40]. Moreover, IL-6 can cross the blood–brain barrier where it can modulate central inflammatory processes and, if its levels are too high, it results in increased neurodegeneration and negatively influences cognitive function [27]. Furthermore, recently-published reviews indicate that high levels of inflammation and IL-6 are related to the negative symptoms of schizophrenia [29].

We now know that the activity generated in the skeletal muscles by exercise is one of the main mechanisms by which the plasma and baseline levels of IL-6 can be modulated [44]. Although there are doubts about the exact mechanism through which this modulation occurs, several studies have shown that as a consequence of exercise there is an acute increase in the plasma levels of IL-6 [40]. This increase occurs as a result of the muscle damage caused by exercise and depends on the different parameters of the exercise performed, including its intensity, duration, and the types of muscle contraction involved, with higher levels of IL-6 resulting from exercise which causes homeostasis to destabilize [50].

Conversely, regularly performing exercise reduces the peak of these increases in IL-6 levels, thus attenuating this acute effect. This means that the final increases in plasma IL-6 levels are a function of the fitness status of each person and their individual level of adaptation to exercise [50]. This decreased IL-6 plasma level peak, seen after only one exercise session in individuals who regularly do exercise, is also observed in the basal levels of IL-6, so that people who regularly exercise have lower baseline levels than those who are sedentary [44].

Therefore, the acute response to a single dose of exercise leads to an increase in plasma levels of IL-6, but the chronic response to regular exercise produces both a minor increase in this acute response and a decrease of the basal levels of IL-6. Regarding the effects of increased levels of IL-6, although most studies refer to its negative effects [39] and its pro-inflammatory properties, several studies also suggest that the moderate increases in IL-6 levels provoked by exercise could lead to

an anti-inflammatory response by inhibiting the production of other pro-inflammatory cytokines such as IL-1b and TNFα, reducing levels of inflammation and promoting a neuroprotective effect [46].

Considering that IL-6 plays a major role in the development and advancement of schizophrenia [29], it is possible that the behavior of IL-6 levels in response to exercise may explain its beneficial effects on this disease which are evident in many studies. These include improved performance in tasks involving working memory, processing speed, and attentional processes [21], as well as a protective effect on brain plasticity resulting in improvements in the symptomatology of schizophrenia [11].

As a stressor, physical exercise would initially cause an acute response leading to an increase in plasma levels of IL-6. Although this may initially be counterproductive in people with schizophrenia (who usually have high IL-6 and inflammation levels), with regular exercise (and the resulting increased adaptative fitness) a chronic response could be achieved. As a result of this chronic response, both the basal IL-6 levels and the peak levels of IL-6 produced during acute responses would decrease in these patients (Figure 3). This double-chronic response would favor both an anti-inflammatory response and a decrease in basal IL-6 levels. This would systemically reduce the state of inflammation which characterizes schizophrenia and favor the exercise-mediated neuroprotective effect on the brain which produces the improvements in cognitive function and symptomatology previously described [11].

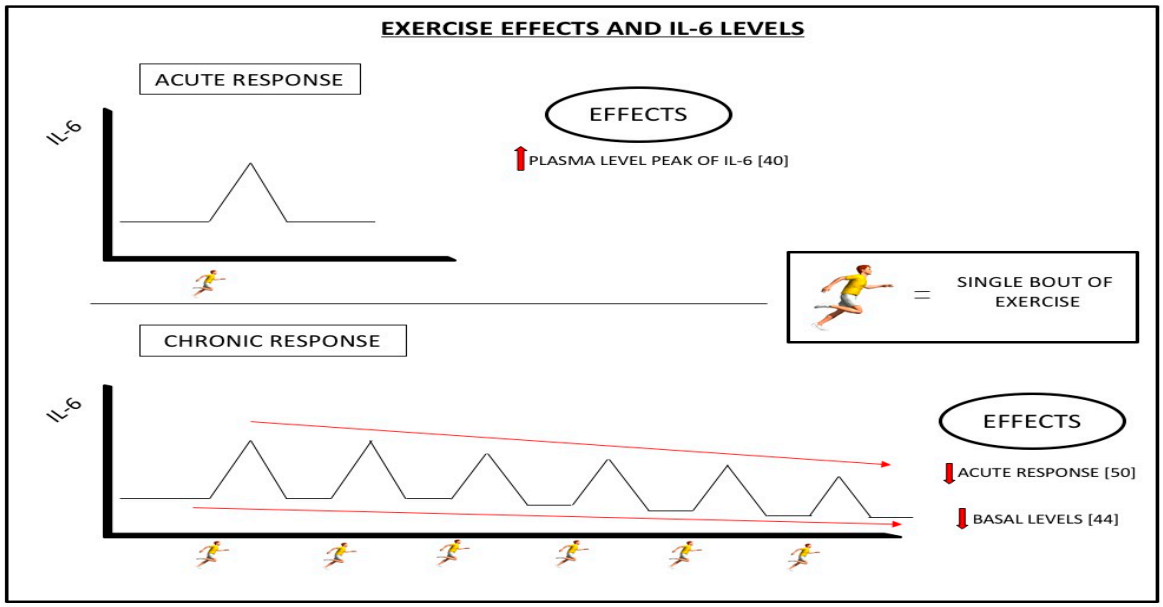

Figure 3. A single bout of exercise causes an increase in plasma levels of IL-6 (ACUTE RESPONSE). However, the regular practice of exercise reduces the acute response to exercise and decreases the basal levels of IL-6 (CHRONIC RESPONSE).

Regarding the most appropriate type of exercise, it is still unknown how the different changes in the parameters of the exercise (intensity, duration, density...) specifically affect the levels of IL-6 [13]. Despite this ignorance, recent studies have observed that high-intensity exercise (anaerobic exercise or strength exercise) causes a greater increase in IL-6 levels than low intensity exercise [47], and that eccentric exercise stimulates a greater production of IL-6 than concentric exercise [40].

Currently, most studies use aerobic exercise protocols or concurrent protocols (protocols in which both aerobic and anaerobic exercises are used) [13]. The problem with concurrent protocols is that we cannot know if the effects on IL-6 are due to aerobic exercise or anaerobic exercise.

Because the increase in levels of IL-6 is related to the amount of muscle damage caused by exercise and also because patients with schizophrenia have a state of permanent inflammation, we should stimulate the production of IL-6 progressively with the aim that the consequences of the acute response are not negative.

Therefore, we should start with an aerobic exercise protocol that causes the least possible muscle damage and, consequently, a controlled increase in the levels of IL-6. When patients with schizophrenia adapt to aerobic exercise protocols, we should progressively change to anaerobic exercise protocols (or strength exercise protocols), first with concentric exercise and later with eccentric exercise. With this progression we will achieve that the protocols always provoke a stimulation of IL-6.

6. Conclusions

There is now ample evidence for the positive effects that exercise has on schizophrenia. The important role that IL-6 plays in the development and symptomatology of schizophrenia, as well as how exercise can modulate the IL-6 response, is also starting to be understood. Therefore, given the information reviewed here, encouraging the implementation of a regular exercise program with the appropriate training parameters (regarding intensity, duration, type of muscle contractions, etc.) as a treatment for schizophrenia could help to modulate IL-6 levels in these patients, resulting in positive effects by improving both cognitive function and symptomatology owing to cerebral structural changes. Influencing the modulation of IL-6 levels through exercise as a treatment in people with schizophrenia is especially relevant not only because it is an economical and accessible treatment option, but also because standard medication treatments have not so far achieved good results for the negative symptoms of the disease—an outcome which is partially achievable through such exercise programs. More studies will be required to help us understand how the variation in the different exercise parameters affects both the acute and chronic plasma levels of IL-6.

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References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disoreders, Fourth Edition(DSM-IV)*; American Psychiatric Organisation: Washington, DC, USA, 1994.
2. Nielsen, R.E.; Levander, S.; Kjaersdam Telléus, G.; Jensen, S.O.W.; Østergaard Christensen, T.; Leucht, S. Second-generation antipsychotic effect on cognition in patients with schizophrenia-a meta-analysis of randomized clinical trials. *Acta Psychiatr. Scand.* **2015**, *131*, 185–196. [CrossRef] [PubMed]
3. Van Kesteren, C.F.M.G.; Gremmels, H.; de Witte, L.D.; Hol, E.M.; Van Gool, A.R.; Falkai, P.G.; Kahn, R.S.; Sommer, I.E.C. Immune involvement in the pathogenesis of schizophrenia: A meta-analysis on postmortem brain studies. *Transl. Psychiatry* **2017**, *7*, e1075. [CrossRef] [PubMed]
4. Singh, B.; Chaudhuri, T.K. Role of C-reactive protein in schizo- phrenia: An overview. *Psychiatry Res.* **2014**, *216*, 277–285. [CrossRef] [PubMed]
5. Barron, H.; Hafizi, S.; Andreazza, A.C.; Mizrahi, R. Neuroinflam- mation and oxidative stress in psychosis and psychosis risk. *Int. J. Mol. Sci.* **2017**, *18*, 651. [CrossRef] [PubMed]
6. Orsolini, L.; Sarchione, F.; Vellante, F.; Fornaro, M.; Matarazzo, I.; Martinotti, G.; Valchera, A.; Di Nicola, M.; Carano, A.; Di Giannantonio, M.; et al. Protein-C reactive as biomarker predictor of schizophrenia phases of illness? A systematic review. *Curr. Neuropharmacol.* **2018**, *16*, 583–606. [CrossRef] [PubMed]
7. Licastro, F.; Chiappelli, M.; Ruscica, M.; Carnelli, V.; Corsi, M.M. Altered cytokine and acute phase response protein levels in the blood of children with Downs syndrome: Relationship with dementia of Alzheimer's type. *Int. J. Immunopathol. Pharmacol.* **2005**, *18*, 165–172. [CrossRef]
8. Hatzistilianou, M.; Hitoglou, S.; Gougoustamou, D.; Rekliti, A.; Tzouvelekis, G.; Nanas, C.; Catriu, D.; Kotsis, O. Serum procalcitonin, adenosine deaminase and its isoenzymes in the aetiological diagnosis of pneumonia in children. *Int. J. Immunopathol. Pharmacol.* **2002**, *15*, 119–127. [CrossRef]

9. De Berardis, D.; Campanella, D.; Gambi, F.; La Rovere, R.; Carano, A.; Conti, C.M.; Silvestrini, C.; Serroni, N.; Piersanti, D.; Di Giuseppe, B.; et al. The Role of C-Reactive Protein in Mood Disorders. *Int. J. Immunopathol. Pharmacol.* **2006**, *19*, 721–725. [CrossRef]
10. Rodrigues-Amorim, D.; Rivera-Baltanás, T.; Spuch, C.; Caruncho, H.J.; González-Fernandez, Á.; Olivares, J.M.; Agís-Balboa, R.C. Cytokines dysregulation in schizophrenia: A systematic review of psychoneuroimmune relationship. *Schizophr. Res.* **2018**, *197*, 19–33. [CrossRef]
11. Van der Stouwe, E.C.D.; Van Busschbach, J.T.; de Vries, B.; Cahn, W.; Aleman, A.; Pijnenborg, G.H.M. Neural correlates of exercise training in individuals with schizophrenia and in healthy individuals_ A systematic review. *Neuroimage Clin.* **2018**, *19*, 287–301. [CrossRef]
12. Mandolesi, L.; Polverino, A.; Montuori, S.; Foti, F.; Ferraioli, G.; Sorrentino, P.; Sorrentino, G. Effects of Physical Exercise on Cognitive Functioning and Wellbeing: Biological and Psychological Benefits. *Front. Psychol.* **2018**, *9*, 509. [CrossRef] [PubMed]
13. Di Benedetto, S.; Müller, L.; Wenger, E.; Düzel, S.; Pawelec, G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neurosci. Biobehav. Rev.* **2017**, *75*, 114–128. [CrossRef] [PubMed]
14. Thielen, J.-W.; Kärgel, C.; Müller, B.W.; Rasche, I.; Genius, J.; Bus, B.; Maderwald, S.; Norris, D.G.; Wiltfang, J.; Tendolkar, I. Aerobic Activity in the Healthy Elderly Is Associated with Larger Plasticity in Memory Related Brain Structures and Lower Systemic Inflammation. *Front. Aging Neurosci.* **2016**, *8*, 319. [CrossRef] [PubMed]
15. Ostermann, S.; Herbsleb, M.; Schulz, S.; Donath, L.; Berger, S.; Eisenträger, D.; Siebert, T.; Müller, H.-J.; Puta, C.; Voss, A.; et al. Exercise Reveals the Interrelation of Physical Fitness, In ammatory Response, Psychopathology, and Autonomic Function in Patients With Schizophrenia. *Schizophr. Bull.* **2018**, *39*, 1139–1149. [CrossRef] [PubMed]
16. Niemann, C.; Godde, B.; Staudinger, U.M.; Voelcker-Rehage, C. Exercise-induced changes in basal ganglia volume and cognition in older adults. *Neuroscience* **2014**, *281*, 147–163. [CrossRef]
17. Hollamby, A.; Davelaar, E.J.; Cadar, D. Increased physical fitness is associated with higher executive functioning in people with dementia. *Front. Public Heal.* **2017**, *5*, 346. [CrossRef] [PubMed]
18. Pedersen, B.K.; Febbraio, M. Muscle-derived interleukin-6—A possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain Behav. Immun.* **2005**, *19*, 371–376. [CrossRef]
19. Dauwan, M.; Begemann, M.J.H.; Heringa, S.M.; Sommer, I.E. Exercise Improves Clinical Symptoms, Quality of Life, Global Functioning, and Depression in Schizophrenia: A Systematic Review and Meta-analysis. *Schizophr. Bull.* **2016**, *42*, 588–599. [CrossRef]
20. Stubbs, B.; Vancampfort, D.; Hallgren, M.; Firth, J.; Veronese, N.; Solmi, M.; Brand, S.; Cordes, J.; Malchow, B.; Gerber, M.; et al. EPA guidance on physical activity as a treatment for severe mental illness: A meta-review of the evidence and Position Statement from the European Psychiatric Association (EPA), supported by the International Organization of Physical Therapists in Mental Health (IOPTMH). *Eur. Psychiatry* **2018**, *54*, 124–144. [CrossRef]
21. Firth, J.; Cotter, J.; Elliott, R.; French, P.; Yung, A.R. A systematic review and meta-analysis of exercise interventions in schizophrenia patients. *Psychol. Med.* **2015**, *45*, 1343–1361. [CrossRef]
22. Hidese, S.; Matsuo, J.; Ishida, I.; Hiraishi, M.; Teraishi, T.; Ota, M.; Hattori, K.; Kunugi, H. Relationship of Handgrip Strength and Body Mass Index With Cognitive Function in Patients With Schizophrenia. *Front. Psychiatry* **2018**, *9*, 156. [CrossRef] [PubMed]
23. Leiter, O.; Kempermann, G.; Walker, T.L. A common language: How neuroimmunological cross talk regulates adult hippocampal neurogenesis. *Stem Cells Int.* **2016**, *2016*, 1681590. [CrossRef] [PubMed]
24. Hasegawa-Ishii, S.; Inaba, M.; Umegaki, H.; Unno, K.; Wakabayashi, K.; Shimada, A. Endotoxemia-induced cytokine-mediated responses of hippocampal astrocytes transmitted by cells of the brain-immune interface. *Sci. Rep.* **2016**, *6*, 25457. [CrossRef] [PubMed]
25. Levin, S.G.; Godukhin, O.V. Modulating Effect of Cytokines on Mechanisms of Synaptic Plasticity in the Brain. *Biochemistry* **2017**, *82*, 264–274. [CrossRef] [PubMed]
26. McAfoose, J.; Baune, B.T. Evidence for a cytokine model of cognitive function. *Neurosci. Biobehav. Rev.* **2009**, *33*, 355–366. [CrossRef] [PubMed]
27. Marsland, A.L.; Gianaros, P.J.; Kuan, D.C.-H.; Sheu, L.K.; Krajina, K.; Manuck, S.B. Brain Morphology Links Systemic Inflammation to Cognitive Function in Midlife Adults. *Brain Behav. Immun.* **2015**, *48*, 195–204. [CrossRef] [PubMed]

28. Scheinert, R.B.; Asokan, A.; Rani, A.; Kumar, A.; Foster, T.C.; Ormerod, B.K. Some hormone, cytokine and chemokine levels that change across lifespan vary by cognitive status in male Fischer 344 rats. *Brain Behav. Immun.* **2015**, *49*, 216–232. [CrossRef]
29. Goldsmith, D.R.; Haroon, E.; Miller, A.H.; Strauss, G.P.; Buckley, P.F.; Miller, B.J. TNF-α and IL-6 are associated with the deficit syndrome and negative symptoms in patients with chronic schizophrenia. *Schizophr. Res.* **2018**, *199*, 281–284. [CrossRef]
30. Noto, C.; Maes, M.; Ota, V.K.; Teixeira, A.L.; Bressan, R.A.; Gadelha, A.; Brietzke, E. High predictive value of immune-inflammatory biomarkers for schizophrenia diagnosis and association with treatment resistance. *World J. Biol. Psychiatry* **2015**, *16*, 422–429. [CrossRef]
31. van der Geest, K.S.M.; Wang, Q.; Eijsvogels, T.M.H.; Koenen, H.J.P.; Joosten, I.; Brouwer, E.; Hopman, M.T.E.; Jacobs, J.F.M.; Boots, A.M.H. Changes in peripheral immune cell numbers and functions in octogenarian walkers—An acute exercise study. *Immun. Ageing* **2017**, *14*, 5. [CrossRef]
32. Gjevestad, G.O.; Holven, K.B.; Ulven, S.M. Effects of Exercise on Gene Expression of Inflammatory Markers in Human Peripheral Blood Cells: A Systematic Review. *Curr. Cardiovasc. Risk Rep.* **2015**, *9*, 34. [CrossRef] [PubMed]
33. Liu, D.; Wang, R.; Grant, A.R.; Zhang, J.; Gordon, P.M.; Wei, Y.; Chen, P. Immune adaptation to chronic intense exercise training: New microarray evidence. *BMC Genom.* **2017**, *18*, 29. [CrossRef] [PubMed]
34. Kline, R.; Wong, E.; Haile, M.; Didehvar, S.; Farber, S.; Sacks, A.; Pirraglia, E.; de Leon, M.J.; Bekker, A. Peri-Operative Inflammatory Cytokines in Plasma of the Elderly Correlate in Prospective Study with Postoperative Changes in Cognitive Test Scores. *Int. J. Anesthesiol. Res.* **2016**, *4*, 313–321. [CrossRef] [PubMed]
35. Puzianowska-Kuźnicka, M.; Owczarz, M.; Wieczorowska-Tobis, K.; Nadrowski, P.; Chudek, J.; Slusarczyk, P.; Skalska, A.; Jonas, M.; Franek, E.; Mossakowska, M. Interleukin-6 and C-reactive protein, successful aging, and mortality: The PolSenior study. *Immun. Ageing* **2016**, *13*, 21. [CrossRef] [PubMed]
36. Paine, N.J.; Bosch, J.A.; Ring, C.; Drayson, M.T.; van Zasten, J.J.C.S.V. Induced mild systemic inflammation is associated with impaired ability to improve cognitive task performance by practice. *Psychophysiology* **2015**, *52*, 333–341. [CrossRef] [PubMed]
37. Bradburn, S.; Sarginson, J.; Murgatroyd, C.A. Association of Peripheral Interleukin-6 with Global Cognitive Decline in Non-demented Adults: A Meta-Analysis of Prospective Studies. *Front. Aging Neurosci.* **2018**, *9*, 438. [CrossRef] [PubMed]
38. Shivakumar, V.; Debnath, M.; Venugopal, D.; Rajasekaran, A.; Kalmady, S.V.; Subbanna, M.; Narayanaswamy, J.C.; Amaresha, A.C.; Venkatasubramanian, G. Influence of correlation between HLA-G polymorphism and Interleukin-6 (IL6) gene expression on the risk of schizophrenia. *Cytokine* **2018**, *107*, 59–64. [CrossRef] [PubMed]
39. Gruol, D.L. IL-6 regulation of synaptic function in the CNS. *Neuropharmacology* **2015**, *96*, 42–54. [CrossRef] [PubMed]
40. Philippe, M.; Krüsmann, P.J.; Mersa, L.; Eder, E.M.; Gatterer, H.; Melmer, A.; Ebenbichler, C.; Burtscher, M. Acute effects of concentric and eccentric exercise on glucose metabolism and interleukin-6 concentration in healthy males. *Biol. Sport.* **2016**, *33*, 153–158. [CrossRef] [PubMed]
41. Lavratti, C.; Dorneles, G.; Pochmann, D.; Peres, A.; Bard, A.; de Lima Schipper, L.; Lago, P.D.; Wagner, L.C.; Elsner, V.R. Exercise-induced modulation of histone H4 acetylation status and cytokines levels in patients with schizophrenia. *Physiol. Behav.* **2017**, *168*, 84–90. [CrossRef] [PubMed]
42. Borovcanin, M.M.; Jovanovic, I.; Radosavljevic, G.; Pantic, J.; Minic Janicijevic, S.; Arsenijevic, N.; Lukic, M.L. Interleukin-6 in Schizophrenia—Is There a Therapeutic Relevance? *Front. Psychiatry* **2017**, *8*, 221. [CrossRef]
43. Misiak, B.A.; Czykiewicz, B.O.S.; Kotowicz, K.; Rybakowski, J.K.; Samochowiec, J.; Frydecka, D. Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. *Schizophr. Res.* **2018**, *192*, 16–29. [CrossRef]
44. Monteiro-Junior, R.S.; de Tarso Maciel-Pinheiro, P.; da Matta Mello, E.; da Silva, L.F.; Terra, R.; Carneiro, L.S.F.; Rodrigues, V.D.; Nascimento, O.J.M.; Deslandes, A.C.; Laks, J. Effect of Exercise on Inflammatory Profile of Older Persons: Systematic Review and Meta-Analyses. *J. Phys. Act. Health.* **2018**, *15*, 64–71. [CrossRef]
45. Kapilevich, L.V.; Zakharova, A.N.; Kabachkova, A.V.; Kironenko, T.A.; Orlov, S.N. Dynamic and Static Exercises Differentially Affect Plasma Cytokine Content in Elite Endurance- and Strength-Trained Athletes and Untrained Volunteers. *Front. Physiol.* **2017**, *8*, 35. [CrossRef]

46. Huh, J.Y. The role of exercise-induced myokines in regulating metabolism. *Arch. Pharmacal Res.* **2018**, *41*, 14–29. [CrossRef]
47. Cronin, O.; Keohane, D.M.; Molloy, M.G.; Shanahan, F. The effect of exercise interventions on inflammatory biomarkers in healthy, physically inactive subjects: A systematic review. *Int. J. Med.* **2017**, *110*, 629–637. [CrossRef]
48. Pruimboom, L.; Raison, C.L.; Muskiet, F.A.J. Physical Activity Protects the Human Brain against Metabolic Stress Induced by a Postprandial and Chronic Inflammation. *Behav. Neurol.* **2015**. [CrossRef]
49. Firth, J.; Cotter, J.; Carney, R.; Yung, A.R. The pro-cognitive mechanisms of physical exercise in people with schizophrenia. *Br. J. Pharmacol.* **2017**, *174*, 3161–3172. [CrossRef]
50. Reihmane, D.; Dela, F. Interleukin-6: Possible biological roles during exercise. *Eur. J. Sport Sci.* **2014**, *14*, 242–250. [CrossRef]

6.2. Artículo 2

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REVIEW ARTICLE

The Beneficial Effect of Physical Exercise on Inflammatory Makers in Older Individuals

Pablo Gómez-Rubio[1,*] and Isabel Trapero[1]

[1]*Department of Nursing, University of Valencia, Valencia, Spain*

Abstract: Old age is associated with a loss of motor functions and a general progressive decline in cognitive functions. Physical exercise is one of the ways in which inflammatory levels in general can be reduced, and therefore physical exercise can be considered a biological aging decelerator. In this article, we examine the relationships between physical exercise and inflammatory markers reported for the different physical exercise protocols that have been used in studies with older individuals, as well as the effects of these regimens. The different types of exercises programmed, and methods used to implement them were very heterogeneous in the articles we analysed. Both, the aerobic exercise and resistance training protocols produced a decrease in plasma levels of IL-6, CRP and TNF-α, and an increase of IL-10 plasma levels as a chronic effect. However, the acute-response of physical exercise appeared to be an initial increase in IL-6 expression and plasma IL-6 levels. Continuing with these exercise programs usually subsequently achieved a chronic response in which there was a decrease in both the basal levels of IL-6, CRP and TNF-α, and the IL-6 produced as acute responses. Regardless of the type of exercise performed, it seems that the exercise parameters, intensity, duration, subject variables, fitness, and level of inflammation are key factors in achieving the expected balance between pro-inflammatory and anti-inflammatory cytokines.

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1. INTRODUCTION

In addition to being associated with a considerable loss of motor functions, old age also produces a general and progressive decline in learning, memory, and cognitive functions [1, 2]. Numerous models have tried to analyse all the factors that lead to this cognitive and motor decline and have shown the importance of both extrinsic variables (psychological stress, pollution, levels of sedentary behaviour, *etc*.) and intrinsic variables (genetics and mail-to:author@institute.xxxneurotransmitter levels, among others) [3], in explaining neurodegeneration and the decline of cognitive functions that occur in old age [4]. One of the factors currently under study is the interaction between the immune system and inflammatory processes in the development of old age, also known as 'inflamm-aging' [5].

It has also been shown that the balance between pro- and anti-inflammatory cytokines, as well as their overall levels, vary over an individual's lifetime. Higher levels of inflammatory markers such as tumour necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and C-reactive protein (CRP) are found in older people [6] suggesting that decreases in anti-inflammatory cytokines coupled with high levels of inflammatory cytokines, increases the central inflammatory response, which in turn, damages synaptic plasticity [7] and worsens age-associated cognitive performance. In this sense, certain inflammatory biomarkers such as IL-6, TNF-*α*, and CRP are already used to predict cognitive decline [6]. Several diseases in this life stage change the immune system with consequent disturbance in cytokine homeostasis [8].

Cytokines are proteins that respond to pathogens with cells of innate and adaptive immunity and also act on inflammatory processes [9]. Among cytokines, most studies refer to IL-6, TNF-*α*, CRP and interleukin-10 (IL-10). TNF-*α* is the main mediator of the acute inflammatory response in which physiological function is stimulation of leukocytes, signalling to sites of inflammation and activating them in order to eradicate microorganisms and reduce inflammation [10]. IL-6 is synthesized from mononuclear phagocytes, vascular endothelial cells, fibroblasts, and adipose and muscle tissues and its function is the stimulation of neutrophil production by bone marrow progenitors, promoting the synthesis of proinflammatory cytokines and inhibiting TNF-*α*. Thus, the body returns to its resting state as the inflammatory process is eradicated [11]. CRP is a risk marker for cardiovascular disease, which also features an important function in the inflammatory process, especially in the acute phase, when CRP can increase up to 1000 times its normal

*Address correspondence to this author at the Department of Nursing, Faculty of Nursing, University of Valencia, C/ Jaume Roig s/n 46010 Valencia, Spain; Tel: (+34) 963983143; Fax: (+34) 963864310;
E-mail: pagorubio1@hotmail.com

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serum level. This protein is produced and released into the bloodstream by hepatocytes, but it can also be produced by arterial walls. Its synthesis is stimulated by IL-1 and IL-6, which are released by macrophages. Among several functions attributed to CRP, perhaps the most important is the synthesis of phagocytic components, which removes structures from blood circulation and induces an anti-inflammatory effect. These events promote tissue repair [12]. IL-10 is a key anti-inflammatory cytokine that acts on the inhibition of systemic inflammation and is also noted that it plays an important role in the inhibition of TNF-α production. Also, the TNF-α/IL-10 ratio is a good index that represents the imbalance between the pro and anti-inflammatory systems in humans [13].

The fact that IL-6 stimulates CRP synthesis and also inhibits TNF- α proinflammatory properties, may be one of the reasons why it has received special attention in the scientific literature.

Several studies have reported evidence for the role of IL-6 in the inflammatory processes through which the immune system intercommunicates with the central nervous system (CNS). It has been observed that IL-6 levels increase with age and that high levels are associated with the cognitive decline that occurs in old age [14-16]. In addition, IL-6 levels inversely correlate with hippocampal grey matter volume [17] as well as with the integrity of the brain white matter [18]. Interactions between IL-6 and the CNS are one of the mechanisms by which the immune system influences the performance of cognitive functions, and these also play a role in neurodegeneration and cognitive functionality.

On the one hand, there is evidence that high levels of peripheral IL-6 negatively affect memory and learning [17], is associated with a generally poor cognitive performance [19], and that cognitive task performance declines in acute inflammatory processes in the presence of high IL-6 levels [20]. On the other hand, IL-6 is a peripheral inflammatory mediator which can cross the blood–brain barrier to modulate central inflammatory processes, leading to increased neurodegeneration and poorer cognitive function [18]. Specifically, the risk of future cognitive age-related decline is accelerated when basal levels of IL-6 are high [21, 22]. Moreover, increased IL-6 levels have also been observed in diseases characterised by cognitive decline or dementia and in depressive disorders [23]. Thus, the risk of developing neurodegenerative diseases may be lower when less IL-6 is present [24].

Nonetheless, most studies associate IL-6 with proinflammatory responses and some authors have reported that the acute increases in IL-6 levels caused by physical exercise produce an anti-inflammatory response by inhibiting, for example, the activation of the highly proinflammatory tumour necrosis factor alpha (TNF-α) [25] while stimulating the production of other predominantly anti-inflammatory immune system molecules [26].

In aging, strategies to improve muscle and immune function are caloric restriction, antioxidant supplementation, and regular exercise training [27]. Engaging in physical exercise is one of the ways by which inflammatory markers levels in general, can be lowered [28-30] and thus, physical exercise can be considered a biological aging decelerator. Physical exercise in itself is a stressor, which produces inflammation and induces different immune responses associated with the production of interleukins, therefore inducing anti-inflammatory responses. Several lines of evidence have demonstrated that physical exercise is associated with a systemic reduction in inflammation [24]. Hence, maintaining a good balance between pro- and anti-inflammatory cytokines may be an important mechanism through which exercise exerts its immunoprotective and immunoregulatory effects. Additionally, new genetic studies suggest that these effects of exercise may activate both genes involved in the production of leukocytes as well as those that downregulate inflammation [31].

Although this mechanism is not yet fully understood, some work published in the academic literature indicates that exercise parameters such as intensity and duration are important factors in sufficiently regulating cytokines in order to provoke this protective anti-inflammatory response, both in healthy individuals and in those with different pathologies [31]. In this article, we examine the relationships between physical exercise and inflammatory makers levels described for the different physical exercise protocols that have already been tested in older people.

2. METHODS

We searched the CINAHL, Scopus, Web of Science, Embase, Cochrane Library, and PubMed electronic databases from December 2009 until 10 December 2019 using the following keyword search terms: ‘aging’ or 'older people' and ‘exercise’ or ‘physical activity’ and ‘inflammatory maker'. This produced insufficient information and so we also conducted four more searches using the specific inflammatory marker (TNF-α, CRP, IL-6 and IL-10) as the keyword search terms. Google Scholar and the reference lists of the articles retrieved using the previously described search strategy were also checked in order to identify any additional potentially relevant publications.

253 records were identified through database searching described above. However, with the aim of analysing articles in which aerobic exercise protocols as well as resistance exercise protocols in human had been described, excluding articles in animals, sample patients with pathologies and articles without description of the exercise protocol or in which a combination of protocols exercise are used, we analysed 24 studies (Fig. **1**)

3. RESULTS

Resistance exercise is distinct from aerobic exercise in that it focusses on repeatedly overloading muscles during static, isometric, or dynamic contractions, which causes the connections between the musculature and nervous system to become closer and stronger [32].

We found a wide variety of studies using different types of exercise programs and activity protocols. Some studies used aerobic exercise schedules designed to improve cardiorespiratory fitness by increasing the metabolic effort required to complete the exercise, thus requiring the participants to increase their breathing rate, heart rate, and blood

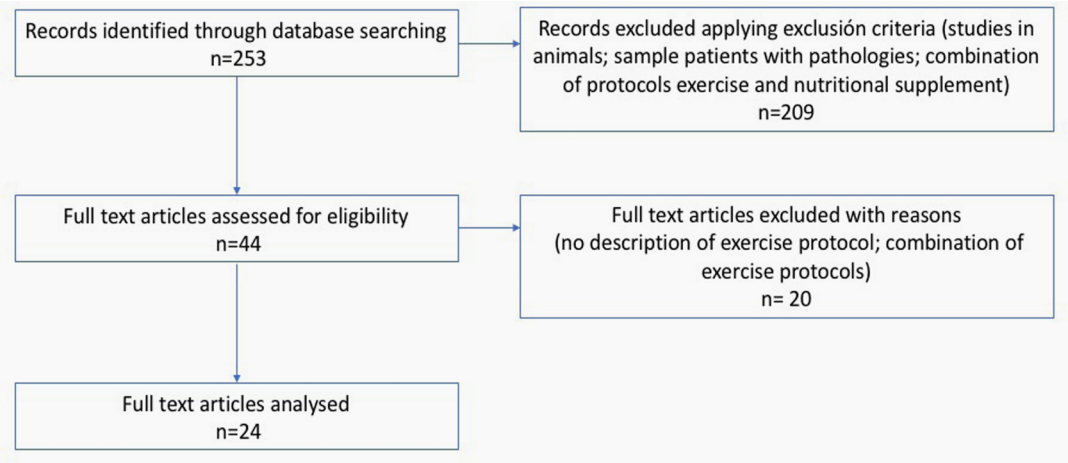

Fig. (1). Flow diagram, process of the review.

flow to match the intensity of the exercise. These aerobic exercise protocols involve activities that require using large muscle groups, such as in the rhythmic propulsion of body masses during movements at different intensities (*e.g.*, walking, jogging, or running) or through activities with a lower mechanical impact (*e.g.*, swimming or cycling). Other studies used resistance training protocols designed to improve muscle strength and endurance via specialised muscular conditioning methods; these normally involved some gym equipment such as free weights, weight machines, elastic bands, and/or body weight exercises.

Most studies analysed the effects of physical exercise on IL-6 in older people used aerobic exercise protocols [33-42]. However, these studies were heterogenous and included populations aged between 40 and 95 years, used different aerobic exercise protocols, lasted between 2 and 12 months, and were preformed between 2 and 5 times per week at an intensity of 45% to 80%. Nonetheless, the results obtained in these different studies usually showed that regular aerobic exercise decreased the presence of inflammatory markers such as IL-6, TNF-α and CRP, and increased Il-10. However, the results were very variable because of the variation in their protocols and inclusion criteria, as also described by other authors [43].

Interestingly, more physical exercise studies with a focus on strength training are now being carried out in populations of older individuals [44-57]. In terms of IL-6, the results from studies which implemented strength training protocols were more contradictory than those from aerobic exercise studies. Strength training tended to produce a lower chronic effect on IL-6, TNF-α, CRP and IL-10 while also resulting in an acute increase in IL-6 levels [56, 57]. However, strength training studies were limited by the fact that they included fewer participants (8 to 17 individuals) than the aerobic exercise studies and because their characteristics were also very heterogeneous, both in terms of the exercises implemented and their intensity, the muscle groups involved, the protocol duration (1–8 months), and exercise frequency (2–3 times per week).

Tables **1** and **2** include information about the author/s and year of publication of the analysed studies; the sample investigated, with details of age, type of exercise, protocol exercise, type of response; and finally, their results regarding inflammatory markers levels.

4. DISCUSSION

There is strong evidence in the scientific literature indicating that exercise has numerous beneficial effects on the CNS by exerting both neuroprotective effects as well as delaying and slowing the decline in cognitive performance produced by age. This data also suggests that engaging in physical exercise improves and optimises different cognitive functions such as learning, memory, and executive functions [58]. Exercise programs are shown to improve skeletal muscle mass and power, aerobic power, and functional capacity in the elderly, attenuating age-related physiological declines [59]. Furthermore, numerous studies have revealed that exercise has an anti-inflammatory effect that positively affects neuroplasticity [14].

Many unresolved questions remain about the specific mechanisms by which exercise mediates these beneficial effects on the cognitive system but there is increasing evidence that the immune system plays a strong role in them. The anti-inflammatory effects of exercise are mediated by multidirectional pathways. Some of them are exerted in adipose tissue, skeletal muscles, immune system cells, and the cardiovascular system. These effects include modulation of anti-inflammatory and pro-inflammatory cytokine profiles [8], achieving an adequate balance between both of them. Pro- and anti-inflammatory cytokines must be properly balanced for the immune system to exert its positive or negative effects on brain neuroplasticity in order to modulate and/or consolidate the reorganisation of neural networks [60].

Given the mounting evidence for the role of exercise in inflammation, there is a strong academic interest in analysing the effect of different physical exercise protocols. Resistance training and/or aerobic training have been pointed out as a practice that could mitigate the deleterious effects of immunosenescence [7]. Evidence of regular aerobic training in older people showed that it caused a decrease in TNF-α plasma concentrations and an increase in the anti-inflammatory cytokine IL-10 [43]. Regarding Resistance Training, the previous evidence showed a reduction in

Table 1. Summary of studies included in this review (Chronic response).

References	Age	Aer (Aerobic Exercise)/RT (Resistance Training)	Protocol	Findings
[33] Irwin and Olmstead, 2012	59-86	Aer: Tai Chi Chih	40 min/session,3 session/week for 16 weeks; Moderate intensity	IL-6 ↓ CRP ⇔
[34] Tartibian *et al*., 2015	50-65	Aer: Walking	25–30 min/day,3–4 days/week with 45–55% HRmax for the first 8 weeks; 40–45 min/day,4–6 days/week with 55–65% HR for the final 8 weeks	IL-6 ↓ CRP ↓ TNF- α ↓
[35] Nishida *et al*., 2015	65-85	Aer: bench step exercise	10–20 min/session,3 times/day, and a goal of 140 min/week for 12 weeks; lactate threshold	IL-6 ⇔ TNF- α ⇔
[36] Abdollahpour *et al*., 2016	50-74	Aer: aerobic exercise	50 min/day,3 days/week for 6 months; 70–80% HRmax	IL-6 ↓ TNF- α ⇔
[37] Nascimento *et al*., 2015	60-70	Aer: aerobic exercise	16 weeks	IL-6 ↓
[38] El-Kader and Al-Jiffri, 2019	60-68	Aer: treadmill	40 min/session, 3 sessions/week for 6 months. 60–80% HRmax	IL-6 ↓ IL-10 ↑ TNF- α ↓
[39] Lee *et al*., 2015	65-70	Aer: walking in treadmill	40 min/session, 3 sessions/week for 8 weeks. 40–70% HRmax	IL-6 ↓ CRP ↓ TNF- α ↓
[40] Lima *et al*., 2015	63-72	Aer: treadmill	20-30 min/session, 3 sessions/week for 30 sessions. 50–80% HRmax	IL-6 ↓ TNF- α ⇔
[41] Muscari *et al*., 2010	65-71	Aer: treadmill	1h/session, 3 sessions/week for 12 months. 70% HRmax	CRP ↓
[44] El-Kader *et al*., 2019	60-67	RT: 9 resistance machines	9 ex; 3x8-12 rep. 60" res/set. 60-80% RM	IL-6 ↓ IL-10 ↑ TNF- α ↓
[45] Ihalainen *et al*., 2019	65-75	RT: whole-body strength training	7-9 ex, 2-5 x 4-12 rep, 6 months, 70-90% RM	IL-6 ↓ CRP ↓
[46] Cao *et al*., 2019	65	RT: 6 resistance machines	6 ex, 2-3 x 10-30 rep, 6 weeks, 40-80% RM	CRP ⇔
[47] Cunha *et al*., 2019	65-74	RT: 9 exercise free weights	9 ex, 1x15 rep, 20min/session, 3 sessions/week 12 weeks	CRP ↓
[48] Alikhani and Sheikholeslami-Vatani, 2019	60-64	RT: 6 resistance machines	6 ex, 4x10 rep, 1h/session, 3 sessions/week, 12 weeks, 75% RM	TNF- α ⇔
[49] Rodriguez-Miguelez *et al*., 2014	68-70	RT: leg press; biceps curl bench; seated peck deck	3 ex; lower limb and chest; vigorous; 3 × 08–12 Rep; 2×/week, 8 weeks.	IL-6 ↓ CRP ↓
[50] Strandberg *et al*., 2015	66-70	RT: leg extension	5 ex; whole body; vigorous; 3 × 15 to 08 Rep; 2×/week, 24 weeks.	IL-6 ↓ CRP ↓
[51] Hagstrom *et al*., 2016	46-60	RT: leg press	8 ex; whole body; vigorous; 3 × 08–10 Rep; 3×/week; 32 weeks.	IL-6 ↓ CRP ↓ TNF- α ⇔
[52] Nunes *et al*., 2016	57-65	RT: leg extension	8 ex; whole body; vigorous; 6 × 08–12 Rep; 3×/week, 16 weeks.	IL-6 ↓ TNF- α ⇔
[53] Tomeleri *et al*., 2018	65-75	RT: chest press; seated row; triceps pushdown; preacher curl; horizontal leg press; knee extension; knee curl; seated calf raised	8 ex; whole body; moderate; 3 × 10-15RM, 3×/week, 12 weeks.	IL-6 ↓ CRP ↓ TNF- α ↓
[54] Ogawa *et al*., 2010	80-89	RT: bands	4 ex,2x10 rep, 1 session/week, 12 weeks	CRP ↓ IL-6 ⇔ TNF- α ⇔
[55] Marques *et al*., 2013	62-73	RT: resistance machines	60 min/session, 3x8 rep, 3 sessions/week, 32 weeks, 75-80% RM	CRP ↓ IL-6 ↓ TNF- α ⇔

Table 2. Summary of studies included in this review (acute response).

References	Age	Aer (Aerobic Exercise)/RT (Resistance Training)	Protocol	Findings
[56] Nascimento *et al*., 2016	60-74	RT: Eccentric resistance exercise	7 sets of 10 repetitions at 110% of 10 RM with a rest of 3 min/set	IL-6 ↓ 48h after
[57] Gmiat *et al*., 2017	40-45	RT: High Intensity Circuit Training	10 exercises, 30 seconds/exercise with 10 seconds rest. 3 times with a rest of 2 min/time	IL-6 ↓ 48h after
[42] Windsor *et al*., 2018	67-77	Aer: cycling	24 min at 40%	IL-6 ↓ 48h after IL-10 ⇔ TNF- α ⇔

resting levels of TNF-α and CRP [54], contributing, in the long-term, to create an anti-inflammatory scene. Resistance exercise has numerous benefits for the elderly, since it can attenuate the loss of muscle mass during normal aging, and increase muscle strength [7]. Skeletal muscle activity resulting from physical exercise is one of the main mechanisms modulating both plasma and baseline levels of IL-6 [61]. Several studies have observed a negative association between the amount of physical activity individuals usually engage in and the levels of plasma IL-6, such that the more activity they perform, the lower the levels of IL-6, and conversely, high levels of plasma IL-6 are related to physical inactivity [62].

Studies analysing the interaction between physical exercise and inflammatory makers levels can be subdivided into those observing the effects after completing one exercise session (acute effect) and those that measure the effects of several sessions over time (chronic effect). The response to the muscle damage caused by one exercise session (acute effect), as a function of different exercise parameters (intensity, duration, muscle types involved, *etc.*), appears to be an acute increase in inflammatory makers plasma levels which, in turn, destabilises homeostasis [25, 63]. The analysed studies show that IL-6 is the inflammatory marker most influenced by the effects of only one exercise session. Also, an increase in its plasma levels was noted. This increase in IL-6 levels produced by aerobic exercise protocols is greater after engaging a single session, while the decrease in IL-6 levels (even below baseline) resulting from a single session of resistance training is stronger after 48 hours of recovery. In other words, in terms of acute effects, IL-6 levels increase more with aerobic exercise and only fall below basal levels at least 48 hours after completing a resistance training session.

On the other hand, a result of a regular physical activity (chronic effect) is a decrease of TNF-α, IL-6 and CRP plasma levels as well as an increase of IL-10 plasma levels. Based on those results, it is highlighted the fact that regular physical exercise attenuates the acute effect by reducing these increases in IL-6 levels (chronic effect). Thus, the final increases in plasma IL-6 levels are a function of the fitness status of each person and their level of adaptation to physical exercise [63]. This decrease in IL-6 plasma levels, observed after only one exercise session in individuals who regularly do physical exercise, is also observed in basal IL-6 levels, such that people who regularly exercise have lower baseline levels of this cytokine than those who are sedentary [61]. Therefore, the chronic response to regular physical exercise results in both a minor increase in this acute response and a decrease in basal IL-6 levels. Regarding the differences between all the different physical exercise protocols previously studied, it seems that both aerobic and resistance training leads to a variation in the plasma levels of the inflammatory markers analysed (TNF-α, IL-6 and CRP plasma levels decrease; IL-10 plasma levels increase) as a chronic effect, although the most favourable results were obtained in studies following aerobic exercise protocols.

Although the mechanisms by which these acute and chronic effects occur are not exactly understood, there is evidence that plasma levels of IL-6 could be regulated during exercise by both central and peripheral signalling. At the peripheral level, numerous studies have revealed that both type I and type II muscle fibres produce IL-6 in response to muscle contractions, causing the plasma levels of this cytokine to increase as an acute effect [64]. This increased release of IL-6 is mediated by muscle glycogen so that as the levels of this molecule decreases, more intramuscular IL-6 is produced and plasma IL-6 levels increase. However, as training continues, the intramuscular expression of IL-6 receptors (IL-6R) increases as a chronic effect, suggesting that muscles become more sensitive to intramuscular IL-6, thus causing plasma IL-6 levels not increase as much [40]. Therefore, at a muscular level, the low concentration of muscle glycogen caused by engaging physical exercise stimulates the IL-6 production, which is reflected as an increase in IL-6 plasma levels. However, as an adaptation to performing this physical exercise, sensitivity to intramuscular IL-6 also increases as a result of the upregulation of its receptor (IL-6R) and so, the same physical exercise that had previously caused a decrease in muscle glycogen levels will no longer increase plasma IL-6 levels as much.

At the level of the CNS, plasma IL-6 levels seem to also depend on signalling from the brain. While the release of IL-6 at the muscular level is proportional to the decrease in glucose levels, the brain produces an inverse signal so that when blood glucose levels are high, the brain signals to increase IL-6 plasma circulation, ceasing its release when blood glucose values are low [65]. Thus, fulfilling both signals (muscular and brain-mediated release) creates the homeostatic equilibrium of plasma IL-6 levels. An increase in the release of IL-6 at the central level has been observed during exercise, although this increase in IL-6 is much

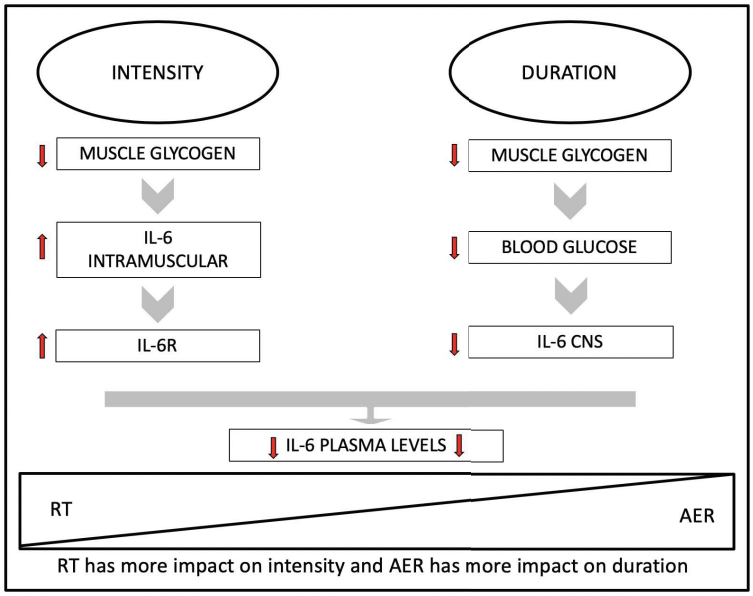

Fig. (2). Exercise parameters *vs.* IL-6 levels. AER: aerobic exercise; RT: Resistance training.

smaller than the release of IL-6 that occurs at the muscular level (Fig. **2**).

None of the studies we reviewed, specified the suitability or superiority of a given protocol type over others to improve inflammatory makers levels in older people. The heterogeneity of the studies available in the scientific literature, both in terms of their participants (*e.g.*, their initial fitness or inflammation levels) and the exercise parameters (duration, intensity, frequency, *etc.*), meant that it was difficult to draw many clear associations from them. However, all these articles noted that exercise intensity and duration, as well as participant fitness levels, were decisive elements in some of the noted improvements. Both studies that used aerobic exercise and those that used resistance training protocols noted a long-term decrease in plasma TNF-α, CRP and IL-6 levels, and increase IL-10 levels (chronic effect). Nonetheless, these levels seemed to fall even further when using aerobic exercise regimens, with the length of time spent exercising being the most decisive parameter.

Regarding the acute effect, we did not find enough evidence on TNF-α, CRP and IL-10 levels to have a strong conclusion about that. However, with respect to Il-6 plasma levels, the most notable increase seemed to occur at the peripheral level (intramuscularly). Continuing exercise programs for longer periods usually resulted in an adaptation mediated by the upregulation of intramuscular IL-6 receptors which consequently, decreased IL-6 output into the blood plasma. Thus, exercise intensity and duration are likely decisive in this reduction in IL-6 because muscles must be depleted of glycogen for this adaptation to occur. This also probably means that the benefits of resistance-based exercise programs (in terms of IL-6 reduction) could be enhanced by designing exercise protocols that focus on muscular engagement.

In addition to the influence that exercise intensity and duration have on decreasing the IL-6 levels of these patients, the participants' baseline fitness and inflammation levels were also important factors. Regardless of whether aerobic or resistance-based training protocols were performed, both the acute (increase in IL-6 levels) and chronic (decrease in baseline IL-6 levels) effects were highest among those with the lowest baseline fitness levels and/or highest initial states of inflammation [45]. Of note, while the intensity and duration of the exercise regimens used in the study protocols we examined were perhaps enough to bring about adaptations in patients not used to physical exercise, they may not have stimulated sufficient muscular stress (and thus, produce the aforementioned adaptations) in individuals who already maintained a daily exercise habit. Regarding the baseline inflammation statuses of the participants, these studies seemed to show that the minor stress stimuli caused by exercise restored the homeostatic inflammatory balance. Therefore, considering that both the exercise parameters and patient baseline characteristics (*e.g.*, their fitness levels) are decisive in how physical exercise protocols will affect IL-6 levels, these regimens must be adequately adapted to suit the individual characteristics of each person (Fig. **3**).

Given that resistance training protocols focus at the muscular level, it is reasonable to infer that engaging in this type of exercise (at appropriate intensity levels) would be preferable. This is because, compared to aerobic exercise, strength-based resistance training would most likely achieve greater glycogen deposit depletion and consequent intramuscular IL-6 receptor proliferation. Hence, although the stimuli generated by this resistance training would lead to an increase in plasma IL-6 levels, regularly engaging in this type of exercise would increase IL-6R expression and decrease the levels of IL-6 reaching the bloodstream. Thus, a chronic adaptation would be achieved that would lower plasma IL-6 levels and increasingly attenuating future spikes in IL-6. Nonetheless, as these adaptations are taking place, resistance exercise should start to be combined with other predominantly aerobic protocols, focusing on duration as the most relevant parameter. This would further enhance

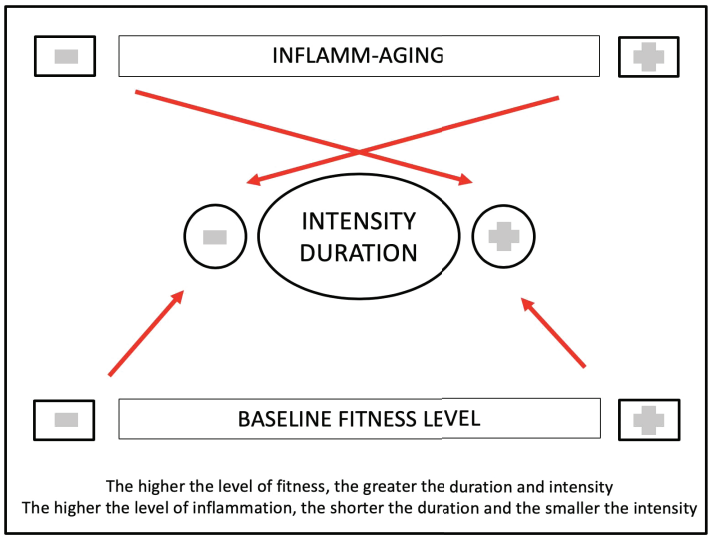

Fig. (3). Subject variables *vs.* exercise parameters.

the decrease in baseline levels of pro-inflammatory cytokines, as observed in longitudinal studies.

This article could have important clinical implications for developing physical exercise protocols in older population because it confirms the importance of adapting the parameters of intensity and duration of exercise to the individual characteristics, physical condition and level of inflammation of the elderly, in order to achieve the expected results. However, some of this article's limitations are that we have not compared the way of assessing the level of physical condition and inflammation level of the individuals in different studies. We have not analysed either the effects of the different inflammatory markers in both types of physical exercise. It becomes evident that more comparative studies are needed to draw conclusions about the suitability of one type of exercise or another.

CONCLUSION

As a stressor, physical exercise initially seems to cause an acute response, leading to an increase in pro-inflammatory cytokines levels. Although at first, this may be counterproductive in older or physically inactive individuals (who usually have high levels of inflammation), they could benefit from the chronic response achieved by engaging in and adapting to regular exercise. Specifically, a reduction in both the basal levels of IL-6 and in the peak acute-response IL-6 levels produced during future physical exercise sessions. This double-chronic response would favour both an anti-inflammatory effect and a decrease in basal pro-inflammatory cytokines levels, therefore reducing the state of systemic inflammation in these patients and favouring the neuroprotective effect that physical exercise has on cognitive functions.

Regardless of whether aerobic or resistance type exercises are performed, it seems that the intensity and duration of this exercise are the most important parameters for producing the desired decrease in pro-inflammatory cytokines levels.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Heneka, M.T.; O'Banion, M.K.; Terwel, D.; Kummer, M.P. Neuroinflammatory processes in Alzheimer's disease. *J. Neural Transm. (Vienna),* **2010**, *117*(8), 919-947. http://dx.doi.org/10.1007/s00702-010-0438-z PMID: 20632195

[2] Forster, M.J.; Dubey, A.; Dawson, K.M.; Stutts, W.A.; Lal, H.; Sohal, R.S. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*(10), 4765-4769. http://dx.doi.org/10.1073/pnas.93.10.4765 PMID: 8643477

[3] Bott, N.T.; Bettcher, B.M.; Yokoyama, J.S.; Frazier, D.T.; Wynn, M.; Karydas, A.; Yaffe, K.; Kramer, J.H. Youthful Processing speed in older adults: genetic, biological and behavioral predictors of cognitive processing speed trajectories in aging. *Front. Aging Neurosci.,* **2017**, *9*, 55. http://dx.doi.org/10.3389/fnagi.2017.00055 PMID: 28344553

[4] DeCarlo, C.A.; Tuokko, H.A.; Williams, D.; Dixon, R.A.; MacDonald, S.W. BioAge: toward a multi-determined, mechanistic account of cognitive aging. *Ageing Res. Rev.,* **2014**, *18*, 95-105. http://dx.doi.org/10.1016/j.arr.2014.09.003 PMID: 25278166

[5] Franceschi, C.; Bonafè, M.; Valensin, S.; Olivieri, F.; De Luca, M.; Ottaviani, E.; De Benedictis, G. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.,* **2000**, *908*, 244-254. http://dx.doi.org/10.1111/j.1749-6632.2000.tb06651.x PMID: 10911963

[6] Scheinert, R.B.; Asokan, A.; Rani, A.; Kumar, A.; Foster, T.C.; Ormerod, B.K. Some hormone, cytokine and chemokine levels that

change across lifespan vary by cognitive status in male Fischer 344 rats. *Brain Behav. Immun.,* **2015**, *49*, 216-232. http://dx.doi.org/10.1016/j.bbi.2015.06.005 PMID: 26093306

[7] Chupel, M.U.; Direito, F.; Furtado, G.E.; Minuzzi, L.G.; Pedrosa, F.M.; Colado, J.C.; Ferreira, J.P.; Filaire, E.; Teixeira, A.M. Strength Training Decreases Inflammation and Increases Cognition and Physical Fitness in Older Women with Cognitive Impairment. *Front. Physiol.,* **2017**, *8*(8), 377. http://dx.doi.org/10.3389/fphys.2017.00377 PMID: 28659812

[8] Di Benedetto, S.; Müller, L.; Wenger, E.; Düzel, S.; Pawelec, G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neurosci. Biobehav. Rev.,* **2017**, *75*, 114-128. http://dx.doi.org/10.1016/j.neubiorev.2017.01.044 PMID: 28161508

[9] Silva, F.O.C.; Macedo, D.V. Physical exercise, inflammatory process and adaptation: an overview. *Brazilian Journal of Cineanthropometry and Human Performance,* **2011**, *13*, 320-328.

[10] Opdenakker, G.; Fibbe, W.E.; Van Damme, J. The molecular basis of leukocytosis. *Immunol. Today,* **1998**, *19*(4), 182-189. http://dx.doi.org/10.1016/S0167-5699(97)01243-7 PMID: 9577095

[11] Locksley, R.M.; Killeen, N.; Lenardo, M.J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell,* **2001**, *104*(4), 487-501. http://dx.doi.org/10.1016/S0092-8674(01)00237-9 PMID: 11239407

[12] Mosca, L. C-reactive protein--to screen or not to screen? *N. Engl. J. Med.,* **2002**, *347*(20), 1615-1617. http://dx.doi.org/10.1056/NEJMe020127 PMID: 12432050

[13] Saraiva, M.; O'Garra, A. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.,* **2010**, *10*(3), 170-181. http://dx.doi.org/10.1038/nri2711 PMID: 20154735

[14] Yaffe, K.; Kanaya, A.; Lindquist, K.; Simonsick, E.M.; Harris, T.; Shorr, R.I.; Tylavsky, F.A.; Newman, A.B. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA,* **2004**, *292*(18), 2237-2242. http://dx.doi.org/10.1001/jama.292.18.2237 PMID: 15536110

[15] Dik, M.G.; Jonker, C.; Hack, C.E.; Smit, J.H.; Comijs, H.C.; Eikelenboom, P. Serum inflammatory proteins and cognitive decline in older persons. *Neurology,* **2005**, *64*(8), 1371-1377. http://dx.doi.org/10.1212/01.WNL.0000158281.08946.68 PMID: 15851726

[16] Mooijaart, S.P.; Sattar, N.; Trompet, S.; Lucke, J.; Stott, D.J.; Ford, I.; Jukema, J.W.; Westendorp, R.G.; de Craen, A.J. PROSPER Study Group. Circulating interleukin-6 concentration and cognitive decline in old age: the PROSPER study. *J. Intern. Med.,* **2013**, *274*(1), 77-85. http://dx.doi.org/10.1111/joim.12052 PMID: 23414490

[17] Kline, R.; Wong, E.; Haile, M.; Didehvar, S.; Farber, S.; Sacks, A.; Pirraglia, E.; de Leon, M.J.; Bekker, A. Peri-Operative Inflammatory Cytokines in Plasma of the Elderly Correlate in Prospective Study with Postoperative Changes in Cognitive Test Scores. *Int. J. Anesthesiol. Res.,* **2016**, *4*(8), 313-321. PMID: 28317003

[18] Marsland, A.L.; Gianaros, P.J.; Kuan, D.C-H.; Sheu, L.K.; Krajina, K.; Manuck, S.B. Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain Behav. Immun.,* **2015**, *48*, 195-204. http://dx.doi.org/10.1016/j.bbi.2015.03.015 PMID: 25882911

[19] Puzianowska-Kuźnicka, M.; Owczarz, M.; Wieczorowska-Tobis, K.; Nadrowski, P.; Chudek, J.; Slusarczyk, P.; Skalska, A.; Jonas, M.; Franek, E.; Mossakowska, M. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immun. Ageing,* **2016**, *13*, 21. http://dx.doi.org/10.1186/s12979-016-0076-x PMID: 27274758

[20] Paine, N.J.; Bosch, J.A.; Ring, C.; Drayson, M.T.; Veldhuijzen van Zanten, J.J. Induced mild systemic inflammation is associated with impaired ability to improve cognitive task performance by practice. *Psychophysiology,* **2015**, *52*(3), 333-341. http://dx.doi.org/10.1111/psyp.12360 PMID: 25366393

[21] Bradburn, S.; Sarginson, J.; Murgatroyd, C.A. Association of Peripheral Interleukin-6 with Global Cognitive Decline in Non-demented Adults: A Meta-Analysis of Prospective Studies. *Front. Aging Neurosci.,* **2018**, *9*, 438. http://dx.doi.org/10.3389/fnagi.2017.00438 PMID: 29358917

[22] Shivakumar, V.; Debnath, M.; Venugopal, D.; Rajasekaran, A.; Kalmady, S.V.; Subbanna, M.; Narayanaswamy, J.C.; Amaresha, A.C.; Venkatasubramanian, G. Influence of correlation between HLA-G polymorphism and Interleukin-6 (IL6) gene expression on the risk of schizophrenia. *Cytokine,* **2018**, *107*, 59-64. http://dx.doi.org/10.1016/j.cyto.2017.11.016 PMID: 29217401

[23] Gruol, D.L. IL-6 regulation of synaptic function in the CNS. *Neuropharmacology.,* **2015**, *96*(Pt. A), 42-54. http://dx.doi.org/10.1016/j.neuropharm.2014.10.023 PMID: 25445486

[24] Thielen, J-W.; Kärgel, C.; Müller, B.W.; Rasche, I.; Genius, J.; Bus, B.; Maderwald, S.; Norris, D.G.; Wiltfang, J.; Tendolkar, I. Aerobic Activity in the Healthy Elderly Is Associated with Larger Plasticity in Memory Related Brain Structures and Lower Systemic Inflammation. *Front. Aging Neurosci.,* **2016**, *8*, 319. http://dx.doi.org/10.3389/fnagi.2016.00319 PMID: 28082894

[25] Philippe, M.; Krüsmann, P.J.; Mersa, L.; Eder, E.M.; Gatterer, H.; Melmer, A.; Ebenbichler, C.; Burtscher, M. Acute effects of concentric and eccentric exercise on glucose metabolism and interleukin-6 concentration in healthy males. *Biol. Sport,* **2016**, *33*(2), 153-158. http://dx.doi.org/10.5604/20831862.1198634 PMID: 27274108

[26] Pruimboom, L.; Raison, C.L.; Muskiet, F.A.J. Physical Activity Protects the Human Brain against Metabolic Stress Induced by a Postprandial and Chronic Inflammation. *Behav. Neurol.,* **2015**, *2015*569869 http://dx.doi.org/10.1155/2015/569869 PMID: 26074674

[27] Kim, J.S.; Yi, H.K. Intermittent bout exercise training down-regulates age-associated inflammation in skeletal muscles. *Exp. Gerontol.,* **2015**, *72*, 261-268. http://dx.doi.org/10.1016/j.exger.2015.11.001 PMID: 26545590

[28] Beavers, K.M.; Brinkley, T.E.; Nicklas, B.J. Effect of exercise training on chronic inflammation. *Clin. Chim. Acta,* **2010**, *411*(11-12), 785-793. http://dx.doi.org/10.1016/j.cca.2010.02.069 PMID: 20188719

[29] Lee, I.M.; Sesso, H.D.; Ridker, P.M.; Mouton, C.P.; Stefanick, M.L.; Manson, J.E. Physical activity and inflammation in a multiethnic cohort of women. *Med. Sci. Sports Exerc.,* **2012**, *44*(6), 1088-1096. http://dx.doi.org/10.1249/MSS.0b013e318242b11a PMID: 22595984

[30] Streese, L.; Deiseroth, A.; Schäfer, J.; Schmidt-Trucksäss, A.; Hanssen, H. Exercise, arterial crosstalk-modulation, and inflammation in an aging population: the ExAMIN age study. *Front. Physiol.,* **2018**, *9*, 116. http://dx.doi.org/10.3389/fphys.2018.00116 PMID: 29515458

[31] Liu, D.; Wang, R.; Grant, A.R.; Zhang, J.; Gordon, P.M.; Wei, Y.; Chen, P. Immune adaptation to chronic intense exercise training: new microarray evidence. *BMC Genomics,* **2017**, *18*(1), 29. http://dx.doi.org/10.1186/s12864-016-3388-5 PMID: 28056786

[32] Miller, K.J.; Gonçalves-Bradley, D.C.; Areerob, P.; Hennessy, D.; Mesagno, C.; Grace, F. Comparative effectiveness of three exercise types to treat clinical depression in older adults: A systematic review and network meta-analysis of randomised controlled trials. *Ageing Res. Rev.,* **2020**, *58,*100999. http://dx.doi.org/10.1016/j.arr.2019.100999 PMID: 31837462

[33] Irwin, M.R.; Olmstead, R. Mitigating cellular inflammation in older adults: a randomized controlled trial of Tai Chi Chih. *Am. J. Geriatr. Psychiatry,* **2012**, *20*(9), 764-772. http://dx.doi.org/10.1097/JGP.0b013e3182330fd3 PMID: 21934474

[34] Tartibian, B.; FitzGerald, L.Z.; Azadpour, N.; Maleki, B.H. A randomized controlled study examining the effect of exercise on inflammatory cytokine levels in post-menopausal women. *Post Reprod. Health,* **2015**, *21*(1), 9-15. http://dx.doi.org/10.1177/2053369114565708 PMID: 25710943

[35] Nishida, Y.; Tanaka, K.; Hara, M.; Hirao, N.; Tanaka, H.; Tobina, T.; Ikeda, M.; Yamato, H.; Ohta, M. Effects of home-based bench step exercise on inflammatory cytokines and lipid profiles in elderly Japanese females: A randomized controlled trial. *Arch. Gerontol. Geriatr.,* **2015**, *61*(3), 443-451. http://dx.doi.org/10.1016/j.archger.2015.06.017 PMID: 26228714

[36] Abdollahpour, A.; Khosravi, N.; Eskandari, Z.; Haghighat, S. Effect of six months of aerobic exercise on plasma interleukin-6 and tumor necrosis factor-alpha as breast cancer risk factors in postmenopausal women: a randomized controlled trial. *Iran. Red Crescent Med. J.,* **2016**, *19,*e27842. http://dx.doi.org/10.5812/ircmj.27842

[37] Nascimento, C.M.C.; Cominetti, M.R.; Pereira, J.R.; Andrade, L.P.; Garuffi, M.; Kerr, D.S.; Forlenza, O.V.; Stella, F. Regular multimodal aerobic exercise reduces pro-inflamatory cytokines and improves BNDF peripheal levels and executive functions in elderly MCI individuals with different BDNF VAL66MET genotypes. *Alzheimers Dement.,* **2015**, *11*(7), 323. http://dx.doi.org/10.1016/j.jalz.2015.07.465

[38] Abd El-Kader, S.M.; Al-Jiffri, O.H. Aerobic exercise modulates cytokine profile and sleep quality in elderly. *Afr. Health Sci.,* **2019**, *19*(2), 2198-2207. http://dx.doi.org/10.4314/ahs.v19i2.45 PMID: 31656505

[39] Lee, J.S.; Kim, C.G.; Seo, T.B.; Kim, H.G.; Yoon, S.J. Effects of 8-week combined training on body composition, isokinetic strength, and cardiovascular disease risk factors in older women. *Aging Clin. Exp. Res.,* **2015**, *27*(2), 179-186. http://dx.doi.org/10.1007/s40520-014-0257-4 PMID: 24997614

[40] Lima, L.G.; Bonardi, J.M.T.; Campos, G.O.; Bertani, R.F.; Scher, L.M.L.; Louzada-Junior, P.; Moriguti, J.C.; Ferriolli, E.; Lima, N.K.C. Effect of aerobic training and aerobic and resistance training on the inflammatory status of hypertensive older adults. *Aging Clin. Exp. Res.,* **2015**, *27*(4), 483-489. http://dx.doi.org/10.1007/s40520-014-0307-y PMID: 25567682

[41] Muscari, A.; Giannoni, C.; Pierpaoli, L.; Berzigotti, A.; Maietta, P.; Foschi, E.; Ravaioli, C.; Poggiopollini, G.; Bianchi, G.; Magalotti, D.; Tentoni, C.; Zoli, M. Chronic endurance exercise training prevents aging-related cognitive decline in healthy older adults: a randomized controlled trial. *Int. J. Geriatr. Psychiatry,* **2010**, *25*(10), 1055-1064. http://dx.doi.org/10.1002/gps.2462 PMID: 20033904

[42] Windsor, M.T.; Bailey, T.G.; Perissiou, M.; Greaves, K.; Jha, P.; Leicht, A.S.; Russell, F.D.; Golledge, J.; Askew, C.D. Acute inflammatory responses to exercise in patients with abdominal aortic aneurysm. *Med. Sci. Sports Exerc.,* **2018**, *50*(4), 649-658. http://dx.doi.org/10.1249/MSS.0000000000001501 PMID: 29210916

[43] Zheng, G.; Qiu, P.; Xia, R.; Lin, H.; Ye, B.; Tao, J.; Chen, L. Effect of Aerobic Exercise on Inflammatory Markers in Healthy Middle-Aged and Older Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Front. Aging Neurosci.,* **2019**, *11*, 98. http://dx.doi.org/10.3389/fnagi.2019.00098 PMID: 31080412

[44] Abd El-Kader, S.M.; Al-Shreef, F.M.; Al-Jiffri, O.H. Impact of aerobic exercise versus resisted exercise on endothelial activation markers and inflammatory cytokines among elderly. *Afr. Health Sci.,* **2019**, *19*(4), 2874-2880. PMID: 32127863

[45] Ihalainen, J.K.; Inglis, A.; Mäkinen, T.; Newton, R.U.; Kainulainen, H.; Kyröläinen, H.; Walker, S. Strength Training Improves Metabolic Health Markers in Older Individual Regardless of Training Frequency. *Front. Physiol.,* **2019**, *10*, 32. http://dx.doi.org/10.3389/fphys.2019.00032 PMID: 30774600

[46] Cao Dinh, H.; Njemini, R.; Onyema, O.O.; Beyer, I.; Liberman, K.; De Ddobbeleer, L.; Renmans, W.; Vander Meeren, S.; Jochmans, K.; Delaere, A.; Knoop, V.; Bautmans, I. Strength Endurance Training but Not Intensive Strength Training Reduces Senescence-Prone T Cells in Peripheral Blood in Community-Dwelling Elderly Women. *J. Gerontol A Biol. Sci. Med. Sci.,* **2019**,*74*(12), 1870-1878. http://dx.doi.org/10.1093/gerona/gly229 PMID: 30285092

[47] Cunha, P.M.; Ribeiro, A.S.; Nunes, J.P.; Tomeleri, C.M.; Nascimento, M.A.; Moraes, G.K.; Sugihara, P.; Barbosa, D.S.; Venturini, D.; Cyrino, E.S. Resistance training performed with single-set is sufficient to reduce cardiovascular risk factors in untrained older women: The randomized clinical trial. Active Aging Longitudinal Study. *Arch. Gerontol. Geriatr.,* **2019**, *81*, 171-175. http://dx.doi.org/10.1016/j.archger.2018.12.012 PMID: 30594892

[48] Alikhani, S.; Sheikholeslami-Vatani, D. Oxidative stress and antioxidant responses to regular resistance training in young and older adult women. *Geriatr. Gerontol. Int.,* **2019**, *19*(5), 419-422. http://dx.doi.org/10.1111/ggi.13636 PMID: 30811775

[49] Rodriguez-Miguelez, P.; Fernandez-Gonzalo, R.; Almar, M.; Mejías, Y.; Rivas, A.; de Paz, J.A.; Cuevas, M.J.; González-Gallego, J. Role of Toll-like receptor 2 and 4 signaling pathways on the inflammatory response to resistance training in elderly subjects. *Age (Dordr.),* **2014**, *36*(6), 9734. http://dx.doi.org/10.1007/s11357-014-9734-0 PMID: 25427999

[50] Hagstrom, A.D.; Marshall, P.W.; Lonsdale, C.; Papalia, S.; Cheema, B.S.; Toben, C.; Baune, B.T.; Fiatarone Singh, M.A.; Green, S. The effect of resistance training on markers of immune function and inflammation in previously sedentary women recovering from breast cancer: a randomized controlled trial. *Breast Cancer Res. Treat.,* **2016**, *155*(3), 471-482. http://dx.doi.org/10.1007/s10549-016-3688-0 PMID: 26820653

[51] Strandberg, E.; Edholm, P.; Ponsot, E.; Wåhlin-Larsson, B.; Hellmén, E.; Nilsson, A.; Engfeldt, P.; Cederholm, T.; Risérus, U.; Kadi, F. Influence of combined resistance training and healthy diet on muscle mass in healthy elderly women: a randomized controlled trial. *J. Appl. Physiol.,* **2015**, *119*(8), 918-925. http://dx.doi.org/10.1152/japplphysiol.00066.2015 PMID: 26338453

[52] Nunes, P.R.; Barcelos, L.C.; Oliveira, A.A.; Furlanetto Júnior, R.; Martins, F.M.; Orsatti, C.L.; Resende, E.A.; Orsatti, F.L. Effect of resistance training on muscular strength and indicators of abdominal adiposity, metabolic risk, and inflammation in postmenopausal women: controlled and randomized clinical trial of efficacy of training volume. *Age (Dordr.),* **2016**, *38*(2), 40. http://dx.doi.org/10.1007/s11357-016-9901-6 PMID: 26984105

[53] Tomeleri, C.M.; Souza, M.F.; Burini, R.C.; Cavaglieri, C.R.; Ribeiro, A.S.; Antunes, M.; Nunes, J.P.; Venturini, D.; Barbosa, D.S.; Sardinha, L.B.; Cyrino, E.S. Resistance training reduces metabolic syndrome and inflammatory markers in older women: A randomized controlled trial. *J. Diabetes,* **2018**, *10*(4), 328-337. http://dx.doi.org/10.1111/1753-0407.12614 PMID: 29031002

[54] Ogawa, K.; Sanada, K.; Machida, S.; Okutsu, M.; Suzuki, K. Resistance Exercise Training-Induced Muscle Hypertrophy Was Associated with Reduction of Inflammatory Markers in Elderly Women. *Mediators Inflamm.,* **2010**.171023. http://dx.doi.org/10.1155/2010/171023 PMID: 21253481

[55] Marques, E.A.; Mota, J.; Viana, J.L.; Tuna, D.; Figueiredo, P.; Guimarães, J.T.; Carvalho, J. Response of bone mineral density, inflammatory cytokines, and biochemical bone markers to a 32-week combined loading exercise programme in older men and women. *Arch. Gerontol. Geriatr.,* **2013**, *57*(2), 226-233. http://dx.doi.org/10.1016/j.archger.2013.03.014 PMID: 23623588

[56] Nascimento, Dda.C.; Navalta, J.W.; Durigan, J.L.Q.; Marqueti, Rde.C.; Tibana, R.A.; Luiz Franco, O.; de Almeida, J.A.; Camarço, N.F.; Neto, I.V.S.; Prestes, J. Acute eccentric resistance exercise decreases matrix metalloproteinase activity in obese elderly women. *Clin. Physiol. Funct. Imaging,* **2016**, *36*(2), 139-145. http://dx.doi.org/10.1111/cpf.12207 PMID: 25523909

[57] Gmiat, A.; Micielska, K.; Kozłowska, M.; Flis, D.J.; Smaruj, M.; Kujach, S.; Jaworska, J.; Lipińska, P.; Ziemann, E. The impact of a single bout of high intensity circuit training on myokines' concentrations and cognitive functions in women of different age. *Physiol. Behav.,* **2017**, *179*, 290-297. http://dx.doi.org/10.1016/j.physbeh.2017.07.004 PMID: 28687176

[58] Mandolesi, L.; Polverino, A.; Montuori, S.; Foti, F.; Ferraioli, G.; Sorrentino, P.; Sorrentino, G. Effects of Physical Exercise on Cognitive Functioning and Wellbeing: Biological and Psychological Benefits. *Front. Psychol.,* **2018**, *9*(509), 509. http://dx.doi.org/10.3389/fpsyg.2018.00509 PMID: 29755380

[59] Li, F.H.; Sun, L.; Zhu, M.; Li, T.; Gao, H.E.; Wu, D.S.; Zhu, L.; Duan, R.; Liu, T.C.Y. Beneficial alterations in body composition, physical performance, oxidative stress, inflammatory markers, and adipocytokines induced by long-term high-intensity interval training in an aged rat model. *Exp. Gerontol.,* **2018**, *113*, 150-162. http://dx.doi.org/10.1016/j.exger.2018.10.006 PMID: 30308288

[60] Levin, S.G.; Godukhin, O.V. Modulating Effect of Cytokines on Mechanisms of Synaptic Plasticity in the Brain. *Biochemistry (Mosc.),* **2017**, *82*(3), 264-274. http://dx.doi.org/10.1134/S000629791703004X PMID: 28320267

[61] Monteiro-Junior, R.S.; de Tarso Maciel-Pinheiro, P.; da Matta Mello Portugal, E.; da Silva Figueiredo, L.F.; Terra, R.; Carneiro, L.S.F.; Rodrigues, V.D.; Nascimento, O.J.M.; Deslandes, A.C.; Laks, J. Effect of Exercise on Inflammatory Profile of Older Persons: Systematic Review and Meta-Analyses. *J. Phys. Act. Health,* **2018**, *15*(1), 64-71. http://dx.doi.org/10.1123/jpah.2016-0735 PMID: 28771081

[62] Fischer, C.P. Interleukin-6 in acute exercise and training: what is the biological relevance? *Exerc. Immunol. Rev.,* **2006**, *12*, 6-33. PMID: 17201070

[63] Reihmane, D.; Dela, F. Interleukin-6: possible biological roles during exercise. *Eur. J. Sport Sci.,* **2014**, *14*(3), 242-250. http://dx.doi.org/10.1080/17461391.2013.776640 PMID: 24655147

[64] Pedersen, B.K.; Febbraio, M. Muscle-derived interleukin-6--a possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain Behav. Immun.,* **2005**, *19*(5), 371-376. http://dx.doi.org/10.1016/j.bbi.2005.04.008 PMID: 15935612

[65] Nybo, L.; Møller, K.; Pedersen, B.K.; Nielsen, B.; Secher, N.H. Association between fatigue and failure to preserve cerebral energy turnover during prolonged exercise. *Acta Physiol. Scand.,* **2003**, *179*(1), 67-74. http://dx.doi.org/10.1046/j.1365-201X.2003.01175.x PMID: 12940940

6.3 Artículo 3

diagnostics

MDPI

Article

Salivary IL-6 Concentration Is Associated with Frailty Syndrome in Older Individuals

Pablo Gómez-Rubio [1], Isabel Trapero [1], Omar Cauli [1,2,*] and Cristina Buigues [1,2]

[1] Department of Nursing, University of Valencia, 46010 Valencia, Spain; pagorubio1@hotmail.com (P.G.-R.); isabel.trapero@uv.es (I.T.); cristina.buigues@uv.es (C.B.)
[2] Frailty and Cognitive Impairment Research Group (FROG), University of Valencia, 46010 Valencia, Spain
* Correspondence: Omar.Cauli@uv.es

Abstract: Background: One of the physiological changes that is most closely associated with frailty is the increase in pro-inflammatory cytokines, and IL-6 in particular. Most studies have demonstrated this association using blood samples. We analyzed the relationship between frailty syndrome, individual frailty criteria, and IL-6 levels obtained by saliva tests. Methods: A cross-sectional pilot study was performed among women institutionalized in nursing homes. Frailty was defined as having three or more of the following components: low lean mass, weakness, self-reported exhaustion, low activity level, and slow walking speed; prefrailty was defined as having one or two of those components. Results: There was a significant and positive correlation between the frailty score and salivary IL-6 concentration. Regarding the associations between IL-6 and individual dichotomized frailty criteria, there were significant differences in salivary IL-6 concentration in two frailty criteria: weight loss ($p = 0.002$) and low physical activity ($p = 0.007$). Receiver operating characteristic curve analysis showed that IL-6 concentration significantly ($p < 0.05$) (although moderately) discriminated patients that progressed in the frailty syndrome (the area under the curve value was 0.697 with 95% CI 0.566–0.827). Conclusions: Salivary IL-6 concentration can be used as potential biomarker of frailty syndrome and as a tool to monitor the effects of interventions in frail individuals.

Keywords: salivary biomarkers; frailty; IL-6; fatigue; weight loss; physical activity

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1. Introduction

Frailty is a crucial age-related clinical syndrome that is commonly defined as a state of increased vulnerability to external stressors, and occurs as a consequence of the cumulative decline in many physiological systems over a lifetime [1]. This syndrome describes the physical and functional decline that occurs as a consequence of certain diseases (e.g., cancer and chronic infection) but which can also occur in the absence of disease [2]. In recent years, frailty has been recognized as an emerging public health priority and the need for early recognition and intervention for frailty is pressing [3]. This is because it is widely accepted as a critical issue for aging populations worldwide, as it is relevant to multiple adverse outcomes, including hospitalization, institutionalization, and premature mortality [4].

An internationally accepted definition of frailty has still not been reached despite the importance of this clinical syndrome. The most commonly used definition considers frailty to be a phenotype comprising five indicators: unintentional weight loss of 5% or 10 pounds within one year, slow gait speed, reduced grip strength, exhaustion, and physical inactivity. Frailty is clinically diagnosed by the presence of three or more of these indicators and is preceded by a prodromal stage known as prefrailty, defined by having one or two indicators [5].

The pathophysiological changes underlying and preceding frailty are not clearly known. The etiology of frailty is not well understood, but it has been associated with changes in several physiological systems, including inflammation, coagulation, hematological, and endocrine systems [6]. Inflammation is one such potential pathophysiological

change, which may be closely linked with frailty [7]. Pro-inflammatory cytokines may influence frailty either directly, by promoting protein degradation, or indirectly by affecting important metabolic pathways [8]. Researchers have reported that increased levels of inflammatory markers, such as interleukins-6 (IL-6), tumor necrosis factor-α (TNF -α), and C-reactive protein (CRP) are associated with frailty syndrome [9]. In particular, several studies have found a significant association between IL-6 in blood and frailty syndrome in older individuals [10–16]. The fact that IL-6 stimulates CRP synthesis and inhibits TNF-α, may be one of the reasons why it has received special attention in the scientific literature. IL-6 is synthesized from mononuclear phagocytes, vascular endothelial cells, fibroblasts, and adipose and muscle tissues. Its concentration is modulated by numerous factors, including proinflammatory agents, viral and bacterial pathogens, neurotransmitters and second messengers, and its function is stimulation of neutrophil production by bone marrow progenitors, promoting synthesis of proinflammatory cytokines and inhibiting TNF-α [17,18]. Several studies have reported evidence for the role of IL-6 in the inflammatory processes through which the immune system intercommunicates with the central nervous system (CNS). It has been observed that IL-6 levels increase with age and that high levels are associated with the cognitive decline that occurs in old age [19–21]. In addition, IL-6 levels inversely correlate with hippocampal grey matter volume [22] as well as with the integrity of the brain white matter [23]. Interactions between IL-6 and the CNS are one of the mechanisms by which the immune system influences the performance of cognitive functions, and these also play a role in neurodegeneration and cognitive functionality [24].

Owing to its easy application in clinical practice and prognostic value in therapeutic decisions in medical specialists, the identification of a frail status is essential in older adults. Biomarkers may help identify patients who are at a risk of becoming or being frail [10]. Biomarkers provide objective, measurable indices for normal and/or pathogenic states as well as responses to therapeutic interventions [25]. They are useful for detecting subclinical changes that lead to observable indicators of frailty and testing intervention protocols for frailty, as they reflect changes in physiological processes that underlie and/or relate to frailty [26].

In order to better understand the etiology, time course, and consequences of inflammatory processes and to develop better interventions, there is a need for new methods for assessing inflammation that can be conducted in ecological contexts, across time, and at multiple moments throughout the day [27]. Blood has been the usual biological fluid in which many analytes are measured for clinical and research purposes. This is reasonable, because blood is a systemic fluid that reaches all parts of the body and can influence the biological behavior of tissues or on the other hand, receive and transport tissue-derived components that reflect what is taking place in bodily tissues [28]. Although the detection of serum and blood biomarkers is a standard practice, the use of noninvasive samples is desirable.

When studying other methods, some authors have reported that saliva is a potential diagnostic tool because of its ease of collection and noninvasiveness. [29]. Human saliva contains proteins, peptides, hormones, and enzymes, each of which can easily be used to assess different diseases [30]. Approximately 1400–2000 proteins have been identified in saliva [31]. The number of proteins in saliva shows its diversity, and each of these proteins can be used as a simple tool to assess toxicity, infection, and immunological and hormonal levels. Human saliva represents whole body images and is also known as the "mirror of the body". A biomarker is a measurable nano-protein, which is used to predict a biological state [32]. Some studies have analyzed salivary IL-6 levels in patients not only with common oral pathologies [33], but also in systemic diseases such as polycystic ovary syndrome [34], bladder pain syndrome sleep disorders [35], in sepsis [29], Huntington's disease [36], diabetes [37], in stroke [38], Hashimoto's disease [39], and inflammatory diseases [40]. However, the association between salivary IL-6 levels in patients with frail syndrome is unknown. There is a consensus about the role of blood IL-6 levels and its

correlation with frailty syndrome in older individuals. This study aims to analyze the relationship between frailty syndrome and IL-6 levels obtained through saliva tests.

2. Materials and Methods

2.1. Study Population

A cross-sectional study with a residential profile was conducted in 2019, among institutionalized older individuals living in long-stay centers (GeroResidencias La Saleta, Valencia, Spain) in the province of Valencia (Spain). Exclusion criteria were dementia, severe psychiatric illness (schizophrenia, bipolar disorder, etc., which will have not permitted answering to questions) or blindness (which will have not permitted the measurements of all frailty criteria). In order to rule out the contribution of known inflammatory processes to modify IL-6 levels, residents with acute infections requiring antibiotic treatment, known cancer, inflammatory and autoimmune diseases were also excluded. None of the patients were receiving corticoid or immunosuppressive drugs at the time of saliva sampling.

This research was conducted according to the requirements of the Declaration of Helsinki, and the entire study protocol was approved by the local ethical committee. Written informed consent was obtained from all participants. We measured the socio-demographic characteristics, and the five frailty criteria (involuntary weight loss, low energy or exhaustion, slow mobility, muscle weakness, and low physical activity) based on Fried's frailty criteria [5], and performed geriatric assessments using the Tinetti index, mini-mental score examination (MMSE) test, Yesavage scale, Barthel index, and Athens insomnia scale (AIS) to estimate the severity of insomnia.

2.2. Measurement of Frailty Criteria

Frailty was measured by assessing the presence or absence of five measurable criteria of the frailty syndrome (Fried criteria [5]) which were defined as follows:

(1) Weight loss. Weight loss was defined as the unintentional loss of 4.5 kg or more in the past year.
(2) Self-reported exhaustion. Exhaustion was indicated if the subjects responded positively to either of the statements on the Centre for Epidemiological Studies depression scale [41]: "I felt that everything I did took a lot of effort at least three or four days a week" and "I felt that I could not keep doing things at least three or four days a week", when referring to the previous week, or if the subject answered "Often" or "Most of the time" to the question "How often in the last week did you feel that everything you did was an effort?", which is also included on the Centre for Epidemiological Studies depression scale [42]. All burnout criteria were estimated to be present.
(3) Physical activity (PA). Low PA was quantified using the Spanish adaptation of the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ) readapted for women [43,44]. The MLTPAQ was administered by a trained interviewer who was provided with precise instructions and a detailed list of PA. The participants received a list of activities and were asked to tick those they had performed during the past year. To avoid recall bias as much as possible, activities performed in the last week were compiled first, followed by those performed in the last month, the last quarter and finally the last year, always including the first periods. For validation purposes, only the information referring to the last year was used. The total leisure time PA energy expenditure (EEPA) was calculated from this questionnaire and used to quantify PA [45].
(4) Slowed motor performance. Slowness was assessed using the 4–6 m walking speed test, adjusted for sex and height according to the standards of the Brief Physical Performance Battery [46]. A low walking speed was estimated to correspond to the worst quintile for sex and height of the group.
(5) Weakness. To determine weakness, strength was measured with a Jaymar hydraulic dynamometer, according to the standards of the Hispanic Established Populations for the Epidemiologic Studies of the Elderly [47].

People who met three or more criteria were classified as frail, those who met 1 or 2 were pre-frail, or those who did not meet any of the criteria were robust.

2.3. Measurement of IL-6

Saliva samples were collected from the patients described above in the morning, with at least 2 h of fasting (between 9–11 h in the morning) using the Salivette® Cortisol system (Sarstedt, Germany). 100 µL of each sample were centrifuged and the supernatant was aliquoted into Eppendorf tubes and frozen (−80 °C) until further analysis. The samples were brought to room temperature, and immunoassay ELISA analysis was performed using the High Sensitivity Human Elisa Kit for IL-6 (AbCam ab46042) according to the manufacturer's instructions. Changes in color intensity and the absorbance at 450 nm and 490 nm was read using the ELISA microplate reader, and a standard curve was prepared by plotting absorbance readings of standards against their concentrations using the Graphpad program.

2.4. Statistical Analysis

The continuous variables were expressed as the mean and standard deviation, and the categorical variables as the absolute value with their percentage. The normal distribution of each variable was assessed with the Shapiro–Wilk test in order to determine whether a parametric or non-parametric test should be applied. The correlation between quantitative variables was determined by Spearman's (for non-normal data distribution) or Pearson's (for normal data distribution) correlation test. The differences between the two groups were analyzed with the non-parametric Mann–Whitney U test or the parametric Student t-test. We evaluated linear correlations between continuous variables using the Spearman's rank test. The discrimination accuracy of the predictive model was calculated using C-statistics (area under the receiver operating characteristic curve; AUC). Statistical significance was set at a p-value of less than 0.05. All statistical analyses were performed using the SPSS software package (version 24.0; SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Study Population, Frailty Score, Geriatric Assessment

A total of 72 subjects (84.7% female) living in nursing care centers located in the province of Valencia (Spain) were recruited for the study. A total of 8.5% of the patients were found to be robust, 54.9% were prefrail, and 36.6% were frail. The most common Fried's criterion in the sample was poor physical activity (75%) followed by slow walking speed (68.1%), exhaustion (29.2%), weakness (22.5%), and weight loss (8.3%). The psycho-geriatric assessments are shown in Table 1.

Table 1. Characteristics of the study sample.

Category	Mean Value	Range
Age (years)	83.3 ± 6.9	66–99
Yesavage scale (depressive symptoms)	3.5 ± 3.2	0–11
Athens insomnia scale	4.6 ± 4	0–16
Barthel index (basic activities of daily life)	74.1 ± 22	25–100
Tinetti balance index	10.5 ± 3.6	1–16
Tinetti gait index	8.0 ± 2.8	
Frailty criteria	0 criteria = 6 individuals 1 criterion = 18 individuals 2 criteria = 22 individuals 3 criteria = 19 individuals 4 criteria = 7 individuals	0–12

Mean ± standard deviation and range for each value/scale. Geriatric assessment was evaluated by validated scales: Tinetti gait and balance index to determine the risk footfalls, mini-mental score examination test (MMSE), Yessavage scale for geriatric depression, Barthel index to measure the activities of daily living and mobility, Athens insomnia scale (AIS) to estimation of the severity of insomnia.

3.2. Evaluation of the Relationship between Salivary IL-6 Concentration and Geriatric Scales and Frailty Criteria

IL-6 was not significantly associated with any of the geriatric assessment scales (Tinetti gait index: $r = 0.18$; $p = 0.12$; Tinetti balance index: $r = 0.22$; $p = 0.064$, Spearman); Barthel index to measure the activities of daily living and mobility ($r = 0.91$; $p = 0.44$, Spearman); MMSE test for cognitive impairment ($r = -0.15$; $p = 0.20$); Yesavage scale for geriatric depression ($r = -0.23$; $p = 0.08$, Spearman); or the Athens insomnia scale for severity of insomnia ($r = 0.17$; $p = 0.14$, Spearman).

No significant correlations were observed between IL-6 count and self-reported exhaustion ($r = 0.23$; $p = 0.848$, Spearman), hand grip strength ($r = 0.13$; $p = 0.280$, Spearman), or slowed motor performance ($r = 0.13$; $p = 0.267$, Spearman).

However, there was a significant and positive relationship between the number of fulfilled frailty criteria with IL-6 ($r = 0.26$; $p = 0.025$, Spearman) (Figure 1A) and a negative relationship between the specific physical activity criteria ($r = -0.31$; $p = 0.007$, Spearman) (Figure 1B).

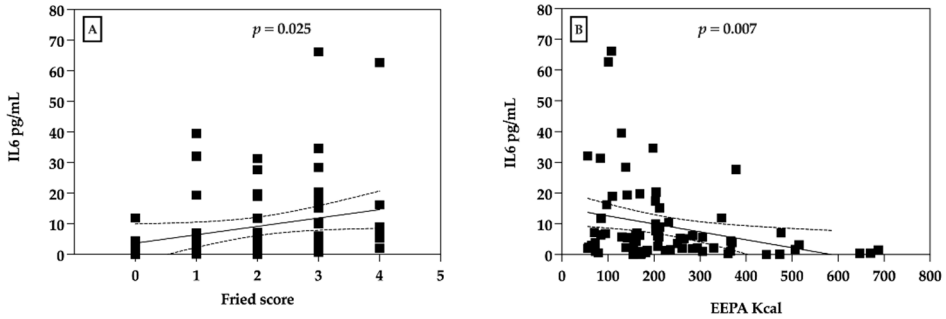

Figure 1. Correlation between IL-6 and Fried score and physical activity. (**A**) Correlation between IL-6 concentration in saliva and frailty score. (**B**) Correlation between IL-6 concentration in saliva and total leisure time PA energy expenditure (EEPA).

There were no significant differences ($p = 0.07$, Kruskal–Wallis) for the IL-6 count between different levels of frailty. There were no significant differences between IL-6 values and sex ($p = 0.496$, Mann–Whitney U) and no significant correlation between IL-6 values and age ($r = -0.053$; $p = 0.658$).

There were significant differences between robust and frail individuals in the concentration of salivary IL-6 ($p < 0.043$, Mann–Whitney U) (Figure 2).

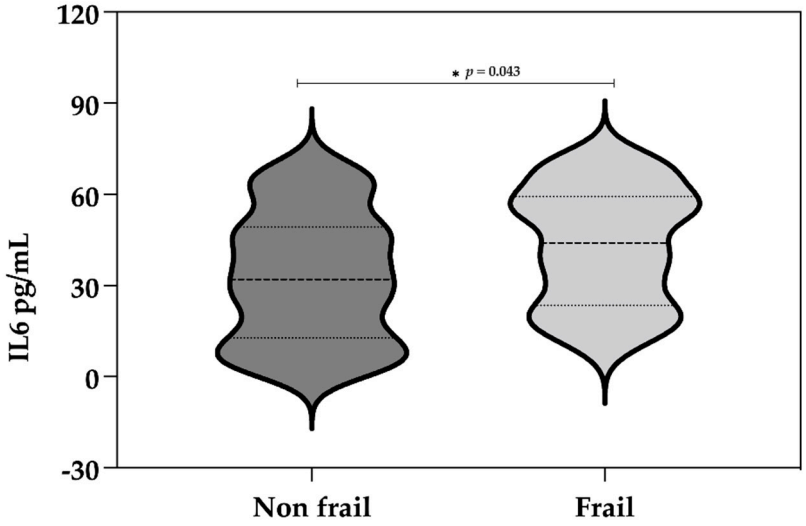

Figure 2. Difference between frailty syndrome and IL-6 (pg/mL). Robust and pre-frail patients were grouped into the non-frail group. Frail patients were those who met 3 or more of the frailty criteria.

For each of the frailty criteria, there were significant differences in IL-6 between those subjects who met the frailty criterion related to the reduction in physical activity and those who did not (p = 0.007, Mann–Whitney U) (Figure 3A). Regarding the frailty criterion "Involuntary weight loss" there was a significant difference in salivary IL-6 concentration between the individuals that fulfilled this criterion compared to those did not (p = 0.002, Mann–Whitney U) (Figure 3B).

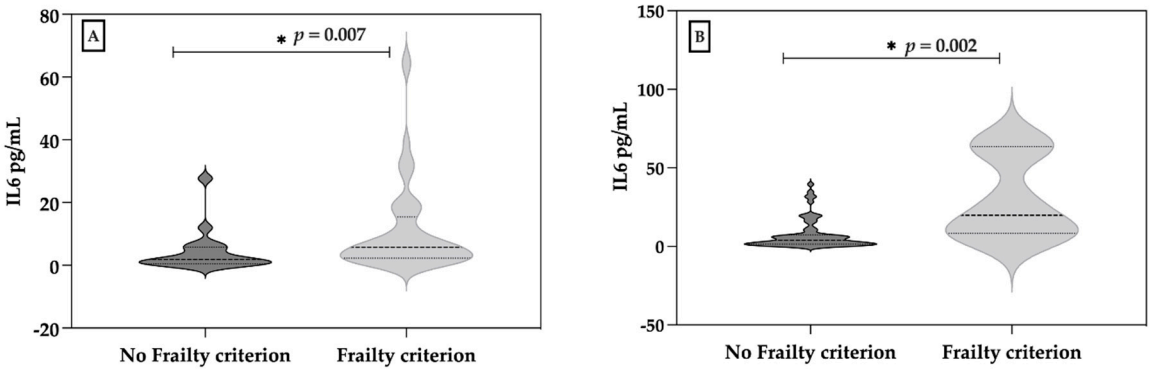

Figure 3. (**A**) Difference between physical activity frailty criteria and (**B**) IL-6 (pg/mL) and weight loss frailty criteria and IL-6 (pg/mL).

In order to analyze the sensitivity and the specificity of salivary IL-6 concentration to discriminate frail versus non frail (robust) individuals, we performed the receiving operating curve (ROC) analysis (Figure 4). The area under the curve value was 0.697 with 95% CI (0.566–0.827) and the cut-off value 3.74 pg/mL has a sensitivity of 69.0% and a specificity of 52.8%.

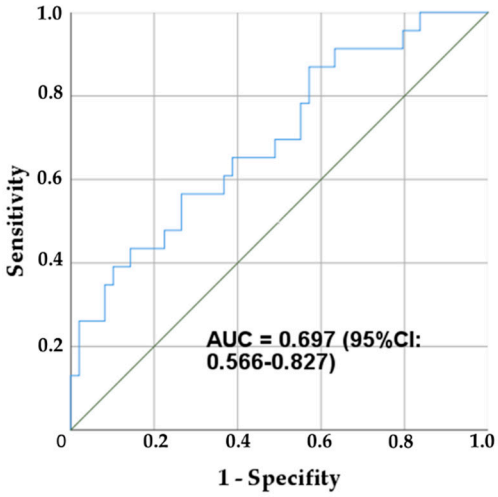

Figure 4. Receiver operating characteristic (ROC) curve for IL-6 concentration and the ability to discriminate between frail individuals versus robust plus pre-frail individuals.

4. Discussion

Frailty has been recognized an emerging public health priority [3]. In the development of the investigation of frailty, many authors have concluded that inflammation is a potential pathophysiological change, which may be closely linked with this syndrome [7]. Fried hypothesized that high levels of cytokines may induce skeletal muscle loss and aggravate neuroendocrine dysregulation, resulting in the muscle loss and weakness seen in the frailty phenotype [5]. This study showed that the IL-6 levels in saliva were higher in participants classified as frail. This is consistent with the observation by other authors who reported a higher IL-6 serum concentration associated with frailty [48]. Researchers

have indicated that increased levels of inflammatory markers such as IL-6, TNF-α, and CRPin blood are associated with reduced muscle strength, poor physical function, frailty, and mortality [49–51]. Several studies have reported evidence for the role of IL-6 in the inflammatory processes through which the immune system intercommunicates with the central nervous system (CNS) [52]. Interactions between IL-6 and the CNS are one of the mechanisms by which the immune system influences the performance of cognitive functions, and these also play a role in neurodegeneration and cognitive functionality. It is well established that IL-6 is a potential biomarker for frailty, which could be evaluated as a target for intervention [53] and is considered a predictive marker for incidents of frailty [54].

Regarding to each of the frailty criteria, in this study we demonstrated for the first time that there are significant differences in salivary IL-6 between subjects who are frail compared to pre-frail and robust ones. In addition, and in particular, the frailty criterion regarding reduction in PA was significantly associated with changes in salivary IL-6 concentration in individuals that met this criterion compared with those that did not meet the criterion. There is a significant body of evidence in the literature regarding levels of inflammatory cytokines in IL-6 people and the level of regular exercise in their daily life. In fact, at all ages, individuals engaging in regular physical activities show lower systemic inflammatory markers in blood, including lower baseline IL-6 levels [55]. A negative association between the amount of physical activity scores and the levels of plasma IL-6 has frequently been reported, so that the more PA they perform the lower their levels of IL-6, and conversely, high levels of plasma IL-6 are related to physical inactivity [56]. The activity generated in the skeletal muscles by exercise is known to be one of the main mechanisms by which the plasma and baseline levels of IL-6 can be modulated [57,58]. IL-6 is also known to induce hepatic glucose output [59] and to induce lipolysis [60]. Physical exercise in itself is a stressor which produces inflammation and induces different immune responses associated with the production of interleukins, thereby inducing anti-inflammatory responses. Maintaining a good balance between pro- and anti-inflammatory cytokines may be an important mechanism through which exercise exerts its immunoprotective and immunoregulatory effects. Furthermore, new genetic studies suggest that these effects of exercise may activate both genes involved in the production of leukocytes and those that downregulate inflammation [61]. Several lines of evidence have demonstrated that physical exercise is associated with a systemic reduction in inflammation [62]. A study performed in older men aged 65–74 years demonstrated that regular PA, independent of disease and disability, alter the levels of pro-inflammatory cytokines in blood [63] i.e., the very physically active group has lower levels of IL-6 than the less active group. In a sample of healthy men with a range of physical activity profiles including older people, this variable was shown to be associated not only with anthropometric variables such as body fat percentage, lean mass, and mass, but also with plasma IL-6 concentration. In addition, age showed an indirect effect on IL-6 through body composition, but no direct effect. The results suggest that PA improves the inflammatory profile through the improvement of body composition, but that there are other pathways as well [64].

Another criterion of frailty in which significant differences were found in this study was weight loss. Interleukin-6 (IL-6) is a cytokine implicated in the regulation of energy metabolism [65]. Elevated circulating levels of IL-6 influence both glucose homeostasis [66] and lipid metabolism [67]. While the effects of IL-6 on glucose homeostasis are conflicting [68], there is greater consensus regarding its role in regulating lipid metabolism. Infusion of IL-6 into healthy individuals stimulates lipolysis and fatty acid oxidation [60]. In vitro studies of adipocytes and myotubes have confirmed the effects of IL-6 on lipolysis and fatty acid oxidation, and evidence shows that IL-6 induces these effects by increasing AMP-activated protein kinase (AMPK) [69]. Finally, studies in rodents support a regulatory effect of IL-6 on adipose tissue mass. Whole-body IL-6 knockout mice develop mature onset obesity and an overall increase in fat mass, a phenotype partially reversed by injection of IL-6 [65].

In this study, for IL-6, the area under the curve value was 0.697 with 95% CI (0.566–0.827) (Figure 3), and the cut-off value 3.74 pg/mL has a sensitivity of 69.0% and a specificity of 52.8%. We demonstrated that higher serum IL-6 levels were significantly associated with the higher levels of frail syndrome. Similar results were found for IL-6 serum concentration [9]. As for IL-6, at a concentration > 1.79 pg/mL, there was a sensitivity of 0.68 and specificity of 0.68 for identification of frail patients, but the sensitivity and specificity were not high.)

Although the results obtained in this study that relate IL-6 with frailty and specifically to levels of PA are well established in the literature, the fact that these results are obtained through saliva suggest this technique can be used as a potential diagnostic tool. Similar results have been obtained in salivary IL-6 values in patients diagnosed with inflammatory diseases such as diabetes [37], sepsis [29] and Huntington's disease [36]. More recently, an increasing number of studies have suggested that salivary biomarkers can be useful for the diagnosis and follow-up of some diseases or conditions, regardless of their blood concentrations [28]. Some studies have obtained results indicating that salivary IL-6 is highly correlated with blood levels [70]. Saliva testing can therefore be a non-invasive tool for frailty diagnosis, without the sampling technique entailing added stress for the patient which implies changes in levels which could occur as a result of the stress associated with drawing blood [35], and it is inexpensive, can be conducted in ecological contexts across time, and at multiple moments throughout the day [27]. Saliva tests could therefore be used for the diagnosis and to indicate the existence or the risk of developing a disease, as well as the response to a particular therapy. Salivary diagnostics is a rapidly emerging field that is dependent on the development of sensitive and specific biomarkers that can be employed in large scale clinical settings.

The main limitation of our study is the small sample size. Despite it being a homogeneous sample because subjects are institutionalized older individuals living in long-stay centers, and therefore with healthy and controlled habits (no smoking, no alcohol consumption), we have not been able to eliminate the influence of all confounding factors that could modify IL-6 levels. There are mixed reports in the literature about the relationship between salivary IL-6 and dental and periodontal disorders. A lack of correlation between IL-6 concentration in saliva and the presence of periodontal diseases has been already demonstrated. Dogan et al. measured periodontal parameters, including gingival index and plaque index, and did not find any significant association between these parameters and IL-6 concentration in saliva [71]. In contrast, in patients with calcified dental plaque composed of calcium phosphate mineral salts and surrounded by a non-mineralized bacterial layer (called dental calculus), the salivary IL-6 concentration was higher with associated chronic periodontitis [72]. In totally edentulous patients with implant-supported overdentures, the concentration of IL-6 in saliva can be used as markers of peri-implant disease [73]. Significant longitudinal associations between oral health indicators and frailty that highlight the importance of oral health as a predictor of frailty in older age [74]. Frailty syndrome is independently associated with the presence of dental caries and oral status [75]. However, periodontal disease has a weaker association with frailty compared with number of teeth [76], demonstrating no significant association between periodontitis and the frailty criterion grip strength [77,78]. Future research should investigate the role of potential mediating factors in the association between salivary IL-6 and frailty syndrome." Moreover, our study being a cross-sectional study, does not allow inference about cause–effect relationship between IL-6 and frailty syndrome; therefore, future longitudinal studies with larger samples will be necessary in order to know the relationship and confounding factors.

More research is needed to differentiate the IL-6 levels between frail and pre-frail individuals and acquire an early identification of frail status, essential for clinical practice and the therapeutic decisions to curb the syndrome. It is reasonable to assume that future clinical studies will progressively demonstrate the usefulness of salivary biomarkers related to inflammatory molecules, such as IL-6, for the diagnosis and follow-up of some diseases, and specifically for the diagnosis of the frailty.

In conclusion, this study shows a relationship between frailty syndrome and IL-6 levels obtained through saliva tests. Level of IL-6 is higher in frail individual compared non-frail group as well as it is higher in those subjects who met the frailty criterion related to the reduction in physical activity and the individuals that fulfilled the criterion "Involuntary weight loss" compared to those who did not. These results suggest that salivary IL-6 tests can be useful to distinguish between fragile and non-fragile people and therefore this technique can be used as a potential diagnostic tool.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Human Research Ethics Committee of the University of Valencia (Valencia, Spain) (procedure number H20190330195344 dated 4 April 2019).

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

Data Availability Statement: Data will be available upon specific request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hubbard, R.E.; Woodhouse, K.W. Frailty, inflammation and the elderly. *Biogerontology* **2010**, *11*, 635–641. [CrossRef] [PubMed]
2. Fernández-Garrido, J.; Navarro-Martínez, R.; Buigues-González, C.; Martínez-Martínez, M.; Ruiz-Ros, V.; Cauli, O. The value of neutrophil and lymphocyte count in frail older women. *Exp. Gerontol.* **2014**, *54*, 35–41. [CrossRef] [PubMed]
3. Wang, J.; Maxwell, C.A.; Yu, F. Biological Processes and Biomarkers Related to Frailty in Older Adults: A State-of-the-Science Literature Review. *Biol. Res. Nurs.* **2018**, *21*, 80–106. [CrossRef]
4. Fried, L.P.; Ferrucci, L.; Darer, J.; Williamson, J.D.; Anderson, G. Untangling the Concepts of Disability, Frailty, and Comorbidity: Implications for Improved Targeting and Care. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2004**, *59*, M255–M263. [CrossRef]
5. Fried, L.P.; Tangen, C.M.; Walston, J.; Newman, A.B.; Hirsch, C.; Gottdiener, J.; Seeman, T.; Tracy, R.; Kop, W.J.; Burke, G.; et al. Frailty in Older adults: Evidence for a phenotype. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2001**, *56*, M146–M157. [CrossRef] [PubMed]
6. Walston, J.; McBurnie, M.A.; Newman, A.B.; Tracy, R.P.; Kop, W.J.; Hirsch, C.H.; Gottdiener, J.; Fried, L.P. Frailty and Activation of the Inflammation and Coagulation Systems with and without Clinical ComorbiditiesResults from the Cardiovascular Health Study. *Arch. Intern. Med.* **2002**, *162*, 2333–2341. [CrossRef]
7. Leng, S.; Chen, X.; Mao, G. Frailty syndrome: An overview. *Clin. Interv. Aging* **2014**, *9*, 433–441. [CrossRef] [PubMed]
8. Lang, P.O.; Michel, J.-P.; Zekry, D. Frailty Syndrome: A Transitional State in a Dynamic Process. *Gerontology* **2009**, *55*, 539–549. [CrossRef]
9. Lai, H.-Y.; Chang, H.-T.; Lee, Y.L.; Hwang, S.-J. Association between inflammatory markers and frailty in institutionalized older men. *Maturitas* **2014**, *79*, 329–333. [CrossRef]
10. Ma, L.; Sha, G.; Zhang, Y.; Li, Y. Elevated serum Il-6 and adiponectin levels are associated with frailty and physical function in Chinese older adults. *Clin. Interv. Aging* **2018**, *13*, 2013–2020. [CrossRef]
11. Furtado, G.E.; Chupel, M.U.; Minuzzi, L.; Patrício, M.; Loureiro, M.; Bandelow, S.; Hogervorst, E.; Ferreira, J.P.; Teixeira, A.M. Exploring the potential of salivary and blood immune biomarkers to elucidate physical frailty in institutionalized older women. *Exp. Gerontol.* **2020**, *129*, 110759. [CrossRef]
12. Pollack, L.R.; Litwack-Harrison, S.; Cawthon, P.M.; Ensrud, K.; Lane, N.E.; Barrett-Connor, E.; Dam, T.-T. Patterns and Predictors of Frailty Transitions in Older Men: The Osteoporotic Fractures in Men Study. *J. Am. Geriatr. Soc.* **2017**, *65*, 2473–2479. [CrossRef]
13. Leng, S.X.; Cappola, A.R.; Andersen, R.E.; Blackman, M.R.; Koenig, K.; Blair, M.; Walston, J.D. Serum levels of insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S), and their relationships with serum interleukin-6, in the geriatric syndrome of frailty. *Aging Clin. Exp. Res.* **2004**, *16*, 153–157. [CrossRef]
14. Leng, S.X.; Tian, X.; Matteini, A.; Li, H.; Hughes, J.; Jain, A.; Walston, J.D.; Fedarko, N. IL-6-independent association of elevated serum neopterin levels with prevalent frailty in community-dwelling older adults. *Age Ageing* **2011**, *40*, 475–481. [CrossRef]
15. Qu, T.; Yang, H.; Walston, J.D.; Fedarko, N.S.; Leng, S.X. Upregulated monocytic expression of CXC chemokine ligand 10 (CXCL-10) and its relationship with serum interleukin-6 levels in the syndrome of frailty. *Cytokine* **2009**, *46*, 319–324. [CrossRef]
16. Soysal, P.; Stubbs, B.; Lucato, P.; Luchini, C.; Solmi, M.; Peluso, R.; Sergi, G.; Isik, A.T.; Manzato, E.; Maggi, S.; et al. Inflammation and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res. Rev.* **2016**, *31*, 1–8. [CrossRef]

17. Locksley, R.M.; Killeen, N.; Lenardo, M.J. The TNF and TNF Receptor Superfamilies: Integrating Mammalian Biology. *Cell* **2001**, *104*, 487–501. [CrossRef]
18. Gruol, D.L.; Nelson, T.E. Physiological and pathological roles of interleukin-6 in the central nervous system. *Mol. Neurobiol.* **1997**, *15*, 307–339. [CrossRef] [PubMed]
19. Yaffe, K.; Kanaya, A.; Lindquist, K.; Simonsick, E.M.; Harris, T.; Shorr, R.I.; Tylavsky, F.A.; Newman, A.B. The Metabolic Syndrome, Inflammation, and Risk of Cognitive Decline. *JAMA J. Am. Med. Assoc.* **2014**, *292*, 2237–2242. [CrossRef]
20. Dik, M.G.; Jonker, C.; Hack, C.E.; Smit, J.H.; Comijs, H.C.; Eikelenboom, P. Serum inflammatory proteins and cognitive decline in older persons. *Neurology* **2015**, *64*, 1371–1377. [CrossRef]
21. Mooijaart, S.P.; Sattar, N.; Trompet, S.; Lucke, J.; Stott, D.J.; Ford, I.; Jukema, J.W.; Westendorp, R.G.J.; De Craen, A.J.M.; The PROSPER Study Group. Circulating interleukin-6 concentration and cognitive decline in old age: The PROSPER study. *J. Intern. Med.* **2013**, *274*, 77–85. [CrossRef] [PubMed]
22. Kline, R.; Wong, E.; Haile, M.; Didehvar, S.; Farber, S.; Sacks, A.; Pirraglia, E.; de Leon, M.J.; Bekker, A. Peri-Operative Inflammatory Cytokines in Plasma of the Elderly Correlate in Prospective Study with Postoperative Changes in Cognitive Test Scores. *Int. J. Anesthesiol. Res.* **2016**, *4*, 313–321. [CrossRef]
23. Marsland, A.L.; Gianaros, P.J.; Kuan, D.C.-H.; Sheu, L.K.; Krajina, K.; Manuck, S.B. Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain Behav. Immun.* **2015**, *48*, 195–204. [CrossRef]
24. Trapero, I.; Cauli, O. Interleukin 6 and cognitive dysfunction. *Metab. Brain Dis.* **2014**, *29*, 593–608. [CrossRef] [PubMed]
25. Colburn, W.; DeGruttola, V.; DeMets, D.; Downing, G.; Hoth, D.; Oates, J.; Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* **2001**, *69*, 89–95. [CrossRef]
26. Corwin, E.J.; Ferranti, E. Integration of biomarkers to advance precision nursing interventions for family research across the lifespan. *Nurs. Outlook* **2016**, *64*, 292–298. [CrossRef]
27. Slavish, D.; Graham-Engeland, J.E.; Smyth, J.; Engeland, C.G. Salivary markers of inflammation in response to acute stress. *Brain Behav. Immun.* **2015**, *44*, 253–269. [CrossRef]
28. Chávez-Alderete, J.; Gochicoa-Rangel, L.; Del-Río-Hidalgo, R.; Guerrero-Zúñiga, S.; Mora-Romero, U.; Benítez-Pérez, R.; Rodríguez-Moreno, L.; Torre-Bouscoulet, L.; Vargas, M.H. Salivary concentrations of cytokines and other analytes in healthy children. *Cytokine* **2021**, *138*, 155379. [CrossRef] [PubMed]
29. Galhardo, L.F.; Ruivo, G.F.; De Oliveira, L.D.; Parize, G.; Dos Santos, S.S.F.; Pallos, D.; Leão, M.V.P. Inflammatory markers in saliva for diagnosis of sepsis of hospitalizes patients. *Eur. J. Clin. Investig.* **2020**, *50*, e13219. [CrossRef]
30. Khurshid, Z.; Naseem, M.; Sheikh, Z.; Najeeb, S.; Shahab, S.; Zafar, M.S. Oral antimicrobial peptides: Types and role in the oral cavity. *Saudi Pharm. J.* **2016**, *24*, 515–524. [CrossRef]
31. Xiao, H.; Wong, D.T. Proteomics and its applications for biomarker discovery in human saliva. *Bioinformation* **2011**, *5*, 294–296. [CrossRef]
32. Gröschl, M.; Köhler, H.; Topf, H.-G.; Rupprecht, T.; Rauh, M. Evaluation of saliva collection devices for the analysis of steroids, peptides and therapeutic drugs. *J. Pharm. Biomed. Anal.* **2008**, *47*, 478–486. [CrossRef]
33. Melguizo-Rodríguez, L.; Costela-Ruiz, V.J.; Manzano-Moreno, F.J.; Ruiz, C.; Illescas-Montes, R. Salivary Biomarkers and Their Application in the Diagnosis and Monitoring of the Most Common Oral Pathologies. *Int. J. Mol. Sci.* **2020**, *21*, 5173. [CrossRef] [PubMed]
34. Özçaka, Ö.; Ceyhan, B.Ö.; Akcali, A.; Biçakci, N.; Lappin, D.F.; Buduneli, N. Is There an Interaction Between Polycystic Ovary Syndrome and Gingival Inflammation? *J. Periodontol.* **2012**, *83*, 1529–1537. [CrossRef]
35. Ibáñez-Del Valle, V.; Navarro-Martínez, R.; Ballestar-Tarin, M.L.; Cauli, O. Salivary Inflammatory Molecules as Biomarkers of Sleep Alterations: A Scoping Review. *Diagnostics* **2021**, *11*, 278. [CrossRef]
36. Corey-Bloom, J.; Fischer, R.S.; Kim, A.; Snell, C.; Georgia, M.; Parkin, G.M.; Granger, D.A.; Granger, S.W.; Thomas, E.A. Levels of Interleukin-6 in Saliva, but Not Plasma, Correlate with Clinical Metrics in Huntington's Disease Patients and Healthy Control Subjects. *Int. J. Mol. Sci.* **2020**, *21*, 6363. [CrossRef] [PubMed]
37. Agho, E.T.; Owotade, F.J.; Kolawole, B.A.; Oyetola, E.O.; Adedeji, T.A. Salivary inflammatory biomarkers and glycated haemoglobin among patients with type 2 diabetic mellitus. *BMC Oral Health* **2021**, *21*, 101. [CrossRef] [PubMed]
38. Maciejczyk, M.; Mil, K.M.; Gerreth, P.; Hojan, K.; Zalewska, A.; Gerreth, K. Salivary cytokine profile in patients with ischemic stroke. *Sci. Rep.* **2021**, *11*, 17185. [CrossRef]
39. Morawska, K.; Maciejczyk, M.; Zięba, S.; Popławski, Ł.; Kita-Popławska, A.; Krętowski, J.; Zalewska, A. Cytokine/Chemokine/Growth Factor Profiles Contribute to Understanding the Pathogenesis of the Salivary Gland Dysfunction in Euthyroid Hashimoto's Thyroiditis Patients. *Mediat. Inflamm.* **2021**, *2021*, 3192409. [CrossRef] [PubMed]
40. Grisius, M.M.; Bermudez, D.K.; Fox, P.C. Salivary and serum interleukin 6 in primary Sjögren's syndrome. *J. Rheumatol.* **1997**, *24*, 1089–1091.
41. Orme, J.G.; Reis, J.; Herz, E.J. Factorial and Discriminant Validity of the Center for Epidemiological Studies Depression (CES-D) Scale. *J. Clin. Psychol.* **1986**, *42*, 28–33. [CrossRef]
42. Radloff, L.S. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Appl. Psychol. Meas.* **1977**, *1*, 385–401. [CrossRef]

43. Elosua, R.; Garcia, M.; Aguilar, A.; Molina, L.; I Covas, M.; Marrugat, J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish Women. Investigators of the MARATDON Group. *Med. Sci. Sports Exerc.* **2000**, *32*, 1431–1437. [CrossRef]
44. Ruiz Comellas, A.; Pera, G.; Baena Díez, J.M.; Mundet Tudurí, X.; Alzamora Sas, T.; Elosua, R.; Torán Monserrat, P.; Heras, A.; Forés Raurell, R.; Fusté Gamisans, M.; et al. Validación de Una Versión Reducida En Español Del Cuestionario de Acti-vidad Física En El Tiempo Libre de Minnesota (VREM). *Rev. Esp. Salud Publica* **2012**, *86*, 495–508.
45. Jacobs, D.R., Jr.; Ainsworth, B.E.; Hartman, T.J.; Leon, A.S. A simultaneous evaluation of 10 commonly used physical activity questionnaires. *Med. Sci. Sports Exerc.* **1993**, *25*, 81–91. [CrossRef] [PubMed]
46. Guralnik, J.; Simonsick, E.; Ferrucci, L.; Glynn, R.; Berkman, L.; Blazer, D.; Scherr, P.; Wallace, R. A Short Physical Perfor-mance Battery Assessing Lower Extremity Function: Association with Self-Reported Disability and Prediction of Mortality and Nursing Home Admission. *J. Gerontol.* **1994**, *49*, M85–M94. [CrossRef]
47. Ottenbacher, K.J.; Branch, L.G.; Ray, L.; Gonzales, V.A.; Peek, M.; Hinman, M.R. The reliability of upper- and lower-extremity strength testing in a community survey of older adults. *Arch. Phys. Med. Rehabil.* **2002**, *83*, 1423–1427. [CrossRef] [PubMed]
48. Marcos-Pérez, D.; Sánchez-Flores, M.; Proietti, S.; Bonassi, S.; Costa, S.; Teixeira, J.P.; Fernández-Tajes, J.; Pásaro, E.; Laffon, B.; Valdiglesias, V. Association of inflammatory mediators with frailty status in older adults: Results from a systematic review and meta-analysis. *GeroScience* **2020**, *42*, 1451–1473. [CrossRef] [PubMed]
49. Schaap, L.A.; Pluijm, S.M.; Deeg, D.J.; Visser, M. Inflammatory Markers and Loss of Muscle Mass (Sarcopenia) and Strength. *Am. J. Med.* **2006**, *119*, 526.e9–526.e17. [CrossRef] [PubMed]
50. Roubenoff, R.; Parise, H.; Payette, H.A.; Abad, L.W.; D'Agostino, R.; Jacques, P.F.; Wilson, P.W.F.; Dinarello, C.A.; Harris, T.B. Cytokines, insulin-likegrowthfactor1, sarcopenia, and mortality in very old community-dwelling men and women: The Framingham heart study. *Am. J. Med.* **2003**, *115*, 429–435. [CrossRef]
51. Schaap, L.A.; Pluijm, S.M.F.; Deeg, D.J.H.; Harris, T.B.; Kritchevsky, S.B.; Newman, A.B.; Colbert, L.H.; Pahor, M.; Rubin, S.M.; Tylavsky, F.A.; et al. Higherinflammatorymarkerlevelsinolder persons: Associations with 5-year change in muscle mass and muscle strength. *J. Gerontol. A Biol. Sci. Med. Sci.* **2009**, *64*, 1183–1189. [CrossRef]
52. Di Benedetto, S.; Müller, L.; Wenger, E.; Düzel, S.; Pawelec, G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neurosci. Biobehav. Rev.* **2017**, *75*, 114–128. [CrossRef]
53. Tembo, M.C.; Holloway-Kew, K.L.; Bortolasci, C.C.; Brennan-Olsen, S.L.; Williams, L.J.; Kotowicz, M.A.; Pasco, J.A. Association between serum interleukin-6 and frailty in older men: Cross-sectional data. *Eur. Geriatr. Med.* **2021**, *12*, 887–892. [CrossRef]
54. Semmarath, W.; Seesen, M.; Yodkeeree, S.; Sapbamrer, R.; Ayood, P.; Malasao, R.; Siviroj, P.; Limtrakul (Dejkriengkraikul), P. The Association between Frailty Indicators and Blood-Based Biomarkers in Early-Old Community Dwellers of Thailand. *Int. J. Environ. Res. Public Health* **2019**, *16*, 3457. [CrossRef]
55. Kapilevich, L.V.; Zakharova, A.N.; Kabachkova, A.V.; Kironenko, T.A.; Orlov, S.N. Dynamic and Static Exercises Differentially Affect Plasma Cytokine Content in Elite Endurance- and Strength-Trained Athletes and Untrained Volunteers. *Front. Physiol.* **2017**, *8*, 35. [CrossRef] [PubMed]
56. Fischer, C.P. Interleukin-6 in acute exercise and training: What is the biological relevance? *Exerc. Immunol. Rev.* **2006**, *12*, 6–33.
57. Monteiro-Junior, R.S.; de Tarso Maciel-Pinheiro, P.; da Matta Mello, E.; da Silva, L.F.; Terra, R.; Carneiro, L.S.F.; Rodrigues, V.D.; Nascimento, O.J.M.; Deslandes, A.C.; Laks, J. Effect of Exercise on Inflammatory Profile of Older Persons: Systematic Review and Meta-Analyses. *J. Phys. Act. Health* **2018**, *15*, 64–71. [CrossRef]
58. Wärnberg, J.; Cunningham, K.; Romeo, J.; Marcos, A. Physical activity, exercise and low-grade systemic inflammation. *Proc. Nutr. Soc.* **2010**, *69*, 400–406. [CrossRef] [PubMed]
59. Keller, C.; Steensberg, A.; Pilegaard, H.; Osada, T.; Saltin, B.; Pedersen, B.K.; Neufer, P.D. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: Influence of muscle glycogen content. *FASEB J.* **2001**, *15*, 1–15. [CrossRef] [PubMed]
60. Van Hall, G.; Steensberg, A.; Sacchetti, M.; Fischer, C.; Keller, C.; Schjerling, P.; Hiscock, N.; Moller, K.; Saltin, B.; Febbraio, M.A.; et al. Interleukin-6 Stimulates Lipolysis and Fat Oxidation in Humans. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 3005–3010. [CrossRef]
61. Liu, D.; Wang, R.; Grant, A.R.; Zhang, J.; Gordon, P.M.; Wei, Y.; Chen, P. Immune adaptation to chronic intense exercise training: New microarray evidence. *BMC Genom.* **2017**, *18*, 29. [CrossRef]
62. Thielen, J.-W.; Kärgel, C.; Müller, B.W.; Rasche, I.; Genius, J.; Bus, B.; Maderwald, S.; Norris, D.G.; Wiltfang, J.; Tendolkar, I. Aerobic Activity in the Healthy Elderly Is Associated with Larger Plasticity in Memory Related Brain Structures and Lower Systemic Inflammation. *Front. Aging Neurosci.* **2016**, *8*, 319. [CrossRef] [PubMed]
63. Jankord, R.; Jemiolo, B. Influence of Physical Activity on Serum IL-6 and IL-10 Levels in Healthy Older Men. *Med. Sci. Sports Exerc.* **2004**, *36*, 960–964. [CrossRef]
64. Fitzgerald, L.; MMacey, P.; Brecht, M.L. Pathways to interleukin-6 in healthy males and serious leisure male athletes: Physical activity, body composition and age. *PLoS ONE* **2012**, *7*, e40513. [CrossRef]
65. Wallenius, V.; Wallenius, K.; Ahren, B.; Rudling, M.; Carlsten, H.; Dickson, S.L.; Ohlsson, C.; Jansson, J.O. Inter-leukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* **2002**, *8*, 75–79. [CrossRef] [PubMed]
66. Febbraio, M.A.; Hiscock, N.; Sacchetti, M.; Fischer, C.P.; Pedersen, B.K. Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. *Diabetes* **2004**, *53*, 1643–1648. [CrossRef]

67. Petersen, E.W.; Carey, A.L.; Sacchetti, M.; Steinberg, G.; Macaulay, S.L.; Febbraio, M.A.; Pedersen, B.K. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *288*, E155–E162. [CrossRef]
68. Pedersen, B.K.; Febbraio, M.A. Point: Interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. *J. Appl. Physiol.* **2007**, *102*, 814–816. [CrossRef]
69. Carey, A.L.; Steinberg, G.R.; Macaulay, S.L.; Thomas, W.G.; Holmes, A.G.; Ramm, G.; Prelovsek, O.; Hohnen-Behrens, C.; Watt, M.J.; James, D.E.; et al. Interleukin-6 Increases Insulin-Stimulated Glucose Disposal in Humans and Glucose Uptake and Fatty Acid Oxidation In Vitro via AMP-Activated Protein Kinase. *Diabetes* **2006**, *55*, 2688–2697. [CrossRef] [PubMed]
70. Byrne, M.L.; O'Brien-Simpson, N.; Reynolds, E.; Walsh, K.A.; Laughton, K.; Waloszek, J.M.; Woods, M.J.; Trinder, J.; Allen, N.B. Acute phase protein and cytokine levels in serum and saliva: A comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain Behav. Immun.* **2013**, *34*, 164–175. [CrossRef]
71. Doğan, G.E.; Toraman, A.; Şebin, S.Ö.; Doğan, Ç.; Güngör, A.; Aksoy, H.; Asutay, H. Salivary IL-6 and IL-10 levels in subjects with obesity and gingivitis. *Am. J. Dent.* **2016**, *29*, 261–265.
72. Batool, H.; Nadeem, A.; Kashif, M.; Shahzad, F.; Tahir, R.; Afzal, N. Salivary Levels of IL-6 and IL-17 Could Be an Indicator of Disease Severity in Patients with Calculus Associated Chronic Periodontitis. *BioMed Res. Int.* **2018**, *2018*, 8531961. [CrossRef]
73. Liskmann, S.; Vihalemm, T.; Salum, O.; Zilmer, K.; Fischer, K.; Zilmer, M. Correlations between clinical parameters and interleukin-6 and interleukin-10 levels in saliva from totally edentulous patients with peri-implant disease. *Int. J. Oral Maxillofac. Implant.* **2006**, *21*, 543–550.
74. Hakeem, F.F.; Bernabé, E.; Sabbah, W. Association between oral health and frailty: A systematic review of longitudinal studies. *Gerodontology* **2019**, *36*, 205–215. [CrossRef] [PubMed]
75. Valdez, E.; Wright, F.A.C.; Naganathan, V.; Milledge, K.; Blyth, F.M.; Hirani, V.; Le Couteur, D.G.; Handelsman, D.J.; Waite, L.M.; Cumming, R.G. Frailty and oral health: Findings from the Concord Health and Ageing in Men Project. *Gerodontology* **2020**, *37*, 28–37. [CrossRef] [PubMed]
76. Hakeem, F.F.; Bernabé, E.; Sabbah, W. Association Between Oral Health and Frailty Among American Older Adults. *J. Am. Med. Dir. Assoc.* **2021**, *22*, 559–563.e2. [CrossRef]
77. Aravindakshan, V.; Hakeem, F.F.; Sabbah, W. Periodontal Disease and Grip Strength among Older Adults. *Geriatrics* **2020**, *5*, 46. [CrossRef]
78. MacEntee, M.I.; Donnelly, L.R. Oral health and the frailty syndrome. *Periodontology 2000* **2016**, *72*, 135–141. [CrossRef]