Does exercise protect from cognitive decline by altering brain cytokine and apoptotic protein levels? A systematic review of the literature

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ABSTRACT

Regular exercise is thought to provide protection against age-related cognitive decline and possibly reduce risk of dementias. The mechanisms for the exercise protective effects are not known although changes in inflammatory cytokine levels may be involved. We conducted a systematic review of the literature to assess 1) the effects of exercise on cytokines in the brain, 2) the methodological rigour of studies which have examined these exercise effects and 3) the potential role of regular exercise in reducing the pro-inflammatory cytokine milieu that may contribute to dementia. We also reviewed the effects of exercise on concurrent pro and anti-apoptotic protein expression in the brain as related to cytokine changes. Five databases were searched until January 2010 with an initial 630 articles identified; 61 articles were retrieved of which 10 met study inclusion criteria. Investigations of both acute and chronic (training) exercise were assessed for methodological quality using a modified PEDro scale. Two studies were carried out with human participants and eight with mouse or rat models; studies differed markedly in design and methodological rigour, the types, intensities and durations of exercise, the cytokine and apoptotic proteins measured, and the regions of the brain (or proxy compartments) sampled. Despite variations in design, specific cytokine outcomes, and exercise type, the 10 studies provide limited evidence that acute strenuous exercise increases and exercise training decreases pro-inflammatory cytokines centrally. Two animal studies relate training associated decreases in pro-inflammatory cytokines with improved cognitive function using behavioural assessments such as the Morris maze. Recommendations for the design of future research on exercise, central cytokines, and cognition are offered.

Key Words: pro-inflammatory cytokines; apoptotic proteins; dementia; training; acute exercise

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INTRODUCTION

In Canada the average life expectancy at birth is approximately 78.3 and 83.0 years for men and women, respectively (52). In 2009, an estimated 15.2% of the Canadian population was over 65 years and this percentage is projected to increase to 24.5% by 2036 (11). Comparable estimates are found for the United States and most Western European countries. These demographic patterns suggest that public health practitioners, basic science researchers, and policy makers must focus on understanding diseases of the aged, including cognitive impairment and dementia (37). Dementias represent a significant challenge for medical care systems in terms of financial and human costs. This challenge will only become more pronounced as it is predicted that by 2020 about 29 million people worldwide will have some form of dementia (18).

It once was widely accepted that dementia and its associated conditions were a natural consequence of aging (5). The identification of risk factors for dementia (e.g., high blood pressure, diabetes, social isolation, family history) (13) have not only de-normalized the condition but have brought clinical attention to lifestyle modalities to decrease risks and improve treatments (29; 48). In order to identify the risk factors for developing dementia, it is important to understand the characteristics of normal cognitive slowing. Cognitive processing speeds and memory retrieval speeds decline by as much as 20% by age 40 and 40-60% by age 80 (14; 48). Crystallized cognitive abilities, however, increase well into the later decades of life and only appear to decline at very advanced ages (14).

Dementias, although varied and complex, share multiple common brain abnormalities including accumulation of amyloid proteins, neurofibrillary plaques and tangles, neurodegradation, decline in endogenous neurotransmitters and synaptic density, increase in cellular oxidative damage, and increase in central inflammatory processes (48). Dementias are also characterized by a range of cognitive and behavioural symptoms such as difficulty performing familiar tasks, problems with word retrieval, disorientation to time and place, impaired judgement, problems with abstract thinking, apathy, and memory loss affecting daily function (2).

There is a large body of evidence suggesting that pro- and anti- inflammatory immune mechanisms contribute to the pathogenesis of many dementias (32). Grammas and Ovase (22) found that Alzheimer's disease (AD) patients had significantly higher levels of the pro-inflammatory cytokine TNF-α in the microvessels of frontal, temporal, and parietal cortices compared to non-AD controls. High levels of TNF-α in brain regions with AD-type pathologies were indicative of inflammatory dysregulation. The finding of inflammatory cytokine involvement in AD neuropathology was confirmed by Alvarez et al (1); serum IL-1β was elevated in AD patients compared to non-AD patients and peripheral concentrations were correlated with the magnitude of cognitive impairment. The overproduction of IL-1 was of particular interest as this cytokine promotes the expression of the β-amyloid precursor protein (β-APP) gene and the production of abnormal proteins in neurofibrillary tangles and plaques (7). Other inflammatory biomarkers detected in the cerebral spinal fluid (CSF) of AD patients include: α1-antichymotrypsin, monocyte chemoattractant protein-1, transforming growth factors α and β (35) and C-reactive protein (34). Activated microglia and astroglia are pres-

ent in regions surrounding amyloid plaques (35; 19) providing further evidence for the role of inflammation in the etiology and/or pathogenesis of dementia. Thus, the inflammatory response may be an early factor in AD development and dementia (4).

With the identification of inflammation as a potential contributory mechanism to cognitive decline comes the realization that population-level primary and secondary prevention strategies may reduce the risk of dementia (29). In the face of many expensive pharmaceutical treatment approaches, exercise is a low cost, attractive alternative and contributes greatly to overall quality of life (6; 3; 27). Regular exercise improves health across the age spectrum (17) including enhanced cardiovascular fitness (30), increased immune function (44), and improved functional performance in older adults (24). Noteworthy is the anecdotal observation that regular aerobic exercise is "good" for the health of the brain; exercise is thought to improve and maintain cognitive performance with age (56). This is also true for older adults with pre-existing cognitive impairments (24) though the greatest benefit to cognition occurs for lifetime exercisers (51). Regular exercise can influence dementia risks and even decrease the symptoms of those with pre-existing dementia (57). However, it is not definitively known how exercise mediates positive effects on cognitive function. Likely, exercise influences cognitive performance though complex vascular, immune, and epigenetic mechanisms. Regardless of the aetiology, regular exercise is not only neuro-protective but also may improve cognition independent of the specific disease state (24).

Given the potential role of inflammation in contributing to dementia, exercise training may confer protection by inducing anti-inflammatory cytokines in neural tissue and, thereby, alleviate disease progression. However, short term, high-intensity exercise also elicits a pro-inflammatory, immune-activating/aggravating response, at least systemically (25; 20). When considering the aetiology of AD and dementia pathologies, it is reasonable to hypothesize that the pro-inflammatory cytokine effects observed in response to high-intensity exercise could exacerbate inflammation contributing to AD and dementia development. An acute bout of exercise promotes the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) including superoxide, hydrogen peroxide, hypochloric acid and nitric oxide within leukocytes (59; 54; 28; 61). Thus, acute exercise may affect ROS/RNS generation in immune cells (including those in the brain), increase the damaging effects of oxidative stress in muscle tissue and leukocyte DNA, and increase the activation of transcription factors for inflammatory cytokines (42). It is important to note, however, that these lymphocyte apoptotic effects are not observed as a response to long term moderate intensity exercise in well-trained endurance athletes (46). Exercise training enhances antioxidant and reduces TNF-α expression in intestinal lymphocytes and in peripheral tissues (25; 45). Exercise-induced IL-6 production, which stimulates other anti-inflammatory cytokines such as IL-1ra (interleukin-1 receptor antagonist) and IL-10 (interleukin-10), inhibits the production of the pro-inflammatory cytokine TNF-α (43). Reductions in TNF-α expression can promote cell survival through inhibition of the extrinsic pathway of apoptosis. Typically TNF-α binds to a TNFR1 death receptor at the cell surface; this initiates the formation of a "death domain" and subsequent activation of caspase-3, leading to downstream proteolysis and cell death (58). Preventing TNF-α production would potentially prevent

this signal cascade from developing. It is through exercise-induced cytokine alterations that regular physical activity may provide protection against the development of AD, other dementias, and cognitive decline.

Purpose of the Review

Given 1) that regular physical activity has beneficial effects on cognitive function, 2) inflammatory processes are linked to the pathophysiology of dementia and cognitive deficits associated with such disorders, 3) the protective role of exercise training on inflammatory cytokines and related apoptotic processes, and 4) the role that physical activity has in improving health and preventing many forms of chronic diseases, there were three objectives in conducting this systematic review. These were: to summarize the literature on the health effects of exercise on cytokines in the brain; to evaluate the methodological rigour of studies which have examined these exercise effects; and to evaluate the potential role of regular exercise in reducing the pro-inflammatory cytokine milieu that may contribute to dementia. Specifically this review examined studies on the effect of exercise and training on neuro-inflammation and cognition. In light of the public health importance of dementias and cognitive declines with aging and the widespread adoption of physical activity as preventive medicine, the underlying rationale was prompted by the lack of research into the effects of exercise and training on central cytokines and the relationship to brain health.

METHODS

A. Search Strategy and Selection Criteria

Literature Search

For this review we were interested in peer-reviewed articles examining the effects of exercise on neuro-immune or neuroinflammatory factors. A systematic review of medical databases was undertaken based on Oxman and Guyatt (40). Relevant articles up to and including January 2010 were identified using an *a priori* defined

Table I: Database search strategies used in review

Databases	Search Strategy	Results	Selected
PUBMED/MEDLINE	(heat shock protein[MeSH] OR cytokines[MeSH] OR anti-apoptotic proteins[MeSH] OR inflammation[MeSH] OR neuroinflammation[TIAB]) AND (exercise[MeSH] OR exercise training[TIAB] OR running[TIAB]) AND (brain[MeSH] OR cognition[MeSH] OR memory[MeSH])	**N=51**	**N*=9+1 ID by hand search**
WEBofKNOWLEDGE	(cytokines OR inflammation OR neuroinflammation) AND (exercise OR exercise training OR running) AND (brain OR memory OR cognition)	**N=214**	**N*=8**
CINAHL	(exercise OR exercise training OR running) AND (cytokines OR inflammation OR neuroinflammation) AND (brain OR cognition OR memory)	**N=97**	**N*=0**
SCOPUS	("exercise" AND "cytokines" AND "brain")	**N=89**	**N*=5**
EMBASE	(exercise OR exercise training OR running) AND (cytokines OR inflammation OR neuroinflammation) AND (brain OR cognition OR memory)	**N=179**	**N*=5**

search string in the following computerized databases: PUBMED-Medline (n=51; N*= 9); Web of Science (n=214; N* = 8), CINAHL (n=97; N* = 0), EMBASE (n = 179; N* = 5), and Scopus (n=89; N* = 5) where (*) identified selected articles.

The following search string was entered into each database using MeSH terms, appropriate alternatives, and Boolean operators: (cytokines OR heat shock protein OR anti-apoptotic proteins OR inflammation OR neuroinflammation) AND (exercise OR exercise training OR running) AND (brain OR cognition OR memory). Variations of the above search strategy (Table I) were used to search the databases other than Medline. The retrieval of full text articles was determined by the abstracts from these citations. Bibliographies from existing articles were hand searched and screened for additional studies. A search of the grey literature was not included in this review process.

Selection Criteria

The review included studies examining the effects of acute or chronic exercise on markers of inflammation in brain tissue in human or animal populations. The outcome measures of interest included cytokines (IL-1β, IL-1ra, IL-6, TNF-α, IFN-γ, MIP-1a), heat shock proteins (HSP72, HSP70), chemokines (CXCL1, CXCL12),

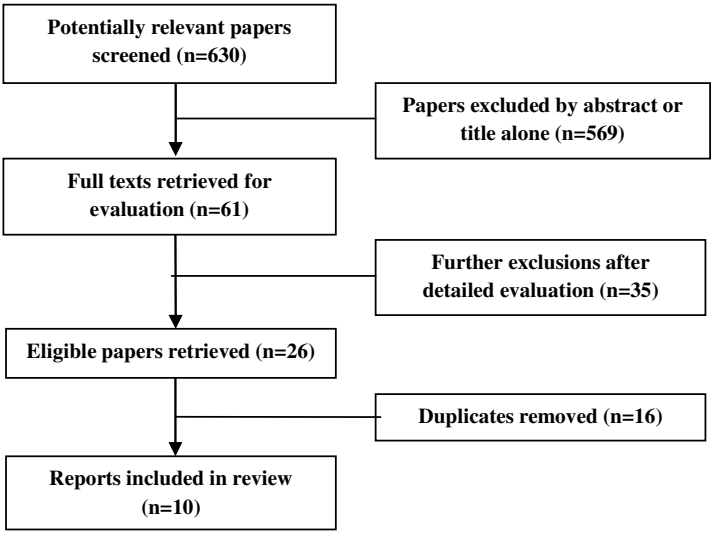

Figure I: Literature review flow diagram. The figure outlines the process of the literature search

CD markers of brain macrophages (CD11b, CD11c, perivascular/meningeal macrophages) and apoptotic proteins (cytochrome c, caspase-9/3, Bax, Bcl-2) from various brain regions (CSF, pituitary, hypothalamus, brain stem, cortex, midbrain, frontal cortex, hippocampus, cerebellum, internal jugular venous to

arterial, global cerebral blood flow). The retrieval of full-text articles occurred if the terms *"inflammation or neuro-inflammation or cytokines or chemokines"* and *"physical activity or exercise"* and *"cognition or memory or brain tissue"* appeared in at least one of the article title, abstract, or keywords. When the title and abstract indicated that the study was potentially relevant for review inclusion, a full-text copy of the article was obtained. A flow chart highlighting the search for relevant review articles is shown in Figure I. To summarize, articles were included in the review if they met the following criteria: 1) were provided in English, 2) included exercise, physical activity or fitness training as a experimental intervention, 3) focused on the measures of cytokines, pro- or anti-apoptotic proteins, heat shock proteins, or other inflammatory or neuro-inflammatory immune markers, and 4) examined these indicators in brain tissue. Studies were excluded if they did not use exercise as an intervention or experimental treatment, did not measure inflammatory factors, or did not focus the outcome measures on brain tissue or the central nervous system (CNS). Control groups were population-based, matched controls, or pre or post treatment subjects. Study designs included pre-post control studies, randomized controlled trials, randomized matched control studies, and true experimental designs. Trials and studies without identification of the method of patient or subject randomization were also included.

B. Data Extraction and Quality Assessment

Data Collection and Study Evaluation Procedures

For each study the following characteristics were collected: author, title, date of publication, study design, exercise type, exercise intensity, sample size, age range, inclusion of controls, outcome measures (cytokines and exercise verifiers), laboratory methods and analytical procedures used, and study results. Table II shows this information.

Studies were scored for methodological quality using a modified PEDro scale (33). Other criteria specific to this review were added. From the original PEDro scale the following items were used: 1) eligibility criteria were specified, 2) subjects were randomly allocated to groups, 3) the groups were similar at baseline regarding the most important prognostic indicators, 4) measurements of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups, 5) the results of between-group statistical comparisons are reported for at least one key outcome, 6) the study provides both point measurement and measurements of variability for at least one key outcome. The additional criteria included for methodological assessment were: 7) reporting of the physical activity intervention in sufficient detail to allow study replication, 8) description of the immunologic laboratory methods used for sample collection and analysis, 9) results reported in sufficient detail to allow conclusions to be drawn, 10) description of the statistical methods used, 11) validation of training effects were appropriate to the study, 12) presentation of a hypothesis or research question, and 13) specific brain regions or proxy tissues identified for sampling and assessment. Each satisfied item (with the exception of the first item) contributed 1 point to the total PEDro score (range 0–12 points). Three researchers independently scored the studies for the quality assessment. Disagreements regarding a study's eligibility or quality assessment were resolved by discussion until consensus was reached.

TABLE II. – General Characteristics of Studies Included for Review

First Author	Title	Year	Sample Size	Species	Ages	Exercise Type	Outcomes Studied and Methods Used	Design
Steensberg	*Cerebrospinal fluid IL-6, HSP72, and TNF-alpha in exercising humans.*	2006	**Total:** N = 24; **Group: (1)** Ex-PLA = 8; **(2)** Ex-CHO = 8; **(3)** CONT = 8.	Human Males	20-29 Years	**Type:** Cycle Ergometer; **Intensity:** 60%$VO2_{max}$; **Duration:** 2-hrs; **Acute.**	**ELISA:** IL-6; TNF-α; HSP72; Glucose	True Experimental Design
Chennaoui	*Effects of physical training on IL-1β, IL-6 and IL-1ra concentrations in various brain areas of the rat.*	2008	**Total:** N = 20; **Group: (1)** EX = 10; **(2)** SED = 10.	Male Wistar Rats	4 Weeks	**Type:** Treadmill; **Intensity:** 15-25m/min, 7% gradient; **Duration:** 7 wks, 60-120 min/day, 5x/wk; **Chronic/Training.**	**ELISA:** IL-1β; IL-6; IL-1ra; **RIA:** Corticosterone; Leptin; Prolactin	True Experimental Design
Nichol	*Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid.*	2008	**Total:** N = 56; **Group: (1)** Tg2576 = 29; **(2)** C57BL6/SJL = 27.	Tg2576 (TG) & C57BL6 (WT) Mice	17-19 Months	**Type:** Wheel Running; **Intensity:** Voluntary; **Duration:** 3 wks; **Chronic/Training.**	**ELISA:** IL-1β; TNF-α; IFN-γ; MIP-1α; $Aβ_{40}$; Soluble & fibrillar Aβ. **Western Blot:** CD40; MHC-II. **Immunohistochemistry:** CD11c; CD11b; CD68	True Experimental Design
Um	*Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model.*	2008	**Total:** N = 20; **Group: (1)** SED WT = 5; **(2)** EXE WT = 5; **(3)** SED Tg = 5; **(4)** EXE Tg = 5.	NSE/APPsw (TG) & Non-NSE/APPsw (WT) Mice	13 Months	**Type:** Treadmill; **Duration:** 16 wks; **Intensity**: 10min/day at 11m/min or 60 min/day at 22cm/sec & 0% grade for 5 d/wk; **Chronic/Training.**	**Western Blot:** Cytochrome C; SOD-1; HSP-70;Catalse; BDNF; GLUT-1; Bcl-2; Bax; Caspase 9 & 3; Aβ-42; GRP-78. **Biochem:** Glucose; cholesterol. **ELISA:** Insulin	True Experimental Design
Nybo	*Interleukin-6 release from the human brain during prolonged exercise.*	2002	**Total:** N = 8; **Group: (1)** Pre and **(2)** Post groups (n = 8).	Human Males	27 ± 2 Years	**Type:** Cycle Ergometer; **Duration:** Two 1-hr exercise bout; **Intensity:** 170 ± 4 W (50%$VO2_{max}$); **Acute.**	**ELISA:** IL-6	Pre-Post Control Study
Parachikova	*Short-term exercise in aged Tg2576 mice alters neuroinflammation and improved cognition.*	2008	**Total:** N = 24; **Group: (1)** Tg_{RUN} = 6; **(2)** Tg_{SED} = 6; **(3)** WT_{RUN} = 6; **(4)** WT_{SED} = 6.	Tg2576 & C57BL6/SJL Mice	15-19 Months	**Type:** Wheel Running; **Duration:** 3 wks; **Intensity:** Voluntary; **Chronic/Training.**	**Western Blot:** APP; **ELISA:** $Aβ_{40}$; $Aβ_{42}$; CXCL1; CXCL12	Randomized Matched Control Study
Carmichael	*Recovery of running performance following muscle-damaging exercise: relationship to brain IL-1 beta.*	2005	**Total:** N=129; **Group:** DH_1=9; UH_1=9; DH_{2a}=6; UH_{2a}=6; DH_{2b}=6; UH_{2b}=6; DH_{2c}=6; UH_{2c}=6; DH_{3a}=15; UH_{3a}=15; Con=15; DH_{3b}=15; UH_{3b}=15.	Male C57BL6 Mice	8 Weeks	**Type:** Wheel Running; down/uphill Treadmill; **Duration:** RTE & 7 day training; **Intensity:** 36 m/min, 8% grade; **Acute.**	**Plasma:** Creatine Kinase; **ELISA:** IL-1β	True Experimental Design
Carmichael	*Role of brain macrophages on IL-1 beta and fatigue following eccentric exercise-induced muscle damage.*	2010	**Total:** N = 70; **Group: (1)** UH_{SAL}; **(2)** DH_{SAL}; **(3)** UH_{CLD}; **(4)** DH_{CLD}.	Male C57BL6 Mice	8 Weeks	**Type:** Treadmill; **Duration:** 150 min; **Intensity:** 36m/min, 8% grade; **Acute.**	**ELISA:** IL-1β	True Experimental Design
Carmichael	*Role of brain IL-1 beta on fatigue after exercise-induced muscle damage.*	2006	**Total:** N = 64; **Group: (1)** Ex = 32; **(2)** Con = 32.	Male C57BL6 Mice	8 Weeks	**Type:** Wheel Running & Treadmill; **Duration:** RTE & Voluntary; **Intensity:** RTE; **Acute.**	**ELISA:** IL-1β	True Experimental Design
Haack	*Exercise reverses chronic stress-induced Bax oligomer formation in the cerebral cortex.*	2008	**Total:** N = 35; **Group: (1)** Chronic Restraint, Voluntary Ex = 28; **(2)** Acute Restraint = 7.	Sprague-Dawley Rats	3 Months	**Type:** Wheel Running; **Duration:** 21 days; **Intensity:** Voluntary; **Chronic/Training.**	**ELISA:** Corticosterone; Bax	True Experimental Design

TABLE III. Modified PEDro scale (Assessment of Methodological Quality)

Authors	Eligibility Criteria	Random Allocation	Baseline Comparisons	Outcomes from >85%	B/t group comparisons reported	Point & variability measurements	Exercise protocol properly described	Description of Immunologic laboratory methods used	Sufficient result reporting	Power and Statistical Analysis Methods	Validation of training effect	Hypothesis Present	Specific Brain Regions	Score/12
Human Studies														
Steensberg	+	+	+	+	+	+	+	+	+	+	-	+	-	10
Nybo	+	NA	NR	+	+	+	+	+	+	+	NA	+	-	8
Animal Studies														
Um	+	NR	-	+	+	-	+	+	-	+	NR	-	-	5
Chennaoui	+	+	-	+	+	+	+	+	+	+	+	-	+	10
Parachikova	+	-	-	+	+	NR	+	+	-	+	NR	+	-	6
Carmichael (2005)	+	+	-	+	+	-	+	+	-	+	+	+	+	9
Carmichael (2010)	+	+	-	+	+	-	+	+	-	+	-	+	+	8
Carmichael (2006)	+	+	-	+	+	-	+	-	-	+	-	+	-	6
Haack	+	+	-	+	+	-	+	+	-	+	+	+	+	9
Nichol	+	+	-	+	+	-	-	+	-	-	-	-	+	5

Legend: NR, Not Reported; NA, Not Applicable; +, Sufficient Reporting; -, Insufficient Reporting. See text for descriptions of each characteristic.

TABLE IV. – Effects of Exercise on Brain Cytokines, Immunological Outcomes, and Apoptotic Markers

Author	Exercise Type	Measure Point	Methods	Tissues Sampled	Cytokines, Immunological Outcomes, and Apoptotic Markers			Non-Immunological Outcomes		
					Group	CONT	EXP	Group	CONT	EXP
Steensberg	**Acute:** Cycle Ergometer	0, 60, 120 min	ELISA	CSF	**TNF-α (ns) (pg ml^{-1})**	0.0±0.0	0.0±0.0	**Glucose (ns) (mmol L^{-1})**	2.7±0.1	3.0±0.1
					IL-6 (ns) (pg ml^{-1})	1.2±0.3	1.4±0.2			
					HSP72 (ns) (ng ml^{-1})	0.3±0.1	0.3±0.1			
Chennaoui	**Chronic:** Treadmill	Pre & Post Exercise Period	ELISA; RIA	Hypothalamus; Pituitary; Hippocampus; Cerebellum; Frontal Cortex	**IL-6* (pg ml^{-1}) Cerebellum**	14.8±1.3	10.7±1.0	**Corticosterone (ns) (ng ml^{-1})**	144.2±31.4 Serum	98.5±19.7 Serum
					IL-1ra* (pg ml^{-1}) Pituitary	328.0±17.7	245.0±14.31	**Prolactin** (ng ml^{-1})**	15.0±2.0 Serum	25.5±3.0 Serum
					IL-1β* (pg ml^{-1}) Hippocampus	1.0±0.1	0.7±0.2	**Leptin** (ng ml^{-1})**	1.6±0.5 Serum	0.3±0.1 Serum
Nichol	**Chronic:** Running Wheel	Post Exercise Period	ELISA; Western Blot; Immunohistochemistry	Hippocampus; Cortex	**TNF-α (ns) (pg ml^{-1})**	Decreased with exercise		**Soluble Aβ** (pg ml^{-1})**	Decreased $A\beta_{40}$ with EX No-effect on $A\beta_{42}$ with EX	
					IL-1β (pg ml^{-1})**	Decreased with exercise		**Insoluble Aβ (ns) (pg ml^{-1})**	(ns) differences between $A\beta_{40}$ or $A\beta_{42}$ or decreases with exercise	
					IFN-γ (ns) (pg ml^{-1})	Increased with exercise		**Aggregated Aβ (ns) (pg ml^{-1})**	35% decrease (ns) in means with exercise	
					MIP1α* (pg ml^{-1})	Increased with exercise		**Swim Speed (m/s) (ns)**	0.2±0.0 WT 0.1±0.0 TG	0.2±0.0WT 0.2±0.0 TG
					CD40 (a.u.)**	Increased with exercise				
					MHC-II* (a.u.)	Increased with exercise				
Parachikova	**Chronic:** Running Wheel	Post Exercise Period	ELISA; Western Blot	Whole Brain	**CXCL1* (pg mg^{-1})**	2.1 fold inc in EX vs SED		**APP (ns) (mean plaque #)**	(ns) difference b/t EX & SED	

					CXCL12* (pg mg^{-1})	1.4 fold increase in EX vs SED	**$A\beta_{40}$ & $A\beta_{42}$ (ns) (pg µg^{-1})**	(ns) difference b/t EX & SED
							Radial Arm Water Maze	Improved performance with EX*
Um	**Chronic:** Treadmill	Pre & Post Exercise Period	ELISA; Western Blot; BioChem	Brain; Lung; Heart; Liver; Kidney; Intestine	**Bcl-2 (relative levels)**	Increase with EX in WT** Increase with EX in TG (ns)	**GLUT-1* (relative levels)**	Increase with EX in TG** Increase with EX in WT**
					Cytochrome C* (relative levels)	Decrease with EX in TG* Decrease with EX in WT (ns)	**BDNF** (relative levels)**	Increased with EX in TG* Increased with EX in WT*
					SOD-1 (relative levels)**	Increase with EX in WT** Increase with EX in TG**	**GRP-78* (relative levels)**	Increase with EX in TG** Increase with EX in WT**
					HSP-70 (relative levels)**	Increase with EX in TG** Increase with EX in WT**	**$A\beta_{42}$ (relative levels)**	Decrease in TG with EX** Reduced in WT with EX*
					Caspase 3 (relative levels)**	Decrease in TG with EX** Decrease in WT with EX**	**Insulin (pmol L^{-1})**	Decrease in TG with EX** Decrease in WT (ns)
					Caspase 9 (relative levels)	Decrease in TG with EX** Decrease in WT with EX*	**Glucose (mg dl^{-1})**	Decrease in TG with EX** Decrease in WT with EX**
					Bax (relative levels)	Decrease with EX in TG** Decrease with EX in WT (ns)	**Water Maze Tests**	Decrease in escape distance and in escape latency in TG with EX**
					Catalase (relative levels)	Increase with EX in TG** Increase with EX in WT (ns)	**Cholesterol (mg dl^{-1})**	Decrease in TG with EX** Decrease in WT (ns)
Nybo	**Acute:** Cycle Ergometer	Pre & Post Exercise Bouts	ELISA	Venous Cerebral Flow	**IL-6 (ng mL^{-1})**	No IL-6 at rest; small release at end of 1st EX bout; Significant* increase at end of 2nd bout	**Glucose (ns) (mM)**	Rest: 5.2±0.2 | 1st: 5.0±0.2 2nd: 4.9±0.2
Carmichael (2005)	**Acute:** Running Wheel	Post Training Period	ELISA; Plasma	Whole Brain	**IL-1β (pg µg^{-1})**	DH: elevated in cerebellum and cortex when compared to UH and Control*	**Creatine Kinase (units liter^{-1})**	Plasma CK was elevated* in DH compared to UH and Control
Carmichael (2010)	**Acute:** Treadmill	Post Training Period	ELISA	Whole Brain	**IL-1β (pg µl^{-1})**	Increase with EX* in DH vs. UP_{SAL} & CON_{SAL}		
Carmichael (2006)	**Acute:** Running	Post Training	ELISA	Whole Brain	**IL-1β (pg µl^{-1})**	IL-1β injection decreased wheel running**		
Haack	**Chronic:** Running Wheel	Post Training Period	ELISA	Left Motor Cortex	**Bax (mitochondrial fraction total^{-1})**	No stress effect with EX; 2-fold increase in Bax with chronic stress*, but not with acute stress		

* Significant effect ($p < 0.05$); ** Significant effect ($p < 0.01$); (ns) = non-significant effect; EX = exercise group; SED = sedentary group; TG = transgenic mice; WT = wildtype mice; Decr = decrease; Incr = increase; DH = downhill; UH = uphill; DH_{SAL} = downhill with saline; UH_{SAL}: uphill with saline; a.u. = arbitrary units; relative levels = no units given.

RESULTS

A. Search Results

A total of 630 publications were identified in the initial database searches. Review of titles and abstracts revealed that 569 did not meet the inclusion criteria. The full texts of the remaining 61 articles were retrieved for more detailed evaluation. Of these, 35 were excluded after detailed evaluation and 16 were excluded because they were duplicate studies; 10 articles were included in this review based on the study criteria (Figure I), of which 2 involved human populations (53; 39) and 8 utilized animal models (12; 38; 55; 41; 8; 10; 9; 23). Scoring for methodological quality of each study is reported in Table III. All included studies examined the effects of exercise (both acute exercise and exercise training) on inflammatory/immune markers of neural health in specific brain tissues or proxy tissue (i.e., CSF).

B. Excluded Studies

Of the 61 articles reviewed in detail, 51 were excluded because they described the effects of the environment on neural excitability (n=4), exercise interventions and only serum or peripheral tissue (not central) cytokine levels (n=4), cytokines and sickness behaviour or fatigue (n=9), drug treatment and markers of neural health (n=5), central cytokine administration and peripheral fat oxidation or enzyme activity (n=4), exercise and disease states other than dementia or AD or disease states and exercise capacity (n=9).

C. Description of Included Studies

Exercise Regimens

Each study provided aerobic exercise in one of three modalities: cycle ergometry for human participants (53; 39) and treadmill running (12; 55; 10) or running wheels (38; 41; 8; 9; 23) for animal subjects. Characteristics of these studies and their findings are reported in Table II.

Five studies used acute exercise protocols. Steensberg et al (53) used a one-time maximal cycle ergometry test and a 2 hr cycling exercise bout at an intensity of 60% VO_2 max. Nybo et al (39) used two 1 hr cycle ergometry sessions at approximately 50% VO_2 max. Carmichael et al (10) provided a single treadmill run (22 m/min at a -14% or +14% incline) for 150 min in experimental animals. Carmichael (8; 9) used a combination regimen consisting of an acute treadmill run (22 m/min at a -14% or +14% incline) for 150 min together with 7 days of voluntary freewheel access; VO_2 max or VO_2 peak were not specified in the Carmichael studies.

The remaining five animal studies used training (chronic exercise) protocols rather than acute exercise for a minimum 3 week period. Chennaoui et al (12) gave mice treadmill exercise at an intensity of 15-25 m/min and at a 7% gradient for 60 to 120 min/day for 7 weeks. Nichol et al (38), Parachikova et al (41), and Haack et al (23) provided 3 week access for mice to in cage running wheels. Um et al (55) used a 16 week treadmill training protocol for 60 min/day at 22 cm/sec and 0% gradient for 5 days/week.

The human studies included monitored aerobic exercise as well as a resistance component (39; 53). Comparison of exercise was complicated by the differ-

ences in intensity and duration used. The animal studies provided aerobic exercise in an unsupervised (voluntary wheel running) protocol (38; 41; 23), a fully supervised (forced treadmill) protocol (55; 12; 10), or both supervised (forced treadmill) and unsupervised (voluntary wheel running) exercise protocols (8; 9). There were large variations in exercise intensities in these studies, again making direct comparisons difficult.

Study Designs (Human / Animal Studies)

The two studies with human subjects differed in experimental design. One used a pretest-posttest acute exercise design (39). The other was a randomized control design (53). The animal studies were all true experimental design with mice or rats randomly allocated to exercise treatment or control groups (12; 38; 55; 41; 8; 9; 10; 23).

Sample Sizes and Statistical Methods (Human / Animal Studies)

The two human studies had a combined study population size of 24 individuals in the exercise condition and 16 individuals in the control condition including the pre-exercise subjects from Nybo et al (39) (Table II). The sample sizes ranged from 8 participants in the exercise condition and without separate controls (39) to 16 participants in the exercise group and 8 controls (53). The 8 animal studies resulted in a combined study population size of 439 experimental subjects and 149 controls (Table II). The sample sizes of the animal studies ranged from 10 in the exercise and 10 in the no exercise conditions (12) to 114 mice in the exercise condition and 15 in the control condition (8). All studies, with the exception of Nichol et al (38), described the statistical methods used. None reported the power or effect size for significance.

Subjects (Human / Animal Studies)

Both human studies included healthy men without dementia or other disease conditions (53; 39). Participants were young adults ranging in age from 20-29 years (Table II). Information about the control group was reported in the study by Steensberg and colleagues (53) which included age, weight, height, and BMI of controls. Participants in the study by Nybo et al (39) served as their own controls using baseline (pre) exercise values.

Six of the eight animal studies used inbred mouse strains and two used rats (Table II). The inbred strains were Tg2576 mice (38; 41), NSE/APPsw mice (55), C57BL6 mice (38; 55; 8; 9; 10), C57BL6/SJL mice (41), Sprague-Dawley rats (23) and Wistar rats (12). Three of the eight animal studies (38; 41; 55) used models of Alzheimer's disease neuropathology (Tg2576 and NSE/APPsw mice). The ages of the animals ranged from 4 weeks (12) to 17-19 months (38).

Brain Tissues Sampled (Human / Animal Studies)

There was considerable variation in which brain region was sampled. The two human studies used CSF or venous blood flow as proxies for neural tissue (53; 39). The eight animal studies sampled neural tissue directly (12; 38; 55; 41; 8; 9; 10; 23). Chennaoui et al (12) excised hypothalamus, pituitary, hippocampus, cerebellum, and frontal cortex. Nichol et al (38) sampled hippocampus and cerebral cortex. Um et al (55) used whole brain (also lung, heart, liver, kidney, and intes-

tine). Parachikova et al (41) and Carmichael et al (8; 9; 10) also used whole brain samples. Haack et al (23) excised tissue from the left motor cortex.

Immunologic Laboratory Techniques Used (Human / Animal Studies)
A variety of laboratory techniques was used to measure cytokines. These were enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), Western blotting, immunohistochemistry, and plasma microplate analysis. ELISA was used in all studies to detect a range of outcomes including IL-6, TNF-α, IL-1β, IL-1ra, IFN-γ, MIP-1a, $A\beta_{40}$, $A\beta_{42}$, insulin, HSP72, glucose, corticosterone, BAX, CXCL1 and CXCL12. Three studies used Western blotting to detect a range of specific proteins: CD11c, CD68, CD40, MHCII, APP, cytochrome C, HSP-70, catalase, BDNF, GLUT-1, Bcl-2, BAX, caspase 3 & 9, $A\beta_{42}$, and GRP-78 (38; 55; 41). Immunohistochemistry for brain tissues was used in one study to identify microglia (CD11c, CD11b, and CD68) (38). These laboratory techniques varied in terms of reported error coefficients, ease of duplication, and sensitivity of detection.

Methodological Evaluation of the Studies
The methodological features of the studies are summarized in Table III. Methodological quality varied between studies, and none of the studies met all PEDro assessment criteria. Using the information reported, two studies met 10 or more quality criteria (53; 12), and 8 studies scored between 5 and 10 on the quality assessment scale (38; 39; 9; 41; 23; 55; 8; 10). None of the studies scored below 5 on the quality assessment scale.

OUTCOMES

A wide variety of cytokines were measured in the brain or in related (proxy) compartments in response to acute exercise and training. Eight studies included one or more pro-inflammatory cytokines or chemokines (IL-1β, IL-6, IFN-γ, TNF-a, MIP-1α, CXCL1, CXCL12) (53; 12; 38; 39; 41; 8; 9; 10). One study assessed anti-inflammatory cytokines (IL-1ra) in neural tissue (12). No other studies examined anti-inflammatory cytokines in either proxy compartments or in the periphery. In addition, multiple markers of apoptotic activity or oxidative stress were measured in neural or peripheral tissue. Three studies included one or more of the heat shock proteins (HSP70, HSP72) or the apoptotic proteins (Bcl-2, BAX, GRP-78, cytochrome c, caspase 3, and caspase 9) (53; 55; 23); one study included antioxidant proteins (catalase, SOD-1) (55). Two cognitive testing paradigms were used for behavioural verification of the immunologic changes observed as a result of exercise training. Parachikova et al (41) assessed exercise-induced cognitive changes via a Morris water maze, and Um et al (55) utilized a modified platform water maze test. A detailed reporting of outcome measures with respect to acute and chronic exercise regimens follows.

A. Acute Exercise Effects
Steensberg et al (53) found that after an acute exercise bout (cycle ergometery, 60% of VO_2 max, 2 hr duration) the levels of IL-6 in CSF in healthy men were 2- and 3-fold higher ($p<0.05$) than plasma levels. During exercise, plasma IL-6

increased from 8 to 18-fold. IL-6 concentrations in the CSF did not change with exercise (PreEx: IL-6 = 1.2±0.3 pg/ml & PostEx: IL-6 = 1.4±0.2 pg/ml). Furthermore, TNF-α concentration in CSF, though below plasma level, remained non-detectable (PreEx: TNF-α = 0.0±0.0 pg/ml & PostEx: TNF-α = 0.0±0.0 pg/ml) throughout exercise. Additional measures of plasma and CSF concentrations of HSP72 indicated that post-exercise levels of plasma HSP72 increased ($p<0.05$) 5-fold when compared to pre-exercise values. CSF HSP72 concentration in humans did not change (PreEx: HSP72 = 0.3±0.1 ng/ml & PostEx: HSP72 = 0.3±0.1 ng/ml) in response to acute exercise. The authors suggest that CSF is segregated from the blood (plasma cytokine changes are not mirrored in the CSF) as acute exercise-associated changes in circulating cytokines do not influence CSF cytokine expression. Finally, HSP72 proteins are unable to cross the blood brain barrier (BBB) within the 2 hr exercise bout, which suggests the release of this heat shock protein from the human brain during exercise is not via CSF efflux.

The cerebral IL-6 response (as determined by internal jugular venous to arterial IL-6 differences and global cerebral blood flow) was assessed in healthy men who underwent 15 or 60 min of exercise (39). There was no change reported in IL-6 release after the 15 min exercise session; however, 60 min of exercise resulted in a small but insignificant release of IL-6 (0.1±0.0 ng/min); at the end of the second exercise bout, cerebral IL-6 release was 5 times greater ($p<0.05$: 0.3±0.1 ng/min). This finding suggests the duration (or intensity) of exercise influences cerebral IL-6 response. However, the duration of the exercise-induced IL-6 effect is unknown since multiple post-exercise measures of IL-6 were not determined.

Cerebellum and cortex IL-1β concentration and recovery of running performance in mice were measured by Carmichael et al (8). Treadmill exercise (22 m/min and -14% [downhill] or +14% [uphill] gradient for 150 min) and run to exhaustion (RTE) times (36 m/min, 8% gradient at 24, 48 and 96 hr post treadmill run) resulted in increased ($p<0.05$) IL-1β concentrations in cortex and cerebellum. Downhill runners demonstrated delayed recovery in treadmill RTE times as compared to uphill runners ($p<0.05$). These findings show elevated brain tissue IL-1β post acute muscle-damaging exercise, and document the role of IL-1β in exercise-induced fatigue.

Brain cytokines and muscle-damaging acute exercise-induced fatigue in mice was the focus of a study by Carmichael et al (9). IL-1β and IL-1ra were injected intracerebroventricularly (icv) and the effects on behaviours associated with acute exercise were monitored. Treadmill exercise (22 m/min and -14% or +14% gradient for 150 min) and IL-1β or IL-1ra injection decreased ($p<0.01$) wheel running activity in uphill and downhill runners, while IL-1ra injection significantly ($p<0.05$) improved or increased activity in downhill runners and IL-1β significantly ($p<0.01$) decreased activity in uphill runners. This observation provided circumstantial support for IL-1β release as a function of acute exercise due to its documented role in muscle fatigue in mice.

Brain macrophage IL-1β expression and exercise-induced fatigue in mice was assessed by Carmichael et al (10). Saline (SAL) or clodronate (CLD) was injected icv and the effects on fatigue and brain IL-1β concentration were measured. CLD depletes perivascular and meningeal macrophages which are major

sources of brain IL-1β (10). Treadmill exercise (22 m/min and -14% [downhill] or +14% [uphill] gradient for 150 min) and CLD injection increased ($p<0.05$ CLD vs. SAL) RTE times in both running groups. CLD icv reduced ($p<0.05$) cortical IL-1β concentration in downhill run mice (10). This finding implicates perivascular and meningeal macrophages in brain IL-1β production; shows IL-1β elevation post acute exercise; and documents the role of IL-1β in exercise-induced fatigue.

B. Chronic Exercise Effects

Chennaoui et al (12) reported that 7 weeks of progressive exercise training (60-120 min/day for 5 days/wk) led to reductions in IL-1β concentrations in the hippocampus (EX: 0.7±0.2 pg/ml vs. SED: 1.0±0.1 pg/mg; $p<0.05$), IL-6 concentrations in the cerebellum (EX: 10.7±1.0 pg/ml vs. SED: 14.8±1.3 pg/mg; $p<0.05$), and IL-1ra concentrations in the pituitary (EX: 245.0±14.3 pg/ml vs. 328.0±17.7 pg/mg; $p<0.01$). Physical training did not affect serum IL-1β, IL-6, or IL-1ra concentrations. These results suggest a decrease in central pro-inflammatory cytokines in response to exercise training. However, interpretation is limited by the lack of measurements at additional time points, making it difficult to assess if the cytokine effects are due to training or a carryover from the last bout of exercise.

Nichol et al (38) used transgenic Tg 2576 mice (i.e., animals with a mutant APP gene that results in Aβ plaques) and found significantly higher IL-1β and TNF-α levels in the hippocampus compared to C57BL6 wildtype (WT) controls ($p<0.05$ and $p<0.01$, respectively). Three weeks of freewheel running in Tg 2576 mice led to a decrease in hippocampal IL-1β and TNF-α to levels observed in wildtype controls. IFN-γ and MIP-1α levels in the hippocampus were lower in Tg 2576 mice than in WT ($p<0.05$ and ns, respectively); when Tg 2576 mice were given freewheel access for 3 weeks, IFN-γ (ns) and MIP-1α ($p<0.05$) increased as did CD40 ($p<0.01$) and MHC II ($p<0.05$) expression in hippocampal cells (38).

Astrocyte expression of chemokines was measured in the study by Parachikova et al (41). Three weeks of freewheel training resulted in a 1.4 fold increase in CXCL12 and a 2.1 fold elevation in CXCL1 mRNA as determined by real time PCR and superarray analysis; protein levels of these chemokines were increased by training (CXCL12, a 1.3 fold increase and CXCL1, a 1.6 fold increase). Both CXCL1 and CXCL12 improve neuronal-glial communication, thus the exercise associated elevations in these proteins were thought to contribute to improvement in learning and memory. In addition, exercise-induced improvements in memory performance were measured using a radial arm water maze with aged Tg2576 mice. Training improved ($p<0.05$) memory and maze performance (particularly working memory) compared to transgenic non-exercised controls. Control and experimental groups did not differ in swim speed or visual latency, measures of physical capability and motivation. These findings indicate that the positive behavioural effects of exercise mirror the exercise-induced increases ($p<0.05$) in neuroprotective chemokines (CXCL1 and CXCL12), despite the null findings for Aβ pathology when compared to sedentary controls.

Um et al (55) did not measure cytokines. However, Tg 2576 mice given 16 weeks of treadmill training (22 cm/sec, 60 min/day, 0% gradient, 5 days/week) had lower whole-brain expression of several apoptotic markers including

cytochrome c ($p<0.05$), caspase-9 ($p<0.05$), caspase-3 ($p<0.001$) and Bax ($p<0.001$) (55). Exercise training also led to the increased expression of the anti-apoptotic protein, Bcl-2 ($p<0.001$), the endogenous antioxidants SOD-1 ($p<0.001$) and catalase ($p<0.01$), and the heat shock protein HSP70 ($p<0.01$) when compared with sedentary animals. These exercise-induced changes in apoptotic markers indicate that exercise might be cyto-protective and conserve neuronal health. Moreover, escape distance and latency were measured using a modified platform water maze test in a study by Um et al (55). Trained mice showed significant ($p<0.05$) reductions in escape distance and latency. These results implied that the behavioural effects of exercise training mirror the neuro-histological (e.g., significant reduction in Aβ-42 deposition) changes and provide converging evidence for exercise-induced improvements in both behavioural and pathological effects in an animal model.

The potential protective effect of training (voluntary freewheel running) in mice against chronic restraint stress-induced changes in brain (left motor cortex) apoptotic protein expression was studied by Hack et al. (23). Daily restraint stress (21 consecutive days, 6 hr/day [0900-1500 hrs], polyethylene tubes [diameter = 9 cm]) increased cerebral cortex Bax expression 2-fold ($p<0.05$). Twenty-one days of voluntary wheel running together with restraint stress prevented the stress-induced cerebral Bax accumulation. Thus, exercise training augments Bax (a marker of neuronal apoptosis) and may increase neuron survival, even in the presence of chronic stress.

DISCUSSION

Physical activity helps to maintain cognitive ability, influence risk factors for dementia, and reduce symptom severity in pre-existing dementia cases (56; 24; 51; 57). This protection may be conferred by the inflammation reducing effects of moderate exercise training, which systemically has been shown to suppress the expression of pro-inflammatory cytokines and reduce lymphocyte apoptosis (25; 43; 45). This potential mechanism has not been examined centrally, where parallel training-related cytokine changes may reduce the inflammatory damage caused by dementia-related pathology. Conversely, acute intense exercise increases pro-inflammatory cytokine expression and formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) capable of inducing oxidative damage (25; 54; 28; 61) within circulating and tissue leukocytes. Such effects in the brain would exacerbate the underlying inflammation in dementia pathology, thus a balance between the beneficial and detrimental effects of exercise needs to be established. The primary objective of this systematic review was to describe the published findings on the effects of acute exercise and training on cytokines and apoptotic markers in the brain in normal and dementia pathology states, and to consider the role of exercise training in the reduction of pro-inflammatory cytokine expression that is associated with dementia development.

A. Acute exercise

Two human studies (53; 39) and three animal studies (8; 9; 10) examined the effects of acute exercise on central pro-inflammatory cytokines.

Both human studies found significant increases in central nervous system (CNS) levels of IL-6 (53; 39) and HSP72 (53) after exposure to acute exercise. Steensberg et al (53) also observed that circulating levels of IL-6 were markedly increased during the exercise session. Taken together the studies show that acute strenuous exercise leads to an increase in pro-inflammatory cytokines centrally that is parallel to systemic changes induced by such exercise protocols. The increase in HSP72 observed by Steensberg et al (53) indicates that acute exercise induces oxidative stress in the central nervous system, as this protein is secreted in order to prevent aggregation of partially-denatured proteins and down-regulate the activation of the pro-apoptotic protein caspase-9 (26).

Three animal studies focused on acute exercise and inflammatory cytokines in the brain, demonstrating significantly increased expression of IL-1 in the cortex and cerebellum (8) and whole brain (10) after acute exercise. Post-exercise IL-1 increases also induced muscle fatigue (9). Carmichael et al (10) suggested that perivascular and meningeal macrophages in brain (as determined by CD markers) were involved in IL-1β production. These changes indicate that acute exercise can elicit increases in pro-inflammatory cytokine expression centrally that are parallel to systemic effects.

Of particular interest is that serum IL-1β is expressed to a greater extent in Alzheimer's disease (AD) patients and its expression results in greater severity of cognitive impairment (1). IL-1β has also been determined to contribute to the formation of neurofibrillary tangles and plaques common in AD and other dementias (7). It can be inferred that acute exercise, which promotes a central pro-inflammatory state in healthy individuals, may be harmful to patients already experiencing inflammatory damage to the CNS. However, details about the exercise intensity and duration that would lead to elevated IL-1β (either systematically or centrally) and the associated cognitive effects in AD patients have not been described. It may be assumed that any exercise which produces significant oxidative and inflammatory stress would result in elevated IL-1β levels and potential cognitive effects.

B. Chronic Exercise (Training)

All of the studies examining the effects of exercise training on central pro-inflammatory cytokines and apoptotic markers were conducted using animal models, two of which detailed changes in healthy animals (23; 12) and three of which explored changes in models of disease states (38; 41; 55).

The two studies that examined exercise training-related changes in healthy animals showed significant reductions in IL-1β in the hippocampus, IL-6 in the cerebellum, and IL-1ra in the pituitary (without any effect on serum concentrations) (12), as well as reductions in Bax expression in the left motor cortex (23). The findings of Chennaoui et al. (12) provide some support for training being "anti-inflammatory" insofar as a decrease in hippocampal IL-1β levels occurred. It is less clear what a training associated decrease in central IL-6 means since this was only observed in the cerebellum, a brain region not normally associated with memory loss (49). Moreover, as many investigators have shown, IL-6 can function as an anti-inflammatory cytokine (43; 45) in a training context and induces IL-10 and IL-1ra (43). Hence, a training associated reduction in central IL-6 levels is difficult to interpret, particularly given Chennaoui and colleagues' findings

of increased serum IL-6 (12). IL-1ra (a potent anti-inflammatory cytokine) expression was lower in the pituitary from trained animals, a finding which is not only in the opposite direction of expected neuroprotection but also in brain tissue not related to memory or cognition (49). Bax is a pro-apoptotic protein that displaces cytochrome c from the mitochondrial membrane and activates the caspase apoptotic cascade (50). A reduction in brain Bax expression could potentially result in increased neuronal survival and protection against apoptosis in the presence of chronic stress. Hence, the results of these studies provide tentative support for the hypothesis that training may be able to reduce inflammatory plaque formation and the damage that is characteristic of dementias.

Interestingly, three of the animal studies used transgenic mice as models of Alzheimer's-like pathologies (38; 41; 55). These types of studies are important as they allow for inferences to be made on the effects that exercise training may have in individuals already diagnosed with dementia or undergoing age-related cognitive decline. Transgenic Tg2576 mice are highly susceptible to Aβ plaque formation and Nichol et al (38) reported transgenic mice to have significantly elevated levels of the pro-inflammatory cytokines IL-1β and TNF-α in the hippocampus in comparison to the wildtype controls. After training, expression of these two cytokines was at levels comparable to the wildtype controls. It was also found that the expression of IFN-γ and MIP-1α in the hippocampus was lower in Tg2576 mice, thus supporting the evidence for inflammatory dysregulation. These levels increased significantly after the training period. This study underscores the fact that Aβ plaque formation is associated with over- and under-production of key pro-inflammatory markers, and that aerobic exercise training can potentially normalize the levels of some of these cytokines. Parachikova et al (41) also used Tg2576 mice and focused on effects of training on the expression of the astrocyte chemokines, CXCL1 and CXCL12, which improve efficiency of neuronal networks. Elevations in the mRNA and protein levels of these chemokines were identified, suggesting that exercise training may be linked with improved cognition. The observation that astrocyte chemokines are affected by training is important for understanding the exercise-AD relationship for several reasons. First, CXCL12, a potent inducer of lymphocyte chemotaxis expressed on microendothelial cells, has anti-inflammatory properties and prevents mononuclear cell proliferation and activation (36). Antagonism of CXCL12 receptors results in AD-like cognitive declines (41). CXCL1 is constitutively expressed on macrophages, neutrophils and epithelium and acts through CXCR2 via regulation of cell growth and inflammation (16). Elevated CXCL1 may prevent apoptosis of hippocampal neurons in response to Aβ pathology (60). Parachikova and colleagues used a behavioural assessment to argue for the link between improved cognition, central chemokines, and exercise training, and found that the training protocol improved working memory and maze performance in Tg2576 mice exposed to a radial arm water maze task. Um et al (55) also employed a cognitive assessment procedure using a modified platform water maze test. Tg2567 mice that underwent long-term training had significantly lower escape distances and latency. Not only did this improve cognitive performance, but also reduced the whole-brain expression of a battery of pro-apoptotic markers, such as cytochrome c, caspase-9, caspase-3, and Bax, and increased expression of the anti-apoptotic protein Bcl-2 compared to sedentary Tg2567 mice. This is of interest because the

regulation of apoptosis depends on the ratio of initiating proteins (e.g., Bax) to inhibiting proteins (e.g., Bcl-2). Increased expression of the latter results in low mitochondrial permeability, thereby preventing the release of cytochrome c and activation of caspase-9; caspase-3 activation and the proteolytic cascade are thus inhibited (47). The effects of training on the pro- and anti-apoptotic markers reported by Haack et al (23) and Um et al (55) are in agreement with this sequence of events and suggest a potentially neuroprotective role of exercise training in "disease-state" animals.

C. Limitations of the Review

There are several limitations of this review including the fact that only 10 studies met the inclusion criteria. There were also substantial differences between the characteristics of each of the studies included. Most evident is that only two human studies were included, both of which assessed the impact of acute exercise, rather than exercise training, on central pro-inflammatory cytokines in healthy young adult males. The results of these two studies are difficult to generalize to other populations, and also are difficult to interpret in the context of exercise as a training modality.

The types of exercise protocols in the 8 animal studies also varied, as did the outcome measures (i.e., different cytokines, chemokines, apoptotic markers, and cognitive tasks) and the laboratory techniques utilized. Also important to note was the variation in brain regions where the cytokines were measured. For example, several of the animal studies included whole brain or motor cortex or cerebellum which (unlike the hippocampus and frontal cortex) are not areas specifically involved with memory and higher order cognition (15). In addition, only one of the studies measured anti-inflammatory cytokine (IL-1ra) expression, and thus it is unclear whether exercise (either acute or chronic) influences anti-inflammatory levels centrally. Not all animal studies compared transgenic mice (with AD-like pathology) to wildtype controls, increasing the variation between the included investigations. Furthermore, the species and age of the animals differed across studies. It is also difficult to equate training responses in 4 week old Wistar rats (12) to those of 17-19 month old Tg2567 mice (38). Another source of variation between studies was the number of subjects in each.

Sample sizes for the human studies were low, and the study by Nybo et al (39) did not use an independent control group. Steensberg et al (53) had twice as many participants in the exercise group compared to the control group. The number of animals reported in each study in this review was also highly variable (and often unbalanced in terms of experimental and control groups). Effect sizes were not provided for any of the studies, thus making it more difficult to aggregate the results of each into a formal meta-analysis.

Finally, the methodological quality of the studies differed. Four evaluation criteria were largely underrepresented in the studies: baseline comparisons, point and variability measurements, sufficient results reporting, and validation of training effect. Steensberg et al (53) was the only study to report baseline comparisons. Only three studies (53; 39; 12) included point and variability measurement and sufficient results reporting. Also noteworthy that of the 8 animal studies, only Chennaoui et al (12), Carmichael et al (8), and Haack et al (23) included data about validation of training effect. The omission of these criteria is important to

consider for several reasons. Baseline measures are needed in order to interpret the immunological outcomes (and other associated measures), especially in the absence of between group comparisons. Point and variability measurements and sufficient results reporting are crucial for proper study and results interpretation and for estimation of treatment effects. Validation of training is necessary to determine whether observed cytokine changes are actually due to training or are confounded by other unrelated factors.

D. Future Research

Despite heterogeneity of exercise protocols and cytokine measures across the studies, there were some general patterns observed suggestive of consistent biological effects. In the acute exercise studies, there were increases in central pro-inflammatory cytokines (and pro-apoptotic factors) as a result of strenuous exercise exposure in healthy subjects. In contrast, the exercise training studies tend to suggest reductions in pro-inflammatory cytokine expression (and consistently for IL-1β) and pro-apoptotic proteins, and/or increases in anti-apoptotic proteins. In addition, the two studies that assessed behavioural measures of cognition were in agreement that exercise training improves cognitive ability. Although different modalities and measures were utilized, the studies were consistent in the direction of effects exerted by acute and chronic exercise exposure.

Additional human studies will be needed to elucidate the cytokine pathways that may exist between exercise training and reduced risk for dementia. These studies would be important to replicate the findings about exercise on central pro-inflammatory processes. Moreover, studies should not be limited to acute exercise protocols as were the two included in this review. Instead, training interventions should be considered to address the question of whether long term moderate exercise reduces CSF pro-inflammatory cytokine levels. Such studies need not be limited to a healthy population; patients with mild dementia and early stage AD could be recruited, allowing for observations of reductions in disease pathology or symptoms. Relationships between changes in inflammatory markers and clinical status of dementia would have to be established before clinicians could safely prescribe personalized exercise regimens to patients.

With respect to animal studies, we suggest that the use of transgenic animals be done alongside appropriate wildtype or normal controls. This will enable investigators to determine whether the effects observed are truly indicative of the treatment or a by-product of genetic differences in the knock-out group. There are also issues regarding the exercise training modalities used. Forced treadmill exercise can exacerbate stress; this is typically not the case in voluntary freewheel running. It is, therefore, important to include physiological measures of stress (e.g., plasma corticosterone) if forced exercise paradigms are used. Many of the studies did not include behavioural assessments, although they are necessary for correlations between exercise-associated immunological change and improved cognitive ability. It is recommended that future research in this area include behavioural as well as immunological outcomes.

In this systematic review, there was only one study that investigated the effects of exercise on anti-inflammatory cytokine expression (namely, IL-1ra). Therefore, a major consideration is that future research includes anti-inflammatory cytokines, since they influence the expression of pro-inflammatory cytokines.

Treadmill training, for instance, leads to increases in circulating IL-10, a Th2 cytokine known to counteract the effects of TNF-α in coronary heart disease patients (21). In addition, IL-10 concentration in white adipose tissue increased after treadmill training in rats (31). Similar increases in anti-inflammatory cytokines in the brain would allow for a clearer understanding of the cytokine pathways (decreased pro-inflammatory, increased anti-inflammatory, altered balance of Th1 and Th2 cytokines, etc.) through which exercise training may slow age related cognitive declines and the development of dementias. Finally, studies are needed that rigorously define durations and intensities of exercise on the persistence and magnitude of cytokine, chemokine, and apoptotic marker expression using animal models of AD-like pathologies.

CONCLUSION

The effects of acute exercise and exercise training on brain pro-inflammatory cytokines, chemokines, apoptotic markers, and measures of cognition in a small number of human and animal studies were evaluated in this systematic review. Overall, the studies indicated that acute exercise increases central pro-inflammatory cytokine levels and pro-apoptotic protein expression. The limited number of studies, all using animal models, suggested that exercise training reduced central levels of some pro-inflammatory cytokines, decreased expression of pro-apoptotic proteins, increased expression of anti-apoptotic proteins, and improved cognitive abilities in behavioural tests. There were large differences across studies in the exercise modalities and cytokine and apoptotic protein outcome measures; the studies differed in the types of design and the quality of methodologies used. Nevertheless, there was a trend in all studies to support the hypothesis that regular physical activity confers some protection against cognitive loss (and development of dementia) by reducing the central expression of pro-inflammatory cytokines, most notably IL-1β, a pro-amyloidogenic cytokine. However, what is evident from this review is that there are major gaps in understanding the relationship between exercise, CNS cytokine immunology, cognition, and dementia disease severity. Collaboration between exercise immunologists, gerontologists, and behavioural scientists will be essential given the public health importance of cognitive changes with age and the growing health care burden of dementia.

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