Degree project

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Intermittent Fasting Improves Cognitive Abilities in Alzheimer's Disease

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Student: Hanna Ek

Supervisor: Sakari Kallio

Examiner: Joel Gerafi

Abstract

Alzheimer's disease is the most common dementia disease and the main cause of death. The hallmark is neurofibrillary tangles (abnormal aggregates of tau protein) and beta-amyloid (Aβ) neuritic plaques that leads to impaired cognitive function such as memory loss and learning difficulties. Researchers have discovered that intermittent fasting improves these cognitive abilities, even though eating regularly is recommended for good cognition. This systematic review aims to investigate further if intermittent fasting improves cognitive function in Alzheimer's disease and if levels of Aβ and tau pathology explain these changes in cognitive function. The research question is: does intermittent fasting improve cognitive abilities in Alzheimer's disease and does the levels of Aβ and tau pathology explain these cognitive changes? A literature search for articles was performed on three electronic databases: Pubmed, Web of Science, and WorldCat which gave n=744 articles. The cognitive tests showed a trend toward improved memory, learning, and exploratory behavior in Alzheimer's disease from intermittent fasting. However, the effects on the levels of Aβ and tau pathology were inconsistent, which invites the possibility of a more prominent, underlying issue of Alzheimer's disease.

Keywords: intermittent fasting, ketogenic diet, Alzheimer's disease, cognition

Intermittent Fasting Improves Cognitive Abilities in Alzheimer's Disease

The elderly population has grown dramatically worldwide. About 13 percent of the population of 962 million people in 2017 is over 60 years old. This group accounts for a higher percentage of total healthcare costs than the younger population; frailty is one important factor (Bock et al., 2016). Cognitive decline is a biological consequence of frailty that decreases independence and increases mortality risk (Anton & Leeuwenburgh, 2013). The domains concerned in cognitive decline are complex attention, executive functioning, learning, memory, language, perceptual motor/visuospatial function, and social cognition. The symptoms manifest in disturbances in cognitive abilities such as difficulty in completing ordinary tasks, holding and using information, remembering recent events or conversations, finding words and phrases, multi-tasking, orienting, and behaving appropriately. The degree of difficulty in everyday activities depends on the severity of the cognitive decline (Hugo & Ganguli, 2014).

The range of cognitive decline in the elderly population includes normal cognitive decline due to aging, Subjective Cognitive Impairment, Mild Cognitive Impairment, and dementia (Jongsiriyanyong & Limpawattana, 2018). Mild Cognitive Impairment is an intermediate state between normal cognition and dementia and affects approximately 6.7% to 25.2% of adults older than 60 (Petersen et al., 2018). The functional abilities are intact to the extent that the patient can be independent in everyday life. However, Mild Cognitive Impairment can progress to dementia. Dementia is characterized as a severe cognitive impairment that affects social and/or working life (Jongsiriyanyong & Limpawattana, 2018). The disease has a high mortality rate, is expensive to treat, and has a detrimental impact on the well-being of caregivers. After 65, the dementia risk doubles every five years. In 2010, 36,5 million people suffered from the disease, and the number is estimated to increase to 115,4 million in 2050 (Hugo & Ganguli, 2014).

Alzheimer's Disease

Alzheimer's disease is the most common dementia disease (Chandra et al., 2019) and has the highest mortality rate of all death causes (Murphy et al., 2013). Usually, the disease is diagnosed at the age of 80-90 but the onset can occur as early as the age of 50. The duration of survival after onset is approximately 10 years but the severity of the cognitive decline and comorbid diseases may impact the life expectancy (Brookmeyer et al., 2002; Helzner et al., 2008). Alzheimer's disease is an etiologic subtype of Mild Neurocognitive Disorders (comparable to Mild Cognitive Impairment) and Major Neurocognitive Disorders

according to the Fifth Edition of The Diagnostic and Statistical Manual of Mental Disorders (DSM-5). To diagnose Mild Neurocognitive Disorders in Alzheimer's disease, the patient must show a modest cognitive decline in at least memory. The patient is still independent in daily activities, although with greater effort. To diagnose Major Neurocognitive Disorders (dementia) in Alzheimer's disease, the patient must show a significant cognitive decline in at least two cognitive areas in which one of the domains concerns memory. The patient has difficulty performing everyday tasks as the disease now hinders independence (Hugo & Ganguli, 2014). Memory loss is one of the first symptoms of Alzheimer's disease. The memory process consists of encoding, storing, and retrieving information about internal and external stimuli. The brain creates representations of the received information from the sensory systems, which need to be constantly updated to allow effective interaction with the changing surroundings.

Neural circuits play an important role in learning and memory (Cajigas et al., 2010). A neural circuit is a group of neurons connected by synapses to perform a certain function during activation. A neuron, also known as a nerve cell, interacts with other cells through synapses, which are specialized connections between cells (Azarfar et al., 2018). Ramón y Cajal (1894) suggested that the strengthening of synaptic connections between neurons, also known as synaptic plasticity, is the underlying mechanism of memory storage. This progress requires protein synthesis: the process by which cells compensate for protein loss by generating new proteins. Proteins are essential for cell function. The nervous system's ability to adapt to changes in the environment, through learning and memory, is a result of protein modification as well as changes in gene transcription, protein synthesis, and protein degradation (Ryan et al., 2015).

There are different categories of memory: short-term memory/working memory and long-term memory. Short-term memory can hold a limited amount of information for seconds to minutes. Long-term memory seems to store an unlimited amount of information for a long period. Long-term memory can be further divided into subcategories: explicit/declarative memory and implicit/non-declarative memory. Explicit memory consists of episodic memory (memory of contextual information such as time and location) and semantic memory (memory of general knowledge such as facts and concepts); (Jahn, 2013). Implicit memory consists of priming (a stimulus impacts the response to a later stimulus) and procedural memory (memory for performing a task automatically); (Hayes et al., 2012). Semantic memory is the first domain to be affected in Alzheimer's disease and leads to diminished ability to form sentences and find words (Jahn, 2013). Semantic memory loss can occur years before onset (Verma & Howard, 2012).

Impairment to working memory and attention affects executive functioning such as problem-solving, planning, and goal-directed behavior. Impairment to executive functioning correlates with difficulty in performing everyday tasks and demonstrates full dementia. Visuoconstructional/perceptual-motor functions, language functions, and social cognition are affected later in the disease progression (Hugo & Ganguli, 2014). The brain region primarily associated with memory formation is the hippocampus (Tonegawa et al., 2015). Previous research concluded that the medial temporal lobe, which encompasses the entorhinal cortex and the hippocampus, was involved in episodic memory during direct electrophysiological stimulation of the region (Penfield et al., 1950). Further studies demonstrated severe amnesia for episodic memories in humans missing large portions of the medial temporal lobe (Scoville & Milner, 2000). Behavioral studies on rodents indicate that the hippocampus is a key brain region for storing and retrieving contextual memory (Moser & Moser, 1998). Patients with Alzheimer's disease show the most decreased hippocampal volume compared to patients with other forms of dementia (Delli Pizzi et al., 2016).

Memory performance in humans is measured by cognitive tests such as the Montreal Cognitive Assessment (MoCA); (Jankovic et al., 2021) and the Animal Naming test (Campagna et al., 2017). The MoCA explores executive functioning, immediate and delayed memory, visuospatial ability, attention, working memory, language, as well as time and place orientation. The overall score ranges from 0 to 30, with a cut score of 26 demonstrating great specificity and sensitivity (by accurately identifying healthy subjects); (Jankovic et al., 2021). Participants of the Animal Naming test are asked to list as many animals as possible on a time limit of one minute. The objective of this test is to achieve a score of at least 14, which implies naming at least 14 animals in less than a minute (Campagna et al., 2017).

The Neuropathological Structure behind Alzheimer's Disease

The pathology behind Alzheimer's disease consists of neurofibrillary tangles and beta-amyloid (Aβ) neuritic plaques, which are positively correlated with synaptic and neuronal loss (Chandra et al., 2019). The neuronal damage affects cognitive abilities such as the sensory, motor, and cognitive function and results in Alzheimer's disease (Hamos et al., 1989). Neurofibrillary tangles are tangled neurofibrils, i.e., threadlike proteinaceous fibers in the cytoplasm of a nerve cell that extend into axons and dendrites (DeTure & Dickson, 2019). The tangles consist of abnormal aggregates of hyperphosphorylated tau protein (Chandra et al., 2019) which impairs neuronal function by compromising the tau's ability to support the cell (Chen et al., 2016). Beta-amyloid (Aβ) is a peptide derived from amyloid precursor

protein that forms amyloid plaques, i.e., extracellular deposits of amyloids around the cells (Silva et al., 2019). The production of beta-amyloid causes oxidative and inflammatory stress resulting in neuronal damage (Martin et al., 2006). Beta-amyloid has two isoforms, i.e., proteins: the 42-residue Aβ42 and the 40-residue Aβ40 which are elevated in Alzheimer's patients (Chandra et al., 2019).

Neuropathology and Metabolic Function

An inactive lifestyle, excessive caloric intake, and subsequent insulin resistance may lead to a chronic positive energy balance (Kapogiannis & Mattson, 2011; Xu et al., 2011). The excessive energy intake leads to the storage of excess energy in the white adipose tissue and seems to be a risk factor for Alzheimer's disease (Kapogiannis & Mattson, 2011; Xu et al., 2011). Metabolic syndrome is a combination of conditions such as obesity and diabetes (Van Dyken & Lacoste, 2018). The blood-brain barrier controls the entry of toxins, immune cells, and pathogens into the brain and is essential for neural function. The systemic inflammation induced by metabolic syndrome leads to an impaired blood-brain barrier which activates neuropathologies. As a result, glial and neuronal cells are disturbed, causing hormonal dysregulation, enhanced immune sensitivity, and/or cognitive impairment (Van Dyken & Lacoste, 2018). Studies have shown that diabetes (Takeda et al., 2010) and a high-fat diet aggravate cognitive impairment and Aβ pathology (Julien et al., 2010).

Non-pharmacological Interventions for Alzheimer's Disease

There is no cure for Alzheimer's disease or any other form of dementia. Once the disease has developed, it can only be delayed but not prevented (Mosley & Spencer, 2013). The symptoms of neurodegenerative illnesses can be alleviated through symptomatic treatments. However, disease-modifying treatments that inhibit the progression of the disease are unavailable (Hugo & Ganguli, 2014). Research has increased in recent years on ways to improve the quality of life for Alzheimer's patients using non-pharmacological interventions along with pharmaceutical treatments. Non-pharmacological interventions in areas of cognition, psychology, physiology, psychosociology, nutrition, gut health, and fasting have been demonstrated to improve cognitive abilities in dementia (Chalfont et al., 2020) such as increased memory function and learning (Erickson et al., 2011).

Intermittent Fasting in Alzheimer's Disease

Fasting decreases a variety of risk factors for diseases such as high waist measurement, weight, insulin production, blood sugar, blood fats, blood pressure, as well as the risk of suffering a heart disease (including myocardial infarction and stroke), cancer, and dementia (Mattson et al., 2017). Intermittent fasting is scheduled cycles of fasting and eating. There are different types of intermittent fasting such as alternate-day fasting, periodic fasting, time-restricted eating (Mosley & Spencer, 2013), and time-restricted feeding (Shin et al., 2018). Ketone bodies are produced due to the lack of glucose during fasting, and serve as an alternative fuel for brain cells. They mimic some insulin-like actions and reduce insulin resistance. Changes in metabolism, cellular activity, and circadian mechanisms occur which can lead to anatomical and functional improvements in the brain of humans and animals (Sato et al., 1995). Ketone bodies may suppress the neuropathology associated with the cognitive impairment (Van der Auwera et al., 2005). Intermittent fasting is considered a successful intervention for dementia patients (Mosley & Spencer, 2013). Ooi et al. (2020) detected improved cognitive functioning in older adults with Mild Cognitive Impairment who consistently carried out intermittent fasting for three years compared to peers who fasted inconsistently and peers who did neither. Furthermore, animal models of Alzheimer's disease demonstrated ameliorated cognitive impairment from intermittent fasting (Johnson et al., 2007; Sterniczuk et al., 2010).

Animal Models in Alzheimer's Disease

Researchers in the field of Alzheimer's disease often use transgenic animal models, i.e., genetically modified models, as well as non-transgenic models as a control group to study the underlying functions and mechanisms of a disease. Examples of different animal models are the triple-transgenic mouse model of AD (3xTg-AD); (Sterniczuk et al., 2010), the amyloid precursor protein/presenilin 1 (APP/PS1) double-transgenic mice model (Lok et al., 2013), amyloid precursor protein knock-in mouse model (AppNL-G-F); (Hamaguchi et al., 2019), and mitochondrial deacetylase sirtuin 3 knockout (SIRT3 KO) mice (Sidorova-Darmos et al., 2018). Specifically bred laboratory rats include: Sprague Dawley rats and Wistar rats (García-López et al., 1996). Non-genetically modified wild-type mice include the C57BL/6 mice (Song & Hwang, 2017). Researchers using animal models in Alzheimer's disease inject β-amyloid peptides into the hippocampus in mice brains to resemble human Alzheimer's, divide them into groups, and let one of the groups undergo intermittent fasting to measure anatomical and functional changes (Shin et al., 2018).

The anatomical changes in animal models in Alzheimer's research include changes in the levels of Aβ and tau pathology which can be detected by using immunoblotting

(also known as Western Blot), immunohistochemistry, or Enzyme-Linked Immunosorbent Assay (ELISA). Immunohistochemistry is a widely used application of immunostaining that aims at detecting and measuring pathologically relevant proteins in the dissected hippocampus by exposing the fixed cells or tissues to antibodies, i.e., protective proteins used by the immune system. Within the tissue, the antibodies bind specifically to antigens, i.e., substances that initiate immune responses (Maity et al., 2013). Immunoblotting also detects relevant protein through an antibody that recognizes the protein of interest exposed on the membrane (Litovchick, 2020). ELISA detects and quantifies the amount of a protein by using enzyme reactions (Ma et al., 2011).

Functional changes in animal models from intermittent fasting are detected using different behavioral tests during intermittent fasting and an ad libitum diet, i.e., eating as they please. Examples of behavioral tests are the Morris Water Maze (Shin et al., 2018), Barnes Maze test (Pitts, 2018), Y Maze test (Fink et al., 2012), Passive/Inhibitory Avoidance test (Ferrandez & Teasdale, 1995), Elevated Plus Maze test (Watson et al., 2017), Open Field test (Gould et al., 2009), Locomotor Activity tests (measured by an electronic animal activity meter); (Carter & Shieh, 2010), and Rotarod test (Caston et al., 1995). The Morris Water Maze test evaluates spatial learning and memory. The rodent is placed in a pool of water to acquire and spatially localize important visual cues to find a hidden platform below the surface of the water. The following aspects are measured: the amount of time the animal stays in various regions of the water, the amount of time it took to locate the platform, and the distance the animal had to swim before it reached the platform. The test includes three trials: 1) a cued version with a visible platform, 2) a non-cued version with a hidden platform, and 3) a probe trial without a platform (Shin et al., 2018).

The Barnes Maze test also measures spatial learning and memory and is conceptually comparable to the Morris Water Maze test. It includes several daily trials carried out over several days. The elevated circular platform has 40 holes that are placed on the outside. An escape tube is installed beneath one of the holes. The existing bright light and wide spaces are motivating factors for the animals to flee. Rodents often use a combination of three different search tactics: random, serial, and spatial to discover the location of the escape tunnel. The escape tunnel is removed after sufficient acquisition training, and a probe trial is used to measure spatial reference memory (Pitts, 2018).

The Y-Maze test measures spatial learning and memory and is divided into two sessions, each lasting around 30 minutes. It is made up of three similar arms with high walls arranged in a "Y" pattern. One arm is blocked during the initial 15-minute session, but the

mouse can investigate the other two open arms. All arms are exposed during the second session. A tracking system is frequently used to keep track of the animal's position in the maze. The analyzed parameters are: The first arm entered, the number of entries into each arm, and the duration spent in each arm. Mice with intact spatial memory will generally make the first entrance into the previously blocked arm during the second session and around 60%–70% of the time is spent in the new arm. The arm entries are not significantly different in mice with spatial memory problems, likely due to a lack of capacity to distinguish between previously viewed and new items (Fink et al., 2012). The Passive/Inhibitory Avoidance test also measures short-term in addition to long-term memory. The animal is placed in a brightly illuminated enclosure of the test box. The animal travels into a dark chamber, where a shock is given. During the test phase, which normally lasts 24 hours, the researchers observe whether the animal returns to the dark compartment, which may indicate an age-related decline (Ferrandez & Teasdale, 1995).

The Elevated Plus maze test measures anxiety and is a four-armed elevated maze with two open arms and two arms with walls. The animals are situated at the crossway of the four arms of the labyrinth, facing an open arm. The ratio of time spent in the open arms to time spent in the closed arms is used to assess anxiety behavior in rodents. Increased open arm activity indicates decreased anxiety behavior and thus improved cognition (Watson et al., 2017). The Open-Field test also measures anxiety through exploratory behavior and activity in mice and rats. The enclosure has surrounding walls preventing escape. Outcome of interest is ambulation, i.e., movement, which can be modified by a variety of factors such as exploratory drive or freezing or other fear-related behavior. The measured parameters are: distance traveled, time spent moving, and change in activity over time. Rats and mice have a natural sensitivity to lighted open spaces. They do, however, exhibit a strong desire to investigate potentially dangerous stimuli. Increased anxiety causes decreased exploratory behavior and a tendency for staying near the field's perimeter (Gould et al., 2009).

Locomotor activity, i.e., animals' spontaneous movement from one location to another, can be measured through infrared beams that are sent across the cage. When an animal travels around the cage, it breaks the beam, and the time and position are recorded by a computer. Spontaneous locomotor activity can be measured using an electronic animal activity meter (Carter & Shieh, 2010). The Rotarod test measures motor coordination and consists of a circular rod turning at a constant or increasing speed. Animals placed on the rotating rod try to avoid falling onto the platform below. Since the cerebellum is important for motor control, a reduced time in remaining on the rod indicate cerebellar deficits (Caston et al., 1995).

Different Theories on Fasting's Effect on Cognition

Research highlighting intermittent fasting's positive impact on cognition contradicts the general guidance on a healthy lifestyle that states skipping meals compromises daily performance and productivity (Mosley & Spencer, 2013). Meanwhile, numerous societies have observed the positive effects of fasting on health over the centuries, either due to food scarcity or religion (Martin et al., 2006). Millennia of famine have led to the assumption that the human body was designed to fast and therefore respond well to periods without food (Mosley & Spencer, 2013). Previous studies suggest that neurofibrillary tangles and beta-amyloid (Aβ) neuritic plaques impair cognitive abilities in Alzheimer's disease (Chandra et al., 2019; Martin et al., 2006) whereas intermittent fasting improves them (Johnson et al., 2007; Ooi et al., 2020; Sterniczuk et al., 2010). In light of the different theories, this systematic review aims to investigate further if intermittent fasting improves cognitive function in Alzheimer's disease and if the levels of Aβ and tau pathology explain these changes in cognitive function. The research question is: does intermittent fasting improve cognitive abilities in Alzheimer's disease and does the levels of Aβ and tau pathology explain these cognitive changes? I will approach this question by focusing on the cognitive tests, changes in cognitive function, and Aβ and tau pathology.

Methods

Search Strategy

A literature search for articles from peer-reviewed journals was performed on three electronic databases: PubMed, Web of Science, and WorldCat on 15 March 2022 with the terms "intermittent fasting", "ketogenic diet", "Alzheimer's disease", and "cognition". The search strategy included AND and OR Boolean search operators and a truncation symbol (*) to receive variations of the word: "cognition" (such as cognitive abilities/function/improvements). The search string included: ("intermittent fasting") OR ("ketogenic diet") AND ("Alzheimer's") AND ("cogniti*") across all databases. No year of publication was specified. The database WorldCat was electronically searched for unpublished work and every article was considered regardless of language and statistically significant results to reduce the risk of publication bias. The selection process was not handled by a second reviewer. The reference management tool: Endnote was used to collect and organize references. The search process was registered by the systematic review protocol: Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA); (Moher et

al., 2009) to provide a flow diagram of the number of studies excluded and included at the different stages of the search process: identification, screening, eligibility, and inclusion.

An initial literature search was performed at the identification stage with the search string: ("intermittent fasting") OR ("ketogenic diet") AND ("Alzheimer's") AND ("cogniti*") across all databases. The initial search gave n=744 hits (see Figure 1). No additional records were identified through other sources (n = 0). Duplicates were removed and the remaining records were screened at the screening stage (n = 282). The articles were scrutinized by reading the title and/or abstract to exclude irrelevant articles that did not match any of the inclusion criteria (n = 194). If the title and/or abstract of the remaining records indicated that the article may meet the inclusion criteria, the full text was read carefully with a focus on method and results at the eligibility stage (n = 88). Reasons for excluding full-text articles included secondary sources such as meta-analysis or systematic reviews (n = 42), no relevant intervention such as physical activity or nutrition (n = 25), no relevant outcome (n = 2), no focus on Alzheimer's disease (n = 1), and no availability for full text (n = 12). Studies included in the qualitative synthesis at the inclusion stage met the inclusion criteria (n = 6).

Inclusion & Exclusion Criteria

Inclusion criteria were studies using the intervention intermittent fasting or fasting in Alzheimer's disease, either as the main focus or as a part of the study. Both human studies and animal models were considered. Only primary sources such as scientific articles, dissertations, research reports, and certain conference proceedings were taken into account. No restrictions concerning time frame, sex, or age. No restrictions concerning which type of intermittent fasting, such as alternate-day fasting, periodic fasting, time-restricted eating, or time-restricted feeding. Exclusion criteria included studies without any focus on the relationship between intermittent fasting and Alzheimer's disease. Secondary sources such as review articles, textbooks, popular-scientific books, articles, and newspapers were not considered.

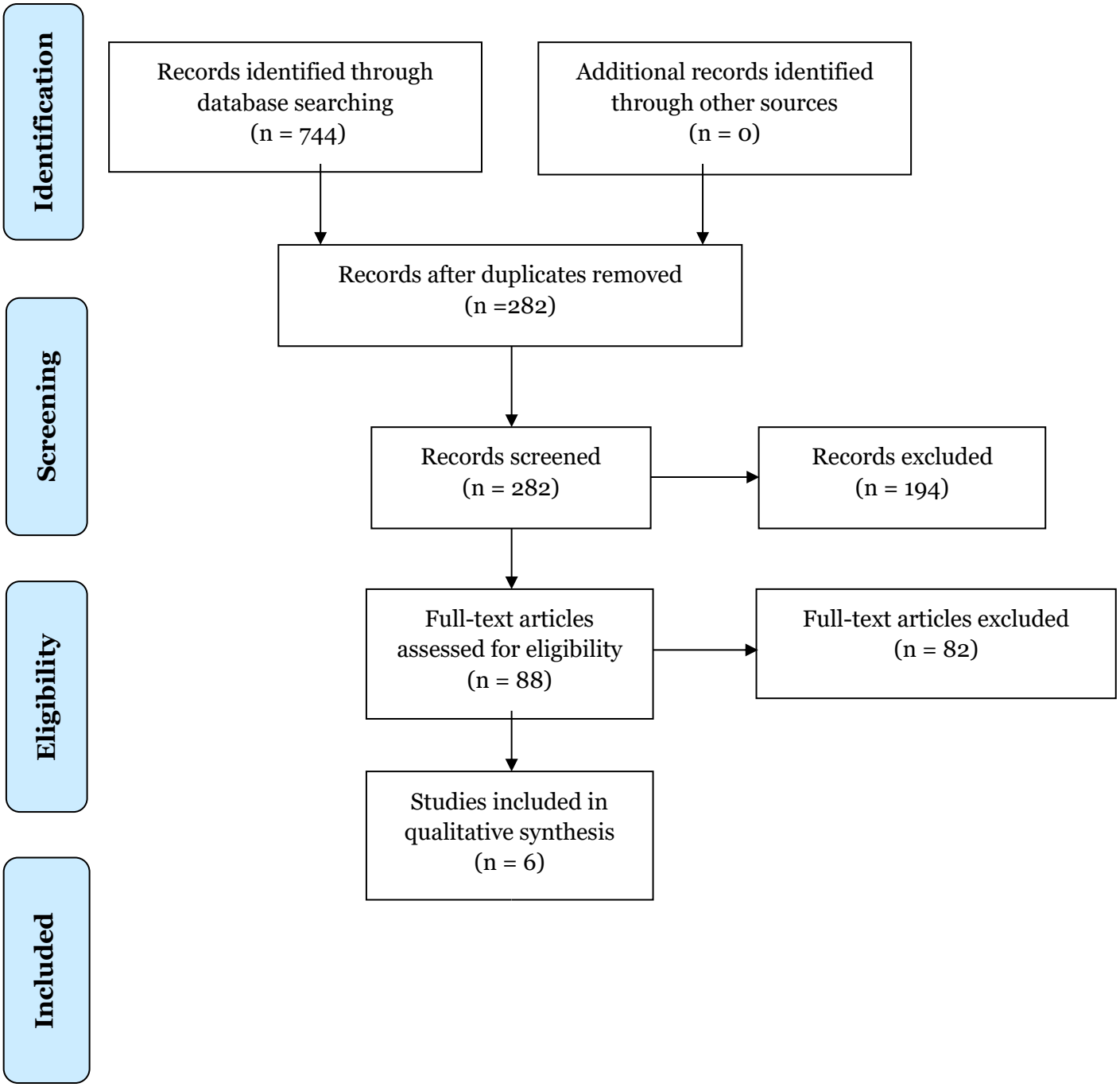
Data Extraction

The main outcomes of the study were anatomical changes in the protein expression and functional changes in the cognitive abilities of humans and/or animals. Outcome measures for anatomical changes included immunohistochemistry (Maity et al., 2013), immunoblotting (Litovchick, 2020), and immunological assays (Ma et al., 2011) to

detect and measure pathologically relevant proteins. Outcome measures for functional changes included measures of mental performance such as the Montreal Cognitive Assessment (MoCA); (Jankovic et al., 2021) and the Animal Naming test (Campagna et al., 2017) for human subjects and behavioral tests such as The Morris Water Maze (Shin et al., 2018), Barnes Maze test (Pitts, 2018), Y Maze test (Fink et al., 2012), Passive/Inhibitory avoidance test (Ferrandez & Teasdale, 1995), Elevated Plus Maze test (Watson et al., 2017), Open Field test (Gould et al., 2009), Locomotor Activity tests (such as the electronic animal activity meter); (Carter & Shieh, 2010) and Rotarod test (Caston et al., 1995) for animal models. A table of information about the included studies presents the following characteristics: authors, publication year, country, study design, study population, intervention, cognitive tests, methods measuring Aβ and tau pathology levels, and outcomes/results.

Figure 1

PRISMA 2009 Flow Diagram.



Note. Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group (2009). Preferred

reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Results

Six articles were found to investigate intermittent fasting's effect on cognitive abilities in Alzheimer's disease. The characteristics of the included articles are summarized in a table (see Table 1). Four studies were based in the USA, one in China, and one in Korea. The studies were published between 2007-2020. Five of the studies were randomized control trials (RCT) and one study was a case study (CS). Five studies used animal models such as mice or rats and one study used a human subject. The studies included interventions such as intermittent fasting, caloric restriction, ketogenic diet, 30 energy percent fat diet, high carbohydrate (starch) diet, exercise, and infused bacterial infection. All studies were primary sources in form of scientific articles.

Table 1

Summary of the following characteristics: authors, publication year, country, study design, study population, intervention, cognitive tests, methods measuring Aβ and tau pathology levels, and outcomes/results of the included articles in the systematic review of intermittent fasting's effect on cognitive abilities and levels of Aβ and tau pathology in Alzheimer's disease.

Authors, publication year, country	**Study design**	**Study population**	**Intervention**	**Cognitive tests**	**Methods for measuring beta-amyloid (Aβ) and tau pathology levels**	**Outcomes/results**
Halagappa et al. 2007 USA	Randomized Controlled Trial	Male and female non-transgenic C57BL/6 mice and a triple-transgenic mouse model (3xTgAD) mice	Intermittent fasting and caloric restriction	Water Maze Test and Open Field test	Enzyme-Linked Immunosorbent Assay (ELISA)	Improved ambulation, locomotion and exploratory behavior without decreasing levels of beta-amyloid (Aβ) and tau pathology
Liu et al. 2019	Randomized Controlled Trial	Wild-type mice, amyloid precursor	Intermittent fasting	Elevated Plus-Maze test, the Open Field	Immunohistochemistry	Improved exploratory behavior, spatial memory and learning without

USA		protein knock-in mouse model (*App*$^{NL-G-F}$) and mitochondrial deacetylase sirtuin 3 knockout (SIRT3 KO) male mice		test, and the Morris Water Maze test		decreasing levels of beta-amyloid (Aβ) and tau pathology
Horner et al. 2020 USA	Case Study	A 59-year-old male with Type 2 Diabetes Mellitus, diabetic neuropathy, and mild Alzheimer's disease with a history of insulin dependency for 11+ years	10-week lifestyle intervention including ketogenic diet, time-restricted feeding window of 5-6 hours (intermittent fasting), and exersice	Montreal Cognitive Assessment (MoCA) and the Animal Naming test	The study did not measure levels of beta-amyloid (Aβ) and tau pathology	Improved memory and cognition. The study did not measure levels of beta-amyloid (Aβ) and tau pathology
Park et al. 2020 Korea	Randomized Controlled Trial	Amyloid-β infused Male Sprague Dawley rats	Ketogenic diet, intermittent fasting, 30-energy percent fat diet and high carbohydrate (starch) diet	The Passive/Inhibitory Avoidance test, Y Maze test, and the Morris Water Maze test	Immunoblotting	Improved spatial memory and learning while decreasing levels of beta-amyloid (Aβ) and tau pathology
Zhang et al. 2017 China	Randomized Controlled Trial	Amyloid precursor protein/presenilin 1 (APP/PS1) double-transgenic mice and wild-type mice	Intermittent fasting	The Morris Water Maze test	Immunohistochemistry	Improved spatial memory and learning while decreasing levels of beta-amyloid (Aβ) pathology. The study did not measure levels of tau pathology
Vasconcelos et al. 2014 USA	Randomized Controlled Trial	Adult 12-week-old male Wistar rats	Intermittent fasting and infused bacterial infection	The Barnes Maze test, an electronic animal activity meter, the Rotarod test	The study did not measure levels of beta-amyloid (Aβ) and tau pathology	Improved spatial memory and learning. The study did not measure levels of beta-amyloid (Aβ) and tau pathology

In a sample of male and female non-transgenic C57BL/6 mice and the triple-transgenic mouse model of Alzheimer's disease (3xTgAD), the researchers wanted to investigate if the ketone-producing diets: 40 percent calorie restriction and intermittent fasting protect against cognitive decline in Alzheimer's disease. Beginning at 3 months of age, groups of 3xTgAD mice were fed ad libitum (the control group), intermittent fasting or with a

40 percent calorie restriction. At 10 months of age, half of the mice in each group were tested with the Morris Water Maze Test and Open Field test, while the other half were tested at 17 months of age. Functional outcomes showed that 3xTgAD mice on the ad libitum diet for 7 or 14 months had considerably less ambulatory activity than the non-transgenic mice fed ad libitum, suggesting less movement in Alzheimer's disease. Intermittent fasting or caloric restriction for 7 months had no effect on ambulation or distance traveled in 3xTgAD mice. However, 3xTgAD mice on these ketone-producing diets for 14 months showed much higher ambulation and greater distance traveled than 3xTgAD mice fed ad libitum (Halagappa et al., 2007).

Furthermore, the Morris Water Maze test showed that 3xTgAD mice on the ketone-producing diets swam faster and had considerably shorter path lengths than 3xTgAD mice fed ad libitum. When the platform was removed from the target quadrant, 3xTgAD mice on the ketone-producing diets showed a stronger tendency to swim in the target quadrant than 3xTgAD mice fed ad libitum. 3xTgAD mice on the ketone-producing diets remembered the location of the platform while 3xTgAD mice fed ad libitum showed poor memory retention. Anatomical outcomes were measured with ELISA. When compared to the control diet group, 3xTgAD animals in the 40 percent calorie restriction group had lower levels of Aβ1–40, Aβ1–42, and tau in the hippocampus, but Aβ and tau levels were not decreased in 3xTgAD mice in the intermittent fasting group. These results suggest that intermittent fasting can improve ambulation, locomotion, and exploratory behavior despite not decreasing Aβ and tau pathology in Alzheimer's disease (Halagappa et al., 2007).

In a sample of Wild type, AppNL-G-F knockin, and SIRT3 KO male mice, the researchers aimed to find out how intermittent fasting affects exploratory behavior during acute food deprivation, as well as memory, learning, and neuropathology. Functional outcomes on exploratory behavior were measured by the Elevated Plus-Maze test and the Open Field test. Male mice were given an acute 24-hour period of food deprivation which causes anxiety-like behaviors. Mice were randomly assigned to either an ad libitum control diet or an alternate-day fasting diet for one month. The results showed that mice on intermittent fasting who were food-deprived for 24 hours had the same amount of time and distance walked in the open arms as non-fasted mice, suggesting that the mice on intermittent fasting were not affected by anxiety from acute food deprivation. Similarly, mice on ad libitum who were food-deprived spent less time and traveled less distance in the open field's central zone than food-deprived mice in the intermittent fasting group. The mice on intermittent fasting were behaviorally adapted to fasting and thus showed decreased anxiety levels and more exploratory behavior (Liu et al., 2019).

Furthermore, they randomly allocated 1-year-old wild type and AppNL-G-F mice to ad libitum or intermittent fasting diets. Functional outcomes on memory and learning were measured by the Morris Water Maze test. The results showed that mice on intermittent fasting showed significantly reduced goal latency times than wild-type mice and AppNL-G-F mice fed ad libitum, suggesting improved memory and learning. The anatomical outcomes were detected by immunohistochemistry. The results showed a non-significant tendency towards decreased Aβ accumulation in AppNL-G-F mice on the intermittent fasting diet. These findings suggest that intermittent fasting improves exploratory behavior as well as spatial learning and memory despite not decreasing levels of Aβ pathology in Alzheimer's disease (Liu et al., 2019).

A case study of a 59-year-old male with Type 2 Diabetes Mellitus, diabetic neuropathy, a history of insulin dependency, and mild Alzheimer's disease, used a 10-week lifestyle intervention including a ketogenic diet, intermittent fasting, exercise, and neuropsychiatric disorder treatment. A part of the study's purpose was to determine if ketones could enhance mitochondrial function and cognition. The functional results were measured through the Montreal cognitive assessment (MoCA) and the Animal Naming test. MoCA showed an improvement in scores from mild Alzheimer's disease: 20/30 to the usual range of 26/30. The 1-minute Animal Naming test was given before and after the intervention. Scores improved from 19 before intervention to 33 after the intervention. The study did not measure changes in levels of Aβ and tau pathology. These findings suggest that intermittent fasting improves memory and cognition in Alzheimer's disease (Horner et al., 2020).

In a sample of Amyloid-β infused Male Sprague Dawley rats, the rats underwent an eight-week ketogenic diet, a 30-energy percent fat diet, a high carbohydrate (starch) diet, and intermittent fasting to determine which ketone-producing diet improves cognitive function, glucose metabolism, and inflammation while also changing the gut flora. Functional outcomes were measured by the Passive Avoidance test, Y Maze test, and the Morris Water Maze test. All rats were placed in a dark room and were given a minor electric shock in the first passive avoidance session. Rats on intermittent fasting had a longer latency to enter the dark room than the other groups in the second trial and did not enter the dark room in the third trial, suggesting that they learned and memorized the location of the shock. In the Y maze test, rats frequently make right turns and then investigate the next arm, rather than returning to the previous arm. The ratio of right turns to the total movements was higher in the intermittent fasting rats and rats on a high carbohydrate (starch) diet than in the 30-

energy percent fat diet, suggesting improved spatial memory since they could distinguish between previous arms and new arms (Park et al., 2020).

Furthermore, the platform was removed in the fourth trial of the Morris Water Maze test. Rats would spend more time in zone five if they remembered the platform area. The intermittent fasting rats and 30-energy percent fat diet rats stayed in zone five for a longer period than the rats on the other diets. The anatomical outcomes were detected by the Western Blot. The results showed that intermittent fasting reduced hippocampal Aβ deposition and tau phosphorylation compared to the 30-energy percent fat diet. These findings suggest that intermittent fasting improves spatial learning and memory as well as decreases levels of Aβ and tau pathology in Alzheimer's disease (Park et al., 2020).

In a sample of APP/PS1 double-transgenic mice and non-transgenic wild-type mice, the researchers wanted to investigate if intermittent fasting protects against Alzheimer's disease by increasing the ketone body β-hydroxybutyrate. Functional outcomes were measured by the Morris Water Maze test. APP/PS1 mice had substantially longer escape latencies than the wild-type mice in escape training sessions. There was a tendency, but not statistically significant, in ameliorating the longer escape latencies for APP/PS1 mice with alternate-day fasting. Furthermore, during the probe trial, APP/PS1 mice showed a substantial decrease in the number of times passing the previous escape platform location and the time spent in the location when compared to wild-type mice. However, alternate-day fasting APP/PS1 animals showed increased entries and time in the previous escape platform location, suggesting ameliorated impaired spatial memory and learning. The anatomical outcomes were detected by immunohistochemistry and showed a substantial decrease in the number of Aβ plaques in alternate-day fasting-treated APP/PS1 mice compared to APP/PS1 mice. These findings suggest that intermittent fasting improved spatial memory and learning as well as decreased Aβ accumulation in Alzheimer's disease (Zhang et al., 2017).

In a sample of adult 12-week-old male Wistar rats with infused systemic bacterial infection, the researchers intended to see how intermittent fasting affected the cognitive impairments from systemic and neural inflammation (which occurs in Alzheimer's disease). The rats were divided into four groups: a control group, a group with infused bacterial infection, an intermittent fasting group, and a group with infused bacterial infection and intermittent fasting. Functional outcomes were measured by the Barnes Maze test, Inhibitory Avoidance test, Locomotor Activity test, and the Rotarod test. The Barnes Maze test showed a decrease in latency to find the escape tunnel beneath the hole with increasing trials in all groups, indicating that all rats learned the location of the escape tunnel. However, when compared to animals with systemic inflammation, the intermittent fasting group and

the intermittent fasting with systemic inflammation group had considerably shorter latencies. On the first evaluation day, systemic inflammation-treated rats did not acquire the information (demonstrating impaired memory and learning) and they performed worse than both intermittent fasting groups (Vasconcelos et al., 2014).

In the Inhibitory Avoidance test, the control group and intermittent fasting groups had significantly longer latencies to return to the room where a shock was given in test sessions than the systematic inflammation group. Furthermore, changes in motor abilities were assessed using an electronic animal activity meter and rotarod device. No significant differences were found in locomotor activity between the groups. These results suggest that intermittent fasting improves learning and memory (specifically long-term memory) in the presence of inflammation. The study did not measure levels of Aβ and tau pathology (Vasconcelos et al., 2014).

Discussion

This systematic review aimed to investigate further if intermittent fasting improves cognitive function in Alzheimer's disease and if the levels of Aβ and tau pathology explain these changes in cognitive function. The research question was: does intermittent fasting improve cognitive abilities in Alzheimer's disease and does the levels of Aβ and tau pathology explain these cognitive changes? All included studies reported improved cognitive function in Alzheimer's disease from intermittent fasting measured by cognitive tests (Horner et al., 2020; Halagappa et al., 2007; Liu et al., 2019; Park et al., 2020; Vasconcelos et al., 2014; Zhang et al., 2017). The underlying process seems to consist of the production of ketone bodies that serve as a fuel source for the brain cells, leading to anatomical changes in the gut microbiome, followed by anatomical and functional changes in the brain of humans and animals (Sato et al., 1995). Nonetheless, different results were found regarding the levels of Aβ and tau pathology in the included studies.

Two studies observed improved cognitive function and decreased Aβ pathology from intermittent fasting (Park et al., 2020; Zhang et al., 2017), where one of them also observed decreased tau pathology (Park et al., 2020). These results suggest that the amelioration of cognitive deficits in Alzheimer's disease from intermittent fasting may be due to decreased levels of Aβ and tau pathology. However, two studies observed improved cognitive function despite not decreasing Aβ and tau pathology (Halagappa et al., 2007; Liu et al., 2019). It appears that intermittent fasting can reduce cognitive deficits in the presence of Aβ and tau accumulation (Halagappa et al., 2007). There may be a mechanism whereby intermittent fasting strengthens neurons' resistance to negative effects from Aβ and tau

pathology and thereby improves cognitive performance (Halagappa et al., 2007). In line with these findings is the study of Vasconcelos et al. (2014) in which intermittent fasting improved cognitive function in the presence of systemic inflammation, indicating that intermittent fasting protects neuronal and synaptic function against inflammatory insult (Van Dyken & Lacoste, 2018). Liu et al. (2019) did not find decreased Aβ accumulation either meanwhile intermittent fasting improved both spatial memory and learning. Additionally, two studies did not even measure Aβ pathology (Horner et al., 2020; Vasconcelos et al., 2014) and three studies did not measure tau pathology (Horner et al., 2020; Vasconcelos et al., 2014; Zhang et al., 2017) while obeserving improved cognitive abilities, indicating that there are other mechanisms that may explain the cognitive changes.

Based on these findings, it seems as if neurofibrillary tangles and beta-amyloid (Aβ) neuritic plaques are not sufficient to cause cognitive decline. Previous research demonstrates that some humans performed well on cognitive tests even though high levels of Aβ and tau pathology were observed (Morris et al., 2014). Halagappa et al. (2007) emphasize that the primary issue of Alzheimer's disease may not even be Aβ and tau pathology, but rather insulin resistance. Genetic mutations, i.e., neuropathology, do not seem to explain the quick growth in Alzheimer's disease prevalence during the last half-decade, as a disease caused by a genetic mutation would be expected to climb progressively over time (Horner et al., 2020). If beta-amyloid plaques were to be blamed for cognitive decline, individuals with no cognitive impairment would lack Aβ accumulation, which is inaccurate (Morris et al., 2014). The hypothesis that insulin resistance is the core cause of Alzheimer's disease is consistent with previous studies emphasizing the link between metabolic syndrome (caused by insulin resistance) and Alzheimer's disease (Van Dyken & Lacoste, 2018). Accordingly, intermittent fasting's positive effects on cognitive function in Alzheimer's disease could be explained by the previously stated negative correlation between increased ketones and decreased insulin resistance (Sato et al., 1995). A probiotic intervention reduces anxiety-like behaviors and Aβ accumulation while improving the gut flora, hippocampal function, and cognitive performance (Bonfili et al., 2017). Even though evidence points at insulin resistance being the primary issue, further research is necessary on the relationship between gut health and brain health: a concept referred to as the "gut-brain axis" (Ticinesi et al., 2018).

Limitations of the Systematic Review

Limitations of this systematic review include the sample size of the systematic review (n = 6) as well as the number of studies measuring Aβ and tau pathology (n = 4). It would have been beneficial to include more studies quantifying Aβ and tau pathology to make

the conclusions of this systematic review more credible. The type of peer-reviewed journals in which the studies were published was also a limitation. The journals focused on cellular and molecular neuroscience, as well as diet and nutrition. Since cognitive neuroscience journals do not focus primarily on neurobiology, it is possible that they would provide more evidence about cognitive changes. Despite this, the articles had sufficient emphasis on cognitive changes and cognitive tests, and could somewhat explain the role of Aβ and tau pathology in the relationship between improved cognitive function in Alzheimer's disease from intermittent fasting. Furthermore, since all of the six included studies were published in peer-reviewed journals, their quality has been evaluated by the research community. The studies appeared to be reliable and valid, in the sense that the science was carried out methodically and correctly using established methods and procedures while measuring what they intended to investigate (Horner et al., 2020; Halagappa et al., 2007; Liu et al., 2019; Park et al., 2020; Vasconcelos et al., 2014; Zhang et al., 2017).

However, one of the most significant limitations is the amount of included studies that used animal models. Concluding insights from experimental animal models to humans should be done with caution (Zhang et al., 2017). The essential, but not established, assumption that the genes, processes, and illnesses in animal models are equivalent to those in humans is a significant problem when studies investigate human diseases in rats or mice. Although individual tissues or pathways have been compared, there is a current research gap regarding studies on the tissue expression or pathway levels to verify this hypothesis (Young et al., 2015). Even in closely related species like humans and chimps, identifying minor changes in pathway regulation may be difficult (Pizzollo et al., 2018). Furthermore, the most commonly utilized animal models, such as the mouse or rat, are significantly less closely linked to humans. Despite the similarity in progression and functions in many genes in humans and animal models, their regulation and interaction might differ (Doncheva et al., 2021). Nevertheless, the findings from studies using animal models offer techniques to prevent or delay human Alzheimer's disease that should be further investigated in humans (Park et al., 2020). Additionally, the health advantages of intermittent fasting have been implicated in human studies as well (Mattson & Wan, 2005).

Societal and Ethical Aspects

Intermittent fasting should be further implied in clinical trials on human subjects with Alzheimer's disease since it would increase the credibility of the research results (Liu et al., 2019; Zhang et al., 2017). Applications of non-pharmacological treatments for Alzheimer’s patients could ultimately decrease the prevalence rate of the disease and the

major societal impacts. The social and/or working life would improve (Jongsiriyanyong & Limpawattana, 2018), as well as the well-being of the Alzheimer's disease patients and their caregivers, and the health economy (Hugo & Ganguli, 2014). Furthermore, studies on human subjects would take into account the animal welfare and the goal of The National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) which implements new technologies and strategies to decrease animal models in scientific research (Lidster et al., 2016). Studies on animal models should at least follow practical guidelines for the procedures to minimize suffering, pain, and distress in the animals. Four of the five included animal studies point out their applications of animal procedures to avoid harm to the animals, such as adhering to guidelines and seeking approval from Animal Care Committees (Liu et al., 2019; Park et al., 2020; Vasconcelos et al., 2014; Zhang et al., 2017).

Conclusion

In conclusion, intermittent fasting is considered an effective treatment for Alzheimer's patients since it ameliorates cognitive deficits (Gudden et al., 2021; Johnson et al., 2007; Mosley & Spencer, 2013; Ooi et al., 2020; Van der Auwera et al., 2005). This systematic review supported the previously observed positive effects of intermittent fasting on cognition in Alzheimer's disease. Yet, it did not serve as conclusive evidence linking decreased Aβ and tau pathology with improved cognitive abilities suggesting that Aβ and tau pathology do not necessarily explain these cognitive changes. It appears that the importance of the relationship between gut and brain health cannot be overstated. Further research on the underlying issue of Alzheimer's disease may not only provide insights to the research community but ultimately improve the quality of life for Alzheimer's patients and even prevent the onset of the disease.

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Wordcount: 7467

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