The Effect of Exercise on Inflammatory Biomarkers

Thesis submitted to the University of Nottingham for the degree of Master of Research

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Abstract

Introduction

Many non-communicable diseases (NCDs) including dementia and some cancers have a component of underlying inflammation within their pathophysiology. Exercise has been shown to reduce morbidity and mortality from NCDs and has been linked with decreased levels of pro-inflammatory cytokines in the plasma of regularly exercising individuals. This project seeks to assess the effect of acute and chronic exercise, including high intensity interval training on inflammatory markers within the cerebrospinal fluid (CSF) and plasma.

Methods

A systematic review and meta-analysis were performed to identify the effects of long term (>6 weeks) exercise on inflammatory markers within the cerebrospinal fluid and plasma. Healthy male volunteers were recruited for a study investigating the effect of an acute high-intensity interval training (HIT) protocol on inflammatory markers (IL-6, IL-10, TNFα and CRP) in CSF. Plasma from volunteers with and without cancer in previous HIT studies was analysed to assess levels of inflammatory biomarkers (IL-6, IL-10, TNFα and CRP) pre- and post- exercise interventions.

Results

A systematic review revealed few statistically significant results showing either increases or decreases in inflammatory markers following a variety of exercise protocols. It was not possible to recruit sufficient volunteers to analyse CSF following exercise and the study was not completed during the time frame of this thesis. The analysis of plasma showed a significant decrease in IL-6 in an elderly population following 4 weeks of HIT, there was also a significant increase seen in a group performing HIT at home.

Conclusions

The data obtained did not support consistent effects of exercise on inflammatory markers in healthy individuals, or in those with cancer. There was some evidence to show that HIT may reduce levels of circulating IL-6 in elderly (>70 years) populations. There may be many cofounders which influence the levels of inflammatory markers within individuals, making a relationship with exercise more difficult to ascertain.

Acknowledgements

I would like to thank the wonderful, brilliant operating department practitioners, nurses and midwives I've had the great fortune to work with over the last year. Without their support, and seemingly endless cups of tea – this thesis would never have been written.

Thank you to Amanda Gates for tirelessly answering my million and one questions and for teaching me the secrets of the CPET bike(!) To Brett Doleman, for uncomplainingly taking me through the statistics for the meta-analysis when it felt as though the results were incomparable and to Joe Bass, for all his help in the laboratory and with the technicalities of the ELISA plates.

Also, to my amazingly supportive boyfriend Andy who has quite literally kept me fed and watered, proof-read and kept me smiling when things were not going as well as I would have liked. Finally, to my supervisors, Beth Phillips and John Williams for their unwavering support and guidance during post- night shift meetings and very late deadlines.

List of abbreviations

1RM	1 repetition maximum
AD	Alzheimer's Disease
BMI	body mass index
CNS	central nervous system
CRP	c-reactive protein
CSF	cerebrospinal fluid
DAMP	danger-associated molecular patterns
GLUT4	glucose transporter type 4
HIT	high intensity interval training
IGF-1	insulin-like growth factor 1
IL-1α	interleukin 1 alpha
IL-1β	interleukin 1 beta
IL-1ra	interleukin 1 receptor antagonist
IL-6	interleukin 6
IL-10	interleukin 10
MDD	minimum detectable dose
NCD	non-communicable disease
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	National Health Service
PAMP	pathogen-associated molecular patterns
PRR	pattern-recognition receptors
SD	standard deviation
SEM	standard error of the mean
SIT	sprint interval training
T2DM	type 2 diabetes mellitus
TLR	toll-like receptors
TNFα	tumour necrosis factor alpha
VO_2 Max	maximum oxygen uptake
VO_2 Peak	peak oxygen uptake

1. Introduction

1.1 Inflammation

The Oxford English Dictionary defines inflammation as: "A morbid process affecting some organ or part of the body, characterized by excessive heat, swelling, pain, and redness; also, a particular instance or occurrence of this" [1], and history records a number of key scientists investigating inflammation within the human body. For example, as early as the 5th century BC, Hippocrates asserted that inflammation occurred as a result of tissue injury [2], while Celsius' famous description of the classical signs of rubor, tumour, calor and dolor appeared in the 1st century AD [3].

While early physicians could only describe the symptoms they encountered, the complex pathophysiology of inflammation has been subject to continuous investigation throughout the history of modern medicine. Current understanding of inflammation has defined it as a hugely complex response to invading pathogens or cell damage [4]. It arises from an activation of the immune system [5] triggering a number of responses within the local immune cells and vasculature, driven by plasma and cell-derived mediators. The exact process is dependent on the initial trigger which may be infectious or non-infectious in nature, but the inflammatory process can be summarised in a series of common steps. Invading pathogens or damaged cells are initially detected by cell surface pattern-recognition receptors (PRRs), by recognition of pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) respectively [6]. The most widely studied cell surface PRRs are the toll-like receptors (TLRs) which initiate a signalling cascade, leading to activation of the effector cells of the innate immune system [7]. The signalling cascade depends on cellular communication via cytokines; an umbrella term including chemokines, interferons, interleukins and growth factors [8]. In particular, interleukin-6

(IL-6) [9] and tumour necrosis factor α (TNF α) activate TLRs, and their own specific receptors (IL-6R, TNFR), triggering further signalling pathways. Cytokine release triggers blood vessel dilation and increases endothelial permeability, producing the typical symptoms of erythema and swelling. Locally produced cytokines migrate into the vascular system and exert effects at various sites including the liver and bone marrow [7, 10]. Specific cytokines play different roles within the inflammatory response and their actions form the complex network of the immune response.

1.1.1: Tumour Necrosis Factor α

TNFα produces systemic effects through interactions in the hypothalamus (fever, anorexia) and liver (increased C-reactive protein synthesis). It is also responsible for the vasodilatation and increased vascular permeability which allows recruitment of immune cells to the site of inflammation [11].

1.1.2: Interleukin 1

Members of the interleukin 1 (Il-1) family, in particular IL-1α and Il-1β are implicated in systemic inflammation [12]. Both L-1α and Il-1β are pro-inflammatory in their effects and are counteracted by Il-1 receptor antagonist (Il-1Ra) [11]. Experiments in animal models lacking in endogenous Il-1Ra have shown worsening of inflammatory conditions and aggressive tumours in response to carcinogen exposure [13]. Pharmacologically, recombinant Il-1Ra is used in the treatment of inflammatory conditions including rheumatoid arthritis and gout [14]. The precursor to the pro-inflammatory IL-1α acts as a DAMP [14], triggering the innate immune system while IL-1α acts via its specific receptor to stimulate an inflammatory response. Il-1β release has been linked to fever, anorexia and increased levels of circulating IL-6 [14]. Both TNFα and Il-1β are known to activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which regulates the expression of genes responsible for the immune response [15].

1.1.3: Interleukin 6

IL-6 has both pro- and anti- inflammatory properties [16] and has been linked to chronic levels of inflammation through dysregulated release from adipose tissues [9]. It is involved in the recruitment of leukocytes through the upregulation of chemokines and cell-adhesion molecules [16], inhibition of T-cell apoptosis and the priming of B cells to secrete antibodies [10]. IL-6 also promotes hepatocyte production of acute phase response proteins such as C-reactive protein, serum amyloid A and fibrinogen [9], via the activation of acute phase response factors [17]. Its release from and actions on skeletal muscle after acute exercise has also led to its labelling as a 'myokine' [18].

1.1.4: Interleukin 10

In order to regulate the inflammatory response, a number of anti-inflammatory cytokines are released. One of the most widely studied is IL-10. It has a range of effects [19], including inhibition of the NF-κB pathway [20], inhibition of the production of TNFα, and other pro-inflammatory cytokines including Il-1 and IL-6 [21].

1.1.5: C-Reactive Protein

While not a cytokine, C-reactive protein plays an important role in the inflammatory response and is easily measured in circulating blood. It is produced in the liver in response to cytokines (especially IL-6, Il-1β and TNFα [22]) and binds to damaged cells in order to activate the complement pathway and promote destruction [23]. It has also been shown to participate in the activation of NF-κB [22]. Alongside release from the liver, CRP expression has also been shown in multiple sites including vascular smooth muscle cells and coronary artery macrophages [24]. The presence of such cytokines provides a method of quantifying the inflammatory response to

various stimuli and have been used alongside other acute phase proteins such as C-reactive protein to measure inflammation [25].

1.2 Chronic Inflammation and Disease

While acute inflammation has a largely positive role in protecting the body from invading pathogens, healing wounds and removing damaged cells there are several instances where inappropriate activation may contribute to disease. A number of common medical conditions share an underlying inflammatory process. Inflammation is associated with a number of recognised chronic medical conditions including arthritis, asthma and inflammatory bowel diseases such as ulcerative colitis [26]. While these conditions have a recognised inflammatory component, and often respond to treatment with immunosuppressant therapy including steroids, chronic inflammation is also seen in patients with other pathologies such as hypertension [27] and diabetes mellitus [28]. Inflammatory processes in such diseases are complex and have been suggested as both cause and effect [29].

1.2.1: Hypertension

In hypertension it has been suggested that levels of chronic inflammation, including the production of cytokines [27], contribute to the development of the disease. Both TNFα and IL-6 have been implicated in hypertension mediated by angiotensin II, itself having pro-inflammatory properties [30] [31] [32] [33]. Hypertension also appears to be an inflammatory condition, contributing to the development of vascular disease by encouraging endothelial cells to increase production of inflammatory messengers [34] [31]. CRP has been heavily implicated in cardiovascular disease [35] and also has been shown to increase monocyte release of IL-6 and TNFα [31], increasing the inflammatory response. CRP is used in risk prediction models for

cardiovascular disease, where it can predict risk of developing hypertension, coronary heart disease and ischemic stroke independent of other risk factors [34].

1.2.2: Type 2 Diabetes

Type 2 diabetes mellitus (T2DM) has been associated with heightened inflammatory states [28]. Like many other conditions, evidence suggests that this link is multifactorial, with inflammation being both cause and effect. A nucleotide polymorphism resulting in reduced level of Il-1Ra is common in patients with T2DM [13] and multiple studies have shown elevated levels of circulating CRP and cytokines including IL-6 [28]. TNFα released by macrophages within adipose tissue has also been implicated in insulin resistance, particularly in obese individuals [28] [36].

1.2.3: Obesity

Obesity is also considered a pro-inflammatory condition, due in part to the inflammatory signals released by macrophages and adipocytes found in obese adipose tissues [16] [28].These cells have been specifically linked to the release of inflammatory cytokines including TNFα and IL-6 (via toll-like receptor pathways) and increased levels of CRP [37] [11] [38]. While the majority of the adipokines (including IL-6 and TNFα) released from excess adipose tissue are pro-inflammatory and potentiate metabolic diseases including T2DM, adiponectin was found to be downregulated in obesity and protective against such pathologies [38]. The complete interaction of inflammation, metabolic and vascular dysfunction seen in obesity is not fully understood; but is the site of ongoing research.

1.2.4: Cancer

The relationship between inflammation and neoplasms is complex. Chronic inflammation related to infectious agents, or particulate matter have long been implicated in carcinogenesis, as seen in

cervical cancer and its association with infection by the human papilloma virus, or gastric cancer and *Helicobacter pylori* [39]. As well as being a result of underlying inflammation, cancer cells create an 'inflammatory microenvironment' which promotes tumour development [40]. In particular the expression of TNFα has been described as important in early stages of cancer proliferation, by '[up]regulating a cascade of cytokines, chemokines…and pro-angiogenic activities' [41]. The NF-κB pathway, and the presence of IL-6, Il-1β and TNFα may all increase the 'invasive capacity of malignant cells' while IL-6 has been suggested to protect tumour cells from therapy-induced damage [42]. Tumour cells have also been shown to produce IL-10, protecting them from the response of cytotoxic T cells and allowing proliferation [41].

Raised levels of inflammatory cytokines have been seen in the plasma of patients with specific cancers. A recent study of 185 breast cancer patients was able to identify them from healthy controls by their plasma cytokine profile [43]. In this study poor prognosis was correlated with elevated levels of Il-17, while other studies have associated disease progression and metastasis with cytokines including IL-6, IL-10 and CRP [44] [45]. Recent studies have also linked raised inflammatory markers and individuals who go on to develop certain cancers [46] [47], with raised levels of CRP associated with lung, breast [47], ovarian, colorectal and renal cancers [48]. Genetic polymorphisms which cause increased expression of inflammatory cytokines such as IL-6 have been linked to the development of cancer [49]. Raised levels of TNFα have also been described in pre-cancerous tissues and have been shown to decrease in disease response to chemotherapy [50].

Given that a chronic inflammatory state may predispose to the development or progression of cancer, many studies have investigated the potential therapeutic and prophylactic use of anti-inflammatory medications. The results of these interventions have been varied, with anti-TNFα

medications such as infliximab and etanercept being used to stabilise disease in metastatic breast cancer, renal cell carcinoma and ovarian cancer [50]. Regular use of non-steroidal anti-inflammatory drugs have also been linked with a reduction in the relative risk for developing colon cancer [49]. However, as with all inflammatory responses there are a number of cofounders, including the duality of many of the cytokines studied. TNFα in particular has been found to play a role in both tumour suppression and proliferation, and concerns have been raised that its suppression may increase the risk of the development of other tumours [50].

1.2.5: Dementia

While other inflammatory conditions can affect any age group, aging itself is associated with increased levels of inflammation. Labelled "inflammaging" [51], it has been suggested that this underlying chronic inflammatory state contributes to many of the diseases associated with older age groups [52] [53]. In particular, large-scale studies have shown that cytokines including IL-6 and TNFaR1 can predict 10-year all-cause mortality [54].

Dementia, and in particular Alzheimer's disease (AD), accounts for £4.3 billion of the annual NHS budget [55]. Alongside neurodegeneration and the development of amyloid deposits, it has also been described as a neuroinflammatory condition [56] [57]. Il-1, IL-6 and TNFα have been shown to cross the blood brain barrier [58] as well as being released from within the cells of the central nervous system (CNS) [59]. It has been suggested that the acute central effects of such cytokines promote a range of responses including fever and 'sickness behaviour' such as reduced physical activity [60]. However, their transmission and effects within the CNS are disputed and may not be inflammatory [61]. Further research is needed to fully understand their actions on the CNS.

It has been demonstrated that cerebrospinal fluid (CSF) levels of TNFα increase linearly with age and this has been suggested as a correlate for brain inflammation [62]. The same study demonstrated a 'U-shaped relationship' between age and IL-10 with lowest levels seen in the CSF of middle-aged participants [62]. A number of studies have investigated the potential links between plasma or CSF levels of (pro- and anti-) inflammatory cytokines and Alzheimer's disease [63] [64]. The most widely investigated cytokines include Il-1, IL-6 and TNFα, however research results are often conflicting; with levels being increased, decreased or unchanged when comparing patients with AD to healthy controls [65] [64] [66].

The combined cost to the NHS of disease associated with inflammation is well documented [67], with rapidly increasing levels of obesity, T2DM and associated cardiovascular complications in the general public. [68] [69] [70] Alongside the use of NHS resources, these conditions also reduce healthy life expectancy [71] and increase dependence on long term care [72]. In order to reduce the burden of such diseases, research focused on interventions which reduce may provide benefit.

1.3 Exercise

One such area of research is physical exercise, levels of which have been shown to correlate with all-cause mortality [73] and healthy aging [74]. In order to reduce the risks of non-communicable diseases (NCDs) including hypertension, type 2 diabetes and coronary heart disease the World Health Organisation guidelines for activity for adults aged 18-64 recommends:

1. *At least 150 minutes of moderate-intensity aerobic physical activity throughout the week or at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity activity.*

2. *Aerobic activity should be performed in bouts of at least 10 minutes duration.*
3. *For additional health benefits…moderate-intensity aerobic physical activity to 300 minutes per week, or engage in 150 minutes of vigorous-intensity aerobic physical activity per week, or an equivalent combination of moderate- and vigorous-intensity activity.*
4. *Muscle-strengthening activities should be done involving major muscle groups on 2 or more days a week* [75].

The benefits of exercise on such pathologies are multifactorial, however there is evidence to suggest that ongoing physical exercise reduces levels of chronic inflammation which may contribute to the reduction of risk of NCDs [49].

Exercise itself has been variously defined and studied. It is often classified into endurance (or aerobic) and resistance (or strength) training, with each modality yielding different effects. Lower intensity or endurance training is associated with improvements in oxygen delivery and consumption within muscle tissue, whereas strength training promotes muscle hypertrophy and strength [76] [77]. More recently high intensity interval training (HIT) and sprint interval training (SIT) have also been studied [78].

1.3.1: Endurance Training

Endurance training is classically described as longer duration, sub-maximal contractions including running, cycling and swimming, which have been shown to promote adaptations including 'mitochondrial biogenesis, fast-to-slow fibre type transformation and shifts in substrate metabolism that favour fat over carbohydrate based fuels' [79] [80]. While also having effects on the cardiovascular and respiratory system, endurance exercise training increases muscle

performance by increasing the energy supply capacity within the cells [81]. A number of mechanisms have been suggested, including increased mitochondrial content [82], electron transport capacity within mitochondria, increasing adenosine triphosphate production and improved respiratory control sensitivity, requiring less ADP for the same oxygen consumption [76] [83]. Endurance training has also been shown to optimise the use of lipid over carbohydrate as a fuel source during endurance exercise. While contracting muscle is viewed as a source of IL-6 production, secretion is known to be downregulated by the ingestion of glucose during exercise leading to the hypothesis that it may be performing as a glucose depletion signal. It has been associated with increased glucose uptake via the GLUT4 transporter. The change from carbohydrate to lipid as a major fuel source may go some way to explaining the results of some studies which show decreased circulating levels of IL-6 in individuals who undertake regular endurance training [84].

1.3.2: Resistance Training

In contrast, resistance training is primarily associated with muscle hypertrophy, thought to be mediated via IGF-1 as well as through satellite cell-mediated repair mechanisms following muscle damage during resistance exercise [85]. Individuals undergoing purely strength based training do not show the mitochondrial changes or fuel utilisation adaptations seen in those performing endurance training [79]. Defining exercise in terms of endurance or resistance training is understandably complex, with some considering them to be opposing ends of a spectrum, rather than discreet entities. Concurrent training, where individuals alternate between endurance and resistance exercise has also been studied with variable results. There is evidence to suggest the two mechanisms may counteract each other, with one preventing the beneficial effects of the other from being fully realised [79]. In practice, concurrent training reduces muscle hypertrophy

(compared to that seen in pure resistance training), without having deleterious effects on aerobic capacity [79].

1.3.3: Interval Training

Alongside the classical modes of endurance and resistance training, recent evidence has suggested that similar adaptations can be produced following shorter sessions of interval training. Again, the nomenclature is variable, but often high-intensity interval training describes activities done at >80% of maximal heart rate, with sprint-interval training being performed at VO2 peak [82]. There is evidence to suggest greater improvements in insulin resistance [86], vascular function, inflammation and insulin sensitivity [87] following HIT compared to control or continuous training. These findings may have significant implications for patients as HIT requires considerably less time commitment than more traditional exercise.

1.3.4: Effect of Exercise on Inflammatory Markers

Acute strenuous exercise has been shown to transiently increase levels of circulating cytokines, especially IL-6 from skeletal muscle [88]. In younger, healthy populations acute exercise related changes in inflammatory cytokines have been demonstrated to be dependent on exercise intensity, duration and muscle mass utilised [89]. The cytokine response to exercise appears to differ from that of the acute inflammatory response to invading pathogens or trauma, as IL-6 predominates, rather than the rapid rise in TNFα and Il-1 [90]. This rise in IL-6 is accompanied by an increase in anti-inflammatory IL-10 and Il-1ra, which is proposed as the mechanism for overall reduction in basal inflammation seen in some long-term exercise programmes [91] [90] [92].

While several large cohort studies [93] [94] [95] have shown statistically significant reductions in inflammatory markers in those partaking in regular exercise compared to sedentary controls,

randomised controlled trials of exercise programmes have described varying results [96] [97]. It has been suggested that the variation in results may be due to the heterogeneity of exercise regimes and populations studied [98] and that the greatest consistency may be found in the CRP levels of older (>60 yrs) populations [99] [96] or those with underlying chronic disease or obesity [97] [100]. In particular, reduction in body mass index (BMI) has been associated with reductions in CRP that is not reflected in levels of IL-6 [99].

Given that long term exercise has been linked with lower levels of inflammatory cytokines in circulating plasma [94], one theory is that this contributes to decreasing the individual's susceptibility to inflammatory conditions including hypertension and type two diabetes [92]. As dementia has also been considered an inflammatory condition [56] [57], it may be theorised that frequent exercise decreases risk through a decrease in inflammatory processes within the brain which may potentially be measured by investigating changes in cytokines in cerebrospinal fluid before and after exercise interventions. Raised levels of inflammatory cytokines have also been observed in patients with malignancies, and elevated levels of specific markers may signal a poor prognosis. There is little known about the effect of exercise on mediating this rise in cytokines and the potential therapeutic benefits of doing so in patients with cancer.

As population demographics continue to trend towards an aging population, the pressure on health care services and resources will increase. By further investigating the relationship between inflammatory markers and exercise, it is hoped that we will better understand the mechanisms for the reductions in mortality seen in those with higher levels of physical activity. In doing so, the application of exercise can potentially be used to mitigate the effects of non-communicable diseases and promote healthy aging.

2. Aims and Hypotheses

Previous work has identified a potential link between increased levels of inflammation and chronic non-communicable diseases, as has been demonstrated in the circulating plasma levels of inflammatory cytokines. As dementia can be considered a neuro-inflammatory condition, it has been suggested that CSF levels of inflammatory cytokines may be used as correlates for brain inflammation.

Exercise has been shown to decrease the risk of developing chronic inflammatory conditions including dementia. While some exercise interventions have been shown to correlate with decreased levels of circulating inflammatory markers in plasma, little is known about the effect of exercise on the cytokine profile in the CSF (and by correlation, the brain). In studying the cytokine levels in the CSF of healthy individuals before and after an exercise intervention it will be possible to investigate the effects of exercise on the inflammatory profile within the CSF and compare this to changes seen in plasma. In addition, while some studies have shown an acute effect of acute high-intensity interval training on inflammatory markers within the plasma further work is required to understand the demographics in which this might be most effective.

Finally, while some studies have shown that worse outcomes in patients with cancer have been correlated with raised inflammatory markers in plasma, little is known about the potential for exercise to ameliorate this pro-inflammatory state.

This project will attempt to test the following hypotheses:

1. Acute high intensity interval training produces measurable changes in inflammatory markers including IL-6, IL-10, TNFα and CRP within the cerebrospinal fluid of healthy individuals.

2. Older participants will show the greatest effects of decreased circulating inflammatory markers after a period of exercise intervention.

3. High intensity interval training improves the inflammatory profile in the plasma of those with a diagnosis of urological or colorectal cancer.

3. Study 1: Investigating the effects of exercise on inflammatory markers in cerebrospinal fluid and plasma: A systematic review and meta-analysis

The pro- and anti- inflammatory effects of exercise have been much investigated [101] and results of studies have shown conflicting results. Some have described no significant changes in levels of inflammatory markers [100], whereas others have shown increases in cytokines (TNFα, IL-6, IL-10) and acute phase proteins (C-reactive protein) immediately after exercise as well as overall reductions in circulating inflammatory markers in in those that exercise long term [98] [92].

The majority of studies of the correlation between exercise and inflammation have focussed on circulating markers found in plasma. However, recent work has investigated the effect on cytokines measured in the CSF in patients with AD [102] and multiple sclerosis [103] but were unable to demonstrate a significant effect after 12-16 weeks of training. This review aims to investigate the literature to compare the effects of resistance and endurance training on the inflammatory biomarker profile of healthy individuals in both CSF and plasma.

3.1 Methods

3.1.1 Search Strategy

All studies evaluating the effect of exercise on inflammatory markers in cerebrospinal fluid were searched in Pubmed, Embase, Cochrane Trials and Reviews. Keywords included exercise, training, inflammation, cytokines, interleukin, cerebrospinal fluid and CSF. Specific inflammatory markers were also included (IL-6, IL-10, TNFα, C-reactive protein). Predetermined inclusion and exclusion criteria were used to prevent bias and references from included studies were searched by hand.

Experiments must have been performed in healthy participants with body mass index not exceeding 35kg/m^2. Exercise protocols including endurance, resistance or combined training of any duration were included. Studies not investigating IL-6, IL-10, TNFα or CRP were excluded.

The initial search strategy provided few results investigating inflammatory markers in cerebrospinal fluid. An additional search was planned to examine the literature for studies investigating the effect of exercise on inflammatory markers in blood. After replacing cerebrospinal fluid with blood or plasma, keywords, inclusion and exclusion criteria remained the same, with the addition of the exclusion of studies with an exercise programme lasting fewer than 8 weeks and those without control groups.

3.1.2 Data Acquisition

Baseline characteristics and exercise protocol data was recorded in addition to the measured IL-6, IL-10, TNFα and CRP in each group.

3.1.3 Quality

Quality of included studies were assessed to quantify risk of bias according to the 6-point Cochrane Collaboration risk of bias tool.

3.1.4 Data Analysis

Statistical analysis was performed as forest plots using Review Manager 5.3 [104]. Due to heterogeneity between studies, a random-effects model was used. Statistical heterogeneity was assessed using I^2, with >50% used as evidence for heterogeneity. Studies were weighted by sample size with statistical significance set at a minimum of $p < 0.05$.

The study results were largely reported using mean ± SD, but where necessary the mean and standard deviation were estimated from median and interquartile range using the Wan [105] and Luo [106] method [107]. A number of studies provided only the difference between experimental and control results. The authors were contacted to provide original data, but this was not received in sufficient time and these studies could not be included in the meta-analysis.

The units used to report results for cytokines and CRP by the studies varied considerably and led to discrepancies when attempting to convert to a standardised unit. Therefore, standard mean difference was used to compare final values in control and experimental groups. The limitations of this method are discussed later.

3.2 Results: Cerebrospinal Fluid

3.2.1 Search results and study characteristics

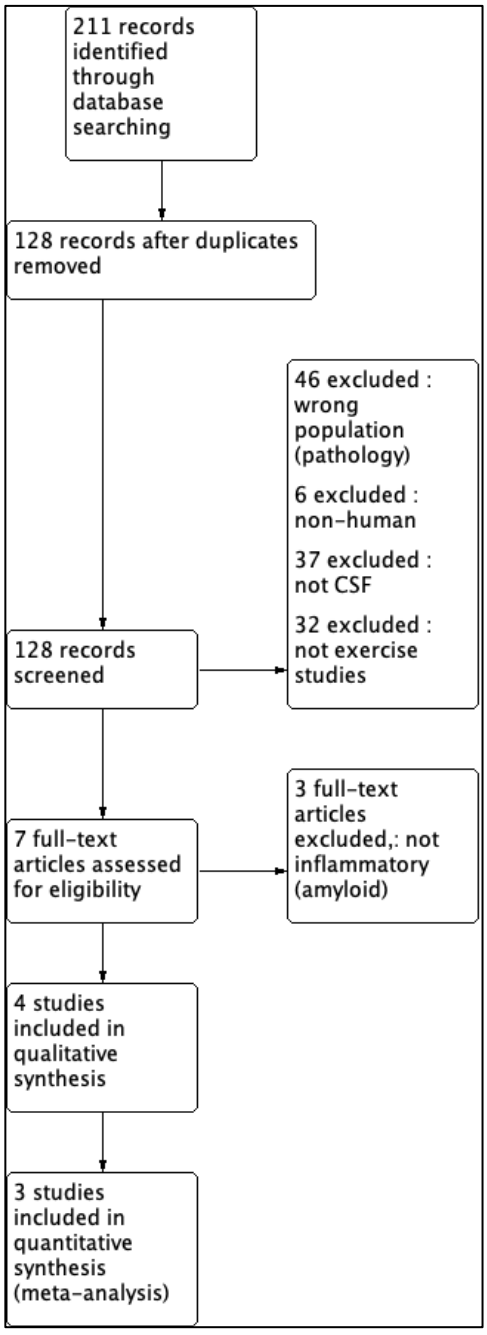


Figure 1: Study Flow Diagram CSF

A total of 221 papers were retrieved of which 83 were duplicates, 46 included participants with pathologies, 6 were in non-human subjects, 37 did not investigate cerebrospinal fluid and 32 did not investigate the effects of exercise. 7 studies remained for full text-analysis, of which 3 did not meet the inclusion criteria. 4 papers remained for inclusion, one study had only an abstract available, however the results were subsequently reported fully in one of the remaining three papers.

Three papers remained for full analysis and both were included into the review [108] [109] [110] with a total of 50 participants studied. All included healthy young volunteers (19.8-29 years). Two studies included mixed gender groups, however there is no available data stratified by sex. The full study demographics are described in Figure 2: Demographics of Study Participants (CSF).

Title	Total	Group	Number	Age	Sex (%)		Height	Weight	BMI	VO2 Max	Protocol	CSF Time
					M	F						
Steensberg, et al	24	REST	8	23(20-25)	100	0	182(178-189)	79(70-88)	23(22-28)	Not given	90 minute run at 75-80% max HR	60 minutes post
		Ex-CHO	8	24(21-27)	100	0	181(172-195)	79(67-90)	23(22-26)			
		Ex-PLA	8	24(21-29)	100	0	180(172-186)	75(63-80)	23(21-25)			
Schon, M et al	11	baseline & exercise	9	22.7±2.9	66.7	33.3	Not given	71.9±7.0	23.1±2.0	42.2±6.0	120 minutes cycling at 60% VO2 Max	<10 minutes post
		exercise only	11	24.6±5.1	54.5	45.5		69.1±8.0	22.7±1.8	41.1±6.7		
Dalsgaard et al	15	exercise	9	26 (23-28)	77.8	22.2	182 (168-192)	80 (66-94)	Not given	Not given	arm and leg ergometer, increasing work rate until exhaustion (av 12 minutes)	2-3 minutes post
		REST	6	24 (22-29)	50	50	180 (174-184)	75 (66-84)				

Figure 2: Demographics of Study Participants (CSF)

3.2.2 Risk of Bias

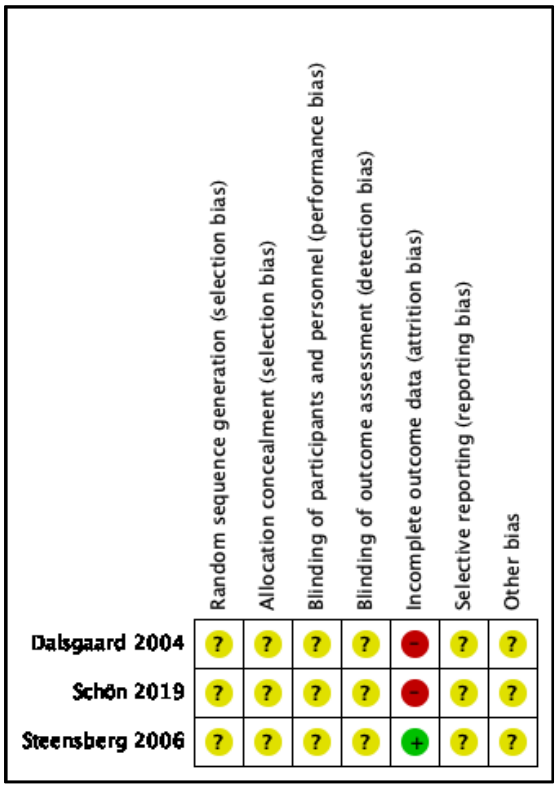


Figure 3: Risk of Bias (CSF)

Assessment of the risk of bias in each study proved difficult due to the lack of information regarding allocation and concealment. In exercise studies it is usually impossible to blind participants to their group, unless through the use of 'sham' exercise in the controls which no study utilised. There was no discussion in any study regarding blinding of the outcome assessors and both Schön et al and Dalsgaard et al appeared to have missing results as the number of participants did not correlate with the number of results presented.

3.2.3 Results

2 experiments investigated the effects of endurance-type exercise, running and cycling while one used a resistance exercise protocol. The intensity of exercise also varied in each experiment, from 60% VO2 max to 75-80% of maximum heart rate.

Samples of CSF were taken at differing time points in each experiment, from 2-3 minutes post exercise to 60 minutes post exercise. Steensberg et al included an exercise group given carbohydrate during the exercise protocol, a substance known to attenuate rises of circulating IL-6 post exercise. Each of the studies investigated inflammatory markers in the CSF with interleukin 6 and TNFα being common to all three. In addition, Schön et al assessed 174 cytokines, finding that only 17 showed significant differences from baseline [108]:

Adipocytokine	Role	Change
Adiponectin	anti-inflammatory, insulin sensitising	⇩21.3%
IL-18Rβ	regulation of feeding in mice	⇩ >10%
PDGF-AA	neuronal plasticity	⇩ >10%
IL5Rα		⇩ >5%
LAP		⇩ >5%
MIG		⇩ >5%
MMP-13		⇩ >5%
TGFβ2	lipid metabolism	⇩ >5%
Tie-1		⇩ >5%
Activin-A		⇩ >5%
IL-18 binding protein α		⇩ >5%
IGF-II	neuroprotection	⇩ >5%
Il-2		⇧ <5%
IL13Rα,		⇩ <5%
TGF-b3,		⇩ <5%
MPIF-1		⇩ <5%
Thrombopoietin		⇩ <5%

Figure 4: Changes in Measured Cytokines in CSF

3.2.4 Meta-analysis

Interleukin 6

All three studies investigated CSF levels of IL-6, with Steensberg and Dalsgaard recording baseline and post exercise levels and Schön et al stating percentage change. No study showed a significant change in any group from baseline to post intervention.

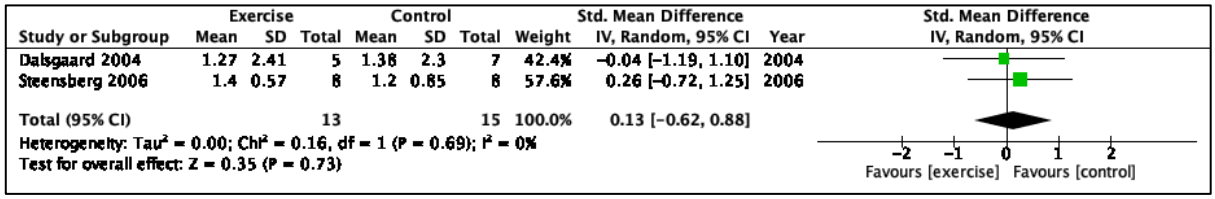


Figure 5: Forest Plot : IL-6 (CSF)

Tumour Necrosis Factorα

Similarly, no significant differences were seen in any of the three studies for TNFα – in the majority of the tests, it was undetectable by the assay used.

Cerebrospinal Fluid TNFα			
Steensberg (pg/mL)	Rest	0.0±0.0*	_
	Ex-CHO	0.1±0.0*	p > 0.05
	Ex-PLA	0.0±0.0*	_
Dalsgaard (pg/mL)	Rest	0**	_
	Post Exercise	0**	p > 0.05
Schön (% change)		0.55	p = 1.58

* Mean ± SEM ** Median (Range)

Figure 6: Changes in TNFα in CSF

Heat Shock Protein 72

Steensberg [110] and Dalsgaard [109] investigated HSP72, but similarly found no significant changes. There was marked heterogeneity in baseline values recorded for HSP72 in CSF despite the similarity of their participants.

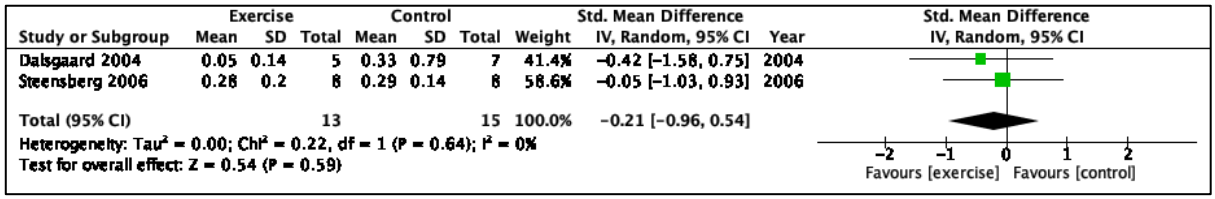


Figure 7: Forest Plot: HSP72 (CSF)

3.3 Results: Plasma

3.3.1 Search results and study characteristics

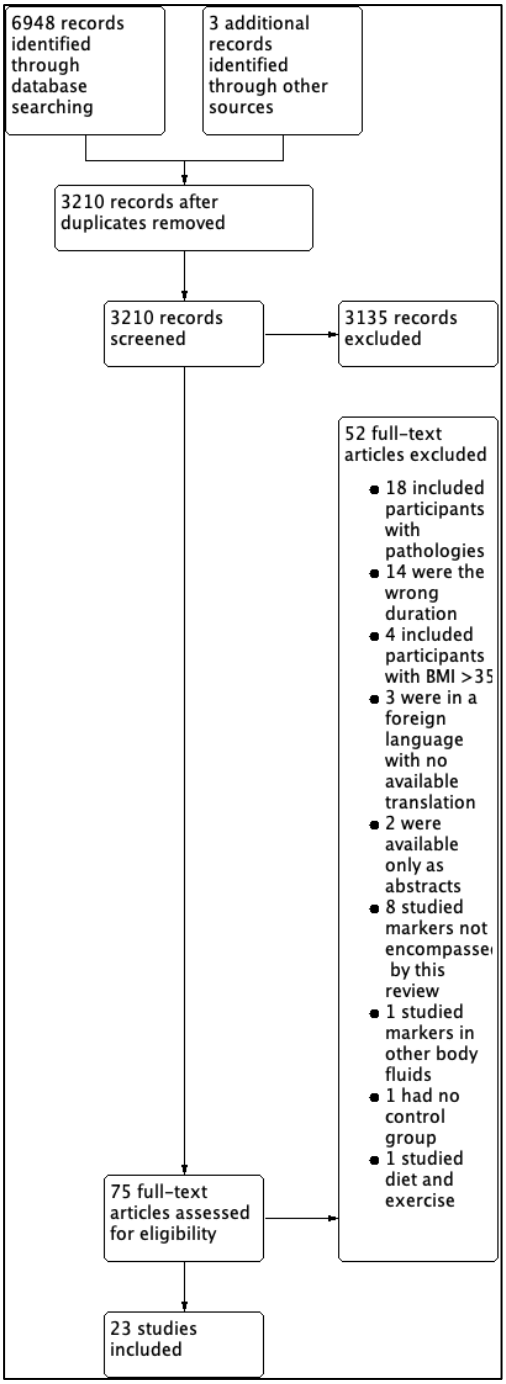


Figure 8: Study Flow Diagram (Plasma)

A total of 6948 records were retrieved from database searches with an additional 3 records from other sources. After duplicates were removed 3135 papers were considered with 75 included in the full text analysis. Of these, 18 included participants with pathologies, 14 had exercise protocols of <6 weeks, 4 included participants with BMI >35 kg/m^2, 3 were in foreign languages with no available English translation, 2 were only available as abstracts, and 11 did not meet inclusion criteria.

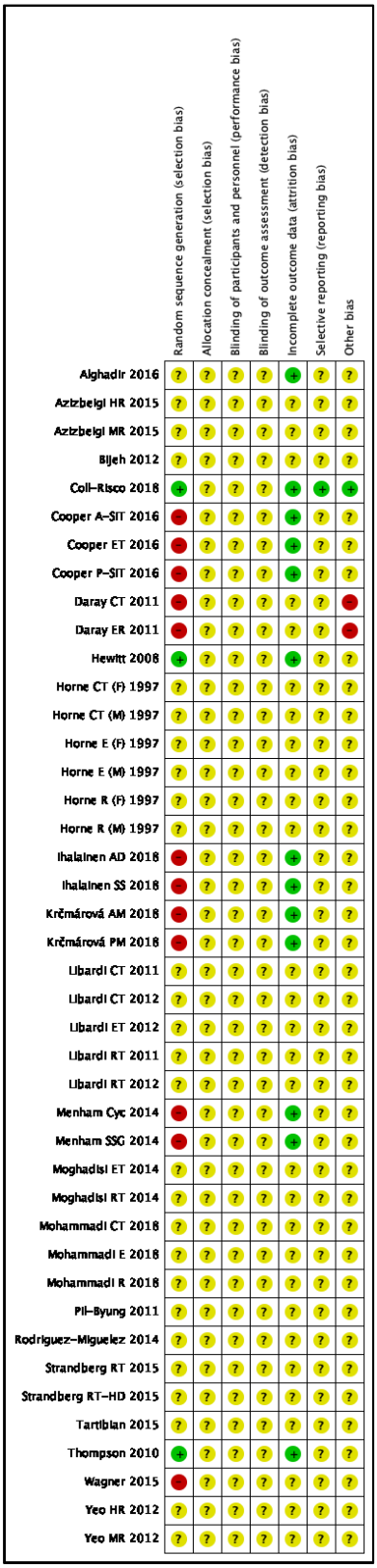
23 studies were included in the final analysis with a total of 1015 participants. The baseline characteristics of the studies can be found in Figure 10: Demographics of Study Participants (Plasma)Figure 10: Demographics of Study Participants (Plasma). 14 authors studied male subjects, 6 studied females with the remaining 4 using mixed populations. The age of participants ranged from 19.3 to 70 years with 9 studies using participants 19-39, 10 aged 40-59 and 4 aged 60 and over. One paper did not disclose the ages of subjects except that they were 'college students' [111], for the purposes of meta-analysis this study was included in the 19-39 year age group. 3 papers provided results only in graphical form, 1 did not report numerical

data from the control group, and 2 papers presented results as change only and so have been excluded from the meta-analysis.

The included papers investigated 35 different exercise groups, with 15 groups [112] [113] [114] [115] [116] [117] [118] [119] [120] [121] [122] [123] performing endurance-type exercise, 11 [124] [125] [126] [127] [118] [128] [123] [111] performing resistance-type exercise and 9 [129] [127] [117] [123] [130] [131] [132] [133] performing combined training. Within the endurance training groups, 2 groups performed interval training, 2 performed endurance and interval training while the remainder used continuous endurance exercise including cycling and running.

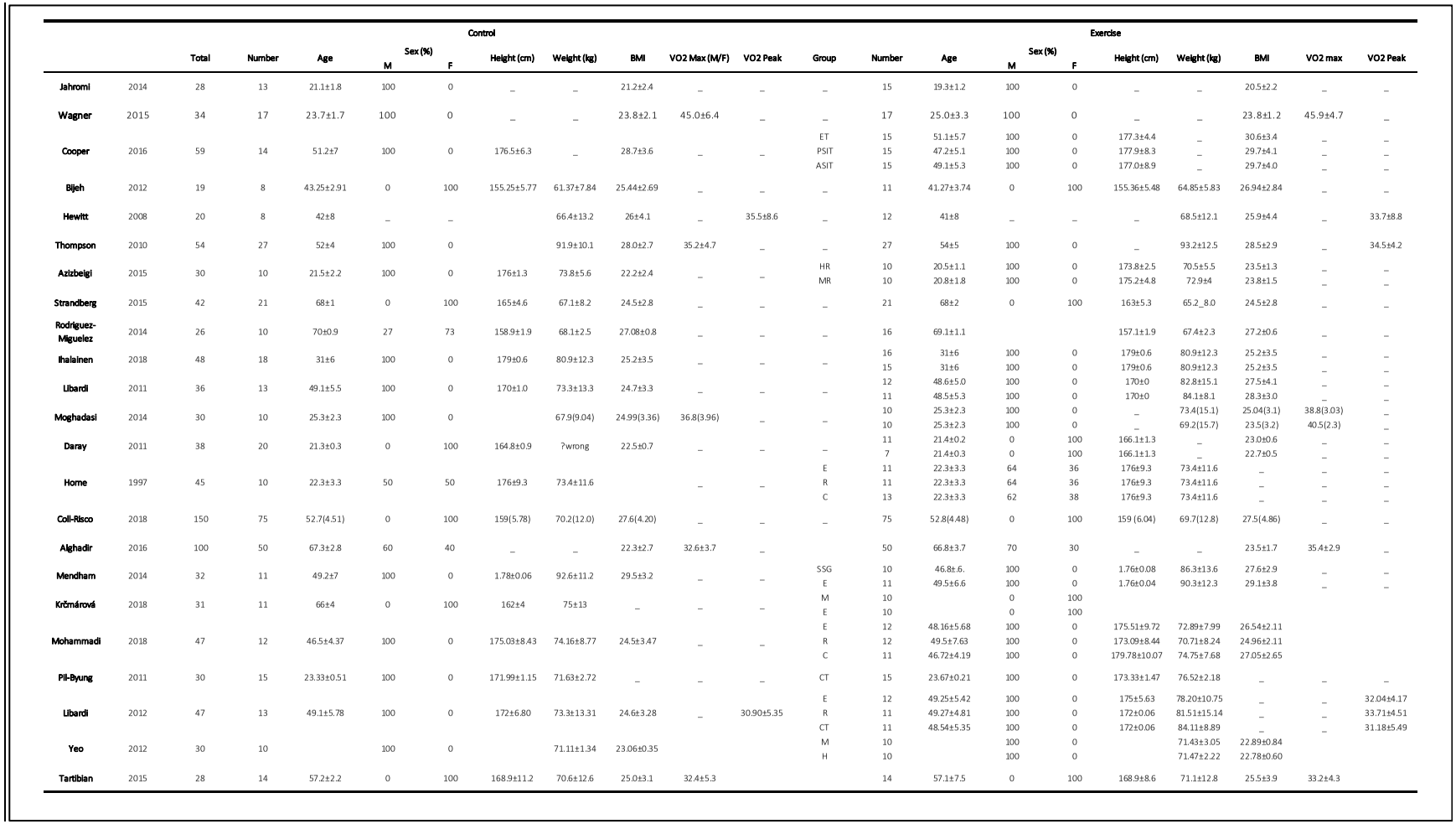
Training duration varied from 8 to 24 weeks with a mean of 14 weeks and inflammatory markers were assessed at a variety of time points. The most commonly studied inflammatory markers included IL-6 (n=11), TNFα (n=9), CRP (n=13) and IL-10 (n=2).

3.3.2 Risk of Bias



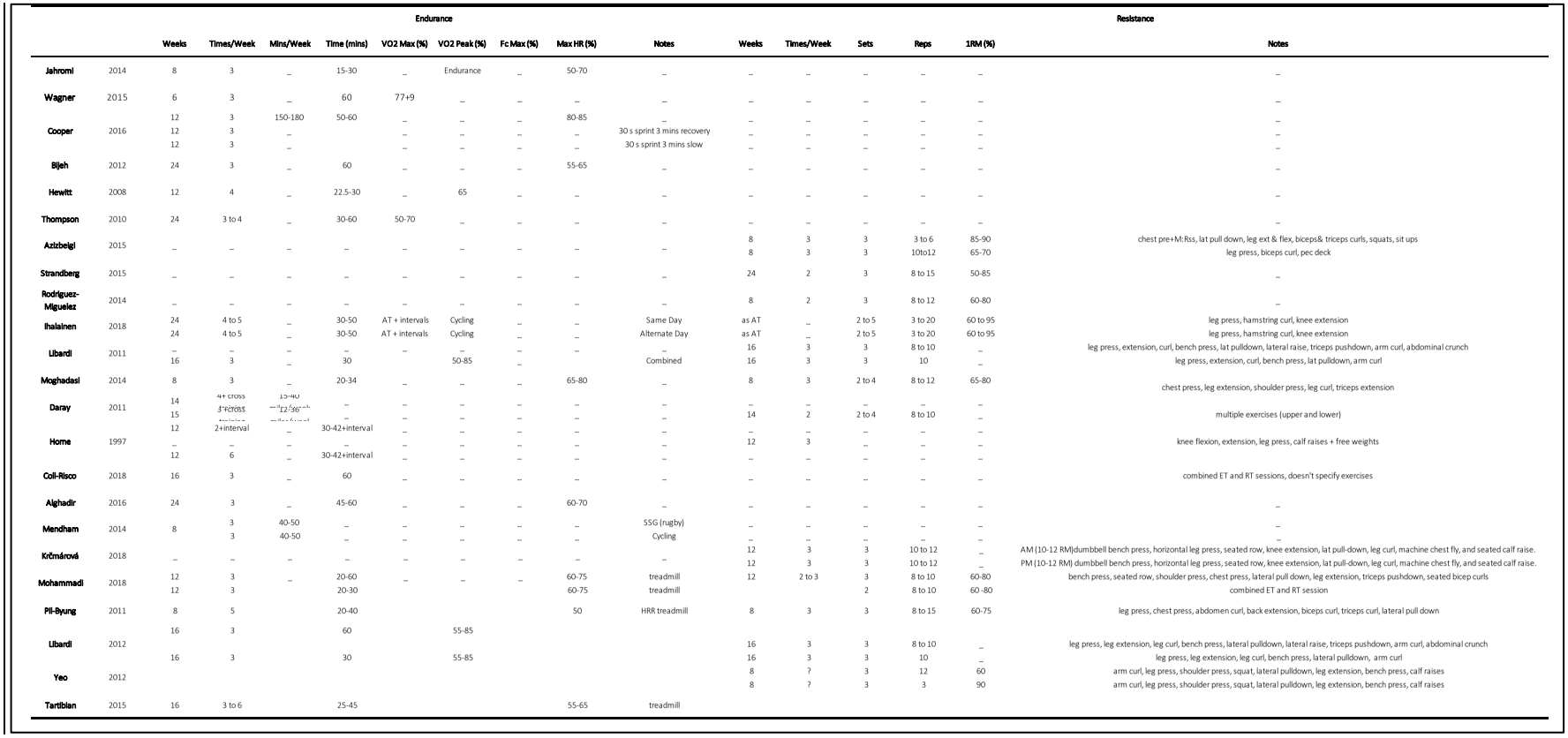
As with the CSF studies, there was universally poor recording of randomisation and allocation processes. Again, no study chose to use 'sham' exercise for their control group and so were not able to blind participants to their allocation arm. There were no reports of blinding of assessors of the data.

Figure 9: Risk of Bias (plasma)



		Control											Exercise							
		Total	Number	Age	Sex (%) M	Sex (%) F	Height (cm)	Weight (kg)	BMI	VO2 Max (M/F)	VO2 Peak	Group	Number	Age	Sex (%) M	Sex (%) F	Height (cm)	Weight (kg)	BMI	VO2 max	VO2 Peak
Jahromi	2014	28	13	21.1±1.8	100	0	_	_	21.2±2.4	_	_	_	15	19.3±1.2	100	0	_	_	20.5±2.2	_	_
Wagner	2015	34	17	23.7±1.7	100	0	_	_	23.8±2.1	45.0±6.4	_	_	17	25.0±3.3	100	0	_	_	23.8±1.2	45.9±4.7	_
Cooper	2016	59	14	51.2±7	100	0	176.5±6.3	_	28.7±3.6	_	_	ET PSIT ASIT	15 15 15	51.1±5.7 47.2±5.1 49.1±5.3	100 100 100	0 0 0	177.3±4.4 177.9±8.3 177.0±8.9	_ _ _	30.6±3.4 29.7±4.1 29.7±4.0	_ _ _	_ _ _
Bijeh	2012	19	8	43.25±2.91	0	100	155.25±5.77	61.37±7.84	25.44±2.69	_	_	_	11	41.27±3.74	0	100	155.36±5.48	64.85±5.83	26.94±2.84	_	_
Hewitt	2008	20	8	42±8	_	_		66.4±13.2	26±4.1	_	35.5±8.6	_	12	41±8	_	_	_	68.5±12.1	25.9±4.4	_	33.7±8.8
Thompson	2010	54	27	52±4	100	0		91.9±10.1	28.0±2.7	35.2±4.7	_	_	27	54±5	100	0	_	93.2±12.5	28.5±2.9	_	34.5±4.2
Azizbeigi	2015	30	10	21.5±2.2	100	0	176±1.3	73.8±5.6	22.2±2.4	_	_	HR MR	10 10	20.5±1.1 20.8±1.8	100 100	0 0	173.8±2.5 175.2±4.8	70.5±5.5 72.9±4	23.5±1.3 23.8±1.5	_ _	_ _
Strandberg	2015	42	21	68±1	0	100	165±4.6	67.1±8.2	24.5±2.8	_	_	_	21	68±2	0	100	163±5.3	65.2_8.0	24.5±2.8	_	_
Rodriguez-Migueiez	2014	26	10	70±0.9	27	73	158.9±1.9	68.1±2.5	27.08±0.8	_	_	_	16	69.1±1.1			157.1±1.9	67.4±2.3	27.2±0.6	_	_
Ihalainen	2018	48	18	31±6	100	0	179±0.6	80.9±12.3	25.2±3.5	_	_	_	16 15	31±6 31±6	100 100	0 0	179±0.6 179±0.6	80.9±12.3 80.9±12.3	25.2±3.5 25.2±3.5	_ _	_ _
Libardi	2011	36	13	49.1±5.5	100	0	170±1.0	73.3±13.3	24.7±3.3	_	_	_	12 11	48.6±5.0 48.5±5.3	100 100	0 0	170±0 170±0	82.8±15.1 84.1±8.1	27.5±4.1 28.3±3.0	_ _	_ _
Moghadasi	2014	30	10	25.3±2.3	100	0		67.9(9.04)	24.99(3.36)	36.8(3.96)	_	_	10 10	25.3±2.3 25.3±2.3	100 100	0 0	_ _	73.4(15.1) 69.2(15.7)	25.04(3.1) 23.5(3.2)	38.8(3.03) 40.5(2.3)	_ _
Daray	2011	38	20	21.3±0.3	0	100	164.8±0.9	?wrong	22.5±0.7	_	_	_	11 7	21.4±0.2 21.4±0.3	0 0	100 100	166.1±1.3 166.1±1.3	_ _	23.0±0.6 22.7±0.5	_ _	_ _
Horne	1997	45	10	22.3±3.3	50	50	176±9.3	73.4±11.6		_	_	E R C	11 11 13	22.3±3.3 22.3±3.3 22.3±3.3	64 64 62	36 36 38	176±9.3 176±9.3 176±9.3	73.4±11.6 73.4±11.6 73.4±11.6	_ _ _	_ _ _	_ _ _
Coll-Risco	2018	150	75	52.7(4.51)	0	100	159(5.78)	70.2(12.0)	27.6(4.20)	_	_	_	75	52.8(4.48)	0	100	159 (6.04)	69.7(12.8)	27.5(4.86)	_	_
Alghadir	2016	100	50	67.3±2.8	60	40	_	_	22.3±2.7	32.6±3.7	_		50	66.8±3.7	70	30	_	_	23.5±1.7	35.4±2.9	_
Mendham	2014	32	11	49.2±7	100	0	1.78±0.06	92.6±11.2	29.5±3.2	_	_	SSG E	10 11	46.8±.6. 49.5±6.6	100 100	0 0	1.76±0.08 1.76±0.04	86.3±13.6 90.3±12.3	27.6±2.9 29.1±3.8	_ _	_ _
Krčmárová	2018	31	11	66±4	0	100	162±4	75±13	_	_	_	M E	10 10		0 0	100 100					
Mohammadi	2018	47	12	46.5±4.37	100	0	175.03±8.43	74.16±8.77	24.5±3.47	_	_	E R C	12 12 11	48.16±5.68 49.5±7.63 46.72±4.19	100 100 100	0 0 0	175.51±9.72 173.09±8.44 179.78±10.07	72.89±7.99 70.71±8.24 74.75±7.68	26.54±2.11 24.96±2.11 27.05±2.65		
Pil-Byung	2011	30	15	23.33±0.51	100	0	171.99±1.15	71.63±2.72	_	_	_	CT	15	23.67±0.21	100	0	173.33±1.47	76.52±2.18	_	_	_
Libardi	2012	47	13	49.1±5.78	100	0	172±6.80	73.3±13.31	24.6±3.28	_	30.90±5.35	E R CT	12 11 11	49.25±5.42 49.27±4.81 48.54±5.35	100 100 100	0 0 0	175±5.63 172±0.06 172±0.06	78.20±10.75 81.51±15.14 84.11±8.89	_ _ _	_ _ _	32.04±4.17 33.71±4.51 31.18±5.49
Yeo	2012	30	10		100	0		71.11±1.34	23.06±0.35			M H	10 10		100 100	0 0		71.43±3.05 71.47±2.22	22.89±0.84 22.78±0.60		
Tartibian	2015	28	14	57.2±2.2	0	100	168.9±11.2	70.6±12.6	25.0±3.1	32.4±5.3			14	57.1±7.5	0	100	168.9±8.6	71.1±12.8	25.5±3.9	33.2±4.3	

Figure 10: Demographics of Study Participants (Plasma)



		Endurance									Resistance					
		Weeks	Times/Week	Mins/Week	Time (mins)	VO2 Max (%)	VO2 Peak (%)	Fc Max (%)	Max HR (%)	Notes	Weeks	Times/Week	Sets	Reps	1RM (%)	Notes
Jahromi	2014	8	3	_	15-30	_	Endurance	_	50-70	_	_	_	_	_	_	_
Wagner	2015	6	3	_	60	77+9	_	_	_	_	_	_	_	_	_	_
		12	3	150-180	50-60	_	_	_	80-85	_	_	_	_	_	_	_
Cooper	2016	12	3	_		_	_	_	_	30 s sprint 3 mins recovery	_	_	_	_	_	_
		12	3	_		_	_	_	_	30 s sprint 3 mins slow	_	_	_	_	_	_
Bijeh	2012	24	3	_	60	_	_	_	55-65	_	_	_	_	_	_	_
Hewitt	2008	12	4	_	22.5-30	_	65	_	_	_	_	_	_	_	_	_
Thompson	2010	24	3 to 4	_	30-60	50-70	_	_	_	_	_	_	_	_	_	_
Azizbeigi	2015	_	_	_	_	_	_	_	_	_	8	3	3	3 to 6	85-90	chest pre+M:Rss, lat pull down, leg ext & flex, biceps& triceps curls, squats, sit ups
											8	3	3	10to12	65-70	leg press, biceps curl, pec deck
Strandberg	2015	_	_	_	_	_	_	_	_	_	24	2	3	8 to 15	50-85	_
Rodriguez-Miguelez	2014	_	_	_	_	_	_	_	_	_	8	2	3	8 to 12	60-80	_
Ihalainen	2018	24	4 to 5	_	30-50	AT + intervals	Cycling	_	_	Same Day	as AT	_	2 to 5	3 to 20	60 to 95	leg press, hamstring curl, knee extension
		24	4 to 5	_	30-50	AT + intervals	Cycling	_	_	Alternate Day	as AT	_	2 to 5	3 to 20	60 to 95	leg press, hamstring curl, knee extension
Libardi	2011	_	_	_	_	_	_	_	_	_	16	3	3	8 to 10	_	leg press, extension, curl, bench press, lat pulldown, lateral raise, triceps pushdown, arm curl, abdominal crunch
		16	3	_	30		50-85	_		Combined	16	3	3	10	_	leg press, extension, curl, bench press, lat pulldown, arm curl
Moghadasi	2014	8	3	_	20-34	_	_	_	65-80	_	8	3	2 to 4	8 to 12	65-80	chest press, leg extension, shoulder press, leg curl, triceps extension
Daray	2011	14	4+ cross 3 circuit	15-40	_	_	_	_	_	_	_	_	_	_	_	
		15		[illegible]	_	_	_	_	_	_	14	2	2 to 4	8 to 10	_	multiple exercises (upper and lower)
Horne	1997	12	2+interval	_	30-42+interval	_	_	_	_	_	_	_	_	_	_	
		_	_	_	_	_	_	_	_	_	12	3	_	_	_	knee flexion, extension, leg press, calf raises + free weights
		12	6	_	30-42+interval	_	_	_	_	_	_	_	_	_	_	
Coll-Risco	2018	16	3	_	60	_	_	_	_	_	_	_	_	_	_	combined ET and RT sessions, doesn't specify exercises
Aghadir	2016	24	3	_	45-60	_	_	_	60-70	_	_	_	_	_	_	_
Mendham	2014	8	3	40-50	_	_	_	_	_	SSG (rugby)	_	_	_	_	_	_
			3	40-50	_	_	_	_	_	Cycling	_	_	_	_	_	_
Krčmárová	2018	_	_	_	_	_	_	_	_	_	12	3	3	10 to 12	_	AM (10-12 RM)dumbbell bench press, horizontal leg press, seated row, knee extension, lat pull-down, leg curl, machine chest fly, and seated calf raise.
											12	3	3	10 to 12	_	PM (10-12 RM) dumbbell bench press, horizontal leg press, seated row, knee extension, lat pull-down, leg curl, machine chest fly, and seated calf raise.
Mohammadi	2018	12	3	_	20-60	_	_	_	60-75	treadmill	12	2 to 3	3	8 to 10	60-80	bench press, seated row, shoulder press, chest press, lateral pull down, leg extension, triceps pushdown, seated bicep curls
		12	3		20-30				60-75	treadmill			2	8 to 10	60 -80	combined ET and RT session
Pil-Byung	2011	8	5		20-40				50	HRR treadmill	8	3	3	8 to 15	60-75	leg press, chest press, abdomen curl, back extension, biceps curl, triceps curl, lateral pull down
Libardi	2012	16	3		60		55-85									
											16	3	3	8 to 10	_	leg press, leg extension, leg curl, bench press, lateral pulldown, lateral raise, triceps pushdown, arm curl, abdominal crunch
		16	3		30		55-85				16	3	3	10	_	leg press, leg extension, leg curl, bench press, lateral pulldown, arm curl
Yeo	2012										8	?	3	12	60	arm curl, leg press, shoulder press, squat, lateral pulldown, leg extension, bench press, calf raises
											8	?	3	3	90	arm curl, leg press, shoulder press, squat, lateral pulldown, leg extension, bench press, calf raises
Tartibian	2015	16	3 to 6		25-45				55-65	treadmill						

Figure 11: Table of Study Exercise Regimes (Plasma)

Interleukin 6

5 of the 11 groups studying IL-6 showed significant changes in levels following the exercise intervention from baseline with 4 groups [122] [121] [116] [126](3 endurance, 1 resistance) recording decreases and 1 group [111](resistance) increasing levels of circulating IL-6. There was heterogeneity of training between the studies, with durations from 8 – 24 weeks. Each of the 4 papers demonstrating significant decreases in circulating IL-6 studied populations from 49-70 years old. Yeo et al demonstrated a rise in IL-6 with 8 weeks of moderate intensity resistance training in a presumed younger age group (college students) [11.9 ± 1.25 to 13.98 ± 0.96 pg.ml-1]. This was not replicated in their high intensity training group.

Tumour Necrosis Factor Alpha

4 of the 9 studies into TNFα (3 endurance, 1 combined) showed significant changes. Two of the studied groups [118] [112] showed increased levels from baseline whereas the remaining groups [122] [129] showed decreased levels. The results recorded by Horne et al appear to suggest the presence of outliers within the group as the standard deviation within the female endurance group is considerably larger than in the male equivalent group. [TNFα pg.ml-1(baseline): Female 5.6±3.7, Male 5.7±4.4; TNFα pg.ml-1 (6weeks): Female: 37.8±24.7, Male: 6±4]

C Reactive Protein

5 [115] [126] [117] [120] [121]of the 13 studies which included CRP in showed a significant decrease in levels following training with one study [127] (resistance) showing an increase. Of those with decreased levels 4 groups participated in endurance training and 1 group in resistance exercise. The duration of exercise and populations of the groups with significant changes were heterogenous with multiple age groups and mixed sexes.

Interleukin 10

Two studies [113] [121] investigated IL-10 and neither group showed significant changes following exercise interventions.

3.3.3 Meta Analysis

Three papers reported only change from baseline [115] [131] [119]and two papers provided only graphical interpretations of their results [129] [134]. The authors were contacted for raw data, however this was not received and these studies were excluded from the meta-analysis.

Most papers reported results as mean ± standard error of the mean (SEM), this was converted into SD. The papers used a variety of units to report cytokine levels which when converted into the same units gave extremely disparate values. For example, levels of IL-6 were reported using either $pg.ml^{-1}$ [112] [116] [135] [121] or $pg.l^{-1}$ [113] [136] [126]. When comparing raw figures, Wagner et al recorded a baseline IL-6 value of 0.48 $pg.ml^{-1}$ in the control group, while in Strandberg et al a value of 1.85 $pg.l^{-1}$ (0.0019 $pg.ml^{-1}$) is given for baseline IL-6 in the control group.

Due to the heterogeneity of units used to report results, the most efficient way to perform a meta-analysis used standard mean difference of the final values from control and exercise groups. There are a number of limitations to this method which will be discussed later in the chapter.

3.3.3.1 Overall analysis

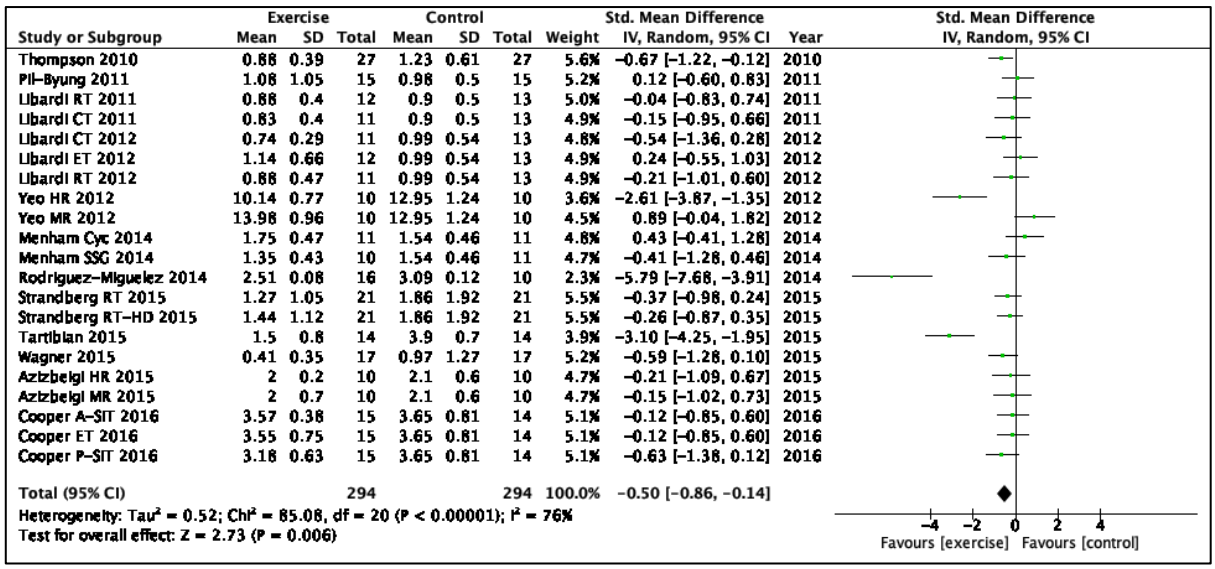


Figure 12: Forest Plot IL-6 (Plasma)

IL-6 was the only cytokine to show a significant change when combining the studies in a meta-analysis (P=0.01). There were three clear outlying studies [111] [126] [122] which all showed a significant decrease in IL-6 in the exercise groups when compared to the controls.

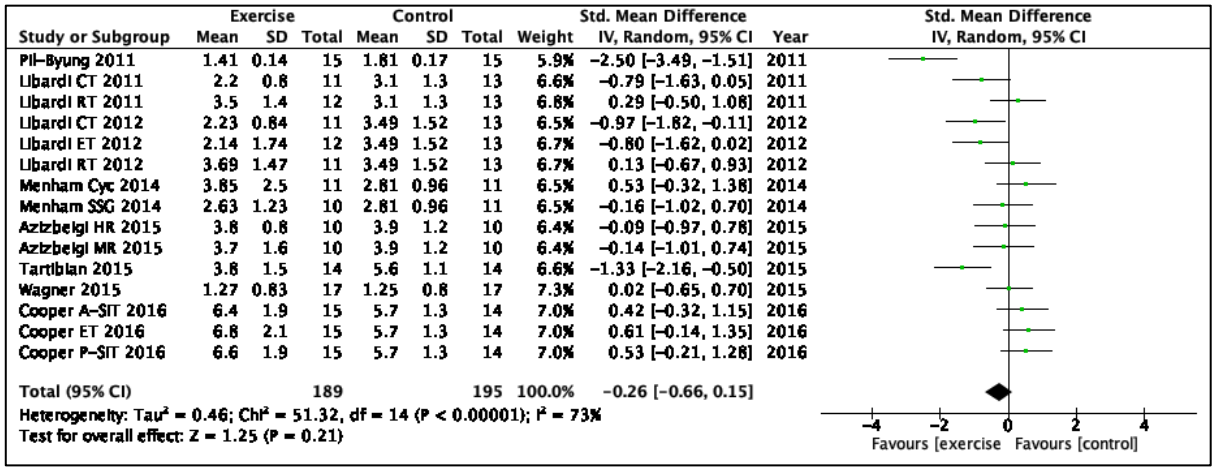


Figure 13: Forest Plot TNFα (Plasma)

A further study [118] was excluded from the meta-analysis of TNFα as there were no detectable levels in the control groups.

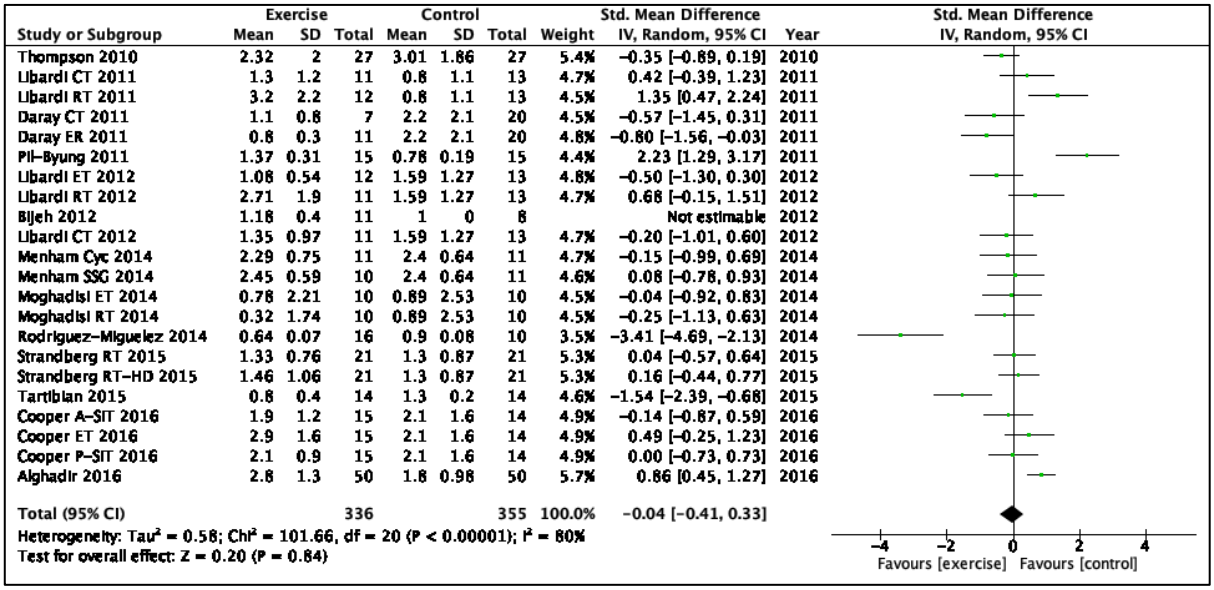


Figure 14: Forest Plot CRP (Plasma)

One further study [128] was excluded from the CRP meta-analysis as there were no baseline plasma values available from the control group. Neither TNFα (P=0.21) nor CRP (P=0.84) showed a significant change between control and exercise groups.

3.3.3.2 Age

Studies were divided into groups according to the age of the participants, 19-39, 40-59 and 60 + years for each of the inflammatory markers studied. Results for IL-6 did not reach overall significance in the 19-39 or >= 60 group, however, in the 40-59 group exercise did show an reduction of IL-6 levels which approached significance (p=0.05).

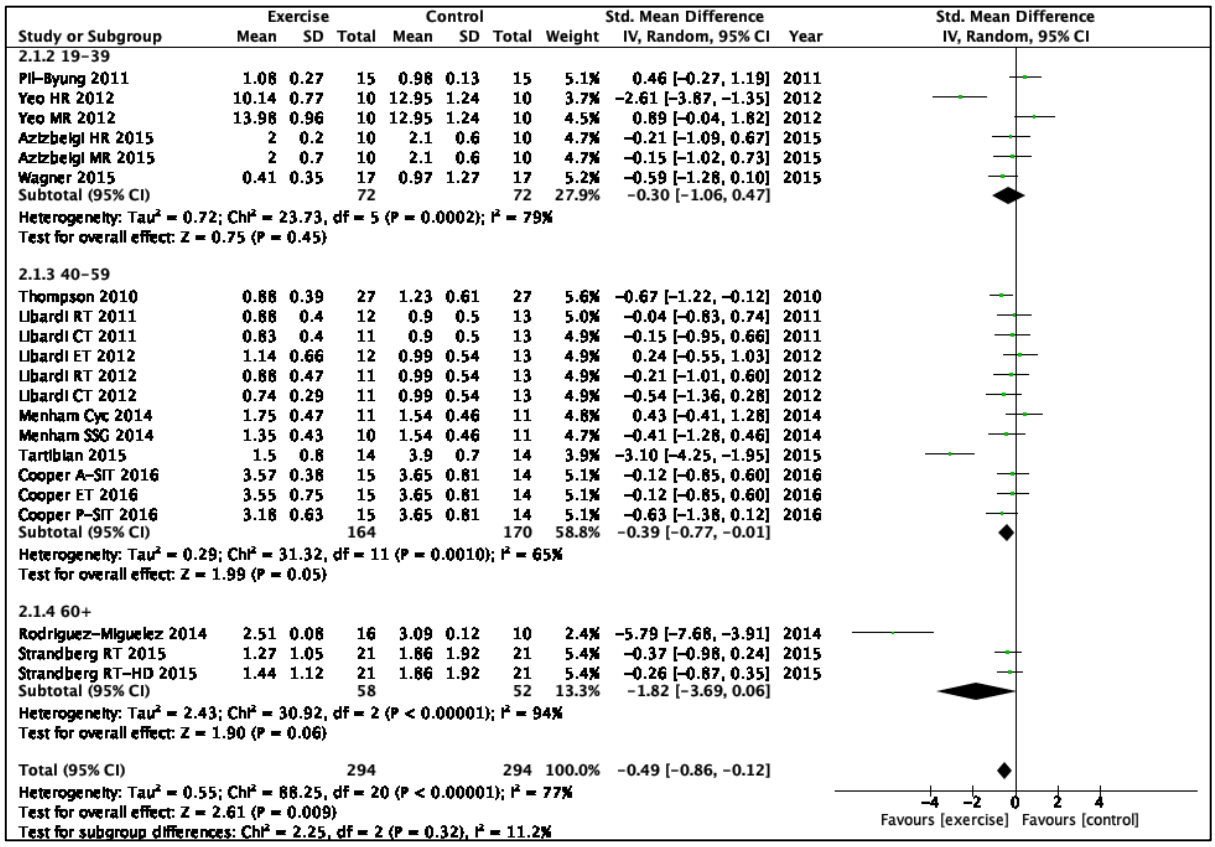


Figure 15: Forest Plot IL-6 by Age Groups (Plasma)

TNFα was not measured in sufficient studies with participants over 60 years and only the lower age groups are included. Neither group showed significant effects of exercise on TNFα levels.

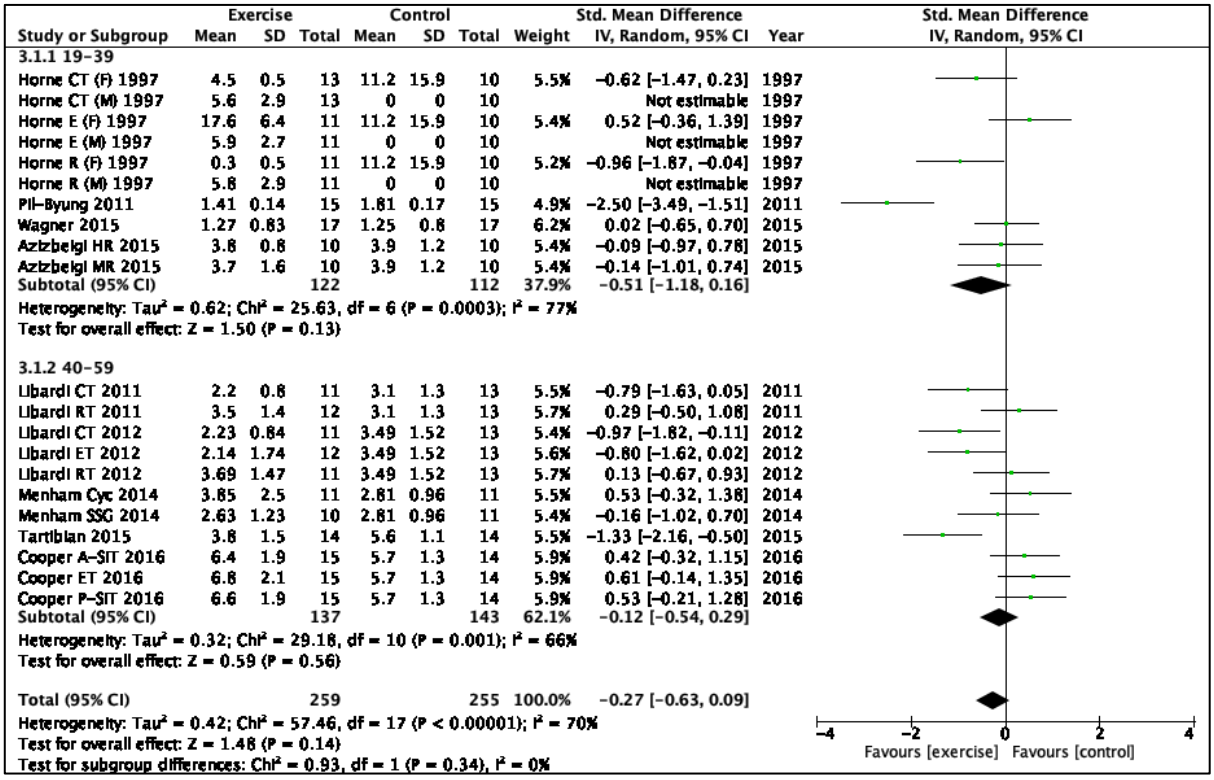


Figure 16: Forest Plot TNF⍺ byAge Groups (Plasma)

Similarly, exercise protocols did not show changes in levels in CRP in any age group.

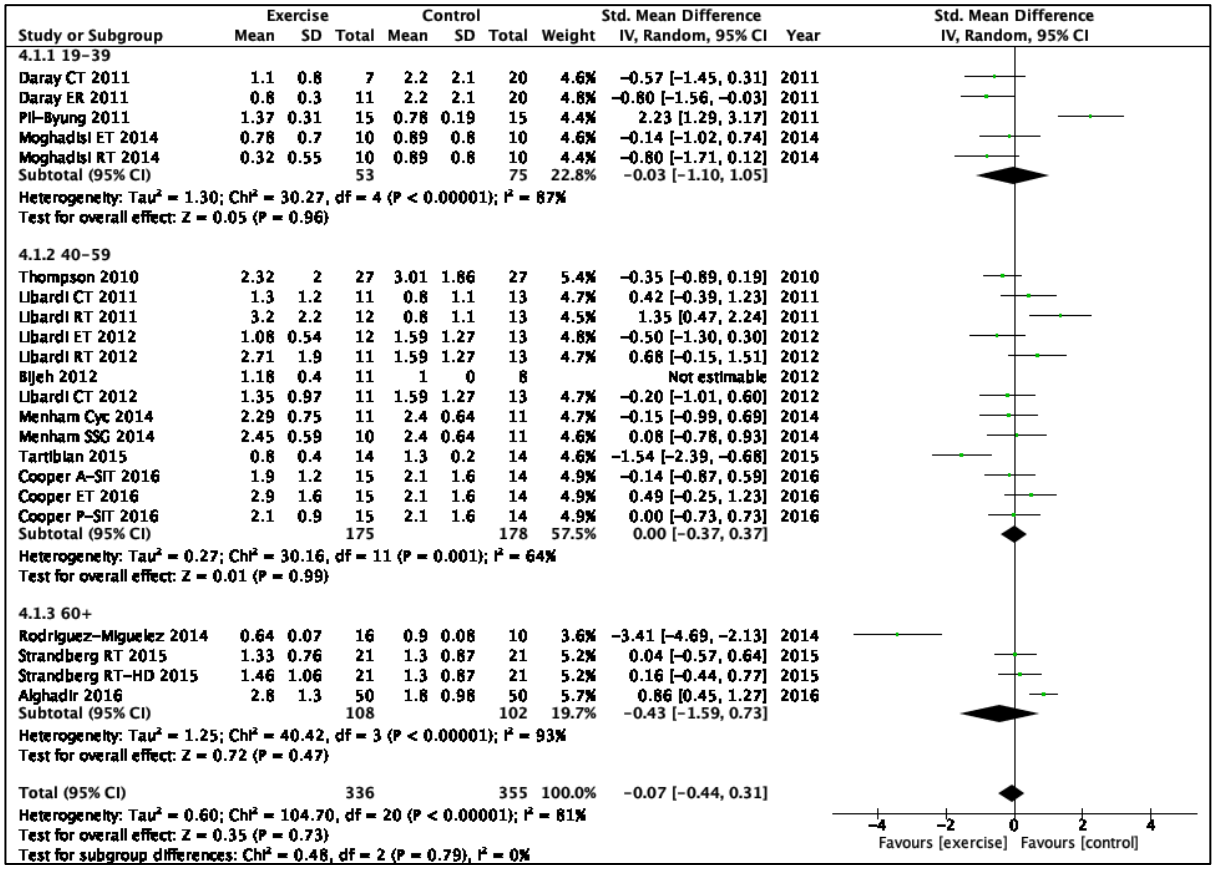


Figure 17: Forest Plot CRP by Age Groups (Plasma)

3.3.3.3 Training Modality

Given the known differences in physiological adaptations to endurance, resistance and combined training, each inflammatory marker was analysed in subgroups performing resistance, endurance and combined training. When comparing levels of IL-6 both resistance and endurance training groups showed an overall decrease which approached significance (p=0.05). The groups which underwent combined training did not show any significant differences (p=0.48).

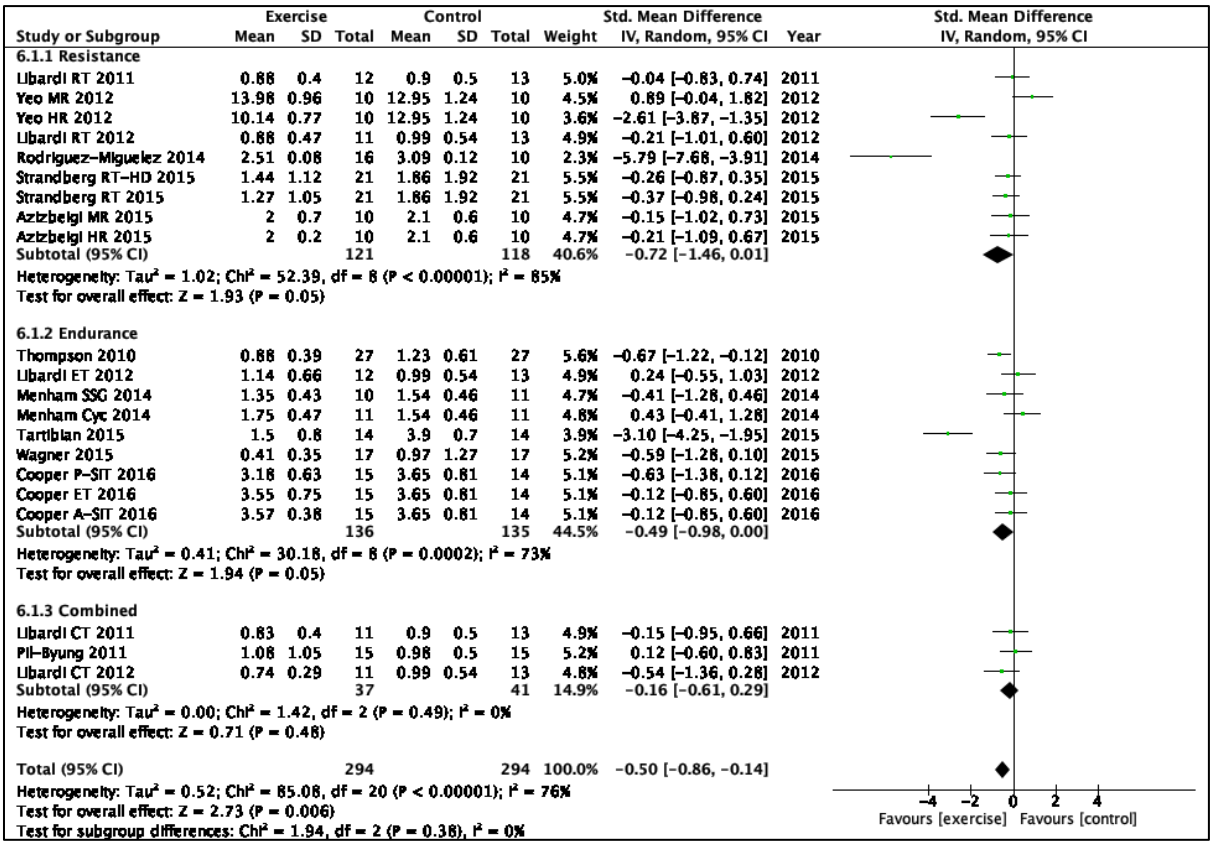


Figure 18: Forest Plot IL-6 by exercise modality (Plasma)

Only combined training programs showed significant effects on levels of TNFα, and although analysis contains results from 4 groups, their interpretation is difficult using a random effects model, as there is limited heterogeneity (I^2=0%) between results when compared to other subgroups.

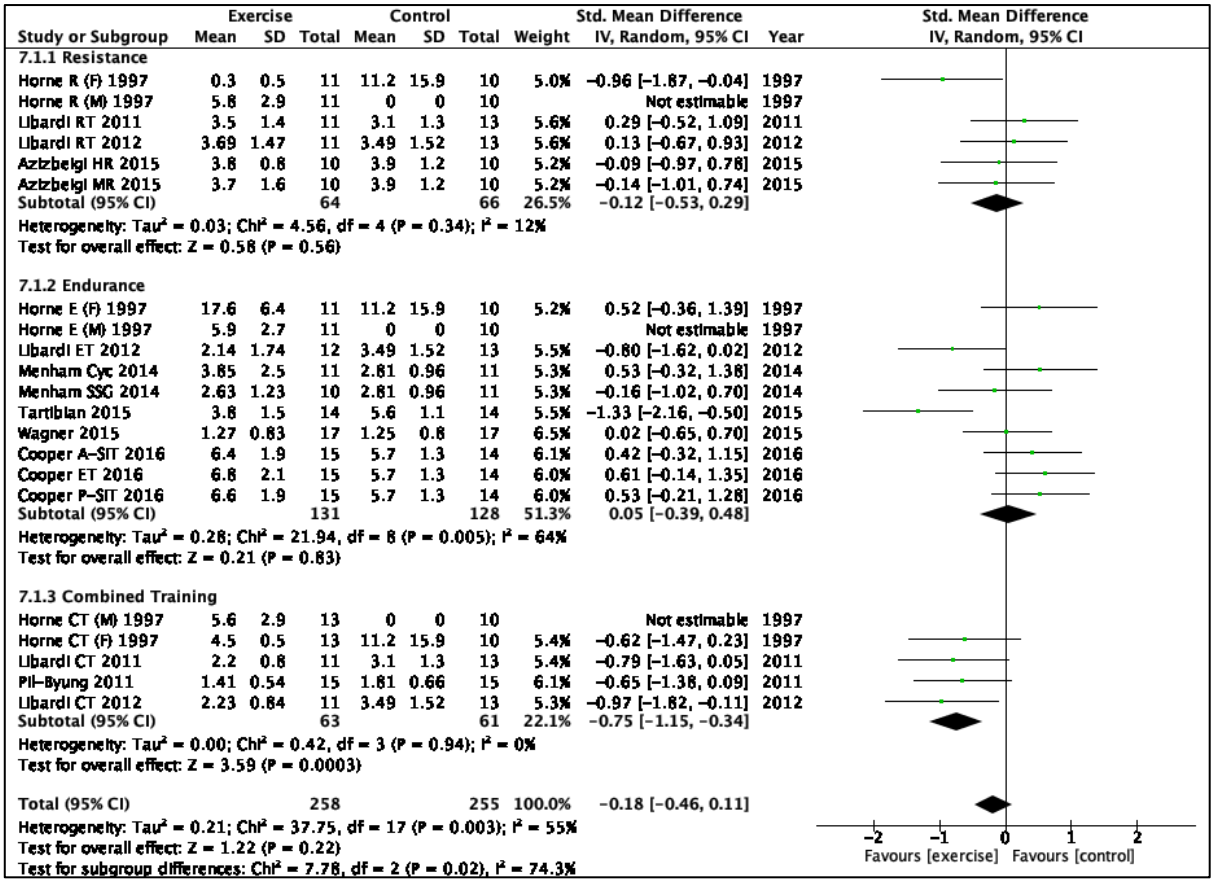


Figure 19: Forest Plot TNFα by exercise modality (Plasma)

Effects on CRP did not reach significance in any subgroup studied, or when combining the overall effect.

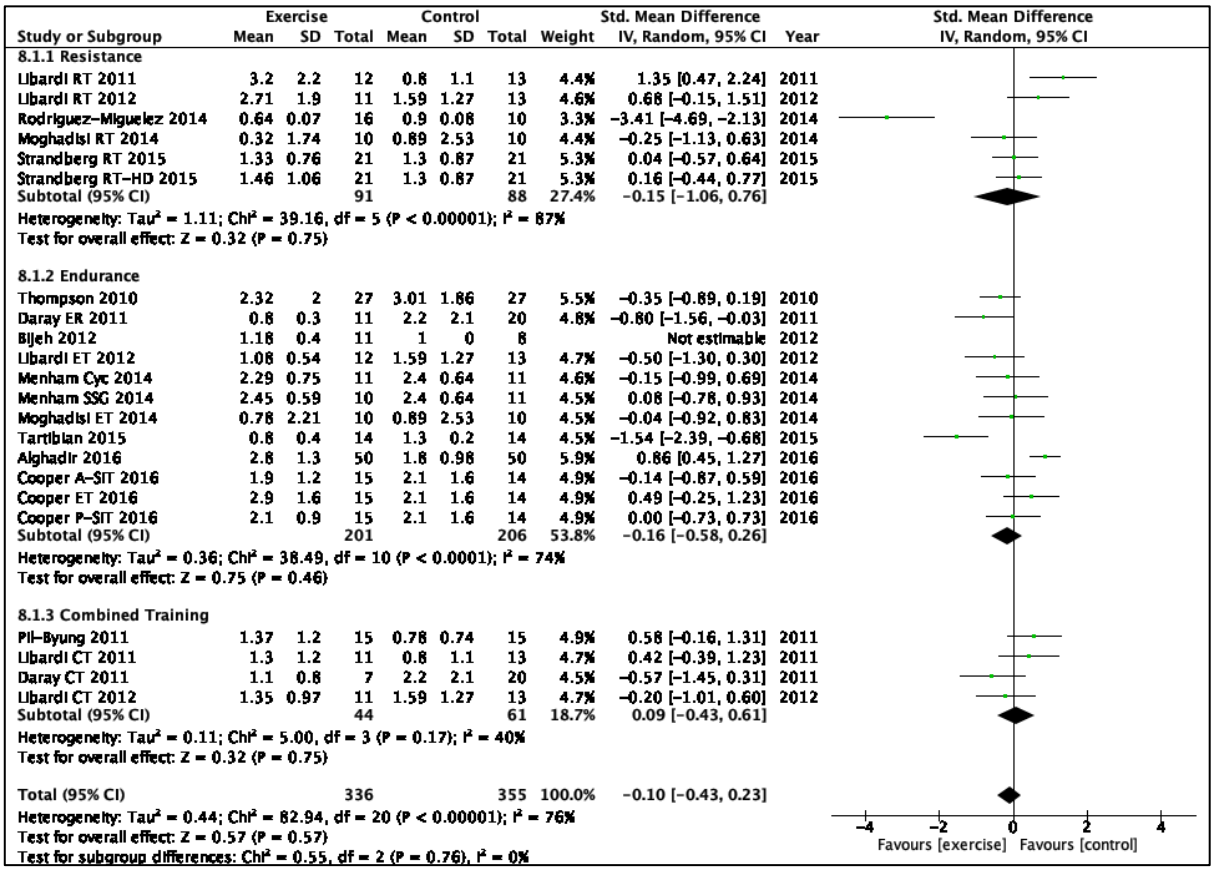


Figure 20: Forest Plot CRP by exercise modality (Plasma)

3.3.3.4 Sex

The final subset of results divided the groups into female, male or mixed sex participants. Again, meta-analysis showed no significant effects of exercise on any group. There were not sufficient groups to provide a meta-analysis for TNFα in mixed groups.

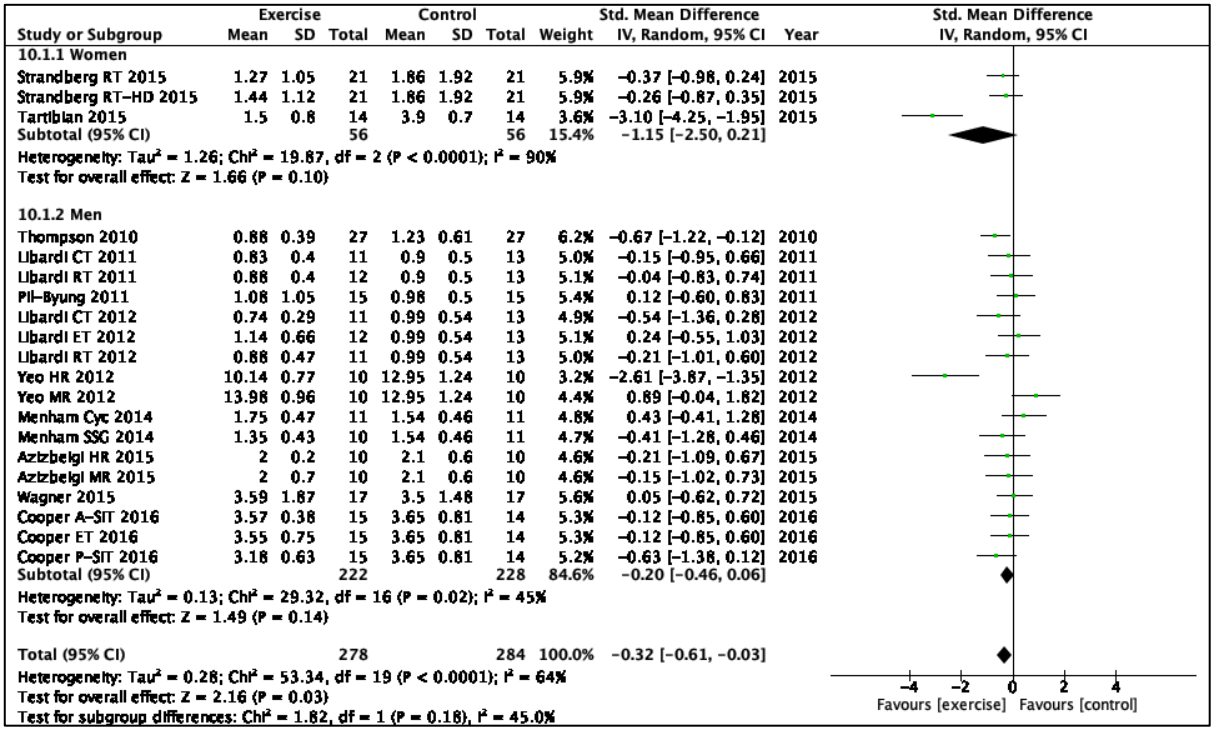


Figure 21: IL-6 by Sex (Plasma)

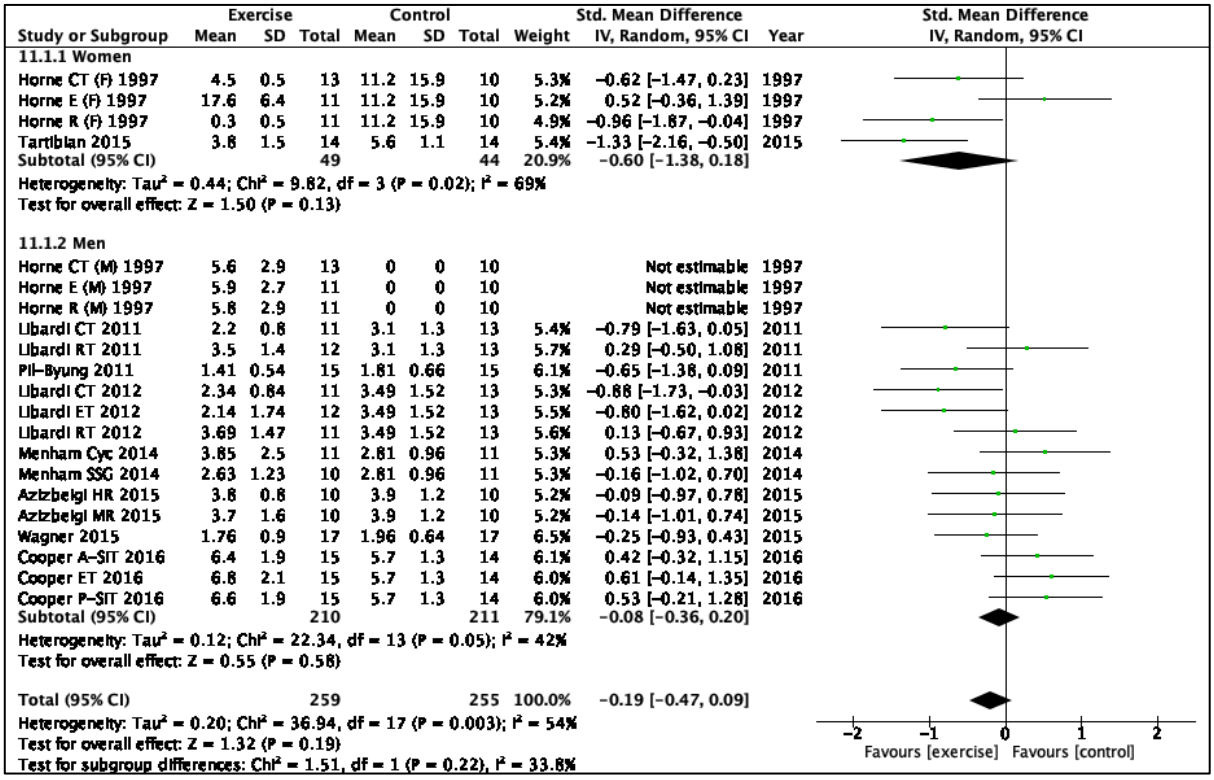


Figure 22: TNFα by Sex (Plasma)

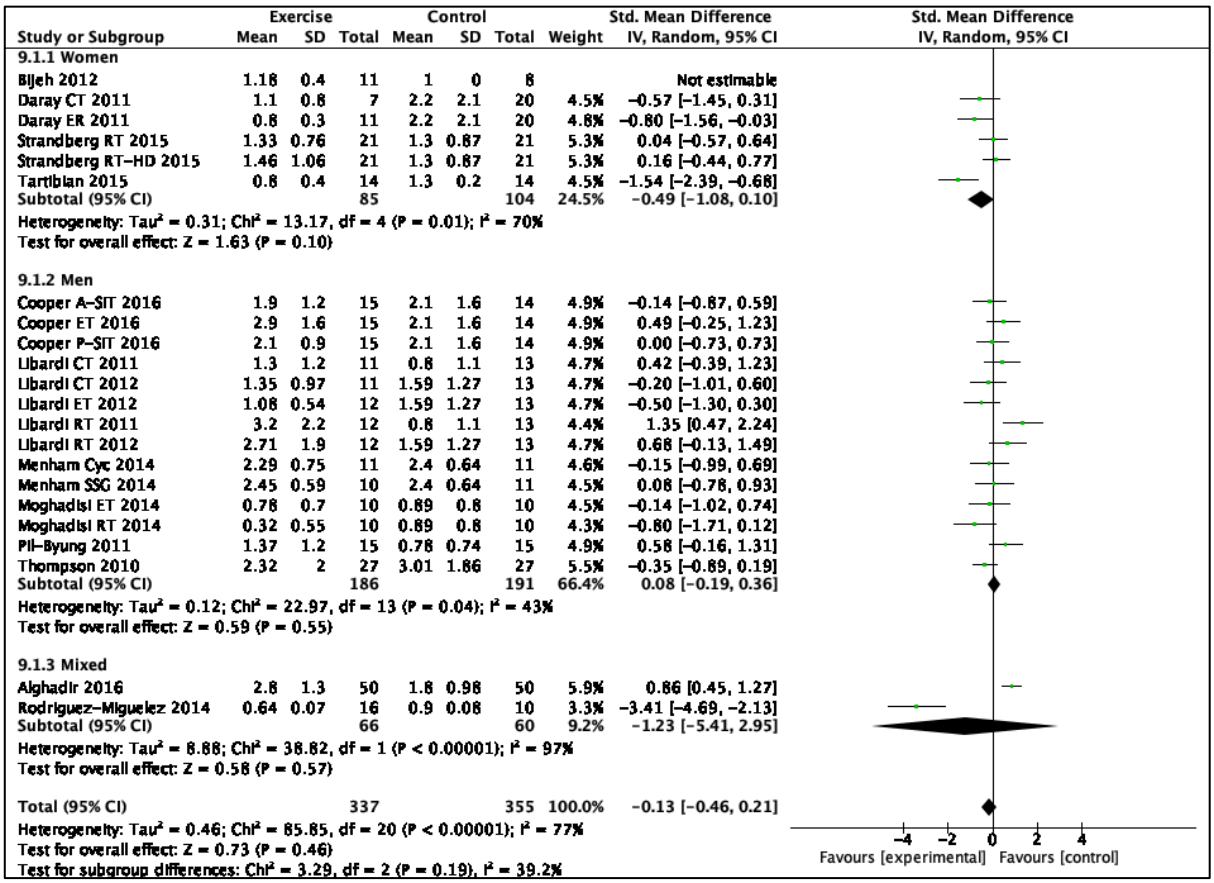


Figure 23: CRP by Sez (Plasma)

3.4 Discussion

3.4.1 Controls, baseline variations and sample sizes

A limitation of the CSF studies was the pairing of volunteers to avoid multiple lumbar punctures. A baseline sample from one volunteer was compared with a post exercise sample from another. This is problematic, as baseline variation in cytokine levels have been shown to vary dramatically between individuals and are dependent on a huge range of factors [137]. This variation in baseline values will also have had an effect on the results of the meta-analysis in the plasma studies. For example, Alghadir et al [120] reported a control group with a baseline mean CRP of 2.6mg.l^{-1} which had reduced to 1.8mg.l^{-1} when repeated after 24 weeks of no intervention. When comparing this with the exercise group with a baseline of 6.8mg.l^{-1} which decreased to 2.8mg.l^{-1} at the end of the study. The authors of the paper report this decrease in the intervention group as a significant result, however it is not seen in the meta-analysis where final control values are compared to final exercise group values. In order to mitigate against the heterogeneity of baseline cytokine levels amongst individuals, significantly larger sample sizes would be required. The studies included in the review had between 19 and 150 participants, with the mean number of participants being 44. The meta-analysis included a total of 1004 participants.

3.4.2 Measurement Techniques

The majority of studies (both CSF and plasma) used enzyme-linked immunosorbent assay (ELISA) to determine cytokine levels, however these were from various manufacturers which may also confound results and may explain the differences in measured values seen across the studies. Reporting of results using different units was commonplace and attempting to convert all results to standardised units gave values which were disparate. This hampered meta-analysis, where standard mean difference of the final values of control and exercise groups were used in order to produce meaningful results.

		Il-6	TNF_{α}	CRP	Il-10
Horne	1997		ELISA (Medgenix diagnostics, Belgium)		
Hewitt	2008	Chemiluminescent sequential immunometric assay (Euro/DPC, Gwynedd, UK)	Chemiluminescent sequential immunometric assay (Euro/DPC, Gwynedd, UK)	Chemiluminescent sequential immunometric assay (Euro/DPC, Gwynedd, UK)	
Thompson	2010	ELISA (Quantikine R&D Systems, Abingdon, UK)		ELISA (DSL, Oxford Bio-innovation, Oxon, UK)	
Libardi	2011	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	
Daray	2011			ELISA (Alpco C-Reactive Protein (hsCRP) Salem, USA)	
Pil-Byung	2011	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	Latex agglutination photometric immunoassay	
Bijeh	2012			Immunoturbidometric Method (Pars Azmoon Co., Tehran, Iran)	
Libardi	2012	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)	
Yeo	2012	Bio-plex pro magnetic cytokine multiplex assay			
Rodriguez-Miguelez	2014	ELISA (Quantikine ELISA Kit R&D Systems, Minneapolis, MN)		ELISA (Quantikine ELISA Kit R&D Systems, Minneapolis, MN)	
Moghadasi	2014			ELISA kits (Diagnostics Biochem Inc., Canada).	
Mendham	2014	ELISA (Quantikine ELISA Kit R&D Systems, Minneapolis, MN)	ELISA (Quantikine ELISA Kit R&D Systems, Minneapolis, MN)	Turbimetric Assay (Dimension Xpand Plus, Siemens)	ELISA (Quantikine ELISA Kit R&D Systems, Minneapolis, MN)
Jahromi	2015		ELISA (Bender Med System, Austria)		
Azizbeigi	2015	ELISA (Bender Med System)	ELISA (Bender Med System)		
Strandberg	2015	ELISA (Quantikine ELISA Kit R&D Systems, Minneapolis, MN)		hsCRP Kit immunoturbidimetric assay (Advia 1800, Chemistry System, Siemens, Germany)	
Tartibian	2015	ELISA Biosource (Nivelles, Belgium)	ELISA Biosource (Nivelles, Belgium)	ELISA Biosource (Nivelles, Belgium)	
Wagner	2015	ELISA (Access Immunoassay Systems, Beckman-Coulter, Germany)	ELISA (Quantikine High Sensitivity Kit, R&D Systems, Minneapolis, MN)		
Cooper	2016	ELISA (Jomar Life Research, Melbourne)	ELISA (Jomar Life Research, Melbourne)	High throughput automated analyser (Dimenson ELISA kits (IBL Inc., USA)	ELISA (Jomar Life Research, Melbourne)
Alghadir	2016				
Ihalainen	2018	Immulite 1000 (Immulite, Siemens, Il)		Immulite 1000 (Immulite, Siemens, Il)	
Coll-Risco	2018			Autoanalyzer (Hitachi-Roche p800; F. Hoffmann-La Roche Ltd,Q3 Basel, Switzerland).	
Krčmárová	2018			Photometric analyzer (Biolis 24i Premium, Tokyo, Japan)	
Mohammadi	2018			Pars Azmoon Kit (Pars Azmoon Co., Tehran, Iran)	

Figure 24: Table of Cytokine Measurement Techniques (Plasma)

This choice of statistical method raises a number of potential problems when interpreting the results. Firstly, there were often variations in the baseline cytokine values of control and exercise groups and this heterogeneity creates difficulty in appreciating changes within groups in the meta-analysis. This is seen in the studies reporting significant effects of exercise intervention as the authors use a comparison of exercise group baseline to final value rather than final values of control and exercise groups.

3.4.3 Timing of Sampling

The timing of blood sampling varied between studies:

		Timing	Fasted	Diet Control
Horne	1997	48-72hrs after training between 4-6pm		
Hewitt	2008	Morning		
Thompson	2010	36hrs after strenuous exercise		
Libardi	2011	7am - 9 am	Fasted	
Daray	2011	24 hours after exercise 5am-9am	Fasted	Same diet for 24 hours
Pil-Byung	2011			
Bijeh	2012	15 minutes at rest		
Libardi	2012	7am - 9 am	Fasted	
Yeo	2012	No details	Fasted	
Rodriguez-Miguelez	2014	5-6 days after exercise between 8am-9am	Fasted	
Moghadasi	2014	at the same time	Fasted	
Mendham	2014	72 hours after exercise between 6am-9am	Fasted	Same diet for 24 hours
Jahromi	2015	24 and 48 hours after exercise		
Azizbeigi	2015	72 hours after exercise between 10am-12pm	Fasted	
Strandberg	2015	24 hours after exercise 7am-9am	Fasted	
Tartibian	2015	24 hours after exercise 7am-8am	Fasted	
Wagner	2015	Immediately after exercise		
Cooper	2016	No details		
Alghadir	2016	Morning	Fasted	
Ihalainen	2018	48 hours after exercise between 7am-9am	Fasted	
Coll-Risco	2018	No details	Fasted	
Krčmárová	2018	Morning	Fasted	
Mohammadi	2018	48 hours after exercise	Fasted	

Figure 25: Table of Blood Sampling Timings (Plasma)

While the majority of studies sampled during the morning, this is not true for all participants. There is evidence to suggest that a number of the targets measured follow a diurnal rhythm and thus levels may be affected by time of sampling [138]. Previous work has also shown that the levels of inflammatory biomarkers change dramatically around exercise, with IL-6 [88] and TNF α [139]showing significant increases following strenuous effort. This varies dependent on the form of IL-6 released as muscle-derived IL-6 shows a peak followed by rapid decline to baseline after cessation of exercise, although pre-exercise levels may not be attained until up to 48 hours after [140]. In contrast, immune-mediated IL-6 (such as may be seen if exercise induces muscle damage) shows a lower but more sustained peak following exercise [141]. As the majority of the subjects

studied were untrained, it would be likely that each sustained a degree of muscle damage during the exercise interventions.

3.4.4 Diet Control

While carbohydrate ingestion before and during exercise has also been shown to attenuate the acute rise in muscle derived IL-6 [142], the majority of the tests were performed on subjects who had been fasting for at least 12 hours. Few studies controlled the diets of their participants, despite nutrition playing an important role in levels of inflammation. Diets which have been linked to lower levels of circulating inflammatory markers include the ingestion of polyunsaturated fatty acids which has been shown to decrease levels of circulating CRP [143]. Conversely, diets high in sugar, saturated and trans-fats may increase activation of the innate immune system and lead to higher levels of inflammatory biomarkers in plasma [144].

3.4.5 Age

Again, there is little agreement between the studies included in the plasma review. Previous work had suggested that older participants may show the most consistent results [99] [96], however studies which included with participants aged over 60 showed little correlation within meta-analysis. While the review attempts to include only participants without comorbidities, some studies were unclear as to whether their older participants had controlled pathologies (e.g. hypertension), stating that they were 'healthy' but not including the specifics of the screening process. In view of previous work suggesting that the greatest change in inflammatory markers post exercise is found in participants with underlying disease [97], this may have affected the results. Interestingly, the study with the oldest participants (70±0.9 years) showed the greatest number of significantly altered biomarkers, with decreases in measured IL-6 and CRP and increased IL-10 [126].

3.4.6 BMI

The review selected studies which included participants with BMI <35. Included studies recorded BMI ranging from 21.2±2.4 to 28.0±2.7. Increased BMI has been linked to increased levels of systemic inflammation [145] and previous work has suggested that reductions in BMI or body fat may produce larger changes in CRP [146]. Within the studies investigated there does not appear to be any correlation between significant changes in biomarkers and BMI with only 2 [126] [116] of the 4 studies [116] [126] [147] [113] with participants of the highest BMI showing significant results. This is perhaps not surprising as the studies included healthy participants with normal BMI. Previous work has shown the largest effects of exercise on CRP in participants with increased BMI (where decreasing body weight and fat percentage correlate with a decrease in CRP) and underlying conditions such as T2DM and cardiovascular disease [146].

3.4.7 Sex

There is considerable variety in the studied populations, with only 10 of the 24 studies including female participants. Of those, 6 were entirely female studies, with only 4 groups of mixed participants. Meta-analysis of these groups showed no significant differences in CRP between exercise and control groups in any sex. There are a number of important differences to consider when comparing results from male and female participants. Pre-menopausal women have been found to have higher levels of CRP and lower levels of IL-6 and TNFα at baseline than their male counterparts due to the action of oestrogen [148]. The effect on CRP is especially pronounced during the follicular phase [149] and may be of significance when considering the results of the two studies which included women of pre-menopausal age [117] [118]. In this analysis, the majority of women were around or above the age of menopause (8/10 studies). A number of previous studies have shown significant decreases in inflammatory markers following exercise

interventions in post-menopausal women, however this has been closely linked to a reduction in adiposity, particularly central adiposity [150]. The participants in these trials all had a BMI <35 which may have limited their ability to reduce adipose tissue as previously discussed.

3.4.8 Training Modality

There appears to be little correlation between endurance training, resistance training or a combined approach and the level of change of inflammatory biomarkers. The duration of training also did not appear to correlate to significant results, with some studies of shorter duration (6-8 weeks) showing multiple significant results with others of the same duration not describing significant effects. Although the review attempted to control for a variation of training intensities in the selection of articles, there remains heterogeneity between the exercise interventions. The measurement of the intensity of the exercise varied between studies (% of VO2 max, % max HR) or was not described at all. Similarly, there was variety in the resistance exercises performed as well as the intensity (50-90% of 1RM). The types of exercise performed in the studies which showed the greatest number of significant results have overlap with other studies which did not show similar results. This heterogeneity of both exercise interventions and results has been commented on in other work [139] and represents a significant barrier to interpretation of meta-analysis.

3.4.9 Outlying Results

There were a number of studies which showed significant results within the meta-analysis. In particular Tartibian et al [122] showed significant decreases across all of the biomarkers measured (IL-6, TNFα and CRP). The study involved healthy women of post-menopausal age doing 16 weeks of endurance exercise at 55-65% of their maximum heart rate. At the peak of their training they were exercising for 45 minutes, 6 times per week. This programme was one of the more strenuous

endurance regimes of all the studies, and resulted in a 10% decrease in body weight, 11% reduction in BMI and 9% loss of body fat in the exercise group. This may account for the large differences seen in this study when compared to others in the review. Another group which showed significant decreases in IL-6 and CRP was Rodriguez-Miguelez et al [126]. Their measurement methods differed from the other studies in the analysis as they sampled participants' blood 5-6 days after the last bout of exercise. The participants in the study were also some of the oldest (70±9 years) which may explain the magnitude of their results as previous work has shown that more significant changes in inflammatory markers are often seen in older subjects [99] [96]. There were three groups who recorded an increase in CRP in the exercise group when compared to the control [133] [120] [127]. There were no clear similarities between the studies in terms of participant demographics, exercise modality or duration. However, when exploring these studies, none include details of the timing of their samples after exercise which may provide an explanation for their results. If the samples were taken immediately or shortly after exercise, a rise in inflammatory markers (including CRP) may be seen [151].

3.5 Conclusion

The review compiled studies in healthy participants over a range of ages who performed both endurance, resistance and combined training programmes of varying duration. None of the studies included showed any significant changes in the chosen inflammatory markers in CSF, however one study has suggested an alternative target in adiponectin, which may have a role in neuroinflammation [108]. The studies investigating changes in biomarkers in plasma have also shown inconclusive results, with only IL-6 demonstrating a significant decrease overall. This inconsistency has been well documented in previous work [96] with heterogeneity of study protocols and participant selection being a key driver. It is also possible that significant changes in systemic inflammation are difficult to measure in participants who already have low levels of

inflammation at baseline. Performing a meta-analysis proved difficult due to the reporting of results in a variety of units, again, standardisation may be useful in order to compare results in the future. In order to draw firm conclusions more investigation is required and may take the form of measuring alternative markers of inflammation to those suggested in this review.

4. Study 2: Investigation of the effect of exercise on inflammatory markers in cerebrospinal fluid

4.1 Ethical Approval

Ethical approval was granted by the University of Nottingham Medical School Research Ethics Committee (135-1809) for recruitment of healthy male participants aged 18-45 years, which was later amended to 18-80 years.

4.2 Participant Recruitment

Posters were created to advertise the study and displayed on noticeboards within the medical school on the Derby and Nottingham campuses as well as within public areas of the Royal Derby Hospital. Students in the medical, nursing and MPT cohorts on the Derby campus were approached via a short presentation of the study at the beginning of lectures.

The initial response was positive with 8 students registering their interest in participation. However, upon reading the participant information sheet, none of the students wished to enrol in the full study. The reasons given centred around the lumbar puncture procedure:

"I don't think I would be comfortable with the actual lumbar puncture procedure"

"I've had a lumbar puncture before…and it was awful"

Further advertising was targeted at members of clubs and sports teams within the University of Nottingham but unfortunately this received no responses.

Upon discussion of the initial problems with recruitment an amendment was submitted and approved by the Research Ethics Committee to increase the age range of participants to 18-80 years with the hypothesis that older participants may be less concerned with the lumbar puncture procedure.

Posters were re-displayed with the updated information and volunteers at the Royal Derby Hospital were approached by the volunteer co-ordinator and again, initial interest was positive with four responses. Of these, two wished to continue with screening after reading the participant information sheet. All subjects gave their written informed consent to participate in the study.

Screening was performed for all participants including a medical questionnaire, physical examination including baseline observations, blood sampling (FBC, U&E, LFT and clotting screen) and resting electrocardiogram. Exclusion criteria are listed in Appendix 8.3.1 CSF: Short Study Protocol and included metabolic, cardiac or respiratory disorders, uncontrolled hypertension, clotting abnormalities or signs of raised intracranial pressure.

Post screening, only one participant was suitable to continue into the study. As no further subjects had been recruited the next study day was postponed until further recruitment had been confirmed which was not possible prior to the submission of this work.

5. Study 3: Inflammatory Biomarkers in Plasma following High Intensity Interval Training in Healthy Individuals and those with Cancer.

Plasma samples were obtained from studies previously performed by the research group. Four studies were identified with suitable protocols:

1. Improvements in aerobic performance following a four-week period of high intensity interval training in the elderly (EHIT) [152].
    - Male and female participants
    - Aged > 70 years

2. A randomised control trial to assess efficacy of preoperative high intensity interval training in older patients scheduled for oncological abdominal surgery (UroHIT) [153].
    - Male and female participants
    - Aged > 65 years

3. Effectiveness of short term, high intensity, interval exercise training in colorectal cancer patients in improving pre-treatment fitness (HITCa) [154] [155].
    - Male and female participants
    - Aged 18-98 years

4. A randomised control trial to assess the efficacy of short-term, home-based high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness (HHIT) [156].
    - Male and female participants
    - Aged > 55 years

	Weeks	Times/Week	Mins/Week	Time (mins)	Watt Max (%)	Notes
EHIT	4	3	49.5	16.5	95-115	Cycling
UroHIT	4	2	36	18	95-115	Cycling
HITCa	4	3	49.5	16.5	95-115	Cycling
HHIT	4	4	60	15	_	Star jumps, mountain climbers, sprint

Figure 26: Table of Exercise Regimes for HIT Studies

5.1 Ethical Approval

Ethical approval for analysis of samples had been previously obtained for each of the studies from the University of Nottingham Medical School Research Ethics Committee.

5.2 Methods

From each study, the first ten participants with sufficient pre- and post- exercise samples were identified and samples retrieved from storage at -80^{o}C. Plasma samples were collected using EDTA as an anticoagulant and centrifuged before storage. All samples and reagents were brought to room temperature before use.

5.3 Sample Analysis

5.3.1 ELISA

Enzyme-linked immunosorbent assays (ELISA) were used to measure IL-6, IL-10, TNFα and CRP in the pre- and post- exercise protocol studies.

IL-6

The Quantikine® (R&D Systems, Minneapolis, USA) Human IL-6 Immunoassay was used. The minimum detectable dose (MDD) of human IL-6 with this assay was <0.70 pg.ml^{-1}.

IL-10

The Quantikine® (R&D Systems, Minneapolis, USA) Human IL-10 Immunoassay was used. The MDD of human IL-10 with this assay was <3.9 $pg.ml^{-1}$.

TNFα

The Quantikine® (R&D Systems, Minneapolis, USA) Human TNFα Immunoassay was used. The MDD of human TNFα ranged from 2.09-6.23 $pg.ml^{-1}$ with a mean MDD of 4.00 $pg.ml^{-1}$.

CRP

The Quantikine® (R&D Systems, Minneapolis, USA) Human C-Reactive Protein Immunoassay was used. The MDD of human C-reactive protein ranged from 0.005-0.022 $ng.ml^{-1}$ with a mean MDD of 0.010 $ng.ml^{-1}$.

5.3.2 Calculation of Results

Results were calculated using GraphPad Prism version 8.2.1 for macOS (GraphPad Software, San Diego, California USA). A standard curve for each assay was created by generating a four-parameter logistic (4-PL) curve fit. The concentration of IL-6, IL-10, TNFα and CRP in each sample was determined from interpolation of this curve. Where levels were undetectable a concentration of zero was used.

5.4.1 Statistical Analysis

Results were analysed using GraphPad Prism version 8.2.1 for macOS (GraphPad Software, San Diego, California USA) and IBM® SPSS® Statistics Version 24.0 for macOS (IBM Corp, Armonk, NY USA). Prior to group comparison, variables were tested and found to be non-normally distributed with the exception of CRP in the UROHIT and EHIT groups. Statistical analyses of pre and post

intervention cytokines and CRP were performed using paired T-tests for normally distributed data and Wilcoxon signed rank for non-normally distributed data. In order to compare baseline values of cancer and non-cancer groups, Mann-Whitney U tests were used for non-normally distributed data. A P value of <0.05 was considered significant. Results are reported as mean ± SD unless otherwise stated.

5.4 Results

5.4.2 Results Table

		IL6 (pg.ml-1)	IL10 (pg.ml-1)	TNF (pg.ml-1)	CRP (ng.ml-1)
EHIT	Pre	6.90±7.64	5.36±11.66	7.53±12.58	21.23±18.93
	Post	1.85±1.43*	2.01±2.11	37.59±110.72	17.17±14.68
UroHIT	Pre	2.68±2.45	2.82±2.26	125.07±214.81	9.13±8.17
	Post	5.29±7.20	3.30±3.31	25.61±48.08	7.34±7.84
HITCa	Pre	2.72±2.82	0.72±1.36	2.00±6.33	36.28±19.19
	Post	3.06±2.14	1.02±1.30	2.80±8.23	35.83±21.48
HHIT	Pre	3.06±2.92	9.34±15.62	0.20±0.60	17.91±15.71
	Post	8.74±8.82**	3.11±3.15	4.00±11.70	15.92±15.30

*significant change within group p = 0.02
**significant change within group p = 0.04

Figure 27: Table of Results for Effect of HIT on Inflammatory Markers

5.4.3 IL-6

The pre- and post- training concentrations of IL-6 were compared in each study. IL-6 was detectable in 73/78 samples.

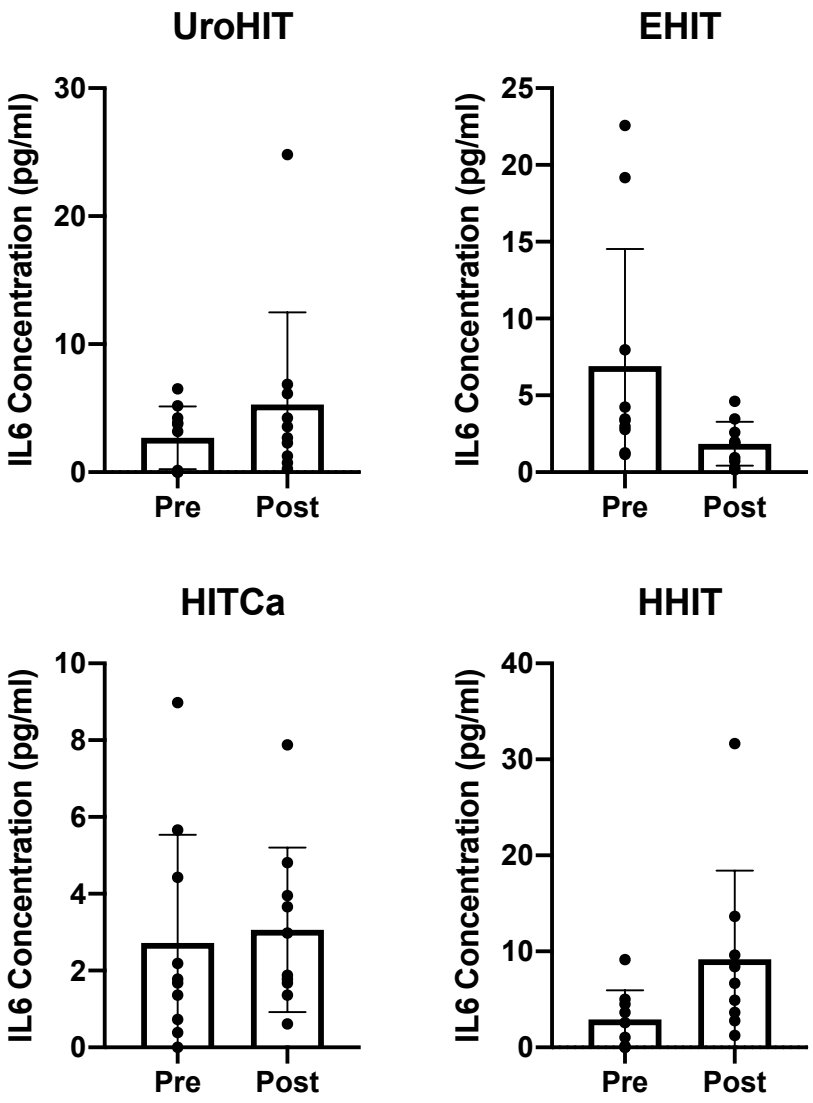


Figure 28: IL-6 Results

There were no significant differences found within either of the cancer groups when comparing pre- training IL-6 concentration with post- training concentrations (UroHIT p=0.49, HITCa p=0.56). Both non-cancer groups showed significant changes in IL-6 concentrations with EHIT decreasing from 6.90±7.64 to 1.85±1.43 (p=0.02) and HHIT increasing from 3.06±2.92 to 8.74± 8.82 (p=0.04).

Baseline IL-6 was compared between cancer and non-cancer groups and appeared to be increased in non-cancer participants, however this did not reach statistical significance (p=0.06). There

appeared to be larger variations in baseline levels within the non-cancer group with a standard deviation of 8.28 compared with 2.50 in the cancer group.

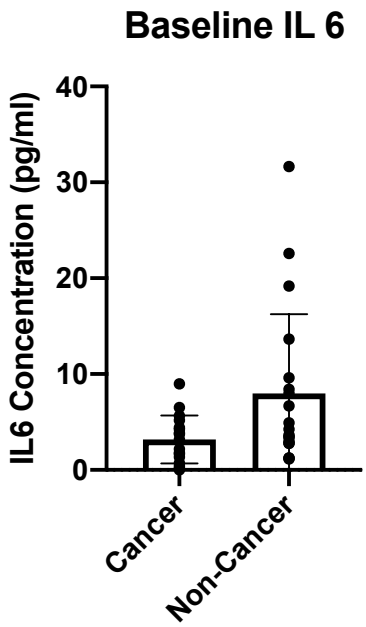


Figure 29: Baseline IL-6 Results

Changes in IL-6 Concentration were compared between cancer and non-cancer groups, with no significant differences were found (P=0.61 and P=0.56).

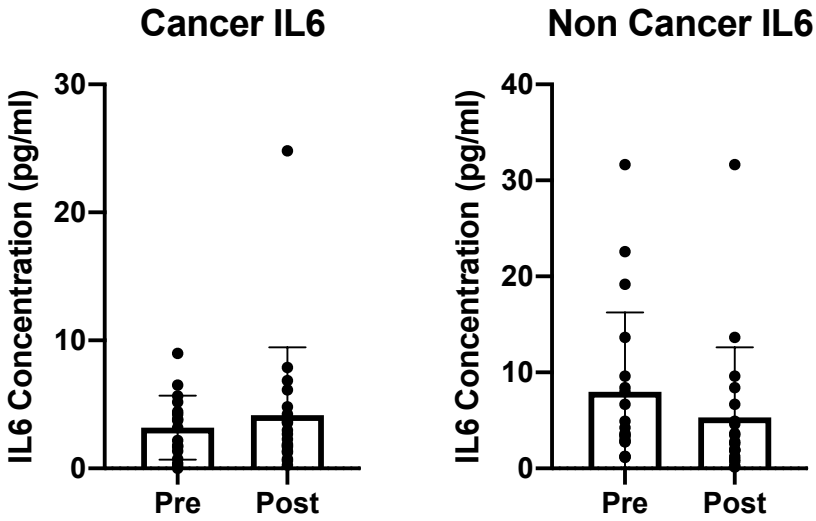


Figure 30: Cancer and Non-Cancer IL-6 Results

5.4.4 IL-10

The pre- and post- training concentrations of IL-10 were compared in each study. IL-10 levels were detectable in 60/78 samples.

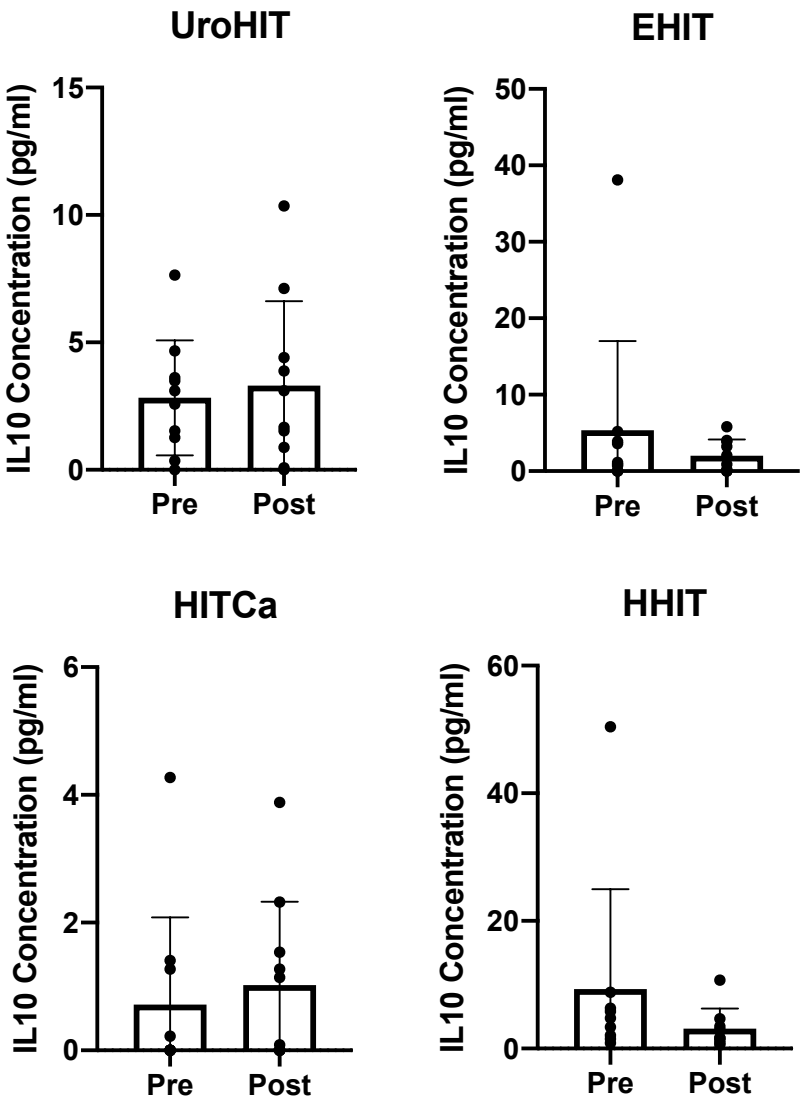


Figure 31: IL-10 Results

There were no significant changes in IL-10 concentration found within any of the groups studied.

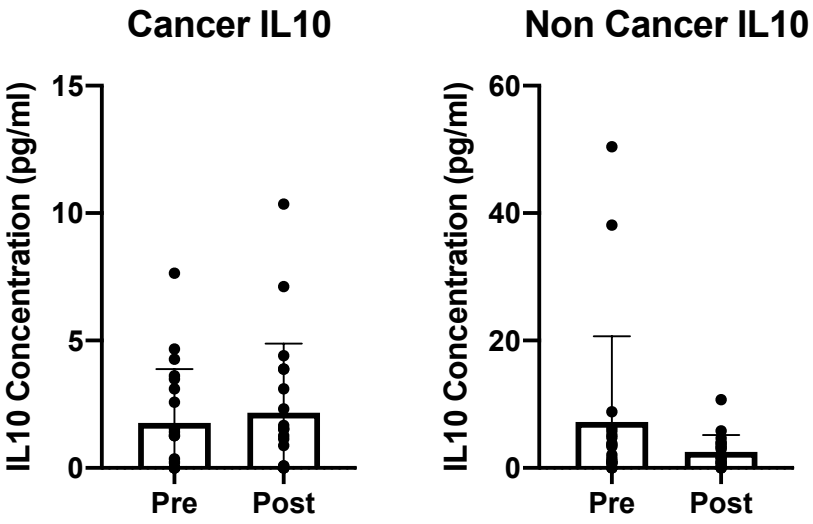


Figure 32: Cancer and Non-Cancer IL-10 Results

When comparing the effect of exercise on IL-10 levels in participants with cancer to those without, no significant changes were seen in the cancer group, but a significant decrease (p=0.02) was seen in the non-cancer group.

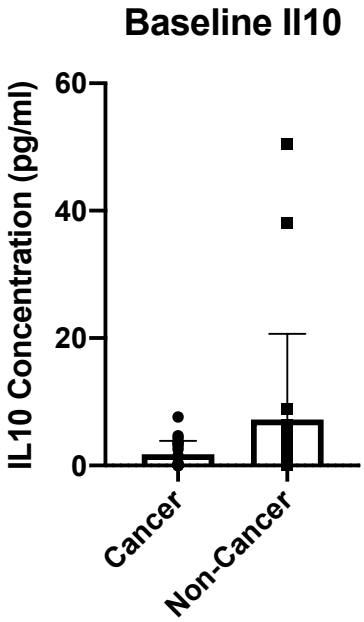


Figure 33: Baseline IL-10 Results

There appeared to be a difference between baseline IL-10 results, with participants with cancers recording lower baseline IL-10 values than non-cancer participants, however this only approached significance (P=0.06).

5.4.5 TNFα

The pre- and post- training concentrations of TNFα were compared in each study. TNFα had the fewest samples (24/78) with detectable levels. No changes in TNFα level reached statistical significance in any group.

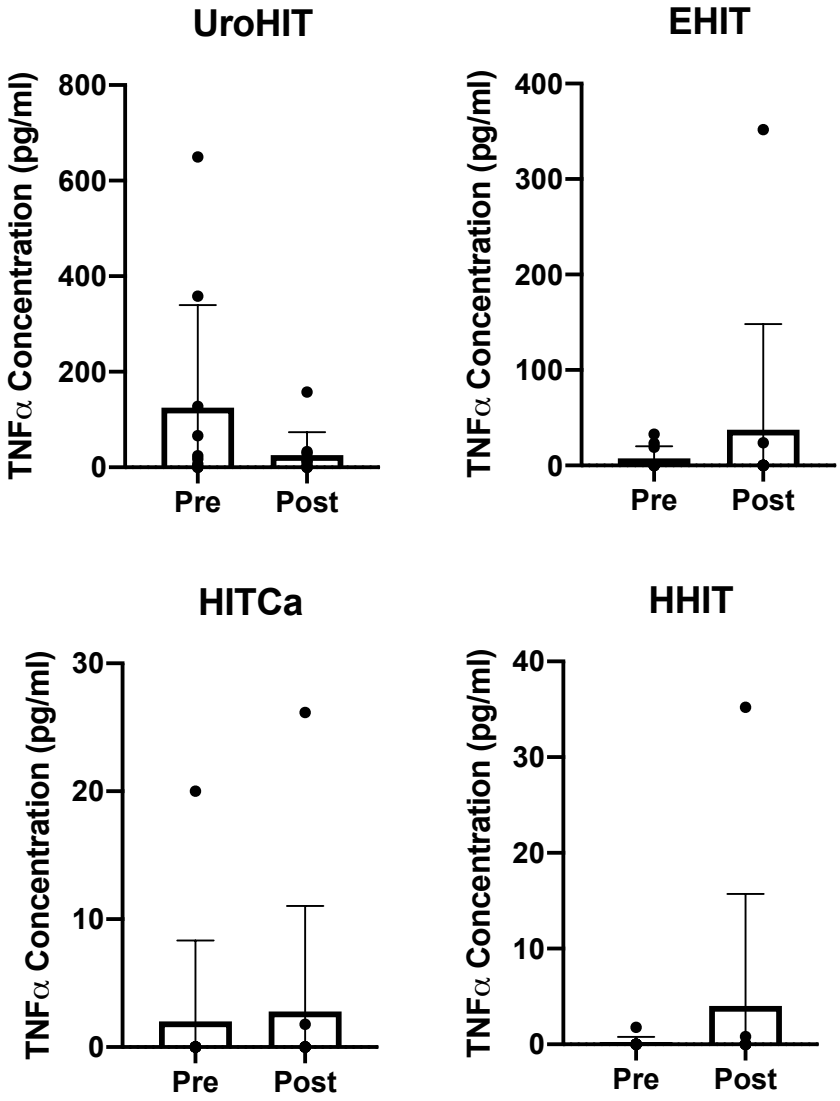


Figure 34: TNFa Results

5.4.6 CRP

The pre- and post- training concentrations of CRP were compared in each study. CRP had the largest number of samples (77/78) with detectable levels. While 3 of the 4 studied groups appeared to show an overall decrease in CRP following training, this did not reach statistical significance in any group ($P > 0.05$).

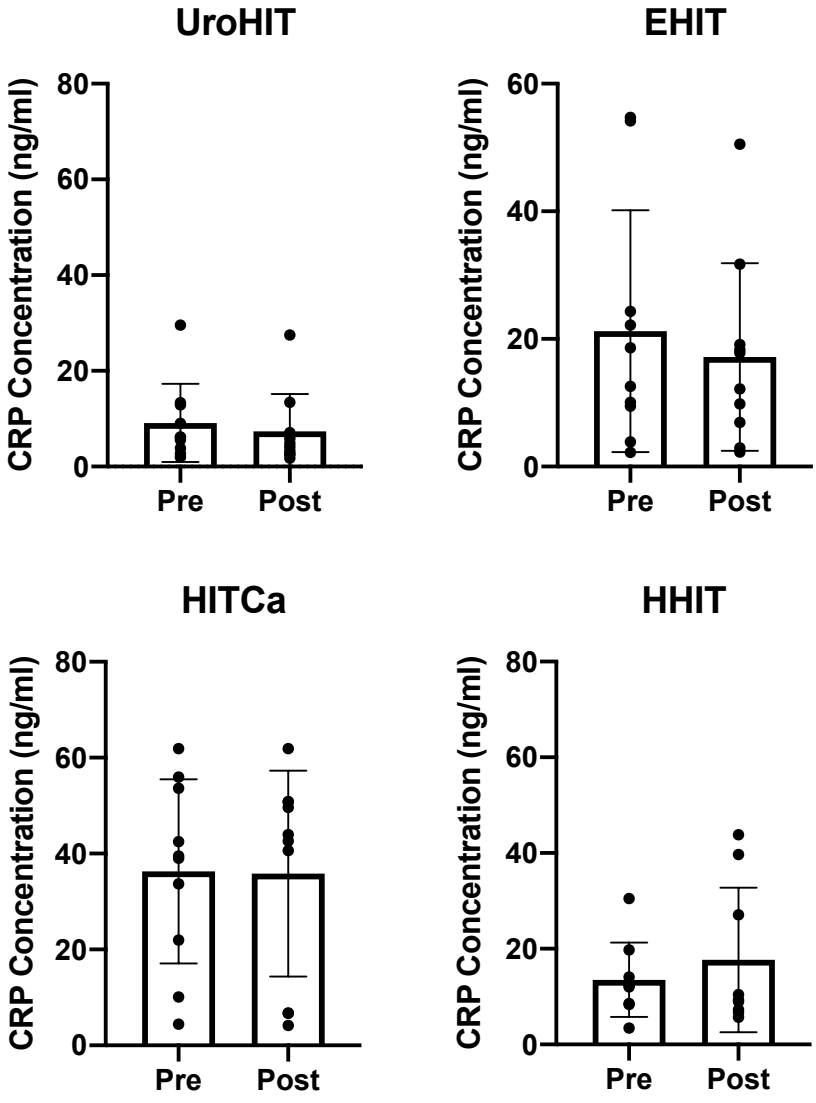


Figure 35: CRP Results

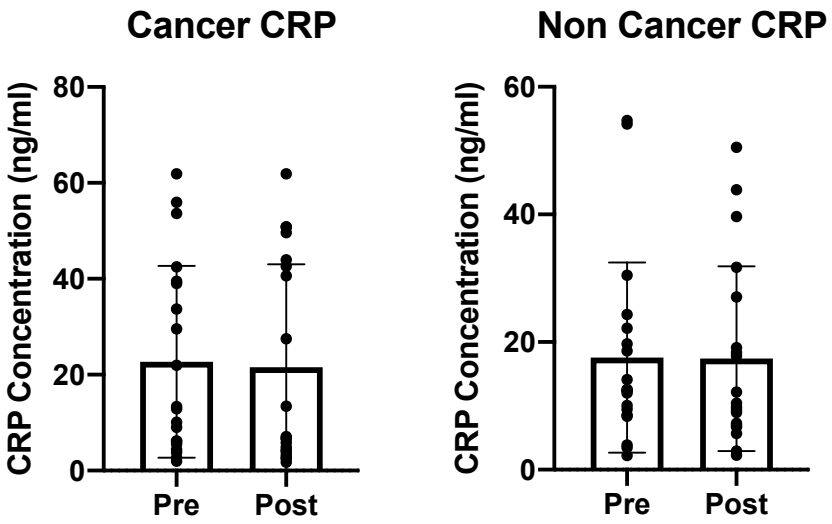


Figure 36: Cancer and Non-Cancer CRP Results

Similarly, when comparing CRP concentrations pre- and post- intervention in participants with and without cancer, there was no significant change seen (P=0.62, P=0.65). Baseline levels of CRP appeared to be increased in participants with cancer, however this was not statistically significant (p=0.73).

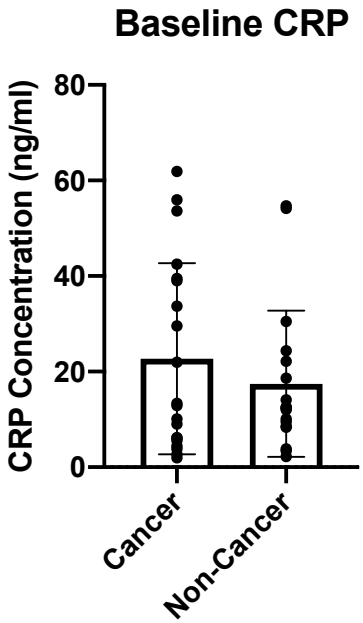


Figure 37: Baseline CRP Results

5.4.7 Outliers

Within each ELISA plate there were outliers at the highest ranges. In the majority of cases these were isolated occurrences, but one (non-cancer) participant had markedly raised IL-6 (22.57pg/mL), IL-10 (38.11pg/mL) and CRP (50.57ng/mL) in the pre- exercise samples which was not seen in the post- exercise samples (1.88pg/mL, 4.01pg/mL and 33.85ng/mL respectively).

5.5 Discussion

5.5.1 Effect of exercise on inflammatory markers

IL-6

Exercise appeared to have an effect on IL-6 concentrations in both non-cancer groups, with the elderly HIT group decreasing mean levels while the home HIT group increased their overall concentrations. The elderly group had the highest levels of IL-6 at baseline (6.90±7.64 pg/mL) compared to the other groups (2.68±2.45, 2.72±2.82, 3.06±2.92) which may be attributed in part to the inflammaging [52] suggested by Franceschi et al. It has been proposed that in groups which consistantly show decreases in inflammatory IL-6 following exercise, baseline levels are often raised due to age or underlying chronic inflammatory conditions such as type 2 diabetes or obesity [96] [97]. It is interesting to note that all groups had higher than average levels of circulating IL-6 (normally around 1 $pg.ml^{-1}$). Alongside aging and inflammatory pathologies, including malignancies, increased levels of IL-6 have been correlated with lower levels of cardiorespiratory fitness [157]. Each of the other groups studied increased levels of IL-6 from baseline, although this only reached significance in the HHIT group. This appears to conflict with the hypothesis that exercise intervention decreases levels of inflammatory cytokines, and a rise in IL-6 was only seen in one study within the systematic review of available literature [111].

As previously discussed, acute bouts of exercise have been shown to increase circulating levels of muscle derived IL-6 increase up to 100 fold [88] which can remain elevated for up to 48 hours [158]. Although the HHIT group were sampled 48 hours after exercise, information was not available for the timing of sampling of the other groups which may go some way to explaining these results. The modality of training (HIT) in these groups also differed from the majority of studies included in the systematic review. HIT has been shown to have a greater effect on acute IL-6 increases than more moderate exercise [159] and the HIT protocol itself can affect rise and duration of elevation [160]. The HHIT group undertook a series of bodyweight exercises rather than the cycling protocol followed by the other groups.

A second role of IL-6 is seen in muscle repair following damage during exercise. As the participants in these studies were not trained, they are likely to have induced muscle damage and also have developed some muscle growth during the study period. IL-6 has been shown to be produced by muscle-infiltrating inflammatory cells, aiding the recruitment of satellite cells and the repair and hypertrophy of muscle [18]. This too may have contributed to the rise in IL-6 seen in the post study period.

Finally, given that the HHIT group performed their exercises at home, they may not have reached the intensity of exercise which the other supervised participants achieved. It is also possible that rather than evenly spacing exercise sessions, participants may have been tempted to perform training on sequential days on the run-up to the reassessment day. While this is entirely speculative, it may offer another insight into the unexpected rise of IL-6.

IL-10

While there appeared to be a trend towards a decrease within the EHIT and HHIT groups and an increase within the groups within the UroHIT and HITCa groups, none produced significant results. However, the decrease in IL-10 within the non-cancer groups became significant when the results were combined (P=0.02).

Like all of the studied cytokines IL-10 is pleiotropic, but is broadly considered to be anti-inflammatory, with a role in muscle regeneration after injury [161]. Acute exercise has been shown to increase levels of IL-10. Previous work has suggested that the intensity of exercise is related to the degree of increase in circulating IL-10 [162], but results of a systematic review are varied, suggesting duration of exercise may have more effect [163]. Work in exercise studies over 12 months have shown no significant effects on IL-10 [164].

One action of IL-10 is the inhibition of inflammatory cytokine release meaning that circulating levels may not clearly reflect the degree of anti-inflammatory effect within the individual. For example, participants with T2DM have been shown to have dysregulated IL-10 effects, with subsequently increased levels of IL-6 and TNFα [165]. In patients with cancer IL-10 becomes more controversial, with some studies showing that high levels of IL-10 correlate with poorer outcomes [166] perhaps due to a 'blunting' of the immune response to tumours. However, IL-10 has also been implicated in tumour regression through its enhancement of the effect of NK cells [166].

TNFα

In previous work, exercise has been shown to decrease levels of TNFα [134] in a generalised setting of reduced inflammation. Within a general population, increased levels of TNFα have been associated with chronic, non-communicable diseases including T2DM [28]. However,

like all the cytokines studied TNFα is pleiotropic, particularly with regards to its effects on cancer cells [50] where it has been seen to exert both anti- and pro- tumour actions. Within this study, TNFα was detected in the lowest number of samples (27/78) which makes interpretation of the results challenging. There were no significant effects seen in any group after the exercise interventions. The group with the highest levels of TNFα was UroHIT, however given the very limited number of results for this cytokine, interpretation of these figures cannot provide a conclusion of any confidence.

CRP

CRP was the most widely detected biomarker, seen in almost every sample within the study. While the other targets (IL-6, IL-10, TNFα) all display pleiotropic effects with regards to inflammation, CRP is most consistently associated with a pro-inflammatory state and is specifically produced by a single organ, rather than being expressed by a wide variety of cells [167].

The highest baseline levels of CRP were found in the HITCa (36.28±19.19) and EHIT (21.23±18.93) groups (P=0.01) which may have been expected, given that older populations have been found to have higher CRP levels [52]. The elevated levels of CRP seen in the patients with colorectal cancer may also be linked with the inflammatory processes surrounding tumour growth [25], although this was not apparent in the participants with urological cancer who had the lowest overall levels of CRP.

While 3/4 groups appeared to show a decrease in overall levels of CRP, this was not statistically significant. While exercise has been linked to reductions in CRP, the most significant changes have been attributed to the loss of adipose tissue during training protocols [168]. This link may explain why there was no significant effect seen within the groups studied. The short duration of training

(4 weeks) did not provide the opportunity for significant decrease in adiposity in non-obese individuals (BMI <35).

5.5.2 Effect of cancer on baseline inflammatory markers

When comparing baseline levels of CRP in cancer and non-cancer participants there appeared to be an increased level in baseline concentrations amongst those with cancer. In contrast, baseline IL-6 was lower in participants with cancer, however, neither was found to be statistically significant.

The highest recorded results for TNFα were both seen in the group with urological cancers at baseline. This might not be surprising given work implicating TNFα and the development of bladder cancer [169], however these elevated levels of TNFα were not seen in post exercise samples from the same participants. It is difficult to ascertain whether this can be attributed to the exercise intervention as it was seen in only 2 participants.

The inflammatory milieu found within the plasma of healthy study participants during exercise is complex and confounded by a range of factors, including BMI and adiposity, age and concurrent infections. The presence of neoplastic cells adds to the complexity of understanding the effect of exercise. While lower overall levels of measured pro-inflammatory markers have been linked to better outcomes, there has been little to ascertain whether exercise can reduce systemic inflammation and provide better outcomes in such patients. Previous work has focused on fatigue during cancer treatment and the beneficial effects of exercise [170] although this has not been shown to alter the inflammatory profile significantly [171].

5.4 Limitations

A number of limitations must be considered when interpreting the results of this study. The limitations can be considered as those relating to the study design and the technology used in the measurement of cytokines.

5.4.1 Technologies and Data Processing

Plasma Samples

Plasma samples were taken from participants in studies previously run by members of the research group from 2016-2018. These samples had been stored at -80^{o}C prior to use in this study, however it is difficult to ascertain whether the samples had been previously thawed and re-frozen. Studies have shown that repeated freeze-thaw cycles can increase the levels of detected cytokines including IL-6 by up to 20% [172]. Due to the limited amount of plasma available for each participant, it was not possible to run the ELISA samples in duplicate, thus increasing the potential for error. In some samples, there was just sufficient plasma available to comply with the ELISA protocol, leading to the possibility of small differences in sample sizes when pipetting.

ELISA standard curves

When plotting the standard curves to allow interpretation of the plate reader output, there were some data points which diverged from the line of fit within the standard samples which may have introduced errors in interpolating concentrations of measured cytokines.

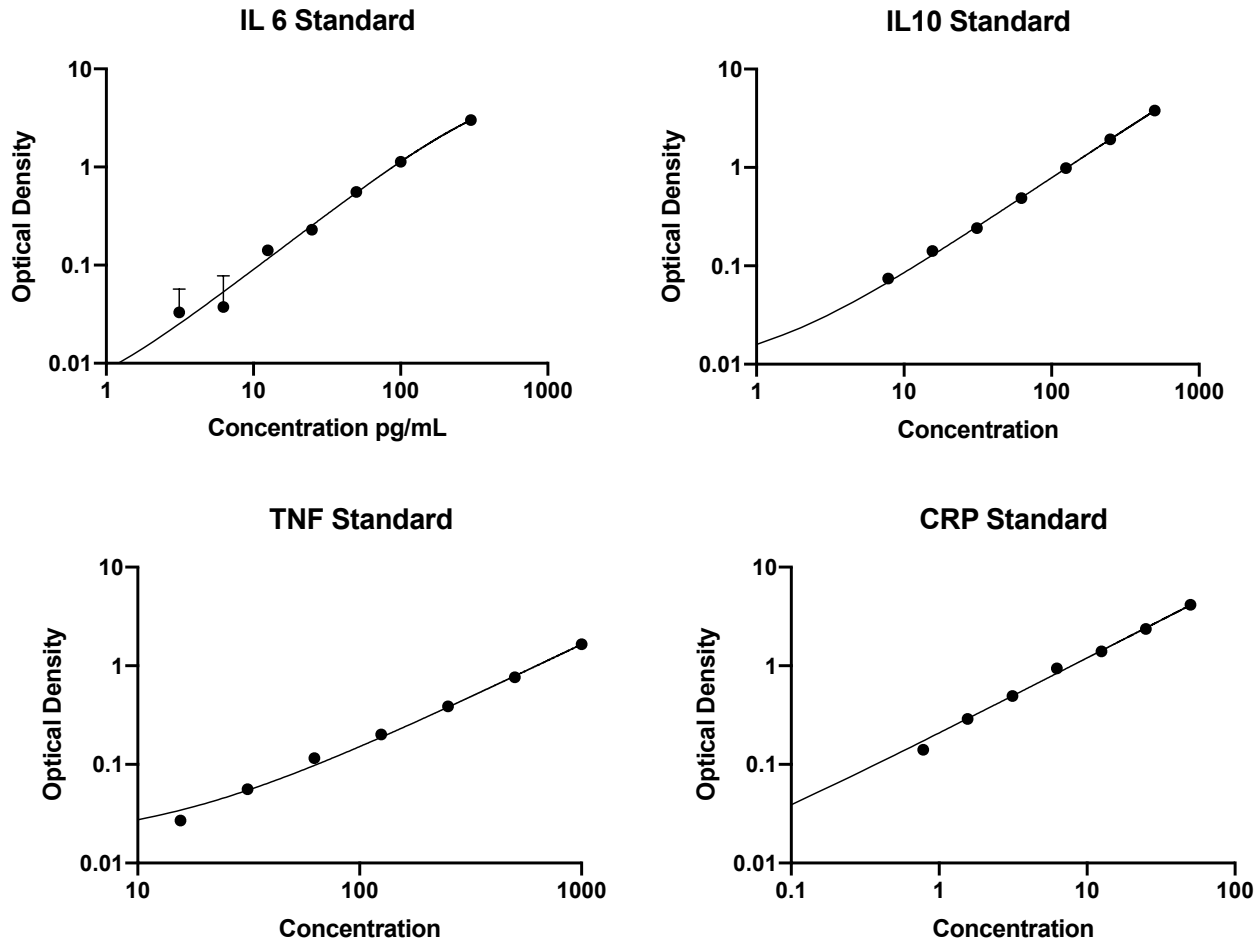


Figure 38: ELISA Standard Curves

5.4.2 Study Design

Participant Selection

Selection was based on the first 10 participants to have suitable pre- and post- exercise training EDTA samples available in sufficient quantities to perform ELISA for all 4 variables. It is possible that this method may have introduced bias in a number of ways. These participants may have been particularly motivated to complete the study, returning for all necessary visits which may represent those with greater overall activity levels, or less disease burden in the case of those with cancer diagnosis.

Study Size

Small group numbers n=10 and n=9 necessarily limit the power of the study. Heterogeneity was seen in the baselines of each group for every variable studied, with outliers having greater influence on overall results.

Physiological Changes

As the original studies were not intended to measure levels of inflammatory markers, there is no record of participants having conditions which may have altered their cytokine levels acutely. For example, a mild respiratory illness or skin infection may present changes in measured cytokines but would not have prevented the participants from being included in the study.

Heterogeneity within groups

As with all human studies there is heterogeneity within the groups studied which may have affected levels of circulating cytokines. For example, participants within the HITCa group had a variety of comorbidities including type 2 diabetes, musculoskeletal conditions and COPD [155] which may have confounded results.

Training Protocols

As the HHIT group performed unsupervised exercise there is potential for non-compliance with the exercise program which may also affect cytokine levels.

Blood Sampling Time

For the HHIT group this was >48 hours after cessation of exercise. The timing of the other samples in other groups may have been taken after a shorter period of rest, which may affect levels of inflammatory markers as previously discussed.

6. Future research

6.1 CSF

Following the work presented in this dissertation, the difficulties of recruiting subjects for cerebrospinal fluid research has become apparent. However, potential participants continue to express interest in the study and recruitment is ongoing in order to complete the study and present more complete results regarding the effect of exercise in inflammatory markers within CSF. One potential area for recruitment is patients undergoing surgery under spinal anaesthesia where samples could be taken at the time of anaesthesia. However, there are potential confounders dependent on whether the surgery is necessitated by an inflammatory condition.

6.2 Plasma

A number of areas for future work have been raised. In order to draw firm conclusions, it may be necessary to restrict inclusion criteria to reduce the heterogeneity within study participants. For example, many of the studies used a BMI <35, but given our understanding of the effect of body composition on inflammatory profile, there may be significant differences between a participant with a BMI of 21 and one of 34. The majority of the studies included use fasting blood samples when measuring cytokines. Given that previous work has shown that carbohydrate ingestion can dramatically affect levels of IL-6 after exercise, standardising the diet of the participants may go some way to avoiding potential confounders.

As with much physiological research, a large proportion of this work is done using only male participants. Studies in women have shown variation in inflammatory cytokines with menstrual cycle, although none of the studies in this project which included younger women appeared to control for this. Future work which specifically includes female participants and are designed to

control for hormonally driven fluctuations are needed to fully understand the inter-sex differences.

Within groups with cancer, raised inflammatory markers are often associated with poorer prognosis. Given that some studies have shown that regular exercise may improve the inflammatory profile of volunteers with raised baseline inflammation, future work may ascertain whether exercise can have a similar effect on those with cancer.

A final, and more complex investigation would be to attempt to differentiate muscle derived IL-6 from that of inflammatory cells. If this was possible, it may allow quantification of the sources of IL-6 seen in participants, and the potential anti- or pro- inflammatory effects of these on the body. This would allow further understanding of the true effect of exercise on the circulating inflammatory profile.

7. Conclusions

'Just one living cell in the human body is more complex than New York City'

~ Linus Pauling

The first, and most important conclusion to draw from this work is the complexity of the human inflammatory profile. Each of the studied targets exhibit significant pleiotropy, with the possible exception of CRP. Furthermore, there is marked heterogeneity in baseline levels within the studied populations which is further compounded by age, sex, underlying disease processes and body composition. There were few statistically significant results found within the review of previous literature or the laboratory study of HIT participants which perhaps alludes to the difficulties and multiple confounders which investigators of inflammatory cytokines encounter.

7.1 Acute high intensity interval training produces measurable changes in inflammatory markers including IL-6, IL-10, TNFα and CRP within the cerebrospinal fluid of healthy individuals

On analysing the available literature, there were few studies which investigated the effect of exercise on inflammatory markers in CSF in healthy populations. From the available data and meta-analysis, it was not possible to draw any firm conclusions. It was also not possible to recruit sufficient participants to perform the study investigating inflammatory markers in cerebrospinal fluid. There were a number of noted barriers, the most significant of which was a concern regarding the lumbar puncture procedure itself.

7.2 Older participants will show the greatest effects of decreased circulating inflammatory markers after a period of exercise intervention.

This hypothesis was based on previous evidence that older participants begin with higher baseline levels of inflammation, showing a more significant decrease than younger participants who may start with lower levels of circulating markers. Within the review of the current literature, the mean age of the oldest age group was 70. When stratified for age there were no significant differences found for any of the studied markers. Amongst the HIT study participants, the oldest group (EHIT) were the only group to show a statistically significant decrease in IL-6, although this was not replicated in the other markers studied. Interestingly, they also had the highest baseline levels of IL-6, supporting the original hypothesis.

7.3 High intensity interval training improves the inflammatory profile in the plasma of those with a diagnosis of urological or colorectal cancer.

This hypothesis was also based on the principal that these groups would have raised levels of baseline inflammation. Although the cancer groups showed a trend towards raised CRP and decreased IL-10, no significant differences found between baseline levels in cancer and non-cancer groups for any marker. Similarly, the HIT exercise interventions did not show significant changes in the inflammatory profile of either cancer group.

7.4 Overall Conclusions

While regular exercise is associated with many benefits this work has shown some of the difficulties which may be encountered when attempting to quantify the effect of exercise on inflammatory markers in otherwise healthy participants. It has shown a gap within the literature of work on the CSF, as well as the challenges of performing such research.

Although some individual plasma studies have shown significant results, when combining the evidence in a meta-analysis these became largely non-significant. In both review and HIT studies IL-6 was the only marker to show significant changes which perhaps speaks to its dual role as both cytokine and myokine.

While this work cannot draw any firm conclusions as to the role of exercise on inflammatory biomarkers, it does raise a number of other questions and avenues for exploration. Clearly, more research is required in order to be able to accurately define these effects and will likely involve larger sample sizes as well as more rigorous standardisation of participants in order to minimise the number of potential confounders.

8. Appendices

8.1 Appendix 1: Ethical Approval

University of Nottingham
UK | CHINA | MALAYSIA

Email: FMHS-ResearchEthics@nottingham.ac.uk

Faculty of Medicine & Health Sciences
Research Ethics Committee
c/o Faculty PVC Office
School of Medicine Education Centre
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Queen's Medical Centre Campus
Nottingham University Hospitals
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20 November 2018

Dr BE Phillips
Assistant Professor
Clinical, Metabolic & Molecular Physiology
Division of Medical Sciences and Graduate Entry Medicine (GEM)
School of Medicine
Royal Derby Hospital Centre,
Uttoxeter Road,
Derby, DE22 3DT

Dear Dr Phillips

Ethics Reference No: 135-1809 – **please always quote**	
Study Title: Exploring central and systemic dynamic responses to exercise: A pilot study	
Chief Investigator/Supervisor: Dr Beth Phillips, Assistant Professor, Clinical, Metabolic & Molecular Physiology, Division of Medical Sciences & GEM	
Lead Investigators/student: Dr Samantha Moore, Anaesthetic Clinical Trainee, Clinical, Metabolic & Molecular Physiology, Division of Medical Sciences & GEM	
Other Key Investigators: Prof Phil Atherton, Prof K Smith, Dr DJ Wilkinson, Assistant Professor, Dr JP Williams, Consultant Anaesthetist & Associate Professor, Clinical, Metabolic & Molecular Physiology, Division of Medical Sciences & GEM.	
Type of Study: Basic Science study involving procedures, medicinal or Food products.	
Proposed Start Date: 01/09/2018	**Proposed End Date:** 30/09/2020 24 mths
No of Subjects: 24	**Age:** 18-45 years

Thank you for submitting this application and the following documents were received:

- FMHS REC Application form and supporting documents version 1.0: 14/09/2018.

These have been reviewed and are satisfactory and the study has been given a favourable opinion.

A favourable opinion has been given on the understanding that:

1. The protocol agreed is followed and the Committee is informed of any changes using a notice of amendment form (please request a form).
2. The Chair is informed of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

University of Nottingham
UK | CHINA | MALAYSIA

Email: FMHS-ResearchEthics@nottingham.ac.uk

Faculty of Medicine & Health Sciences
Research Ethics Committee
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5 February 2019

Dr Samantha Moore
Anaesthetic Clinical Trainee
Clinical, Metabolic & Molecular Physiology
Division of Medical Sciences and Graduate Entry Medicine (GEM)
School of Medicine
Royal Derby Hospital Centre,
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Proposed Start Date: 01/09/2018	**Proposed End Date:** 30/09/2020 24 mths
No of Subjects: 24	**Age:** 18-45 years

Thank you for notifying the Committee of amendment no 1: 12.12.2018 In summary as follows:

Based on test samples from previous studies the dose of D2O suggested in this study may be too low to provide useful results. Request to increase the dose from 8mls/kg to 15mls/kg. Administration of this larger dose will be over a 1 hr period to attempt to mitigate the known potential side effects of dizziness.

and the following documents were received:

- FMHS REC Notice of amendment form dated 12/12/2018.
- **CSF-Ex Ethics Form V2.0: 12/12/2018**
- **CSF-Ex Protocol V2.0: 12/12/2918**

These have been reviewed and are satisfactory and the study amend no 1 12.12.18 has been given a favourable opinion.

A favourable opinion has been given on the understanding that:

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2. The Chair is informed of any serious or unexpected event.
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Yours sincerely

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

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5 February 2019

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Yours sincerely

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

8.2 Appendix 2: Physiology 2019: Poster

The Effect of Exercise on Inflammatory Markers in Cerebrospinal Fluid and Plasma: A Systematic Review

Samantha Moore[1,2], Brett Doleman[1,2], Jon Lund[1,2], Philip J Atherton[1,2], *John Williams[1,2], *Bethan Phillips[1,2]

[1] MRC-ARUK Centre for Musculoskeletal Ageing Research; [2] School of Medicine, University of Nottingham, Derby (*=co-senior authorship)

What effect does exercise have on inflammatory markers in cerebrospinal fluid (CSF) and plasma in healthy adults?

BACKGROUND

- The role of inflammation in dementia is debated, with some evidence that systemic inflammation potentiates neurodegeneration in a range of conditions, including Alzheimer's disease (AD) [1].
- A number of studies have investigated links between levels of inflammatory cytokines in plasma or cerebrospinal fluid (CSF) and AD. However research results are often conflicting [2].
- Exercise has been shown to protect against development of cognitive impairment.
- The majority of studies of the correlation between exercise and inflammation have focussed on plasma. Recent work has investigated the effect of exercise on cytokines measured in CSF [3].

The aim of this systematic review was to explore the effect of exercise on central (CSF) and peripheral (plasma) inflammatory markers in healthy volunteers.

METHODS

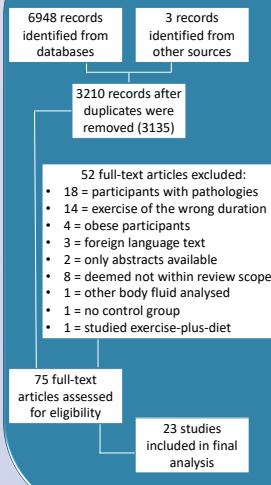

Figure 1: *Study Flow Diagram*

- The literature search was conducted using PubMed, Embase and the Cochrane library before all results were screened.
- Randomised controlled trials (RCT's) recruiting healthy participants investigating the response of IL-6, IL-10, CRP and/or TNF-α to exercise were included.
- In CSF studies any exercise protocol of any duration was accepted.
- In plasma studies, moderate to high intensity exercise (resistance and/or endurance) for at least 8 weeks was included.

RESULTS

- 3 studies investigating CSF (n=50) were identified, with **no significant changes** identified between control and exercise groups.
- 23 RCT's were identified for plasma (n=1123). Plasma results showed significant heterogeneity, with combined results showing no effect on CRP (*Figure 2 (left)*) or TNF-α (*Figure (2 (right)*); **reaching significance only in IL-6** *(Figure 3)*.

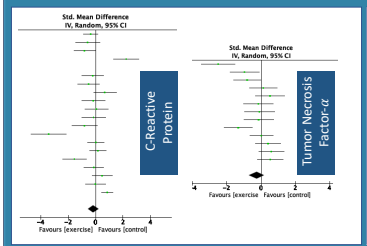

Figure 2: Forest Plot for changes in CRP (left) and TNF-α (right): No significant change in either factor was observed with exercise training.

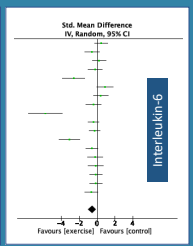

Figure 3: Forest Plot for change in IL-6. A significant decrease in IL-6 with exercise training was observed.

DISCUSSION

- The reporting of results within manuscripts presents statistical challenges for meta-analysis as the heterogeneity of baseline levels of inflammatory markers may have an impact on the changes seen.
- In order to complete meta analysis, final values from control and exercise groups were used, however this has statistical limitations.
- These limitations may explain why the CSF studies did not find significant differences between control and exercise groups, and why plasma experiments only identified significant changes *within* the exercise group (e.g., comparing with their own baseline levels).
- Previously detected effects of exercise on inflammation may be more profound in subjects with underlying pathologies such as diabetes or obesity. Further randomised controlled trials are required to determine the effect of exercise on central inflammatory markers in different groups.

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8.3 Appendix 3: Exclusion Criteria and Study Protocols

8.3.1 CSF: Short Study Protocol

This study aims to explore the effects of both resistance and aerobic exercise on inflammatory markers and protein handling within the CNS and systemic circulation.

Recruitment and Screening:

This study will involve the recruitment of 3 separate groups :

- Group 1: 8 young males (18-45 y; BMI <35 $kg \cdot m^2$) to partake in an aerobic exercise session with blood and CSF sampling
- Group 2: 8 young males (18-45 y; BMI <35 $kg \cdot m^2$) to partake in a resistance exercise session with blood and CSF sampling
- Group 3: 8 young males (18-45 y; BMI <35 $kg \cdot m^2$) to act as the control group with no exercise session and blood and CSF sampling

All subjects will undergo a full medical screening before enrolment into the study. This will involve measurements of height and weight (BMI), a medical history examination, blood tests (for FBC's, U&E's, LFT's, TFT's, lipid profiles and coagulation profiles) and an ECG.

Subjects will be excluded if they display evidence of:

- A BMI <18 $kg \cdot m^2$ or >35 $kg \cdot m^2$
- Active cardiovascular disease
- Cerebrovascular disease
- Respiratory disease (excluding well-controlled asthma)
- Metabolic disease
- Active inflammatory bowel or renal disease
- Active malignancy
- Recent steroid treatment (within 6 mo) or hormone replacement therapy
- Clotting dysfunction
- Musculoskeletal or neurological disorders
- Symptoms of raised intracranial pressure

Any abnormal screening results will first be discussed with the subject and their GP informed.

8 subjects will act as a control arm and will not participate in an acute exercise session. The remaining 16 subjects will be randomized (via sealed www.envelope.com) to perform a single bout of either resistance or aerobic-type exercise during their acute study day.

Pre-study tests:

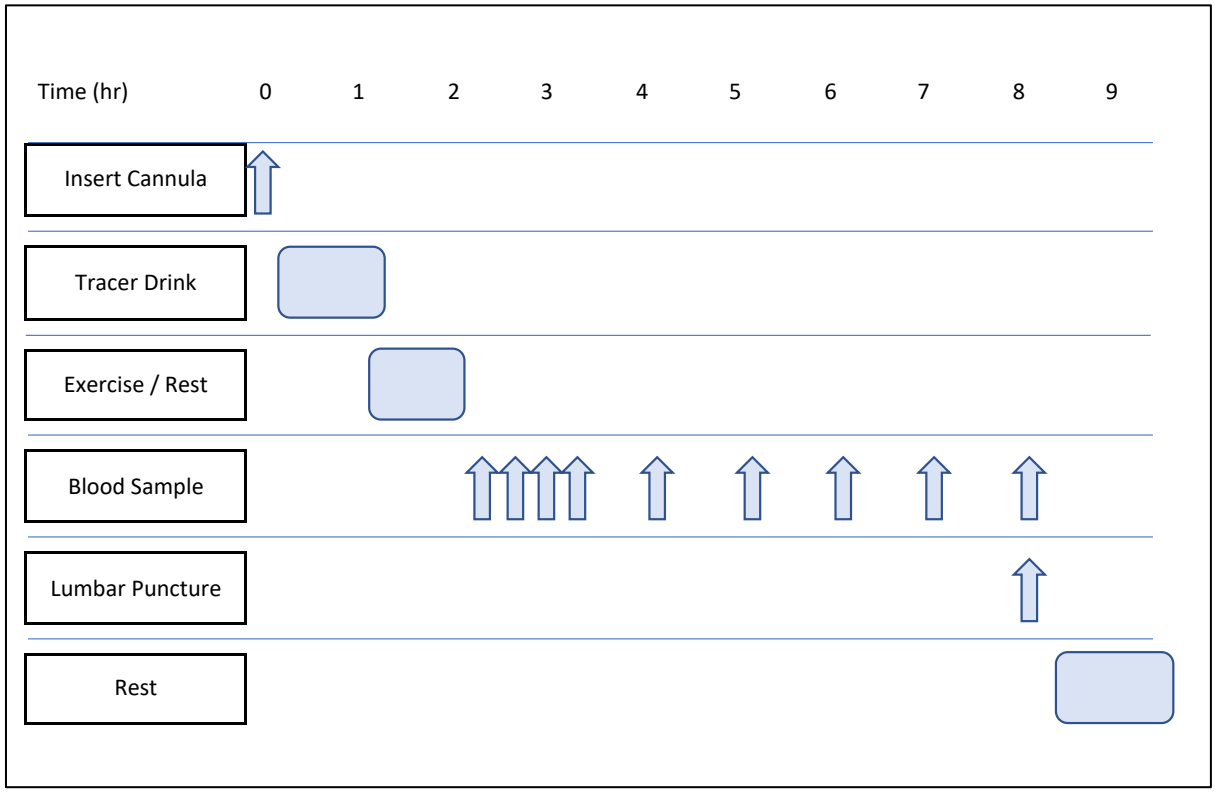
Approximately 72h before (V1) their first acute study visit, all subjects will be asked to attend our clinical facility for assessment of cardiorespiratory fitness (via an incremental cardiopulmonary exercise test (CPET)) and a short series of muscle function tests.

Acute study protocol:

3-7 days after their first visit subjects will attend the unit for their first lumbar puncture (V2) to obtain baseline CSF. Between their first visit and this visit, subjects will be asked not to participate in any moderate to high intensity exercise. This session will last ~2h, 1h for the lumbar puncture procedure (including preparation) and 1h for post-procedure monitoring (by the clinician performing the lumbar puncture).

7-10 days after their first lumbar puncture subjects will attend the unit for their acute study day (V3). Subjects will be asked to report to our clinical facility at ~0800h and this visit will begin will the insertion of a forearm cannula for repeat blood sampling throughout the day. After an initial blood sample is taken subjects will be asked to consume 15mL/kg (1050mL for a 70kg individual) of D_2O "heavy water" tracer for the assessment of CSF protein turnover throughout this day. To minimize the chance dizziness due to alterations in inner-ear equilibrium (which is normally only apparent at higher doses and primarily in older individuals), this D_2O dose will be given in 4 equal portions equally distributed across a 1h time-period. After their final D_2O dose, subjects will perform the bout of exercise that has been assigned to them (or have an equivalent rest period if they are in the control group). For subjects in the aerobic exercise group, they will perform 45min cycling on a stationary cycle ergometer at 60% VO_2 max; the resistance exercise training group will complete 45min whole-

body exercise, including leg-press, leg-curl, leg-extension, lat pull-down, chest press and seated row. After this exercise, all subjects will have small (<3mL) blood samples taken immediately after exercise and then at 15,30,45 and 60min followed by 1h intervals for the assessment of systemic inflammation. A single lumbar puncture will be performed 6h after the end of the exercise to assess central inflammation and protein turnover. Subjects will then remain in the facility for a 1h post-procedure monitoring period as in V2.



8.3.2 Inclusion Criteria : EHIT [152]

EHIT Eligibility Criteria
Volunteers aged over 70 years Males and females
EHIT Inclusion Criteria
Aged over 70 years. Sufficient mobility to be able to exercise on a static exercise bike. Availability for the entire 8 week period Sufficient capacity to consent for the trial
EHIT Exclusion Criteria
Participants under the age of 70 years. Participants with a significant past medical history of: Myocardial infarction (within last 6 months) Unstable Angina Heart failure (NYHA class III/IV) Uncontrolled Hypertension (BP>160/100) Previous stroke/TIA Severe respiratory disease inc. known pulmonary hypertension (>25 mmHg), Forced Expiratory Volume in 1 second <1.5l. Brittle asthma / exercise induced asthma Known cerebral aneurysm or abdominal aortic aneurysm. Cognitive impairment, which may reduce individuals' ability to provide informed consent. Inclusion in a recent study which included any form of exercise, taking a drug or ionising radiation.

Figure 39: EHIT Inclusion and Exclusion Criteria

8.3.2 Study Protocol : EHIT

Over 4 weeks volunteers will attend the unit for 3 HIT sessions each week. This will involve 16.5 minutes static cycling per session,

- 2 minutes warm up
- 60s constant load intensity work out at elicit 95 - 115% Watt max
- 90s recovery
- *Repeat 5 times*
- 2 minutes cool down (to baseline, pre exercise, heart rate)

The first training session will comprise of 5 60second intervals set at 95%, 100%, 105%, 110% and 115% Watt max. The intervals at the following training sessions will be set at the highest completed interval from this initial assessment session.

After 6 training sessions the interval training level will be increased by 10% of the initial baseline maximum power output providing:

- Borg score below 8 (modified BORG (0-10) scale)
- No adverse cardiovascular signs during the first 6 training sessions
- Fully completed all training sessions [152]

8.3.3 Baseline Characteristics : EHIT

Baseline Characteristics	
Age (mean) (y)	81.3
Gender	62% male / 38% female

8.3.4 Inclusion Criteria : UroHIT [153]

UroHIT Inclusion Criteria
Aged over 65 years (no upper age limit). Histologically proven, radiologically or direct visualisation leading to high clinical suspicion of cancer. Sufficient mobility to be able to exercise on a static exercise bike. Ability to give informed consent. Ability to travel to RDH to complete the HIT sessions (may also rely upon dependents/relatives to provide transport).
UroHIT Exclusion Criteria
Adjuvant systemic chemotherapy / radiotherapy treatment for this cancer. Participants with a significant past medical history of: Myocardial infarction (within last 6 months) Unstable Angina Heart failure (NYHA class III/IV) Uncontrolled Hypertension (BP>160/100) Previous stroke/TIA Severe respiratory disease inc. known pulmonary hypertension (>25 mmHg), Forced Expiratory Volume in 1 second <1.5l. Brittle asthma / exercise induced asthma Known cerebral aneurysm. Inclusion in a recent (within 3 months) study which included any form of exercise, taking a drug or ionising radiation.

Figure 40: UroHIT Inclusion and Exclusion Criteria

8.3.5 Study Protocol: UroHIT

Participants will complete a minimum of eight individual HIT sessions at a frequency of three to four per week. Participants will complete a maximum of twelve sessions before retesting prior to their surgery.

HIT sessions consist of a 2 minute lightly loaded pedalling (15W) warm up phase followed by 5 x 1 minute intervals with 90 seconds rest in between. A 3 minute warm down period follows the final interval. The first training session will comprise of 5 x 60second intervals set at 95%, 100%, 105%, 110% and 115% Watt max. The intervals at the following training sessions will be set at the highest completed interval from this initial assessment session.

After 6 training sessions the interval training level will be increased by 10% of the initial baseline maximum power output providing:

- The participants assess their effort on a modified Borg scale at 8 or below
- No adverse cardiovascular signs during the first 6 training sessions
- Fully completed all training sessions [153]

8.3.6 Inclusion Criteria : HITCa [154]

HITCa Inclusion Criteria
Confirmed colorectal cancer
Offered treatment by the Royal Derby Hospital NHS Foundation Trust Colorectal Cancer Multidisciplinary Team
Male and female
18-98 years old
Ability to give informed consent
HITCa Exclusion Criteria
Uncontrolled hypertension (BP > 160/100),
Angina,
Heart failure (class III/IV),
Cardiac arrthymias,
Right to left cardiac shunt,
Recent cardiac event,
Previous stroke/TIA,
Aneurysm (large vessel or intracranial),
Severe respiratory disease including pulmonary hypertension,
COPD/asthma with an FEV1 less than 1.5 l,
Coagulation disorders,
Scarring disorders.
Current neoadjuvant chemo/radiotherapy
Inability to complete the consent process
Pregnancy
Those included in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure or exposure to ionising radiation, will also be excluded.

Figure 41: HITCa Inclusion and Exclusion Criteria

8.3.7 Study Protocol : HITCa

Over 4 weeks volunteers will attend the unit for, an average of, 3 HIT sessions each week, aiming to complete 12 sessions in 31 days. This will involve 16.5 minutes static cycling per session:

- 2 minutes warm up
- 60 second constant load intensity cycling at 90-110% Watt max
- 90 second recovery
- Repeat 5 times
- 3.5 minutes cool down

The first training session will comprise of 5 x 60second intervals set at 95%, 100%, 105%, 110% and 115% Watt max. The intervals at the following training sessions will be set at the highest completed interval from this initial assessment session.

After 6 training sessions the interval training level will be increased by 10% of the initial baseline maximum power output providing:

- The participants assess their effort on a Borg scale at 15 or below
- No adverse cardiovascular signs during the first 6 training sessions
- Fully completed all training sessions [154]

8.3.8 Baseline Characteristics : HITCa [155]

Characteristic	Number of Participants
Age (SD) (y)	67 (8)
Gender	13 male 5 female
BMI (kg/m^2)	28.7 (26.6-31.4)
ASA	Grade 1: 12
	Grade 2: 6

Figure 42: HIT Ca Baseline Characteristics

8.3.9 Inclusion Criteria : HHIT [156]

HHIT Inclusion Criteria
Age > 55 years Able to give informed consent
HHIT Exclusion Criteria
Current participation in a formal exercise regime A BMI < 18 or > 35 kg·m^2 Active cardiovascular disease: • Uncontrolled hypertension (BP > 160/100) • Angina • Heart failure (class III/IV) • Significant arrhythmia • Right to left cardiac shunt • Recent cardiac event Taking beta-adrenergic blocking agents Cerebrovascular disease: • Previous stroke • Aneurysm (large vessel or intracranial) • Epilepsy Respiratory disease including: • Pulmonary hypertension • Significant COPD • Uncontrolled asthma Malignancy Metabolic disease e.g. diabetic patients Clotting dysfunction Significant Musculoskeletal or neurological disorders Family history of early (<55y) death from cardiovascular disease

Figure 43: HHIT Inclusion and Exclusion Criteria

8.3.10 Study Protocol : HHIT

HIT sessions will involve a 2 minute warm up, followed by 5x1min high intensity intervals separated by 90 seconds active recovery (walking on the spot) and then a 2 minute recovery period. The high intensity bouts will be a pyramid of exercises (star jumps, mountain climbers and on-the-spot sprints) with the intensity target set as their heart rate at the end of a HIT session performed on the baseline study day.

The Home-HIT group will perform the same HIT programme at home but will not receive any feedback on their performance. Up to 5 x per week with a maximum of 16 sessions in 4 weeks. [156]

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