Circulating Leptin Levels in Patients with Myalgic Encephalomyelitis Chronic Fatigue Syndrome and/or Fibromyalgia: a Systematic Review

Submitted for the degree of Master of Clinical Science

The University of Adelaide: JBI, Faculty of Health and Medical Sciences

Michael Musker March 2021

Master of Clinical Science Thesis

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Abstract

Title: Circulating Leptin levels in Patients with Myalgic Encephalomyelitis Chronic Fatigue Syndrome and/or Fibromyalgia: a Systematic Review

Objective: The objective of this thesis was to evaluate circulating levels of leptin in cases diagnosed with Myalgic Encephalomyelitis Chronic Fatigue Syndrome and/or Fibromyalgia Syndrome and to investigate the differences compared with healthy controls.

Background: Myalgic Encephalomyelitis Chronic Fatigue Syndrome is a condition that has major symptoms including self-reported fatigue, post exertional malaise and unexplained pain across the body. The widespread pain is measured in a systematic way and is often referred to as fibromyalgia. The two disorders have many similarities but their association with leptin has indicated that leptin may affect the role of proinflammatory cytokines and symptom severity.

Inclusion criteria: This thesis considered observational studies of varying study designs including prospective and retrospective cohort studies, case-control studies, time-series and analytical cross-sectional studies that included both cases and healthy comparators. Cases included a diagnosis of Myalgic Encephalomyelitis, Chronic Fatigue Syndrome and/or Fibromyalgia. Some consider Myalgic Encephalomyelitis to be a different condition from Chronic Fatigue Syndrome, but for the purpose of this thesis we will use a consistent format 'Myalgic Encephalomyelitis Chronic Fatigue Syndrome' without prejudice to the community perspective. Controls are those without this diagnosis, usually healthy participants. Only studies published in English were included due to limited resources for translation.

Methods: This systematic review is reported based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist and follows JBI's methodology for systematic reviews of etiology and risk. A comprehensive search strategy included PubMed, Embase, Scopus, Science Direct, and

PsychINFO. Two reviewers screened, critically appraised eligible articles and extracted data using a standardised data extraction tool informed by the JBI System for the Unified Management, Assessment and Review of Information (SUMARI) software. Data Synthesis: The authors completed a quantitative analysis that produced synthesised findings across studies using pooled effect sizes and confidence intervals of the measures provided.

Results: There were 12 case (n=649) vs control (n=658) studies combined (n=1307) in meta-analysis which demonstrated a small difference favouring a higher level of circulating leptin in cases compared to controls when using a standardised mean difference. There was an overall pooled difference of 0.39, with a 95% Confidence Interval 0.04 to 0.74, which was significant (p=0.029). The meta-analysis showed a high level of heterogeneity I^2=86%. Re-expressing SMDs (Standardised Mean Difference) using the original units of leptin showed a pooled effect of 3.26 ng/mL (CI 0.33 ng/mL to 6.19 ng/mL).

Conclusions: Much of the literature describes the role of leptin as being part of an inflammatory response, but this systematic review did not find that studies could provide convincing evidence that leptin was higher in cases than controls. Although many studies indicated increased leptin in cases, there were two studies that reported lower leptin in cases compared to controls, four studies that showed no discernible difference, and six studies that indicated higher levels of leptin in cases. Individual study methodology varied between studies causing some difficulties in comparison. A series of investigative benchmarks and protocols need to be developed in relation to researching adipokines if exploration of biomarkers in these syndromes is to yield useful results. To provide clarity, clinical investigators need to use similar standardised practices and research methods to aid better comparison of experimental results between studies. A series of international standards and protocols needs to be developed, accepted, and cited across the literature as part of clinical operational procedures if we are to advance this area of research. A series of recommendations for future research has been made in chapter 5.

Keywords: Adipokines; cytokines; myalgic encephalomyelitis chronic fatigue syndrome; fibromyalgia; leptin.

Table 1 Summary of Findings Table

Leptin Cases compared to Controls SMD for Myalgic Encephalomyelitis Chronic Fatigue Syndrome and/or Fibromyalgia

Patient or population: [health problem] Chronic Fatigue Syndrome and/or Fibromyalgia vs Controls (Healthy participants)
Setting: Usually a clinic setting or university / community research recruitment
Intervention: Circulating Leptin levels Cases
Comparison: Controls - SMD also re-expressed as original units

Outcomes	**Illustrative comparative risks*** (95% CI) **Leptin in those without MECFS or FM**	Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
Leptin Levels ng/mL (circulating leptin levels – plasma / serum or cerebrospinal fluid)	The mean difference in leptin levels in cases was SMD **0.39 (0.04 to 0.74) higher in cases than controls. This is equivalent to a re-expressed result of** 3.26 ng/mL higher (0.33 to 6.19) in cases as compared to controls. *		n=1307 (649 Cases vs 658 Controls) (12 observational studies)	⊕◯◯◯ VERY LOW a,b,c,d	All studies used leptin ng/mL but used different fluids and methods of analysis

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).**CI:** Confidence interval; MD Mean Difference
SMD: Standardised Mean difference. SMD and CI taken from SUMARI analysis.

GRADE (Grades of Recommendation, Assessment, Development, and Evaluation) **Working Group grades of evidence**
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations:
a. Downgrade 1 for imprecision Two studies provide evidence of hypoleptinaemia (low leptin), four studies report negligible difference, and 6 studies report hyperleptinaemia (high leptin) in cases.
b. High heterogeneity (I^2 = 86%), downgrade 1.
c. Downgraded 1 for risk of bias. Confounders such as BMI and time of day (circadian rhythmicity) are not considered in all studies, and some have adjusted their results whilst others have not. The results vary significantly, indicating the method of laboratory storage, sample handling, assessment and analysis are different between studies.
d. Downgraded 1 level for inconsistency with wide confidence intervals, and outcome results presented in opposite directions.

Declaration

I, Michael Musker, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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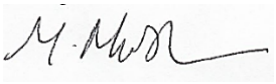
I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Signed: 

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List of Abbreviations

Table 2 Abbreviations

Abbreviation	Definition
BMI	Body Mass Index (unit kg/m^2)
CCC	Canadian Consensus Criteria
CFS	Chronic Fatigue Syndrome
CSF	Cerebrospinal Fluid
ELISA	Enzyme Linked Immunosorbent Assay
FE	Fixed Effect
FM	Fibromyalgia
ICC	International Consensus Criterion
JBI	Joanna Briggs Institute
LIW	Low-intensity walking program
ME	Myalgic Encephalomyelitis
MECFS	Myalgic Encephalomyelitis Chronic Fatigue Syndrome
mL	millilitre
NCBI	National Centre for Biotechnology Investigation
NICE	National Institute of Clinical Excellence
ng	Nanogram
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institute of Health
NW	Nordic walking program
pg	Picogram
RA	Random Effect
RIA	Radioimmunoassay
SD	Standard Deviation
SE	Standard Error
SEID	Systemic Exercise Intolerance Disease
SEM	Standard Error of the Mean
SIDS	Sudden Infant Death Syndrome
SMD	Standardised Mean Difference

CHAPTER 1

INTRODUCTION

1 Introduction

1.1 Context and Purpose of the Review

The author is a senior research fellow at the South Australian Health and Medical Research Institute and is investigating the role of adipokines (biological messengers produced by fat cells) and cytokines (biological messengers produced by immune cells) in relation to Myalgic Encephalomyelitis Chronic Fatigue Syndrome (MECFS). There have been several reviews and publications that have systematically reviewed the role of cytokines in MECFS, but none have specifically focused on the role of adipokines, and more specifically circulating leptin levels. A number of published papers have inferred that leptin plays a role in the symptom severity of both MECFS and fibromyalgia (Ataoglu et al. 2018; Cleare, O'Keane & Miell 2001; Hornig et al. 2015; Stringer et al. 2013). One author implied that the diagnosis of MECFS and fibromyalgia are often confused by clinicians as they have many similarities in syndrome symptoms (Natelson 2019). There are no current biological biomarkers that have been identified in MECFS nor fibromyalgia, hence why both conditions are considered syndromes. A syndrome is a list of symptoms that are typically found in a specific condition or illness but have not yet been fully understood to the point whereby it can be identified as a recognised 'disease' which often has clear biological causative symptoms and treatment pathways (Calvo et al. 2003). Current investigative research therefore is attempting to find specific biomarkers such as cytokines, adipokines, and inflammatory mechanisms that better explain these two disorders MECFS and fibromyalgia. Exploring the biological role of inflammatory cytokines, adipokines, and their interaction, may lead to new therapeutic interventions for people with neuroimmune type disorders (Straub et al. 2013; VanElzakker, Brumfield & Lara-Mejia 2018).

1.2 Statement of Review Question

Leptin, an adipokine, is a measurable element in our blood or spinal fluid and plays a role in the production and circadian pathways of many cytokines and bodily functions such as eating, sleeping, resting and mood (Licinio, Negrao & Wong 2014). The primary objective of this systematic review is to investigate the following question:

Is there a difference in circulating leptin levels in MECFS and/or fibromyalgia that can be used as an outcome measure in cases and controls? Leptin is measured in ng/mL and is assessed through the analysis of blood plasma, blood serum, or cerebrospinal fluid (CSF) levels (ng/mL).

Several authors have identified a correlation of symptom severity and the levels of circulating leptin in the blood (Montoya et al. 2017; Olama, Elsaid & El-Arman 2013; Stringer et al. 2013). For example, symptom severity and circulating leptin levels of participants were tracked over 25 days. Using an algorithm, the authors were able to predict high symptom severity days (Stringer et al. 2013; Younger et al. 2016). Whilst some studies indicate high leptin levels predict higher symptom severity, there are others which indicate the opposite (Olama, Elsaid & El-Arman 2013; Paiva et al. 2017). This systematic review of the evidence attempted to determine whether there is a difference in leptin levels between cases and controls and to assess the quality of that evidence.

1.3 The Science of Evidence Synthesis and Systematic Reviews

A systematic review can use several strategies to review the current findings on a given topic, with the objective of obtaining the best available information to support clinical practice and to guide the direction of future research. One exemplary systematic review looked at the outcome of infant cot deaths (Sudden infant death

syndrome – SIDS) as alarmingly in practice there had been approximately 100,000 infant related cot deaths recorded between 1950 and 1990 (Bornstein et al. 2009). This much needed review indicated that the practice of laying babies on their front side instead of their backs, a popular practice during this period of review may have led to an increase in deaths. The authors of the systematic review concluded that the evidence of using the preferred practice of laying babies on their backs reduced the risk of cot death by up to 50% and may have saved around 10,000 babies had this form of best practice been implemented earlier (ibid.).

Systematic reviews are a relatively new approach to summarise and reflect on the available evidence in the literature. Meta-analysis has been used by psychologists as a method of sifting through the evidence since the 1970's, but "systematic reviews" that use the process of pooling evidence data for analysis was not practiced until around 1995 (Cleophas & Zwinderman 2017). The practice saw the rise of new evidenced based practice centres such as Cochrane and the Joanna Briggs Institute, which now have centres and affiliates across the world. The Cochrane collaboration was named after a British epidemiologist Archie Cochrane and was formally established in 1992 by a so called 'motley crew' led by Ian Chalmers at Oxford University (Ault 2003). The Joanna Briggs Institute (JBI) was established in 1996 by a visionary of evidenced based practice Professor Alan Pearson in a collaboration between the University of Adelaide and the Royal Adelaide Hospital (Stannard & Cooper 2014). The JBI is named after the first Matron of the Royal Adelaide Hospital (1849-1866) (Jordan, Donnelly & Pittman 2006). Both the Cochrane Centre and the JBI officially formed a partnership in 2016 (Cochrane 2016), focusing on a worldwide approach to evidenced based practice, which is shared and disseminated in the journal 'JBI Evidence Synthesis'. The protocol for this systematic review was published in the same journal on 2nd November 2020 (Musker et al. 2020).

1.4 Methodological basis of the chosen approach synthesis

Systematic reviews usually follow an a priori protocol that is based on the literature for that type of review and these often use the PICO (patient, intervention, comparison, outcome) format which was proposed by Richardson et al. (1995) an editorial of American College of Physicians Journal (Eriksen & Frandsen 2018; Richardson et al. 1995):

1) the patient or problem being addressed;

2) the intervention or exposure being considered;

3) the comparison intervention or exposure, when relevant;

4) the clinical outcomes of interest.

When using the above format in the case of MECFS and/or fibromyalgia and the role of leptin, it unfolds like this:

1. Patient: A person with a formalised diagnosis of MECFS or fibromyalgia
2. Exposure: The exposure is the diagnosis of MECFS or fibromyalgia, and the exposure being syndromic symptoms, and this may include how long the person has been diagnosed.
3. Comparator: The comparison is looking at measures of leptin in people who are diagnosed with or without the syndrome (healthy people)
4. Outcome: The outcome is the level of leptin in the persons circulatory system (blood or CSF) and this is often linked to symptom severity.

A review into the role of circulating cytokines in relation to a syndrome is somewhat problematic in that it does not readily fit into a standardised systematic review approach. It best fits with the area of 'etiology and risk' and this is still an emerging framework in the context of the systematic review process (Moola, S. et al. 2015). There are no current diagnostic tests or effective treatments to compare MECFS

and or fibromyalgia studies and so this protocol followed an association review methodology. An association review looks at studies that make links between different types of evidence and may be used to develop an understanding of a disease process, diagnostic method, or in forming a prognosis. This was further elaborated by Riley et al. (2019) p. 2, who stated prognostic variables are "associated with the risk of a subsequent health outcome" for people with a specific condition. A proposed methodology for association studies was described by the JBI in 2015 (Moola, S. et al. 2015). A typical systematic review contains the following typical elements, and where possible these steps were undertaken in this thesis (Munn, Zachary et al. 2019):

(1) Formulating a review question

(2) Defining inclusion and exclusion criteria

(3) Locating studies through searching

(4) Selecting relevant studies for inclusion

(5) Assessing the quality of studies

(6) Extracting data

(7) Analysing and synthesising the results

(8) Presenting and interpreting the results and establishing confidence in the body of evidence (through systems such as GRADE, Grades of Recommendation, Assessment, Development, and Evaluation)

However, this review is somewhat atypical and was tailored to meet the diversity of the literature presented on the role of adipokines in relation to MECFS and/or fibromyalgia. There are three modes by which the research is compared. Firstly, a meta-analysis of specific measures of leptin in bodily fluids (plasma, serum, or CSF) usually recorded in the form nanograms per millilitre (ng/mL); secondly studies may vary dramatically in the way they present their findings, so a tabular synthesis of the results was necessary, and thirdly a narrative synthesis describing the relationship

between the studies and systematic review results were formulated. The above stages are provided in more detail in chapter 2.

1.5 Background

One of the challenges in undertaking this systematic review was that there are no current objective biological clinical indicators that have been identified to support the diagnosis of MECFS or fibromyalgia. The diagnosis for MECFS is completed by the patient self-reporting a series of symptoms against a list of criteria known as the 'Canadian Consensus Criteria' published in 2003 or the 'International Consensus Criterion' established in 2011 (Carruthers 2007; Carruthers et al. 2003; Carruthers et al. 2011). As there are no diagnostic markers, it was not possible to complete a 'diagnostic test accuracy' systematic review, nor are there enough randomised controlled trials that involved the use of leptin changes as a treatment. There is conflicting evidence around the role of leptin in these syndromes in that some researchers reported that circulating leptin is higher in cases than controls, whereas other researchers have claimed the opposite, and these are discussed in more detail later in the thesis. Further investigation is required to help clarify the current evidence in the literature and to support continuing research in this area.

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a syndrome that has some key major symptoms such as new unexplained headaches, self-reported fatigue, myalgias (muscle pain), post exertional malaise and unexplained pain across the body to name but a few (Carruthers et al. 2011). The widespread body pain is measured in a systematic way and is often referred to as fibromyalgia (Natelson 2019).

There is an accepted definition for ME/CFS developed by the US Center for Disease Control, which is often referred to as the 'Fukuda definition' and is frequently referenced in the literature as the mode of classification / diagnosis (Fukuda et al. 1994). However, this definition is now quite outdated and only covers the most basic

symptoms. More recent research will cite the International Consensus Criterion as a minimal standard of diagnosis. There is a comprehensive questionnaire that is lengthy (129 questions) and captures most symptoms and lifestyle issues called the DePaul Symptom Questionnaire – version 2 (Jason & Sunnquist 2018). Fukuda has quite a simple definition which describes fatigue that endures for at least six months, and for some it may last for decades. The Fukuda criterion also state that to meet diagnostic criterion, the person will also report symptoms of a minimum of **an additional four of eight potential symptoms**: Impaired memory or concentration; sore throat; tender cervical or axillary lymph nodes; muscle pain; multi-joint pain; new headaches; unrefreshing sleep; and post exertional malaise (ibid.). The Canadian Consensus Criteria is a series of assessment tools and support for diagnostic guidelines produced as a set of guidance tools for practitioners known to provide assessment advice (Carruthers et al. 2011).

The shortened term 'Chronic Fatigue Syndrome' continues to be controversial, and the use of this term alone is often questioned as to whether it best describes the disease phenomena, due to potential for misinterpretation and the resultant heterogeneity (Cathébras 2016). The heterogenous nature of ME/CFS leads to speculation that there may be several subgroups within the syndrome, which may explain why some people make an apparent recovery and others do not (Williams et al. 2017).

1.6 Fibromyalgia

A major symptom of ME/CFS is fibromyalgia and there is considerable overlap with these conditions, with patients often given both diagnoses. It is suggested that many may be misdiagnosed into either of these classifications (Natelson 2019). The classification criterion determined in 1990 for fibromyalgia is a combination of widespread pain across four quadrants of the body with tenderness in a minimum of 11 of the 18 identified tender points, and this should have been experienced for

at least three months (Clauw 2009; Wolfe, F. et al. 2019). The research in fibromyalgia and leptin may be more advanced, than in MECFS and leptin, because the diagnosis has a clear focus on the experience of physical pain. Fibromyalgia is considered to be a disease of a rheumatic and inflammatory nature and some research has reported higher levels of leptin in sufferers vs controls (Ataoglu et al. 2018). The author has published an online article during the course of this thesis explaining the symptoms of this syndrome in The Conversation "Explainer: what is fibromyalgia" (Musker & Gill 2019).

1.7 Leptin

Leptin is an adipokine, which is a hormone produced by fat cells, that influences the body's appetite by stimulating leptin receptors in the hypothalamus and other areas of the brain (Radic et al. 2003). Leptin produced by fat cells enters the circulatory system and becomes a feedback mechanism to areas of the brain, providing information on current fat storage and availability, allowing the brain to control and manage energy expenditure (Meinders et al. 1998). The effects of leptin are autocrine (effects the cell producing it), paracrine (effects neighbouring cells) as well as endocrine (effects distant cells), but also promotes the production of proinflammatory cytokines for example interleukin 6 (Sun et al. 2018). Leptin's association has been investigated in relation to ME/CFS from as early as 2001, linking the potential aetiology to hypocortisolism and other disorders (Cleare, O'Keane & Miell 2001).

Faulty leptin signalling can cause problems by promoting autoimmunity (whereby the body attacks itself), a relationship with cardiovascular issues like blood pressure changes, and more serious diseases like cancer have been reported (Zabeau, Peelman & Tavernier 2015). It is theorised that these changes in inflammatory mechanisms (the inflammasome) affect the nervous and immune systems and are evidenced by sickness behaviour in the form of fatigue and other pathogenic

symptoms (Arnett & Clark 2012). This type of fatigue like behaviour is also seen in animal research as is interpreted as a behaviour that supports rest, recovery, and recuperation, but in MECFS and/or fibromyalgia this response can unfortunately become prolonged, and often becomes permanent, to the point that it irrevocably changes people's lives for the worse (Fernández et al. 2009; Harden et al. 2015).

In 2013 Stringer et al. (2013) completed a study on 10 ME/CFS participants and 10 healthy controls, tracking their blood samples over a 25-day period. They reported that there was a significant correlation of leptin with those participants with ME/CFS and their self-report of fatigue. Using a computer algorithm to correlate leptin profiles, the team were able to predict which days the person would feel more fatigued. There have been several publications profiling the levels of leptin in humans, but there is no clarity of a 'normal' level or range of leptin. Circulating leptin levels change according to a few factors such as BMI level, the menstrual cycle and other potential confounders. This is problematic if we use a one-off sampling technique, or a frequency of one sample per day to measure leptin levels because they are known to change as part a 24 h circadian cycle (Meinders et al. 1998). Samples are often taken in the morning following a period of fasting usually between 8 and 10 am; however, this is usually the nadir (lowest level) of the leptin cycle and may not be representative of a person's daily circulating leptin levels.

Additionally, a study that examined 56 female normal subjects, aged between 18 and 25 years (26 normal and 30 overweight or obese) reported that there are three phases of mean leptin in these groups that change across the menstrual cycle: the follicular phase (mean and SD serum leptin 9.97 ± 5.48 ng/mL), the ovulatory phase (11.58 ± 6.49 ng/mL), with a peak of leptin in the luteal phase (12.52 ± 6.39 ng/mL) but a further confounder was that leptin levels were up to 50% higher in those who were overweight or obese (Rafique et al. 2018). (see Figure 1)

Figure 1 Leptin Levels in Women (aged 18-25 yrs.)

Menstrual Phase	Normal weight mean ± SD	Overweight or obese mean ± SD	p value between groups
Follicular phase (F)	8.38 ± 4.58	11.35 ± 5.89	0.02
Preovulatory phase (PO)	9.67 ± 5.28	13.24 ± 7.04	0.018
Luteal phase (L)	10.39 ± 4.40	14.37 ± 7.29	0.027

Adapted from Rafique et al. (2018)

This is further evidence that taking a sample at one single time point rather than multiple samples across the day, may not be the best method of calculating an individual's circulating mean leptin. It also indicates the importance of BMI on the experimental outcomes. An important landmark study by Licinio et al. (1997) (n=6 healthy men, aged 23-43) took blood every 7 min, providing objective evidence that normal leptin changes occur across a 24-h period and that leptin levels have a pulsatile nature. Astonishingly they found that leptin could show a seven-fold difference (higher) in obese participants. The frequency of pulses is consistent across weight ranges at 32 ± 1.5 pulses every 24 h which equates to each pulse lasting 32.8 ± 1.6 min. That is, leptin has small peaks and troughs or 'pulsatility' and the overall level of circulating leptin gradually goes up to a zenith (peak) during the evening and overnight (between 8 pm and 4 am) with a nadir (trough) in the early morning (8 am to midday), gradually changing over the 24-h period in an upward trajectory, demonstrating a pattern of diurnal variation (Licinio et al. 1997). The mean plasma leptin across the 6 male participants across the 24-h period was 4.44 ± 1.81 SEM ng/mL with a mean range of 0.62 ng/mL to 13.11 ng/mL. There was also a pulse height provided which had 5.94 ± 2.90 (Mean ± SEM) within the same mean range.

It has been suggested that the pulses seen in leptin levels are effected by extrinsic factors, that is they are not controlled by the adipose tissue but instead they have a complex relationship with, and are influenced by, other circulatory biofeedback mechanisms such as the levels of growth hormone, cortisol and insulin (Koutkia et al. 2003).

In another sex inclusive study that measured both normal men and women and an obese group of men and women, it was noted that serum leptin is higher in obesity, and significantly higher across genders in both normal and obese women (Al-Sultan & Al-Elq 2006). This unique paper provides a good benchmark as it includes age, BMI, gender, and waist-hip ratios as well as blood pressure levels. Here is the data in a table for comparative reference with other studies (See Table 3):

Table 3 Serum leptin in healthy men & women

	Normal Weight		Obese Weight	
	men	women	men	women
n=	20	23	25	21
serum leptin mean ± SD ng/mL	2.2 ± 0.3	8.8 ± 2.1	12.5 ± 2.2	23.0 ± 4.0
Age	25.8 ± 5.3	23.9 ± 1.9	29.4 ± 7.6	28.8 ± 6.2
BMI	23.1 ± 1.4	23.0 ± 1.8	35.5 ± 5.7	35.6 ± 4.4
Waist	81.2 ± 7.3	72.8 ± 8.9	109.8 ± 13.1	100.0 ± 13.4
Hip	100.9 ± 15.6	95.9 ± 7.2	118.7 ± 13.1	119.6 ± 11.7
Waist Hip Ratio	0.818 ± 0.116	0.758 ± 0.066	0.929 ± 0.063	0.836 ± 0.080

The circadian changes in leptin are just one element our body uses to keep time to assist with controlling energy expenditure, sleep, and weight. Perturbances in this system such as disruptions in sleep, work patterns and stress can cause a jet lag like effect throwing these mechanisms into desynchrony and may be the causal link to a variety of health problems (Dibner & Gachon 2015).

1.8 Prevalence and Current Findings

The prevalence of ME/CFS is around 14.8 (95% CI 14.5, 15.1) per 100,000 according to an estimation from an analysis of general practitioner visits in the UK (Collin et al. 2017). A meta-analysis found a pooled prevalence estimate of 0.76% (95% CI: 0.23–1.29) for the general population who were identified through clinical diagnosis for the condition (Johnston et al. 2013). The Center for Disease Control in America indicate there are around 836,000 to 2.5 million people with ME/CFS costing the economy about $24 billion annually (CDC 2019). ME/CFS is a syndrome without any definitive diagnostic test to confirm its presence or to what degree the person is affected by the illness. There is an apparent stigma associated with the illness because there are no obvious biological markers that indicate a prognosis or cause (McManimen et al. 2018; Wessely 2002). Society is more tolerant of other forms of sickness that can be medically identified and have known causes, for example, if we look more closely at other forms of sickness that cause ongoing fatigue like the Epstein Barr virus or glandular fever, these can be easily identified by testing for the specific viral reaction through a diagnostic test that identifies the virus (antibody test – viral load or titres)(Kristiansen et al. 2019). The prevalence of fibromyalgia is estimated to be between 0.2 and 6.6% in the general population (Marques et al. 2017). Both MECFS and fibromyalgia syndromes are considered to be at a higher frequency in women than in men, around 90% being women with fibromyalgia and similar findings have been seen in some MECFS studies (Wolfe, F. et al. 2018). For example one MECFS study has 1309 participants of which only 9.1% (n=119) were men and of these 1309 participants, 29% of the men had fibromyalgia compared to 58% of the women (Faro et al. 2016).

A systematic review was completed by Blundell et al. (2015) to examine if there were any studies of cytokines that showed an association with ME/CFS entitled ‘Chronic fatigue syndrome and circulating cytokines: A systematic review’ (Blundell et al. 2015). The review authors identified 38 papers that reviewed 77 circulatory cytokines reporting the results as a combined effect of whether an investigated

cytokine showed any difference in participants or controls. This review reported the outcomes of cases vs controls based upon one of three possible states; higher cytokine levels in cases; lower in cases; or no significant difference between cases and controls. The pleiotropic cytokine TGF-β was the only major cytokine identified as being higher and this was seen in 63% of cases across the included studies. This is a multipotent cytokine that has both pro- and anti-inflammatory effects on the immune system (Sanjabi et al. 2009). Since the Blundell et al. (2015) systematic review was published four years ago, there have been further reports investigating other potential associations between leptin and MECFS and/or fibromyalgia with an inference that leptin may play a role in influencing proinflammatory cytokines (Montoya et al. 2017). The role of these cytokines has previously been linked with pain, inflammation, depression and autoimmune responses (Miranda et al. 2018). Many studies have examined both leptin and proinflammatory cytokines, so a more in-depth, systematic meta-analysis that focuses on the difference that the measurement of leptin would make is of value, as it is often lost amongst a group of analytes with few details being reported, yet it may be the circadian controller of these analytes.

A preliminary search of PROSPERO, MEDLINE, the Cochrane Database of Systematic Reviews and JBI Evidence Synthesis (formerly the JBI Database of Systematic Reviews and Implementation Reports) was conducted for any similar systematic reviews. A total of 124 systematic reviews were listed in the PROSPERO database (searched 15-10-2019) but the majority were unrelated. One systematic review and meta-analysis comparing patients with controls was commenced in 2015 and had an end date of July 2018 is listed as ‘review ongoing’ (PROSPERO ref: ID=CRD42018065182). This review has now been published (Strawbridge et al. 2019). Our proposed protocol differs in that it only reviewed those studies that focus on the analysis of leptin, and which provide the raw leptin levels in their original units of ng/mL. There were only 3 articles listed in Cochrane for “Chronic Fatigue” and 40 for fibromyalgia; all of these were unrelated to our proposed protocol. Most of the fibromyalgia proposals focused on medication such as pain relief (searched 15-10-2019).

A systematic review was completed in March 2015 (Blundell et al. 2015), but within this review only one cited study mentioned the role of leptin in ME/CFS. The cited study showed that leptin was higher in ME/CFS participants compared to controls, indicating that there was a potential inter-cytokine correlation with other regulatory networks (Hornig et al. 2015). Conversely, a meta-analysis that reviewed 64 cytokines including leptin relied on p-values to compare studies and found the use of cytokines as a biomarker was inconclusive; however, the review did not include studies on fibromyalgia (Corbitt et al. 2019). Another systematic review that reviewed inflammatory proteins in ME/CFS did not include leptin but did find some support to indicate an inflammatory component in CFS (Strawbridge et al. 2019). We did not find a systematic review that compared leptin in both ME/CFS and fibromyalgia as a combined group, but previous researchers have combined the two syndromes to investigate cytokines such as C-Reactive Protein, and others have hypothesised that it is a unified syndrome as there are few differences between them (Abbi & Natelson 2013; Groven, Fors & Reitan 2019).

1.9 The Scope of the Current Literature on MECFS and Fibromyalgia

Clinical researchers have taken a varied approach in tackling the etiology and risk factors of MECFS and fibromyalgia. MECFS is classed as a neurological disease by the World Health Organisation and has been since 1969 (Maes et al. 2013). The name itself holds controversy and has many synonyms, but the most consistent name appears to be a blend of Myalgic Encephalomyelitis and Chronic Fatigue Syndrome. The names used are diverse for example ‘Systemic Exercise Intolerance Disease’ (SEID) which was suggested by the Institute of Medicine in 2015 (Clayton 2015; IOM 2015; Twisk 2016a). Neurasthenia (or nerve weakness) is thought to be an early conceptualisation of MECFS, a condition described by an American physician George Beard as early as 1869 (Bynum 2003).

The name SEID remains controversial with much of the scientific community and consumers seemingly not accepting the label, and it appears to be used more as

another synonym for MECFS, but we have not seen its wholehearted adoption in the literature or the community (Jason & Johnson 2020). A similar reaction occurred around the time of moving from the term 'Myalgic Encephalomyelitis' to using the term 'Chronic Fatigue Syndrome' (Prins, van der Meer & Bleijenberg 2006). To add further confusion, there is also the London Criterion for ME which has been shown to be more stringent than the International Consensus Criterion as ME requires a higher frequency of some symptoms (Sunnquist et al. 2017). Many practitioners even question whether ME or CFS exists and ME seems to have been born as a UK-centric concept but is now widely accepted (Mouterde 2001; van Houdenhove 2001). Debate following the introduction of the term CFS caused great ire, so now both terms are used and are often referred to in the literature as "CFS/ME", "ME/CFS" or MECFS (Clark et al. 2002). At a similar time, controversy around treatment approaches also occurred involving treatments like Graded Exercise Therapy, Cognitive Behaviour Therapy and pacing, widely known as '**The Pace Trial**' (White, Goldsmith, Johnson, Potts, et al. 2011; White, Goldsmith, Johnson, Walwyn, et al. 2011). This was a randomised controlled trial that indicated a combination of graded exercise and Cognitive Behaviour Therapy provide beneficial outcomes, but consumers were left feeling that they were being told that they should just 'do more exercise', and everything will be fine. This made them feel even further disenfranchised and distressed.

Fibromyalgia only has one name, but historically has had other potential candidates like 'fibrosis of the musculature', 'muscular rheumatism' and 'Syndrome of Myalgic Encephalomyelopathy' (Hartrick 2008; Leonhardt 2000; Mehendale & Goldman 2002). One author has described and tabled some of the similarities of fibromyalgia and ME symptoms due to the similarity in nature of the syndromes sharing myelopathic, myopathic, encephalopathic, and neuropathic symptoms (Mehendale & Goldman 2002). The term 'fibromyalgia' is well recognised in the community and is considered to be a rheumatic condition and treatment is centred in the field of rheumatology, but even this diagnosis comes with its own controversies such as suggestions of misdiagnosis and the way people are treated is thought to be affected by cultural and social forces (Natelson 2019; Wolfe, F. et al. 2019).

There are many similarities and shared symptoms between MECFS and fibromyalgia particularly with the neurological issues of fatigue, the ‘Fibro fog’ or ‘brain fog’, and pain sensitivities to name but a few (Farhad & Oaklander 2018). MECFS is often comorbid with Fibromyalgia, one study indicating around 60% (n=30) of 48 MECFS patients, and this combination of symptoms usually leads to a picture of more severe outcomes of pain and increased fatigue in this group which can result in a condition known as kinesiophobia (fear of movement to avoid pain) (Meeus et al. 2016).

Both MECFS and fibromyalgia have been the focus of previous systematic reviews that have examined the role of cytokines, but none of these have reviewed the role of adipokines (Strawbridge et al. 2019; Uçeyler, Häuser & Sommer 2011). The author is unaware of any systematic review that has examined the role of leptin and its association with both MECFS and fibromyalgia combined. There is a study on leptin and ankylosing spondylitis (fusing of small bones in the spine) which provides a similar approach for this type of systematic review, that is it compares the evidence using the difference of leptin levels between patients with ankylosing spondylitis and healthy controls. (Mei et al. 2016). It is worthy of note that none of the articles identified in the full text review of the Mei (2016) analysis have been found in the systematic search in this study, that emphasises that they are unrelated areas of disease. The result of that review found 8 studies which were used in a meta-analysis that showed no significant difference between cases and controls.

Circulating leptin plays a major role in the homeostasis of adipose tissues within the human body, but it also influences several other systems including the brain, immune system, and insulin balance (Friedman & Halaas 1998). It has been investigated for its role in many diseases and the evidence about its effects on the body continue to emerge, such as leptin’s role in energy expenditure or osteo-arthritis (Bi, Loo & Henry 2019; Kelesidis et al. 2010; Yan et al. 2018). Several research studies have reported the association of leptin and MECFS, but they vary in their outcomes, creating confusion for the medical community (Cleare, O'Keane & Miell 2001; Stringer et al. 2013). Similarly, several research studies with fibromyalgia report both hyperleptinaemia (high leptin) and yet other studies the

opposing results were found suggesting hypoleptinaemia (low leptin) in participants with Fibromyalgia (Homann et al. 2014; Olama, Elsaid & El-Arman 2013).

These studies use similar methods in defining protocols and scientific analysis, for example they use blood samples to detect the circulating leptin levels using enzyme linked immunosorbent assays, providing a quantitative measure through spectrophotometry (Aydin 2015), yet there are many nuances between these studies. A simple example is the time of day that the samples were taken, and another is that they use different sample 'fluid types' including serum, plasma, and CSF. One of the leading authors in this field, Keiji Fukuda, expressed the need to investigate these syndromes systematically to avoid the problems of heterogeneity due to its overlapping symptoms with other disorders such as depression and fibromyalgia (Fukuda et al. 1994). The difference in practice and methodologies are explored further and discussed in more detail later in section 1.11.

1.10 Relationship Between Existing Literature and the Proposed Review

There are a number of papers inferring a strong link of symptom severity and leptin, starting with Cleare, O'Keane and Miell (2001), who reported an association with hypocortisolism and circulating leptin, which they investigated using a placebo-controlled crossover study between cases and matched controls. They found no basic differences between cases and controls but did notice that there were changes in leptin levels in response to injections of hydrocortisone, suggesting that this response may provide an avenue for further investigation. This was followed by another significant study from Stringer et al. (2013) who took the novel approach of taking a sample from individuals every day for 25 days, rather than a one off sample. The outcome of their study was that they reported leptin as a potential driver of cytokine changes associated with outcome severity. They use the data to show that self-reported fatigue severity was significantly correlated with leptin levels. They associated the results using a machine learning algorithm to determine the outcome that symptom severity could be predicted by circulating leptin levels. One of the problems with this study is that at no time in the paper do they provide actual raw circulating leptin results, only correlations and diagrams of their association. There

is limited information about the algorithm used to calculate these outcomes. This paper is cited in almost every subsequent MECFS study and according to SCOPUS (searched on 16/11/2020) it has been cited 54 times (37 of those in the last 5 years) and has a journal citation impact factor of 2.18. The corresponding author was contacted by email to see if they could provide any raw leptin results to support their study outcome (Younger, J.W.; Department of Anaesthesiology, Stanford University, School of Medicine, United States; email: jyounger@stanford.edu), however no response has yet been received. A follow up email was attempted to a more contemporary email address but without response (younger@uab.edu). The landmark study was performed 8 years ago.

A later study by Montoya et al. (2017) indicated that MECFS symptom severity had an upward linear trend in association to 17 cytokines, one of which was leptin. This was another example of a project from the team at Stanford University School of Medicine, and again in this study, the authors only provide correlations to these cytokines with no actual raw circulating leptin levels; i.e., plasma levels in nanograms per millilitres (ng/mL) are not stated. An online link to a supplementary index is provided within the article, but this only has a list of spearman correlations and significance values (p). The Montoya paper, according to SCOPUS, is also highly cited with 102 citations (all within the last 5 years - searched on 16/11/2020). The corresponding author was contacted with no reply (Email: mmdavis@stanford.edu.).

Several other more transparent studies do provide the baseline levels of circulating leptin and standard deviations between cases and controls which enabled this meta-analysis to be completed. There are only minimal MECFS papers available to review and compare these types of results (4 studies in MECFS), but auspiciously similar studies have been completed in the area of fibromyalgia (8 studies). The combination of results (12 studies) of these two syndromes provides a more comprehensive picture and more thorough meta-analysis. The symptoms of these two syndromes, MECFS and fibromyalgia have been considered by some authors to be aligned, but no meta-analysis results have been published combining them in this way. There are some published articles identifying that symptoms across the

two syndromes are so similar to the point whereby a diagnosis of either syndrome may be made by a practitioner, inferring that doctors may be misdiagnosing patients (Abbi & Natelson 2013; Natelson 2019). It is not surprising as it has been reported that up to 75% of patients with MECFS also have comorbid fibromyalgia (McManimen & Jason 2017).

There are many examples of studies that combine the two disorders to assess other biological outcomes, but not using leptin as their focus (Alameda Cuesta et al. 2019; Almenar-Perez et al. 2019; Amel Kashipaz et al. 2003). Therefore, it is appropriate to examine the role of inflammatory biomarkers such as leptin across these two syndromes.

1.11 Measuring Human Leptin and Issues of Heterogeneity

The overarching aim of this study was to examine whether there is a difference in leptin levels in people experiencing MECFS and/or fibromyalgia compared to a control group of healthy individuals. Before we can answer this question, we need to look at how the units of circulating leptin are measured and what is the current evidence. This question presents issues with multiple confounding factors because people's leptin levels may differ for many reasons and they interact with other bodily systems (see Figure 2). There are limited studies across ethnicity and culture that have measured leptin but one study of 1,176 subjects demonstrated there was a difference between baseline fasting levels of circulating leptin measured in ng/mL between European n=312 (9.21 $\pm$ 0.85, mean $\pm$ Standard Error of the Mean [SEM]), Chinese n=303 (8.25 SEM 0.77), South Asian n=317 (11.82 SEM 0.94), and Canadian Aboriginals n=244 (11.13 SEM 1.21) and in each ethnicity women had 3 times higher mean leptin than men e.g., European women 13.6 - SEM 1.36 compared to men 5.93 - SEM 0.86 (Mente et al. 2010). Therefore, gender is another major confounder, but this has been handled by most of the included studies by excluding men (7 out of 12) or by including fewer men than women, but this is

inconsistent across articles and across cases (20/32 females) vs controls (18/32) e.g., Cleare, O'Keane and Miell (2001).

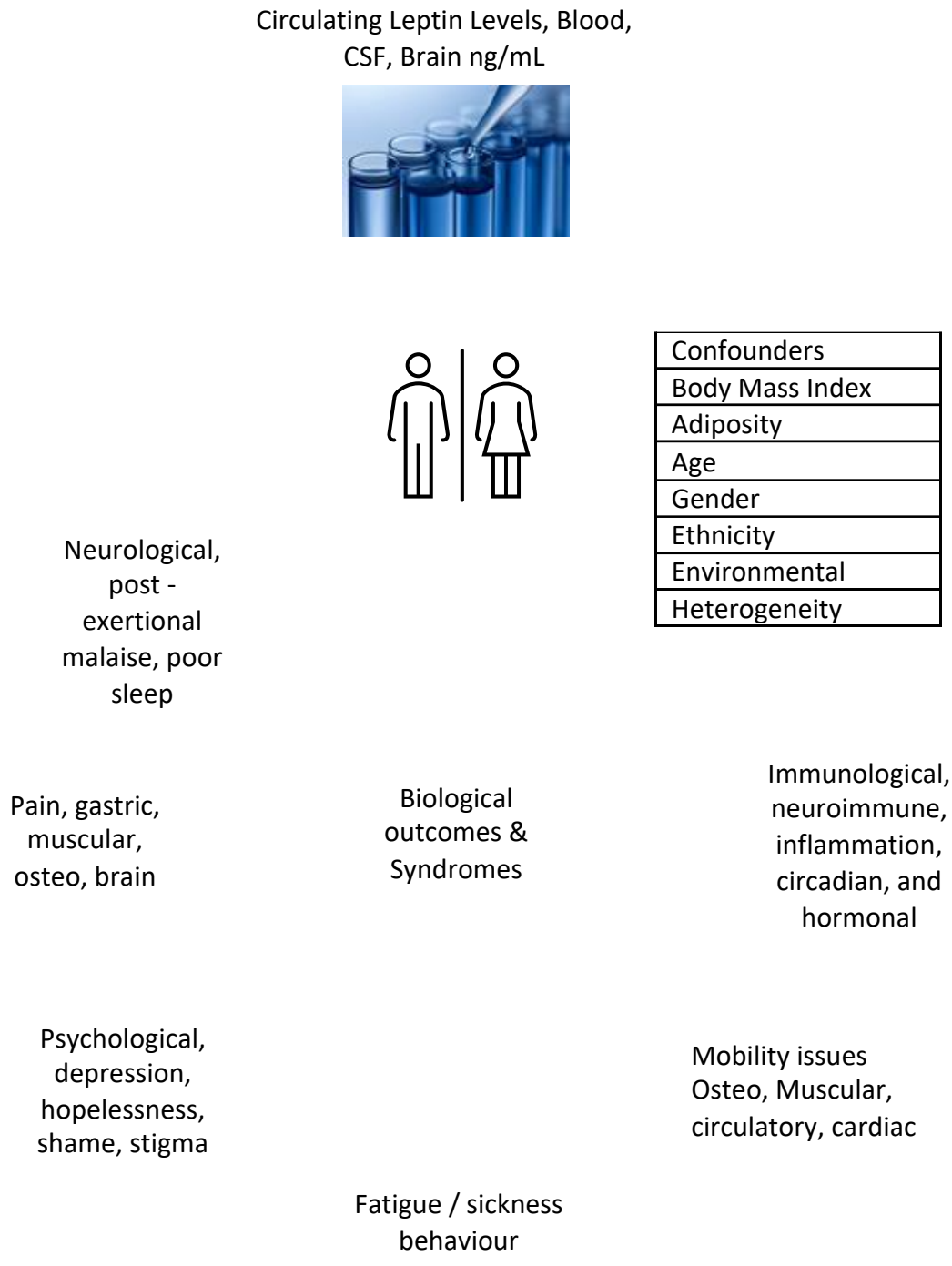


Figure 2 Conceptual Model of Leptin and MECFS and/or Fibromyalgia

The ethnicity study used a specific laboratory technique to measure the levels of leptin in serum called an ‘Enzyme Linked Immunosorbent Assay’ (or the commonly abbreviated acronym ELISA) which comprises a process of using antibodies and antigens to support the detection of leptin levels. Caution is required, however, as some studies have used an older system called radioimmunoassay which may have a more sensitive detection level and therefore provide a different magnitude of results (Kimura, E et al. 2000). Lastly there are the multiplex type assays which combine the analysis of many cytokines on one laboratory plate, making the process very efficient, but not as accurate. These multiplex systems examine between 51 and 72 analytes all at once, but these are thought to provide different results than an individual cytokine/adipokine analysis, potentially being less sensitive, when compared to the other methods (de Koning et al. 2012). It begs the question of whether we can make comparison across such studies.

People who are diagnosed with MECFS and/or Fibromyalgia experience many investigative tests, looking for answers and often receive hundreds of tests to investigate their symptoms (Park, JP 2012). This leads to thoughts of not knowing what is wrong with their body. Their experience makes them wonder if they will ever get to the bottom of what is causing their sickness, fatigue, pain and a myriad of other symptoms such as post exertional malaise (ibid.).

With so many potential differences in results between studies because of the specific methods and laboratory techniques that have been used, results may also be affected by the skills of the laboratory personnel. Laboratory skills include the ability to measure samples accurately using pipettes, micropipettes or by machines using an automated process to provide precise, replicable volumes. An example is the JANUS Automated Workstation (produced by Perkin Elmer) which provides precise dilutions and reduces the potential for human error (Enten et al. 2016). Many hours are required (up to one whole working day (8 hrs) to work through the ELISA process, and includes around 25 process steps or actions (a clear detailed description of these processes is provided below – See Figure 3), but one of the most frequent and important steps is washing the plates between solutions and this can be done in a uniform way by machines or more haphazardly by hand (Chiswick

et al. 2012). These minutiae but important details are not provided in any of the papers investigated in this systematic review, and the practicality of research laboratories using the same techniques, the same antibody solutions, and the same methods of measurement are unlikely to eventuate unless an international standard is outlined. However, if the heterogeneity of results is to be reduced, we must work toward this type of consistency wherever possible.

A Typical Laboratory Practice Protocol – Adapted from (Chiswick et al. 2012)

1. The ELISA plates must first be printed with the capture antibody specific for the cytokines of interest. The printing process is specific to the printer used, and the manufacturer's recommendations should be followed – example follows.

2. Incubate the printed plates overnight at 4°C.

3. Block the plate with 150 µL/well of blocking buffer for 1 h.

4. Standard cocktail preparation: Using dilution buffer, mix each recombinant cytokine / adipokine of interest into a heterogeneous cocktail. A 16-point standard curve (including the blank) that begins at 50,000 pg/mL/cytokine followed by twofold serial dilutions are enough (i.e.: dilutions of 1×, 2×, 4×, n×., 16,384×, or, 50,000–3.05 pg/mL). This may differ according the analyte being measured.

5. Incubate standards, samples, and the detection AB cocktail as with the standard ELISA procedure. For the sake of simplicity, dilute all detection ABs into the cocktail at the same concentration.

6. After the detection cocktail incubation is complete, wash the plate and add the streptavidin dye conjugate diluted in dilution buffer, 50 µL/well, and incubate for 30 min in the dark.

7. Wash the plate and dry thoroughly by spinning upside down in a centrifuge. Alternatively use a paper towel and tap gently on against a hard surface.

8. Inspect surface of plate for fingerprints or other optical obstructions. If necessary, clean the bottom of the plate and then place into the scanner.

9. Scan and analyse as suggested by the specific system used.

10. See Figure 3 for technical sequence – note this is just an example:

Schematic depicting the time arrangement of a simplified sequential ELISA protocol of exemplary targets analyzed in three subsequent cycles

	Previous day capture incubation overnight		
Time (h)	**Regular ELISA cycle # 1**	**Sequential ELISA cycle # 2**	**Sequential ELISA cycle # 3**
0.0	Blocking		
1.0	Samples		
2.0	Incubation	Blocking	
3.0	Detection ⇒TRANSFER SAMPLES[a]	TO CYCLE # 2 ⇒Samples	
4.0	Incubation	Incubation	Blocking
5.0	Streptavidin–HRP	Detection ⇒TRANSFER SAMPLES[a]	TO CYCLE # 3 ⇒Samples
5.5	TMB	Incubation	Incubation
6.0	Reading	Incubation	Incubation
7.0		Streptavidin–HRP	Detection ⇒REMOVE AND STORE SAMPLES[b]
7.5		TMB	Incubation
8.0		Reading	Incubation
9.0			Streptavidin–HRP
9.5			TMB
10.0			Reading

Figure 3 ELISA Protocol Adapted from (Chiswick et al. 2012)

The laboratory methodology above is just one example of the potential differences in technique that can affect the way results are reported and it could be argued that individual results can only be compared within a study or laboratory and not between studies. If everyone were to follow the same exact protocol or at least provide information of what steps were taken in the analysis and use the same equipment, this may enable cross laboratory comparison and reduce heterogeneity in results.

Other potential anomalies which focus on the individuality of the person being investigated, highlight many differences that can explain the increased heterogeneity. One of the most basic differences in people is body mass index (BMI) which is used to calculate the level of fat mass when comparing people as part of a general population. We now use well known internationally recognised BMI subgroups to explain this aspect of heterogeneity. Under 18.5 BMI (kg/m^2) is considered to be underweight, between BMI 18.5 and 24.99 is considered to be the normal weight range, BMI 25 to 29.99 being overweight and BMI 30 to 34.99 being obese class 1 and between BMI 35 to 39.99 is class 2 (severe obesity) and over BMI 40 is class 3 (morbid obesity), and the most extreme is referred to as 'severe morbid obesity' at over BMI 50 (Abdelaal, le Roux & Docherty 2017; AIHW 2017). (See Figure 4)

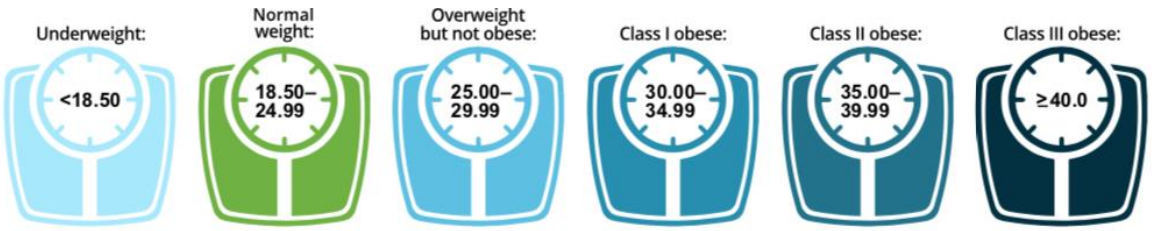


Figure 4 Obesity Scale adapted from the Australian Institute of Health and Welfare (AIHW 2017)

The research on leptin has consistently reported that BMI is positively correlated with leptin levels, because the more body fat you have the greater the potential quantity of leptin those cells are able to produce. The lower the fat mass the lower level of leptin produced (Friedman 2011). Measuring the fat content in your body using BMI, which only requires simple tools like a stadiometer and clinical weighing scales, serves only as an approximation of adiposity. There is a procedure known as Dual Energy X-RAY Absorptiometry (DEXA or DXA), which uses two beams of x-rays at different frequencies (high and low) to highlight the difference between tissues such as bone, muscle and fat (El Maghraoui & Roux 2008; Kuriyan 2018). More accurate studies use this technique rather than just relying on the

approximation using BMI. BMI is weight divided by the square of height (BMI=weight/height^2).

People also differ in their lifestyles including diet, sleep patterns, and activity, all of which affect circulating leptin levels, hormones, and immune responses (Nieman & Pence 2020). Many of the articles identified in the twelve case control studies refer to a 'fasting period' prior to the sample being taken, but also provide a 'time window' of when the sample was taken e.g., between 9 am and 10 am, but not all studies provided these details. These factors are important because leptin is known to have a circadian pulsatile like pattern across a 24-h period, being lowest in the morning, which is the bodies way to signal the need for activity and food seeking, and leptin levels are highest at night which encourages reduced activity, reduced food intake and the need for sleep (Licinio et al. 1997; Licinio, Negrao & Wong 2014). In other words, it provides biological cues of when to get up and be active and when to sleep.

Some studies take samples across different days, but this can be problematic as it has been shown that with healthy subjects the inter-day difference can vary from 10.9% and up to 22.5% (Ma et al. 1996). To counter these pulsatile changes and potential inaccuracy Licinio et al. (1997) estimated that samples are required to be taken every 7 min across a 24-h period.

Other examples that can cause confounding between studies include additional sample processing issues such as how long samples have been stored in refrigerators before analysis, at what temperature they were stored (-20 or -80 °C), and how quickly they were frozen or even defrosted (Mitchell et al. 2005). These issues have been highlighted in the media with concerns over the integrity of Covid 19 Virus Vaccines that have to be stored at -80 °C degrees. There is also the problem of the type of body fluid used. If serum is used, then a period of 30 min at room temperature is required prior to the sample being centrifuged, but the exact time is rarely reported (Rai et al. 2005).

It is important to provide standardised details of all these timings of sample processing such as the time of day that the sample was taken, the amount of time it took to store and freeze samples and other processing factors that may create anomalies (Mischak et al. 2007). It has been shown that even minutiae detail like

the centrifugal force and length of spin can affect the outcome of results and so it is recommended that the standard of <=1300 *g* for 10 min should be used for both plasma and serum to ensure consistent results between studies (Rai et al. 2005). In the articles examined in this review some samples have been frozen at -20 °C and some at -80 °C, but most papers have not indicated how long the samples were stored prior to thawing for analysis and this may affect results and another concern about the ability to compare across studies (Grizzle et al. 2005). Frozen samples may be stored for many years and just like a good wine they can go off (become less representative) during storage. A length of storage prior to analysis should be declared.

It can also make a difference in how much the samples are diluted with buffers prior to using either an ELISA kit or radioimmunoassay kit which can create inaccuracy such as background noise, false positive reactions, and negative results (Waritani et al. 2017). Then there is the type of ELISA kit, microplates, and processing fluids used as there are a number of commercial varieties available (Trask 2018). Very few studies provide the detail about the microplate reader or software that was used, and few have described the logarithmic calculations used to convert the raw reading result to the final actual ng/mL outcome. These conversions are usually completed by software built into the microplate reader or by using an online conversion program.

Many of these laboratory components are described in the NIH (National Institute of Health)'s 'The Assay Guidance Manual' which explains the differences in some of these equipment and methods (Jones, Michael & Sittampalam 2004). This systematic review has only examined the difference in baseline leptin, but clearly the handling of samples and type of samples can cause changes in the results and outcomes of research studies that attempt to explore changes in leptin over time and against environmental variables like smoking, exercise and diet. A systematic review will rarely inter-study to these anomalies.

We have not yet considered the heterogeneity within the cohort with reference to the diagnosis of each syndrome MECFS and/or fibromyalgia. We earlier discussed the differences in these syndromes, but the diagnosis of either of them relies on self-

reported symptoms and not on biological biomarkers or objective physical measures. This alone creates problems with diagnosis and results in a commonly reported level of heterogeneity (Lane et al. 1998; Sullivan et al. 2005; Williams et al. 2017; Wright 1992).

1.12 Heterogeneity

Heterogeneity can be defined as:

"**The extent to which observed effect sizes differ from one another. In meta-analysis, statistical tests allow for the assessment of whether the variability in observed effect size is greater than would be expected given chance (that is, sampling error alone).**" (Cooper, Hedges & Valentine 2009) p.576

It should be noted that there are many explanations for the cause of heterogeneity, this includes clinical, methodological and statistical heterogeneity. The heterogeneity of a meta-analysis is often expressed using I^2 and this is the "proportion of total variation in study estimates that is due to heterogeneity" calculated by a transformation of H (square root of $\chi 2$ divided by the degrees of freedom) (Higgins & Thompson 2002). This is obtained through a series of statistical calculations that require Cochran's homogeneity 'Q statistic' which is a calculation of weight squared deviations, T^2 a measure of the between studies variance squared, T is the between study standard variation, and I^2 which is "the ratio of true heterogeneity to total observed variation" (Bornstein et al. 2009) p105.

Heterogeneity within prognostic studies should be expected because they are generally exploratory in nature so there is no set format unlike other typical systematic reviews that examine the differences in a common treatment effect, frequently using randomised controlled trials (Riley et al. 2019). It is suggested by these authors that a random effects model is invariably used with prognostic types of meta-analysis as the research methods used often differ significantly. When using a random effects model it is common to provide an across study variation

statistic Tau^2 which is an estimate of the standard deviation between studies of the underlying effect across those studies, squared (Biggerstaff & Tweedie 1997; Higgins JPT et al. 2020).

One of the difficulties in studying syndromes like MECFS and/or fibromyalgia is that a diagnosis is made on self-reported symptoms and an assumption that these symptoms equate to an identifiable illness but there are opposing reports of overdiagnosis and underdiagnosis of both syndromes (Fitzcharles & Esdaile 1997; Hauser, Sarzi-Puttini & Fitzcharles 2019; Son 2019; Wolfe, F. et al. 2019). Even more disconcerting is that some general practitioners find the illness contentious and for some patients an inappropriate diagnosis is made. It is not uncommon that patients are mistakenly given a diagnosis of MECFS when the person actually has fibromyalgia, or vice versa (Bayliss et al. 2016; Natelson 2019). Fatigue as a singular symptom is very common, with over 24% of the general population reporting at least 2 weeks of fatigue and with two thirds of those having no identifiable medical cause (Fukuda et al. 1994).

In this systematic review we have combined both disorders MECFS and fibromyalgia, investigating them as similar inflammatory based entities that have similar pathological effects on the neuroimmune system, and it is worthy of acknowledgement that fibromyalgia is a common and comorbid symptom of MECFS (Bjorklund et al. 2020; Kristiansen et al. 2019). These syndromes are both chronic autoimmune diseases that cause unexplained pain, fatigue, psychological changes and significant changes in biology creating potential opportunities to identify diagnostic biomarkers (Giacomelli et al. 2019; Hornig et al. 2017). As described in the previous section, not only is there heterogeneity in the diagnostic population, but also in sub populations, and in the methodologies used to investigate group differences (Williams et al. 2017).

1.13 Biomarkers

Biomarkers, short for biological markers, are the medical signatures produced by the body that enable us to detect disease and also assist in reporting the effectiveness of treatments (Strimbu & Tavel 2010). The NIH is one lead American agency that provides guidance and standards in the form of a handbook for the exploration of biomarkers and their assessment (Auld et al. 2004; Jones, Michael & Sittampalam 2004). The NIH provides advice and information describing the latest research about biomarkers, exposure science, and exposomics which are available online from a sub-branch of the NIH called the National Institute of Environmental Health Sciences (NIEHS 2020).

The area of MECFS and fibromyalgia as previously explained is already plagued with the problem of heterogeneity within the sample population, making it imperative that the science provides solid foundations for researching this field by making inroads to reduce the differences in study design and methodology. MECFS and fibromyalgia are not the only topic areas that suffer from these disparities and there are international efforts to coordinate systematic investigation into biomarkers and blood based analytes (Chiswick et al. 2012; Mitchell et al. 2005).

1.14 Assumptions and Limitations

Assumptions

The author of this review assumed that we can combine the results of studies that examined circulating leptin levels in both MECFS and fibromyalgia as if it were one syndrome. It is also assumed that healthy humans (controls) would have similar mean levels of circulating leptin to enable comparison. There are limited studies that have provided such baseline leptin levels for MECFS participants, with only 8 of the 24 studies selected reporting baseline leptin levels, and just 4 of these were case-control studies. There were 15 of the 24 studies on fibromyalgia with baseline

leptin results, but only 8 of these could be used as case-control studies because the others did not provide a control arm. The remaining studies provided a cumulative group of 12 case-control studies for the meta-analysis. One of the full text studies Kurajoh et al. (2016) only referred to fatigue and didn't refer to MECFS or fibromyalgia other than in a reference. In summary, after separating out the case control studies from the cohort type studies, this resulted in only 12 studies being used in the meta-analysis (**4 MECFS, and 8 fibromyalgia combined**) (See Chapter 3 for the full meta-analysis). There were 9 remaining studies in those with a cohort design (**1 MECFS, and 8 fibromyalgia**) which could not be combined, so their results are tabled individually with a brief narrative reflection on each study. (See Chapter 4 – Cohort Studies).

Limitations

There are no currently accepted objective diagnostic biomarkers for MECFS nor fibromyalgia and so if there were a significant difference between cases and controls then this would only create a starting point for future investigative research. That is, it would support the assumption that inflammatory mechanisms are at play, and that leptin is a worthy biomarker for further investigation. Leptin has varying known effects on other cytokines networks, adipokines, and immune cells, which are reported elsewhere in this thesis. Leptin's influence may therefore have far reaching consequences as a biological mediator in both MECFS and fibromyalgia syndromes that are of interest and are a logical place to start biomarker investigation (Ataoglu et al. 2018; Montoya et al. 2017). The heterogeneity issues have been described in detail in the previous section and will be further highlighted in the final discussion. Many confounders have not been managed with the same detail or consistency across the studies examined and these factors may have consequences for the accuracy and outcome of this meta-analysis.

1.15 Definitions of Terms and the Controversy of Syndrome Names

The condition of Fibromyalgia does not have any other confusing terms and it is a syndrome that was described in the International Classification of Diseases (ICD) 10 in 1994, but remains controversial to the point that strong debate about the diagnostic legitimacy have been called the '**Fibro wars**' (Hauser & Fitzcharles 2018). Not so straight forward, MECFS has had many names across the years and the table below (Table 4) is a list of some of them were presented in the literature. These labels were used as part of the search criterion and are comprehensively and astutely described with a pictorial timeline of their historical relevance by Sharif et al. (2018) (see Figure 5 displaying historical timeline).

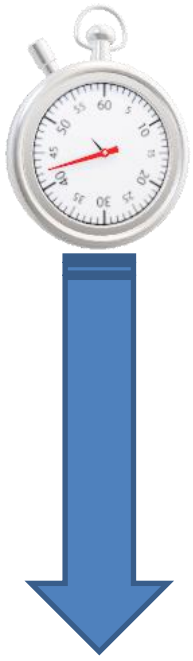
The term MECFS is often used in the form "ME/CFS" or even vice versa "CFS/ME". There have been some derogatory, pejorative and stigmatising labels used to describe MECFS such as 'Yuppie Flu', or 'Bored Housewife Syndrome', exemplifying some of the difficulties associated with this diagnosis (Loblay 1995). People diagnosed with either MECFS and/or fibromyalgia have been the targets of stigma from a clinical, social, and even familial perspective (McManimen et al. 2018; Stahl 2001).

Although we know little about the cause of MECFS or fibromyalgia syndromes, they are potentially an infectious viral disease. There have been many large outbreaks in a number of countries across the world which have been well documented over the last century (Underhill 2015), and are not unlike the random outbreaks of SARS or COVID 19 (Islam, Cotler & Jason 2020). The ICD version 11 uses the term **Post Viral Fatigue** in section 8E49 to describe MECFS, and lists 'benign Encephalomyelitis and Chronic Fatigue Syndrome' as two subheadings. The ICD is regularly updated and the previous version ICD version 10 used the reference G93.3 (WHO 2018). The author has published an article during the course of this thesis published by the ABC News Australia explaining the history of Post Viral Fatigue "What is post-viral fatigue syndrome, the condition affecting some COVID-19 survivors?" (Musker 2020).

Table 4 Terms used for MECFS across time

Names	Explanation of the Term
Myalgic Encephalomyelitis	A term suggested in a letter to the editor of the Lancet in 1956 by Dene E Hicks of Brighton South Australia as benign myalgic encephalomyelitis (Hicks 1956) It is unclear if the author of the letter created the name or that it had already been identified as part of the 1955 outbreak in the Royal Free Hospital in London (Blattner 1956).
Febricula	Cases described as early as 1734 (Sharif et al. 2018). Usually, a transient fever but has been found to occur with CFS (Numata et al. 2020).
Atypical Poliomyelitis	An outbreak in 1934 in Los Angeles Country General Hospital (Sharif et al. 2018). Cases described in the Lancet in 1952 by Gordon Ward (Ward 1953).
Akureyri or Icelandic Disease	1948-1949 an epidemic involving 465 eases occurred in Akureyri, in northern Iceland (Blattner 1956).
Neurocirculatory Asthenia	A chronic condition of fatigue notices in soldiers following wartime campaigns (Paul 1987).
Royal Free	An outbreak that affected 292 medical and admin staff. The outbreak happened on 13 July 1955. (Dawson 1987; Geffen 1957).
postural orthostatic tachycardia syndrome	POTS – as this is a key symptom of MECFS it was used to expand the search. (Dahan, Tomljenovic & Shoenfeld 2016).

Chronic Epstein Barr	Epstein Barr is a common virus that can go on to have chronic effects like chronic fatigue syndrome (Kimura, H & Cohen 2017).
Systemic Exertion Intolerance Disease	A decision by the institute of medicine committee to replace MECFS with SEID (Twisk 2015).
Post Viral Fatigue	The name used by the International Classification of Diseases G93.3 ICD 10.
Tapanui Flu	An epidemic of 28 cases in West Otago New Zealand that had prolonged unexplained fatigue (Levine et al. 1997).



- **1750 Febricula**
- **1879 Neurocirculatory asthenia**
- **1937 Atypical poliomyelitis**
- **1948 Akureyri disease**
- **1955 Royal Free disease**
- **1956 Myalgic encephalomyelitis**
- **1984 Tapanui outbreak**
- **1987 Chronic Fatigue Syndrome**
- **2015 Systemic Exertion Intolerance Disease**

Figure 5 Illustrative Timeline adapted from Sharif et al. (2018)

The first stage of the literature search had to deal with the juxtaposition of numerous names / terms that have been used to describe MECFS. Using the MECFS term alone is controversial as there are some groups that believe that Myalgic Encephalomyelitis and Chronic Fatigue Syndrome are different illnesses that have now been grouped together, so in its written form it is often separated by a forward oblique 'Myalgic Encephalomyelitis / Chronic Fatigue Syndrome' or even written in the reverse order. Fortunately, some of these name issues have been dealt with by previous authors and the 'Medical Subject Heading (MeSH) term 'Fatigue Syndrome, Chronic' which was introduced in 1990, provides some clarity (NCBI 2020; Twisk 2016a). The NCBI has updated the term to include the majority of regularly used synonyms. These accumulating synonyms that are provided by the MeSH database are listed below, showing the complexity of the search and it is comprehensive:

List of current MeSH Entry Terms:

- Myalgic Encephalomyelitis
- Encephalomyelitis, Myalgic
- Chronic Fatigue Syndrome
- Chronic Fatigue Syndromes
- Fatigue Syndromes, Chronic
- Chronic Fatigue-Fibromyalgia Syndrome
- Chronic Fatigue Fibromyalgia Syndrome
- Chronic Fatigue-Fibromyalgia Syndromes
- Fatigue-Fibromyalgia Syndrome, Chronic
- Fatigue-Fibromyalgia Syndromes, Chronic
- Postviral Fatigue Syndrome

- Postviral Fatigue Syndromes
- Infectious Mononucleosis-Like Syndrome, Chronic
- Infectious Mononucleosis Like Syndrome, Chronic
- Royal Free Disease
- Chronic Fatigue and Immune Dysfunction Syndrome
- Chronic Fatigue Disorder
- Chronic Fatigue Disorders
- Fatigue Disorder, Chronic
- Fatigue Disorders, Chronic
- Systemic Exertion Intolerance Disease
- Fatigue Syndrome, Postviral
- Fatigue Syndromes, Postviral

Adapted from NCBI 2020 MeSH Library (NCBI 2020)

The MeSH term covers most of the commonly used names in the literature, but working with the librarian, a more extensive search was completed using many additional terms, such as obscure terms like ‘Akureyri disease’ named after a mystery disease outbreak in New Zealand. Similar outbreaks occurred in a number of other places across the world over the last century, each of them adding to the name list, one of the largest outbreaks being at the Royal Free Hospital in London on 13th July 1955 (Geffen 1957) with over 200 hospital personnel and patients presenting with strange symptoms. Hence the term ‘Royal Free Disease’, another synonym for MECFS. Adding such diverse ‘fatigue’ focused outbreaks and labels for MECFS is a good example of how heterogeneity, inconsistency and imprecision is supported by this ongoing labelling issue.

To avoid such indiscriminate application of these labels, researchers should consistently be using the International Consensus Criterion, which provides clear

symptom descriptors of symptoms that are experienced by consumers, and should help to avoid further ambiguity (Carruthers et al. 2011). This trend has been adopted by the most recent research as the baseline criteria for assessing patients as cases. It is unfortunate that the latest recommended label ‘Systemic Exertion Intolerance Disease / Chronic Fatigue Syndrome’, has not been fully accepted or used as a term in the literature. Most recent articles use MECFS in some form or other and the new term has many challengers (Twisk 2016b). So, the naming convention controversy continues and is unlikely to be settled any time soon. We explore this further in chapter 3 where we provide more detail about how the search was completed.

CHAPTER 2

Methodology

2 Methodology

2.1 Review Question

Question: **Is there a difference in circulating leptin levels in MECFS and/or fibromyalgia that can be used as an outcome measure in cases and controls?** (Leptin is measured in ng/mL and is assessed through the analysis of blood plasma, blood serum, or CSF)

2.2 Objectives

To gather the evidence of scientific studies that have examined the levels of the adipokine leptin in the circulatory system of the body in two syndromes MECFS and fibromyalgia. Then to compare the results between studies, and to complete a meta-analysis of the results to see if there are any differences of the baseline level of circulating leptin in a comparison of cases and controls.

2.3 Criteria for Considering Studies

The aim of this review was to cast a wide net and capture the current available evidence about leptin in MECFS and/or fibromyalgia. The following section will provide in detail the decisions of inclusion and exclusion.

2.4 Types of Studies

This review considered observational studies of varying study designs including prospective and retrospective cohort studies, case-control studies, time-series and analytical cross-sectional studies that include both cases and healthy comparators.

Following the search, all identified citations were collated and uploaded into EndNote v.X9 (Clarivate Analytics, PA, USA) and duplicates were removed. Titles and abstracts were independently screened by two reviewers for assessment against the inclusion criteria (MM & ML). This process was completed using COVIDENCE (Veritas Health Innovation, Melbourne, Australia) and a third reviewer was used to arbitrate discrepancies (AM). Potential, relevant studies were retrieved in full and their citation details imported into the JBI System for the Unified Management, Assessment and Review of Information (JBI SUMARI; JBI, Adelaide, Australia). The full text articles of selected citations were assessed in detail against the inclusion criteria by two independent reviewers (MM and ML). Reasons for exclusion of full text studies that do not meet the inclusion criteria were recorded and reported in this systematic review in the form of a summary table. (See Table 5). The exclusion reasons for the 85 excluded full text articles are recorded in COVIDENCE, and a complete list of reasons is provided in the appendix with a brief abstract about each study (See Appendix 1):

Table 5 Summary of reasons for exclusion – 85 full text articles

26	No results provided
20	No leptin results in paper
15	Wrong patient population
13	Leptin is not discussed in article
5	Another major disease
3	Foreign language
2	Wrong comparator or outcome
1	Sytematic review - exclude

2.5 Types of Participants

The review considers studies that include people of any age diagnosed with MECFS, and/or fibromyalgia. Reference to diagnostic criteria must be detailed; for example, diagnosis by a general practitioner, Fukuda definition, International Consensus Criterion, or other descriptions indicating the method of diagnosis (Carruthers et al. 2011; Fukuda et al. 1994). Attention to the diagnostic method for both MECFS and fibromyalgia is an important factor for heterogeneity. Where no reference or details are provided about diagnostic method, attempts were made to contact the authors where appropriate. Other language terms for MECFS or fibromyalgia were included; for example, if it states that there is ‘‘chronic fatigue’’ that lasts for at least six months, then this warranted further investigation at the full text level. There continues to be debate about which names to use for MECFS and getting a consensus remains controversial.

2.6 Types of Interventions / Exposure of Interest

The exposure is a diagnosis of MECFS and/or fibromyalgia. The diagnosis were described by operational criteria such as a diagnosis from a general practitioner or those described by the Fukuda definition, Canadian Consensus Criterion, or American College of Rheumatology (Carruthers et al. 2011; Wolfe, F. et al. 1990). The level of exposure was not addressed in this review, except to provide information about one cohort study which made references to ‘short’- and ‘long-term’ exposure. The study used a 3-year anniversary cut off period for short-term and beyond this time period, exposure was considered long-term (Hornig et al. 2017). This same study also distinguished between typical and atypical exposure, dividing cases by the pattern of onset and the link to comorbid issues. As part of the critical appraisal process, exposure was considered an all or nothing principle rather than a level of exposure. That is, the person had MECFS or fibromyalgia, or they did not.

2.7 Comparators / Context

The comparator was healthy participants without other physical disorders, such as cancer or medical illness like hepatitis or rheumatoid arthritis, that would likely cause symptoms of pain and fatigue. Different methods of recruitment have been described, where some researchers recruited specifically for a specific study, but others made comparisons to another historical healthy cohort that had previously been studied, for example using results from a clinical biobank.

2.8 Types of Outcome Measures

The outcome is circulating leptin levels, and whether there is a difference between comparators and those with the condition / exposure. Included studies measured levels of plasma leptin or other methods of measuring leptin such as serum levels, or CSF levels. When comparing study outcomes, issues such as the way leptin was measured, the frequency of when samples were taken (hourly, daily or once only), and confounders such as BMI were considered. Where BMI is included in the article, these were recorded and compared across studies in both cases and controls. Leptin levels were used as a comparative outcome; however, the differences in study design proved difficult to compare across studies and resulted in a standardised mean difference being used. This was managed by focusing on the leptin levels being translated into ng/mL and discrepancies between study methods identified. An example included whether they measured serum, plasma, or CSF and whether there was an indication of when (specific time of day) the samples were taken. The circulating leptin levels reported across studies appeared to be on different scales / magnitude, a further reason for the need to use a standardised mean difference. The cohort studies all investigated unique conditions, groups and parameters, making them difficult to compare and resulted in them being reviewed individually or in pairs. A narrative synthesis is provided for each study in chapter 4.

2.9 Review Methods

The systematic review was reported based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist and followed the JBI methodology for systematic reviews of etiology and risk (Moola, S et al. 2019). By including a broad, inclusive review approach assessing any difference of leptin between MECFS and/or fibromyalgia and controls, the results of this review may provide a springboard into the development of future research, treatments, diagnostic tests, or prognostic models for people with MECFS and/ or fibromyalgia. It will also provide a source for new and experienced researchers looking for information on the raw leptin results for participants with MECFS and/or fibromyalgia, assisting with analysis and preparing future research projects. A series of recommendations is provided for future research in Chapter 5.

2.10 Search Strategy

The search strategy aims were to locate both published and unpublished studies. An initial limited search of Scopus (Elsevier), PubMed (NIH National Library of Medicine), and Embase (Elsevier) was undertaken to identify articles on the topic. The text words contained in the titles and abstracts of relevant articles, and the index terms used to describe the articles were used to develop a full search strategy for PubMed, Embase, Scopus, Science Direct (Elsevier), and PsycINFO (Ovid). The search strategy included all identified keywords and index terms, and the format was adapted for each included information source, including unpublished studies such as theses, conference abstracts, and government reports found using Google Scholar. The reference list of all articles selected for critical appraisal was screened for additional studies. Studies were not date limited but the human equivalent of leptin was not discovered until 1994, so naturally there are no studies available prior to this date (Zhang et al. 1994). Only studies published in English were included due to limited resources for translation.

2.11 Assessment of Methodological Quality / Critical Appraisal

Eligible studies were critically appraised by two independent reviewers at the study level for methodological quality in the review. The quality assessment used the relevant study type assessment tool identified by JBI, including the JBI Critical Appraisal Checklist for cohort studies, case-control studies, case series, case reports, and cross-sectional studies, where these studies were identified for inclusion (Moola, S et al. 2019). Any disagreements that arose were resolved through discussion, or with a third reviewer. All studies, irrespective of methodological quality, were included in the review, except those that do not provide leptin as an outcome measure. A table is provided for the two critical appraisal checklists, one for case control studies (See Table 11 in Chapter 3, page 85) and one for cohort studies (See Table 27 in Chapter 4, page 135).

2.12 Date Extraction

The data extracted included specific details about the outcome of interest (circulating leptin levels), plus other information like populations, study methods and outcomes or dependent variables of significance to the review question and specific objectives (Munn, Z., Tufanaru & Aromataris 2014). The extraction tool 'The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews Checklist for Case Control Studies' was used (Moola, S et al. 2019). Demographics and participant type were extracted such as sex, age group, BMI and any context provided in the study for example how the participants were selected, and whether participants were fasted, or if measures were continuous or completed over a number of days, or months in a time series. Corresponding authors of papers were contacted to request missing or additional data where this was required, but only a few of them returned emails. Those that kindly responded with comments or original data were (see next page):

- Dr Samy Metyas; (<drmetyas@gmail.com>)
- Barb Cameron; (<barbcameron55@gmail.com>)
- Vanessa Ribeiro; (<vafisio.ribeiro@gmail.com>)
- Ana Cristina Lacerda; (<lacerdaacr@gmail.com>)

2.13 Data Synthesis

The authors completed a quantitative analysis that synthesised findings across studies using pooled effect sizes and confidence intervals of the measures provided. However, this method could only be used for the case control studies that provided the baseline results for both cases and controls in their original units ng/mL. The cohort studies were quite different in their design and some were completed across a timeline and explored different phenomena such as smoking, exercise, typical and atypical MECFS. Dekkers et al. (2019) recommended that where the experimental design is not exact and it is unclear whether the same phenomenon is being measured, a random effects model should be considered and this advice was adhered to. Standard meta-analysis for continuous outcomes were pooled using the JBI SUMARI platform, and a 'standardised mean difference' rather than 'mean difference' was used which is explained in detail elsewhere. Heterogeneity was assessed using an appropriate method (I^2). An overall effects score that provided a standard mean difference was calculated as per the JBI Manual for Evidence Synthesis (Moola, S et al. 2019). A potential limitation of the study is that details at the level of measurement (e.g., nanograms per mL [or equivalent e.g., picogram mL or mg per 100mL]) was not provided in several studies. Where results were provided for the cohort study, a tabular and narrative synthesis was completed that provides a useful summary of information for each study, but mainly for the cohort design studies (Munn, Z., Tufanaru & Aromataris 2014). See Chapter 4 for more information about the cohort studies included in this review.

2.14 Assessing Certainty of Findings

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach for grading the certainty of evidence was followed, and a Summary of Findings (SoF) was created using GRADEPro GDT (McMaster University, ON, Canada) (Alonso-Coello et al. 2016; Neumann et al. 2016). The SoF is presented with some of the following information: Standardised mean difference for the cases and control and a ranking of the quality of the evidence based on the risk of bias, directness, heterogeneity, precision, and risk of publication bias of the review results. The outcomes reported in the SoF includes the level of heterogeneity, and risk of bias. (See Table 17 in Chapter 3, page 112 for more information.)

CHAPTER 3

Results:

Literature Search & Meta-analysis

3 Literature Search & Meta-analysis

3.1 Literature Search and Results

This chapter will explain the process of the literature search using the platform NCBI PubMed (NIH National Library of Medicine) as the example. It will also describe how the documents found were organised using the Endnote v.X9 (Clarivate Analytics, PA, USA) and COVIDENCE (Veritas Health Innovation, Melbourne, Australia) platforms.

3.2 The Literature Search Explained

A systematic search of the literature was completed using five search platforms Embase, PsycINFO, PubMed, Science Direct, and Scopus on 3rd April 2020 (updated on 27/7/20). All preliminary (3/4/20) and follow up searches (27/7/20) were performed on the same date for all databases. The results for the searches are as follows:

Table 6 Systematic Search Results

Database	Found	Endnote	Screening
Embase	220		
PsycINFO	273		
PubMed	54		
Science Direct	411		
Scopus	51	duplicates	New Total
Total Records Found	1009	196	813

All 1009 selected searches were imported into EndNote v.X9 (Clarivate Analytics, PA, USA) and duplicates removed. This resulted in 813 records being reviewed at the title and abstract level, resulting in 107 articles selected for full text review. As

part of this full text review 2 additional articles were found in the references, resulting in a total of 109 articles being selected for review by two reviewers (MM & ML). The full text review was completed by two independent reviewers using COVIDENCE (Veritas Health Innovation, Melbourne, Australia) and a third reviewer was identified to arbitrate discrepancies (AM). However, the third reviewer was not required, but did provide objective supervision of the process.

An example of how the search was completed is shown in the logic table used in the PubMed search (See Table 7 and 8):

Table 7 Logic Grid Search Terms

MECFS	Fibromyalgia	Leptin
fatigue syndrome, Chronic"[Mesh] OR "Chronic Fatigue Syndrome" OR Myalgic Encephalomyelitis OR ME/CFS OR MECFS OR ME-CFS OR Febricula OR "Neurocirculatory Asthenia" OR "Atypical Poliomyelitis" OR Akureyri OR "Royal Free" OR Tapanui OR "Chronic Epstein Barr" OR "Systemic Exertion Intolerance Disease" OR "postural orthostatic tachycardia syndrome" OR POTS)	Fibromyalgia or Fibromyalgia [Mesh]	(Leptin OR "Leptin"[Mesh])

A MeSH term is assigned by the National Library of Medicine (NLM) and each new term entered in the database is assigned around 10-12 labels associated with that phenomenon (Baumann 2016). Following advice from the librarian, as part of this search MeSH terms were used as well as the individual terms within MeSH, as this ensured the most recent publications were located. A full list of the MeSH terms for MECFS are provided in Chapter 2. The systematic search was updated on 27/7/2020 to ensure the most up to date articles were included in this review. There are many publications about leptin receiving 37,623 hits, with more articles published on the subject of fibromyalgia (n=11766) than Chronic Fatigue Syndrome (8719). The terms were then combined using Boolean operators and with all the terms and synonyms when combined yielded just 54 articles from the PubMed database (Ecker & Skelly 2010).

The same strategy was then completed for the other databases which were not as accurate at pinpointing articles and therefore the net was cast with less precision

than PubMed creating more work for the reviewers. The greatest yield was from Science Direct identifying 411 articles but many of these were unrelated, then PsycINFO with 273, then Embase with 220, and finally Scopus with 51 articles. These searches were all imported into EndNote and the simple process of 'Find Duplicates' was used, which identifies articles that have the same titles enabling the reviewer to check each duplicate (Kwon et al. 2015). This process can miss some duplicates because their indexing may be slightly different in EndNote, but they are then discovered as part of the next phase of the review using the online reviewing software COVIDENCE (Covidence.org 2020).

Table 8 Example Search Strategy

Stepwise example using PubMed, NIH platform search conducted on 27/7/2020

Search Database PubMed NIH	Search Terms – Exact	Comments	Found (prior to duplicates removal
	Leptin		37,623
	Fibromyalgia		11,766
	Chronic Fatigue Syndrome		8,719
Combined Search	((“fatigue syndrome, Chronic"[Mesh] OR “Chronic Fatigue Syndrome” OR Fibromyalgia OR Myalgic Encephalomyelitis OR ME/CFS OR MECFS OR ME-CFS OR Febricula OR “Neurocirculatory Asthenia” OR “Atypical Poliomyelitis” OR Akureyri OR “Royal Free” OR Tapanui OR “Chronic Epstein Barr” OR “Systemic Exertion Intolerance Disease” OR "postural orthostatic tachycardia syndrome" OR POTS) AND (Leptin OR "Leptin"[Mesh]))	Search is exact	54

All searches were then uploaded to COVIDENCE from EndNote, all 1009 which were assessed at title and abstract level by the first author. Articles were excluded at this level if they were clearly investigating another disease or met the exclusion criterion set out in the a priori protocol. Wherever possible, the 'Best Practice Guidelines for Abstract Screening' described by the Cochrane Handbook (version 6.1 online, chapter 4) and others were followed (Higgins JPT et al. 2020; Polanin et al. 2019). COVIDENCE enables the user to define exclusion criterion in the form of a 'TAG' and these tags are then assigned by the reviewers as they include or

exclude articles, providing summary information on the reasons for exclusion at the end of this sorting process. During the full text search, two additional articles were found in the references of other included papers. See PRISMA Diagram (Figure 6) on the next page:

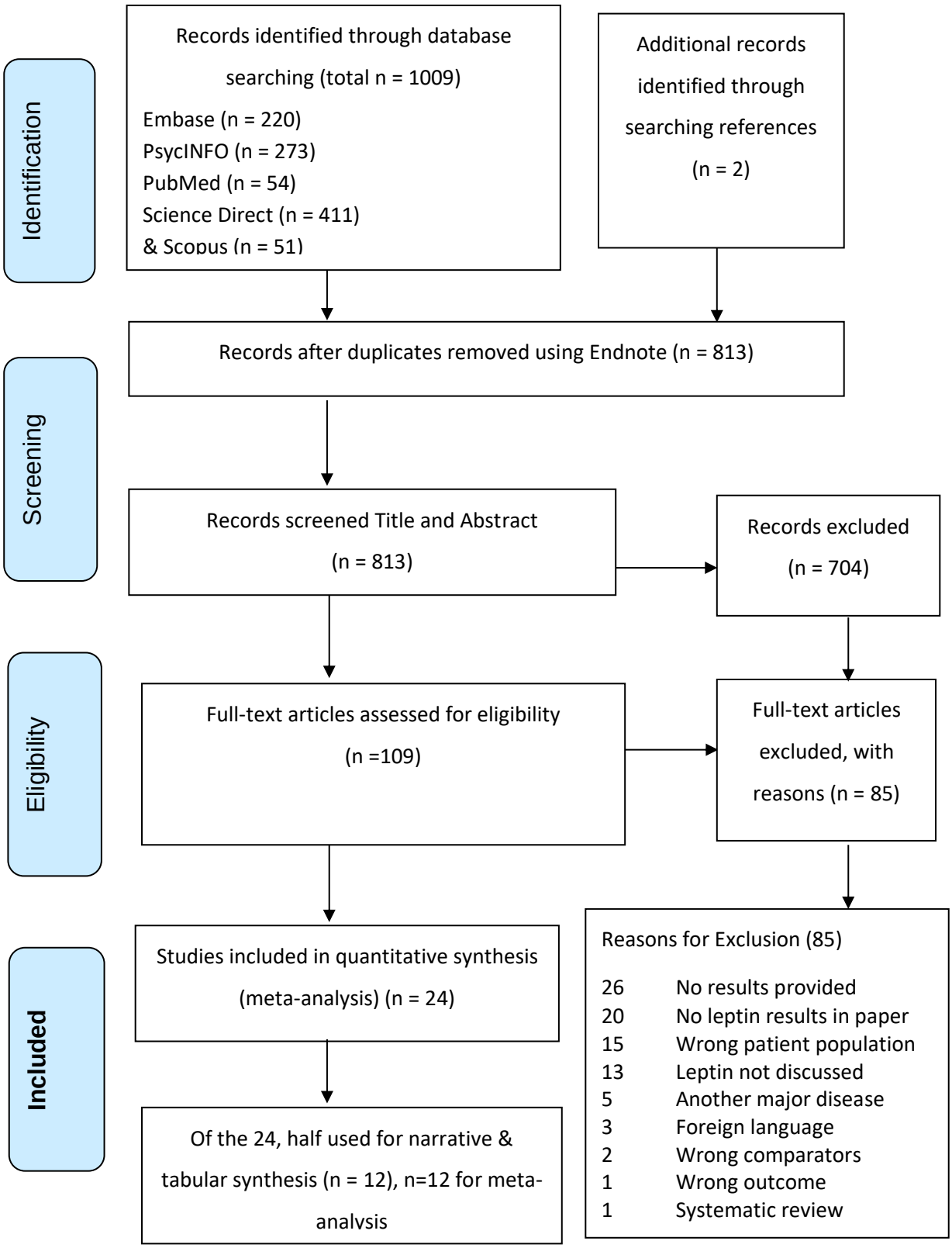


Figure 6 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta- analysis) Diagram adapted (Moher et al. 2009)

Following an initial scan of the literature a series of reasons for common exclusion were entered in the platform COVIDENCE to support the reviewers during the setup process and prior to screening. The first two reasons show that there were **'no results'** published in the article; this often meant that the paper was an explanatory paper about the illness and did discuss leptin but at no point reported any actual results i.e., the first item in the reasons for exclusion table above (See right side of Prisma Diagram Figure 6) **'no results provided'** but the absence of these results could not be determined at the title and abstract phase of the search. Some studies did include clinical results of other cytokines or adipokines but at no time provided leptin results, so these had to be excluded, hence the heading – 'no leptin results' rather than just 'no results provided'. An example of an alternative result that could not be used in this meta-analysis was the circulating levels of adiponectin, another adipokine, which has a similar relationship with adipocytes to leptin but they appear to counter each other in their biological actions (Park, KG et al. 2004).

Whilst some papers mentioned fatigue, for example, they were describing it in the context of a different illness or population such as the elderly therefore, **'wrong population'** was selected as the exclusion criterion. Another example would be an article that described the role of fatigue in cardiac or hepatitis patients. The most apt exclusion criterion for these articles was **'another major disease'** in that the article was clearly talking about a disease like cancer, or heart disease which meant it was part of the exclusion criterion described in the a priori protocol. Again, these exclusions could not be determined at the title and abstract phase. Thirteen articles did not mention leptin in their results and therefore they were not relevant to this review and hence excluded. The reason they got past the title and abstract phase was because some had mentioned leptin as a reference or made a passing remark about leptin in the paper but did not discuss leptin in any detail. Some articles, whilst not useful for this review, did mention other pro-inflammatory cytokines which are important factors such as interleukin 6 or interleukin 1 beta, and these cytokines are thought to have a strong relationship with the levels of circulating leptin (Azim et al. 2018; Phitak et al. 2018). Finally, three articles were downloaded but were in a foreign language and did not provide any translation into English and, as no

resources were available for translation, they were all excluded. For each article excluded, a record is elicited in COVIDENCE and was downloaded into a CSV excel file, which provides all the references reviewed and why they were excluded. At the full text phase two reviewers provided reasons for exclusion and then a consensus was sought where disagreement occurred (MM & ML). There was no requirement for a third reviewer, although a third author was assigned to provide arbitration (AM). See the appendix for full text exclusion details, where a brief extract for each article is provided with the reason for exclusion in the right-hand column of the table. The abstract has been included to support future researchers on this topic area.

3.3 Full Text Articles

Twenty-four articles appeared to provide some detailed data that could be extracted for a systematic review, but only half of these were suitable for comparison in the form of a meta-analysis. Initially 15 papers appeared suitable for data extraction, but on closer inspection, three did not provide enough detail i.e., a baseline level of circulating leptin for cases and controls. Additionally, some papers only provided p values and correlations to symptoms of fatigue, but not with enough detail to calculate the raw units of circulating levels of leptin in ng/mL. Twelve publications were suitable for data extraction, and 3 of the 15 were excluded from this part of the analysis. Specific reasons for their exclusion are provided in Table 12 (section 3.7) with a rationale explained in section 3.7. A list of the 24 included studies are provided in a table below (See Table 9).

Table 9 All 24 Included Studies (case controls, then cohort studies)

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
1	800	Ablin 2012	Evaluation of leptin levels among fibromyalgia patients before and after three months of treatment in comparison with healthy controls	Israel	No	Yes	To analyse potential changes in leptin levels among female fibromyalgia patients compared with healthy controls, and to evaluate the changes in leptin levels during treatment	Case control study	No	Yes
2	766	Ataoglu 2018	The relationship between serum leptin level and disease activity and inflammatory markers in fibromyalgia patients	Turkey	No	Yes	The aim of this study was to investigate the correlation between serum leptin level, disease activity, and markers of inflammation, and to compare the leptin level in patients diagnosed with FMS with that of healthy individuals.	Case control study	No	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
3	689	Cameron 2010	Serum cytokine levels in post-infective fatigue syndrome	Australia	Yes	No	Divided Post Infective patients and controls and measured 3 times across 12 months. Baseline, 3 months and 12 months. We will use 12-month results as baseline for comparison of cases vs controls. Longitudinally collected clinical data and blood samples from participants. Examined the pathophysiology of chronic fatigue syndrome	Case control study	Yes	Yes
4	650	Cleare 2001	Plasma leptin in chronic fatigue syndrome and a placebo-controlled study of the effects of low-dose hydrocortisone on leptin secretion	UK	Yes	No	The effects of hydrocortisone on leptin in CFS patient's vs controls using a placebo-controlled trial. Also included appetite and weight	Case control study	Yes	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
5	634	Covelli 2005	Drug targets in stress-related disorders	Italy	Yes	No	IFN-Gamma leptin, a hormone which regulates food intake, fluctuates within normal ranges in CFS individuals. Quite interestingly, in depressed patients, used as controls, leptinaemia was more elevated than in CFS	Case control study	Yes	Yes
6	815	Hornig 2016	Cytokine network analysis of cerebrospinal fluid in myalgic encephalomyelitis /chronic fatigue syndrome	United States	Yes	No	Analysed cerebrospinal fluid from 32 cases, 40 subjects with multiple sclerosis and 19 normal subjects' frequency-matched for age and sex using a 51-plex cytokine assay. This study uses samples from a biobank and compares 3 groups MECFS / MS / and Controls.	Case control study	Yes	Yes
7	814	Hornig 2015	Distinct plasma immune signatures in ME/CFS are present early in	United States	Yes	No	Cases being short term <=3years and long term MECFS >3 years. "report here distinct alterations in plasma immune signatures ME/CFS (n	Case control study	Yes	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
			the course of illness				= 52) relative to healthy controls (n = 348) that are not present in subjects with longer duration of illness (n = 246)."			
8	546	Montoya 2017	Cytokine signature associated with disease severity in chronic fatigue syndrome patients	United States	Yes	No	To determine whether a signature of serum cytokines could be associated with ME/CFS and correlated with disease severity and fatigue duration, cytokines	Case control study	Yes	Yes
9	474	Homann 2014	Hyperleptinemia independent of body adiposity in women with fibromyalgia	Brazil	No	Yes	The effect of fibromyalgia (patients vs. controls) on the relationship of leptin and acylated ghrelin with anthropometric indicators [body mass index, waist circumference (WC) and WC by height].	Case control study	No	Yes
10	473	Homann 2013	Acylated ghrelin: A potential marker for fibromyalgia?	Brazil	No	Yes	investigated the relationships between these two neuropeptides and sleep and	Case control study	No	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
							various pain characteristics in patients with fibromyalgia			
11	393	Koca 2018	Relationship of leptin, growth hormone, and insulin-like growth factor levels with body mass index and disease severity in patients with fibromyalgia syndrome	Turkey	No	Yes	This study aimed to investigate leptin, growth hormone (GH), and insulin-like growth factor (IGF-1) levels in FMS, and their relationship with body mass index (BMI) and disease severity	Case control study	Yes	Yes
12	195	Olama 2013	Serum leptin in Egyptian patients with fibromyalgia syndrome: relation to disease severity	Egypt	No	Yes	Aim: Recently, a large body of studies has focused on the leptin levels in psychiatric disorders. This study aimed to measure serum leptin levels in fibromyalgia syndrome (FMS) due to a significantly higher prevalence of psychiatric disorder and to determine the	Case control study	No	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
							relationship between leptin and FMS.			
13	189	Paiva 2017	Serum levels of leptin and adiponectin and clinical parameters in women with fibromyalgia and overweight/obesity	Brazil	No	Yes	to evaluate the serum levels of adipokines in women with fibromyalgia with and without overweight/obesity, and to correlate the adipokines levels with clinical parameters associated with fibromyalgia and adipose tissue mass (body fat).	Case control study	No	Yes
14	130	Ribeiro 2018	Inflammatory biomarkers responses after acute whole-body vibration in fibromyalgia	Brazil	No	Yes	The aims of this study were 1) to characterize the intensity of the vibration stimulation in women diagnosed with fibromyalgia (FM) compared to a control group of healthy women (HW) matched by age and anthropometric parameters, and 2) to investigate the effect of a single session of whole-body	Case control study	No	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
							vibration (WBV) on inflammatory responses.			
15	25	Stringer 2013	Daily cytokine fluctuations, driven by leptin, are associated with fatigue severity in chronic fatigue syndrome: evidence of inflammatory pathology	United States	Yes	Yes	To evaluate the role of cytokines daily over 25 days in MECFS	Case control study	No	Yes
16	732	Bjersing 2013	Exercise and obesity in fibromyalgia: Beneficial roles of IGF-1 and resistin?	Sweden	No	Yes	The aim was to examine effects of exercise on fatigue, in lean, overweight and obese FM patients.	Cohort study	No	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
17	718	Bokarewa 2013	Smoking is associated with reduced IGF-1 levels and higher pain experience in patients with fibromyalgia	Sweden	No	Yes	Evaluates the possible relation between pain, cigarette smoking, and levels of IGF-1 in patients with FM.	Cohort study	No	Yes
18	717	Bokarewa 2014	Smoking is associated with reduced leptin and neuropeptide Y levels and higher pain experience in patients with fibromyalgia	Sweden	No	Yes	The effect of cigarette smoking on adipokines and pain parameters, in 62 women with fibromyalgia (FM) pain syndrome with unknown etiology.	Cohort study	No	Yes
19	471	Hornig 2017	Immune network analysis of cerebrospinal fluid in myalgic encephalomyelitis /chronic fatigue syndrome	United States	Yes	No	analysis of cerebrospinal fluid in myalgic encephalomyelitis / chronic fatigue syndrome with atypical and classical presentations	Cohort study	Yes	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
20	406	Katz 2017	Leptin, a hypothalamic signaling hormone, is elevated in fibromyalgia patients	United States	No	Yes	Leptin levels were assessed in 27 fibromyalgia syndrome patients and six rheumatoid arthritis patients	Cohort study	Not stated	Not stated
21	245	Younger 2016	Association of Leptin with Body Pain in Women	United States	No	Yes	To explore the association of Leptin with self-reported body pain in 3 women. Study 2 was a retrospective study of leptin results in postmenopausal women who experience varying levels of pain.	Cohort study	No	Yes
22	142	Quismorio 2014	Elevated serum leptin concentrations in a subset of fibromyalgia patients	United States	No	Yes	To measure inflammatory markers using a method called Vectra DA. This provided a mean baseline Leptin result, 42.3ng/mL with a range of 30-81 ng/mL	Cohort study	Yes	Yes

No.	Cov #	Study Author & Year	Title	Country of study	Contain MECFS	Contains Fibromyalgia	Aim of study stated in article or a summary of	Study type	Male in pop	Female in pop
23	383	Kurajoh 2016	Plasma leptin concentration is associated with fatigue severity in patients with cardiovascular risk factors ,HSCAA study	Japan	No	No	to examine the impact of sleep condition, cardiac autonomic dysfunction, and subclinical atherosclerosis on cardiovascular events. The present study is a sub-analysis of the HSCAA study	Cross sectional study	Yes	Yes
24	731	Bjersing 2017	Benefits of resistance exercise in lean women with fibromyalgia: Involvement of IGF-1 and leptin	Sweden	No	Yes	To evaluate the role of metabolic factors in lean, overweight and obese women during resistance exercise, in relation to symptom severity and muscle strength in women with FM	Other: Cohort study	No	Yes

To enable this review to compare results across studies the authors had to demonstrate that it was appropriate to include them in the comparison. It was necessary for included studies to report a similar methodology and provide data on circulating leptin that could be compared in nanograms per millilitre using both cases and controls. This was not always self-evident, and some studies had to be translated from picograms to nanograms or the authors had to be contacted for clarification about their results.

Another problem encountered was that some papers only provided pictorial graphs of the results, which could not readily be interpreted into the basic mean values. More importantly, they did not provide the standard deviation (SD) of the results, which was necessary to make an effective comparison, and even more difficult to discern from a graph than say the mean average. One study by Hornig et al. (2016) for example provided the standard error of the mean (SEM) and this figure had to be converted back to SD to enable it to be used in the meta-analysis. The calculation for this result is explained below and the converted result SEM to SD, was used in the final analysis. It is suggested that some articles use the SEM format instead of SD because it is always a smaller figure than the SD and may make the evidence look more precise than it actually is (Brown 1982).

The SD tells us the measure of dispersion and can be calculated by first finding the mean of the group, that is by adding the results of each participant, then dividing the total by the number of participants = μ. The SEM is how far the sample mean is from the true population mean; that is how much discrepancy from the mean. We often make assumptions and interpretations of data based on the normal Gaussian distribution or bell curve (Livingston 2004). We know that 95% of the population will fall within 2 SD of the mean (Altman & Bland 2005). You then take each result and work out the difference from the mean (μ-x). Then square the difference for each participant $(\mu\text{-}x)^2$ and add them all together $(\mu_i\text{-}x)^2$. Note all numbers become positive, as when you square a minus figure, this means a minus multiplied by a minus becomes a positive number. Then to obtain the variance σ^2 we find the mean of the sum of the squared differences / divided by the mean ($\sum(\mu_i\text{-}x)^2/n$) which gives the average distance between the results of participants. The SD is then the square root of the variance.

Because we are working on a sample and not the whole population, we must make a correction known as Bessel's correction, which is not just dividing these differences by n, but n-1 which is called 'the sample variance'. This is a complicated correction to remove the risk of sampling error and provides a better estimate of the true standard deviation within a sample population (Biau 2011). Then take the square root of the sample variance to get the sample standard deviation or $\sqrt{\sum}(m-i)^2/(n-1)$. Some studies provide the SEM and this can be calculated by dividing the standard deviation by the square root of the sample (n) or SEM=SD/$\sqrt{n}$ (Curran-Everett 2008). You can also then calculate the SD from the SEM, because you simply reverse the calculation, that is the square root of sample (n) multiplied by the SEM or SD=$\sqrt{n}$ x SEM instead of dividing it. See the example used to convert the Hornig study in this meta-analysis and as you can see the cases SD (55.47) is much larger than the SEM (9.8).

Method for Converting the Hornig (2016) results: SEM to SD

- CASES: CFS 68.2 (SEM 9.8); NB. These are CSF samples - n is 32 (Square root of 32 is 5.66) 9.8 ng/mL x 5.66 = SD 55.47 ng/mL
- Controls 41.6 (SEM 10.2) n = 19 i.e., healthy participants only (Square root of 19 = 4.36) 10.2 ng/mL x 4.36 = SD 44.47 ng/mL

Making comparisons across studies which measure the same continuous outcome, for example levels of circulating leptin in the body, where the experiments have slightly different methodologies would require the use of a Standardised Mean Difference (SMD) rather than just a mean difference. A SMD uses a common metric or Z score across studies to compare differences (Walwyn & Roberts 2017). A Z score represents how many SD from the population mean a raw score is.

3.4 Description of Studies

In this section we explore some of the methodological and quality aspects of the research using the JBI critical appraisal checklist and explain why some studies could not be included in the analysis.

3.5 Methodological Quality of Included studies

Two reviewers used the critical appraisal tool for case controls studies provided in the JBI Manual for Evidence Synthesis [Chapter 7 section 2] (Moola, S et al. 2019). If the study met the criterion then a 'Yes' was given, and these were added together as a cumulative score of +1 (for each yes) up to a total possible score of 9 out of 9. The higher the score reflects the quality of the article in that it contained consistent information that would be expected from this type of case control study. One of the 10 questions was not applicable for this type of case control study, which is 'Was the exposure period of interest long enough to be meaningful'. In this study the exposure was MECFS and/or fibromyalgia, and as we were only interested in the outcome of the baseline measurement of leptin, the period of exposure was not measured and not relevant. It would be unusual for a study to declare how long the person has had the diagnosis for, unless it was part of the study design. Hence only 9 questions were utilised. The purpose of this exercise is to consider the quality of the study and whether studies should be included in the meta-analysis. We did not exclude any studies based on the critical appraisal outcome; however, some analysis was completed to see if low quality studies had an impact on the outcome.

As is made clear in the next section, a series of sub-group meta-analysis were completed based on the critical appraisal score and the outcomes were unaffected by the removal of low scoring studies. As part of this investigation, an iterative process was used to remove two 'low quality' studies from the analysis (Hornig 2016 MECFS scored a critical appraisal score of 2, and Cameron 2010 MECFS scored 2) facilitated by REVMAN (Cochrane's Review Manager software), but it made little

difference to the overall result i.e., SMD REVMAN 0.41 (CI -0.07, 0.89) p=0.10. Further to this process, removing any study with a critical appraisal score lower than 7 was then completed and resulted in the further removal of two more studies Hornig (2016 MECFS, score 6) and Ataoglu (2018 FM, score 6), but still this made little difference to the overall pooled outcome i.e., SMD REVMAN 0.28 (CI -0.17, 0.74) p=0.22. Finally, we then tried using just the four highest quality studies in the analysis which achieved a 'Yes' for all 9-quality criterion i.e., scored 9 out of 9, but even then, the results showed minimal difference to when all 12 studies were included. It reduced the overall difference and significance SMD REVMAN 0.47 (CI -0.19, 1.13) p=0.16. To summarise, when the analysis is reduced to the highest quality studies, the difference between cases and controls is slightly higher but the results also become less significant. All these iterations are provided in the table below (See Table 10):

Table 10 Meta-analysis: removal of low-quality studies using critical appraisal

Type of Analysis	Quality rating score	Total included studies in analysis	Difference in pooled SMD	Lower CI	Upper CI	Significance
SMD/RA	All	12 studies	**0.39**	-0.04	0.82	0.08
SMD/RA	>2	10 studies	**0.41**	-0.07	0.89	0.1
SMD/RA	>6	8 studies	**0.28**	-0.17	0.74	0.22
SMD/RA	=9	5 studies	**0.47**	-0.19	1.13	0.16

3.6 Critical appraisal process - checklist for case-control studies

A critical appraisal checklist was uploaded to COVIDENCE as a series of questions and this was adapted from the JBI Manual for Evidence Synthesis chapter 7: Systematic Reviews of etiology and risk, appendix 7.2 ‘Critical appraisal checklist for case-control studies (Moola, S et al. 2019). The checklist was completed by two reviewers within COVIDENCE and the results transferred to excel. Where there was disagreement about an item, both reviewers discussed the difference and decided upon a consensus. There was no need for a third reviewer as agreement

was obtained following discussion for each item post review. Table 11 is provided on the next page below showing the consensus outcome. Where there is a 'yes' item this meant that the condition was met. At the end of the table all the questions that received a yes were given a score of plus one as described earlier, and this has been summarised as a total score. The higher the score was indicative of the quality of the paper. Only two of the papers scored lower than 6, and 5 papers received the full score of 9 out of a possible 9. These scores were used to try out different analysis in REVMAN, as illustrated in the previous section removing low quality scoring papers made only a small difference to the overall outcome. A table of the results from the critical appraisal checklist is provided in alphabetical order and with total scores displayed in the right-hand column (Table 11).

Table 11 Critical appraisal checklist for case-control studies

Study Author & Year	Q1 of 10 Case-Control Study: Were the groups comparable	Q2 of 10: Were cases and controls matched appropriately?	Q3 of 10: Were the same criteria used for identification of cases and controls	Q4 of 10: Was exposure measured in a standard, valid and reliable way?	Q5 of 10: Was exposure measured in the same way for cases and controls	Q6 of 10: Were confounding factors identified?	Q7 of 10: Were strategies to deal with confounding factors stated?	Q8 of 10: Were outcomes assessed in a standard, valid and reliable way	Q9 of 10: Was the exposure period of interest long enough to be meaningful?	Q10 of 10: Was appropriate statistical analysis used?	If yes. Then add 1 to total up to a total of 9
Ablin 2012	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	N/A	Yes	7
Ataoglu 2018	Yes	No	Yes	Yes	Yes	No	No	Yes	N/A	Yes	6
Cameron 2010	Unclear	Unclear	Yes	Unclear	Unclear	No	No	Unclear	N/A	Yes	2
Cleare 2001	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	9
Homann 2013	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	9
Homann 2014	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	9
Hornig 2015	Yes	Yes	Unclear	Yes	Yes	No	No	Yes	N/A	Yes	6
Hornig 2016	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	Yes	N/A	Yes	2
Koca 2018	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	9
Olama 2013	Yes	Yes	Yes	Yes	Unclear	Yes	No	Yes	N/A	Yes	7
Paiva 2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	9
Ribeiro 2018	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	N/A	Yes	8

3.7 Review Findings/Results and Description of Full Text Studies

There was a total of 24 articles that were initially thought suitable for meta-analysis on the first screening by two reviewers. Fifteen papers were identified as case-control studies and nine as cohort type studies that provided circulating leptin results, but on closer examination 3 of the case-control studies had to be excluded from the meta-analysis (See Table 12 below). These excluded studies with reasons for exclusion are provided below:

Table 12 Full text exclusions case controls

(Covelli et al. 2005)	This paper has a diagram of the results but labelled the results in pg/mL, which would make the results of a different order of magnitude i.e., outside usual measures by approximately 100-fold. If you were to switch the label to ng/mL the results would not fit the analysis. The seeming incorrectly labelled diagram is available in the full text. Another reason for exclusion is that it did not write the results anywhere in the article. Results in the diagram are most likely reporting leptin ng/mL, but this cannot be verified, and the authors did not respond to email queries.
(Montoya et al. 2017)	This article also has a diagram where leptin is shown to be lower in the mild group, whereas moderate and severe cases are closer to controls, indicating that leptin relates to severity of symptoms in an upward trajectory, but no raw results to elucidate this are provided. There are no raw leptin results in the article nor in the supplementary. There are only p values in relation to other factors such as linear trend of disease severity. The linear relationship with severity was statistically significant even after correction for multiple comparisons, even though these 17 cytokines did not distinguish cases from controls overall.

(Stringer et al. 2013)	Another example using correlations of symptom fatigue are provided, but no raw results in ng/mL are provided and resulted in exclusion of this important and highly cited article. The research offers a diagrammatic view of the results for each individual in the study, but only shows correlations comparing standardised leptin levels with fatigue.

Whilst it was tempting to use the leptin results from the Covelli et al. (2005) study by extrapolating the data from the graph (approximately 9 pg/mL SD 13 cases vs 15 pg/mL SD 10 controls), but because of what appears to be incorrect labelling, pg/mL instead of ng/mL, it was decided by both reviewers to exclude this from the analysis but include in the discussion. Mainly because it is of the wrong order of magnitude, and the results are not confirmed in the text. To interpret these results from picograms to nanograms, it is necessary to divide by 1000 i.e., (approximately 0.009 ng/mL SD 0.013 cases vs 0.015 ng/mL SD 0.01 controls). These results would not be valid using these figures, as a mean leptin result is rarely below zero and not to this degree.

The Montoya (2017) study is the most highly cited article (102 citations) but also fails to provide actual circulating leptin results in ng/mL, making it unsuitable for meta-analysis. Stringer et al. (2013) provided impressive graphics showing correlations of symptom severity using a complex algorithm but this was unhelpful in our meta-analysis. These levels closely track symptoms, but we don't know what the circulating leptin levels are. All three studies described above were excluded at this stage.

Twelve studies were used in the final meta-analysis which had a pooled outcome that leptin is slightly higher in cases than controls by approximately 0.39, 95% CI 0.04, 0.74. The 95% confidence interval did not cross the line of significance, making this outcome significant using SUMARI (See Figure 9). The level of significance was lost when using another platform REVMAN (See Figure 8), possibly

due to the differences in weighting used to calculate the pooled outcome, and this is explored later. A series of forest plots clearly highlight that two studies (Olama, Elsaid & El-Arman 2013; Paiva et al. 2017) reported hypoleptinaemia (lower leptin) in cases vs controls as opposed to the other 10 studies which erred toward it being of no difference or higher in cases. Further explanation is provided in the next section.

3.8 Significance of Results & Forest Plots

The following forest plots provide a list of studies that are ordered alphabetically, and they used a standardised mean difference, and random effects model to calculate whether the cumulative outcome shows a pooled difference. Whilst using SUMARI the results showed an overall effect of 0.39 with a 95% confidence interval **(CI) of 0.04,0.74** indicating that the difference is significant (p=0.029) (See Figure 9). However, when the same results were placed in REVMAN 5 (See Figure 8) this provided the same pooled difference of 0.39, but then reported a negative 95% **CI -0.04,0.82** removing the significance of the result from p=0.029 reported in SUMARI (Figure 9) changing to p=0.08 reported in REVMAN (Figure 8).

It also had a slightly higher I^2 result moving from 86% in SUMARI to 91% in REVMAN (See Figure 9). Either way the heterogeneity is very high, which reduces the precision of the results and is not supportive of the precision of these studies. It is explained elsewhere in this thesis that a standardised mean difference was used due to differences in methodology; however, an alternative **'mean difference'**, random effects model meta-analysis was also completed in SUMARI (See Figure 10) and REVMAN (See Figure 11), showing slightly different results. It is notable that the largest study of 646 participants (Hornig 2015 study) is charted very differently when using a 'mean difference' model, when compared to a 'standardised mean difference model'. In the mean difference modelling, this appears in the forest plot to show little difference (actual mean difference of cases vs controls is only 0.51 ng/mL) being plotted on the line of no difference, yet in the standardised mean

difference chart this appears to have a much bigger influence on the charted result (compare Figures 9 SMD and Figure 10 MD for example).

A series of analysis was completed using both REVMAN and SUMARI and a summary table is provided following the forest plot diagrams to provide an easy comparison between all analysis type and platform (See Table 14, page 99). A potential explanation for the differences between REVMAN and SUMARI is the way the software programs 'weight' studies, there is a noticeable difference in the largest study (Hornig 2015) (See Table 13) showing the slight differences in weights between platforms (9.6% compared to 10.28%). The standard mean difference and confidence intervals are identical for each study across both platforms.

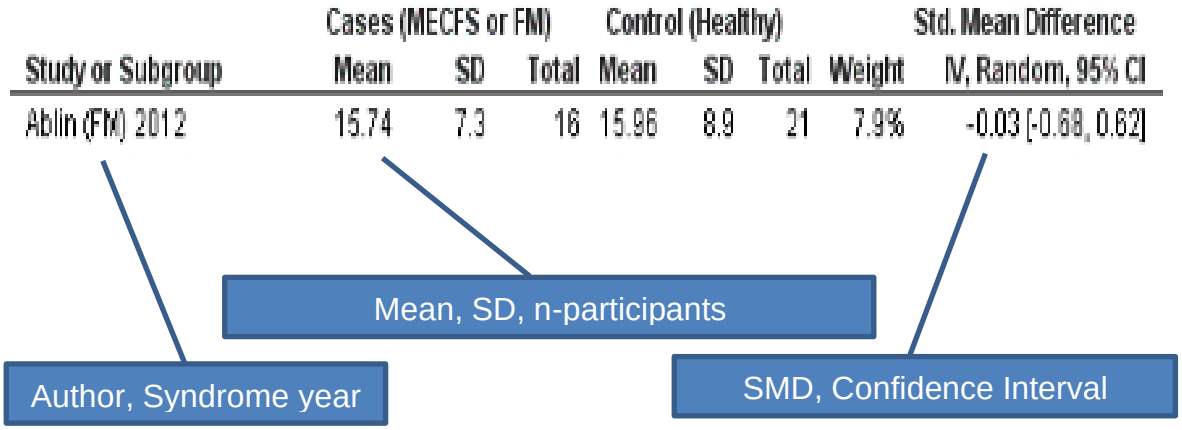
Table 13 Weight comparison between REVMAN vs SUMARI

Study	Weight REVMAN	Weight SUMARI	Weight Difference
Ablin (FM) 2012	7.9%	7.71%	**0.19%**
Ataoglu (FM) 2018	8.8%	9.04%	**-0.24%**
Cameron (MECFS) 2010	6.9%	6.32%	**0.58%**
Cleare (MECFS) 2001	8.6%	8.70%	**-0.10%**
Homann (FM) 2013	7.7%	7.40%	**0.30%**
Homann (FM) 2014	7.3%	6.90%	**0.40%**
Hornig (MECFS) 2015	9.6%	10.28%	**-0.68%**
Hornig (MECFS) 2016	8.3%	8.18%	**0.12%**
Koca (FM) 2018	8.9%	9.21%	**-0.31%**
Olama (FM) 2013	9.0%	9.27%	**-0.27%**
Paiva (FM) 2017	9.0%	9.25%	**-0.25%**
Ribiero (FM) 2018	8.0%	7.75%	**0.25%**

3.9 Explanation of the Following Forest Plots

This series of forest plots provides a graphical view of the comparison of baseline leptin in cases vs control. Cases are those participants who have been diagnosed with either of MECFS or fibromyalgia (FM) syndromes, and controls are healthy participants. They are listed in alphabetical order of author followed by what type of syndrome the paper was reporting i.e., Ablin et al. (2012) is the first author. This information is then followed by the date i.e., Ablin (FM) 2012 (See Figure 7). All full references can be found in the reference section at the end of the thesis.

Figure 7 Example of Study Listed in Meta-analysis



The forest plot provides the raw mean results of cases and then controls, which is juxtaposed to the SD, followed by the number of participants in the group. Each study is then weighted which is calculated by the software used, either REVMAN (in the first forest plot) and SUMARI (in the second forest plot). At the right side of the forest plot, the SMD is listed, then the confidence intervals are in brackets e.g., for Ablin -0.03 is the SMD, and the confidence interval for this study is -0.68 to 0.62 meaning that the actual result may fall anywhere between these intervals.

These results are then plotted on the chart to show both the standardised mean difference in the centre of the confidence intervals which are represented by a line on the graph. This is then repeated for each paper. A summary of the results is provided at the base of each forest plot showing the total population of all studies included for all combined cases (649) and cases (658), followed by the overall SMD (0.39) and finally the overall confidence intervals (0.04 to 0.82).

A list of statistical references reflecting the heterogeneity are also provided: Tau^2 = 0.3, Chi^2 = 118.65, df = 11 ($p<0.00001$); I^2= 86%. Test for overall effect: Z=2.18.

There are six forest plots that follow (13-18). Both the REVMAN and SUMARI version of the forest plot are provided for some comparisons to enable the reader to see the differences in weighting between these 2 software programmes. You will note that the raw means and standard deviations are unchanged across all forest plots. There was a decision to use the SMD due to the difference in methodologies across studies. This further supported the decision to use a random effects model rather than a fixed effects model. In an ideal world all studies would have used the same methodology and the observer could be confident that the same analyte was being measured in a systematic way, then the 'mean difference' and a fixed effect model would have been used. The 'mean difference' forest plot is provided for the curious reader to review the results in the original unit format rather the SMD format. We have also separated studies into just the MECFS studies – See Figure 12 (4 papers, 781 of the total 1307 participants of all 12 studies) in just one forest plot, then another forest plot for 'fibromyalgia only' studies – See Figure 13 (8 papers, 526 participants of the total 1307 participants in all 12 studies). This allows us to review the differences in the two separate syndromes, which, as can be seen from the forest plot results, does not vary much from the whole 12 study outcome. A table is provided at the end of the forest plots allowing the reader to make a quick comparison of all the different comparison results.

Several forest plots are provided in the following order:

1. REVMAN - All 12 studies using Standardised Mean Difference (Figure 8)
2. SUMARI – All 12 studies using Standardised Mean Difference (Figure 9)
3. SUMARI – All 12 studies using Mean Difference (Figure 10)
4. REVMAN - All 12 studies using Mean Difference (Figure 11)
5. REVMAN - MECFS studies only using Standardised Mean Difference (Figure 12)
6. REVMAN - Fibromyalgia studies only using Standardised Mean Difference (Figure 13)
7. Summary table indicating differences in results (Table 14)

Series of Six Forest Plots follow on next few pages:

Forest Plot generated in **REVMAN 5** of all twelve case control studies – **Standardised Mean Difference**

Study or Subgroup	Cases (MECFS or FM) Mean	SD	Total	Control (Healthy) Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Ablin (FM) 2012	15.74	7.3	16	15.96	8.9	21	7.9%	-0.03 [-0.68, 0.62]
Ataoglu (FM) 2018	30.32	57.25	48	11.5	7.06	36	8.8%	0.43 [-0.01, 0.86]
Cameron (MECFS) 2010	2.67	2.65	10	2.75	2.9	10	6.9%	-0.03 [-0.90, 0.85]
Cleare (MECFS) 2001	13.8	13.33	32	10.2	8.37	32	8.6%	0.32 [-0.17, 0.81]
Homann (FM) 2013	22.4	10.6	17	13.3	17.9	16	7.7%	0.61 [-0.09, 1.31]
Homann (FM) 2014	22.4	10.6	17	9.1	6.7	15	7.3%	1.44 [0.65, 2.23]
Hornig (MECFS) 2015	1.51	0.08	298	1.4	0.1	348	9.6%	1.20 [1.04, 1.37]
Hornig (MECFS) 2016	68.2	55.47	32	41.6	44.47	19	8.3%	0.51 [-0.07, 1.08]
Koca (FM) 2018	10.3	6.4	60	6.2	3.3	42	8.9%	0.76 [0.35, 1.17]
Olama (FM) 2013	7.1	6.8	50	9.4	4.5	50	9.0%	-0.40 [-0.79, 0.00]
Paiva (FM) 2017	12.81	8.62	50	18.32	10.19	50	9.0%	-0.58 [-0.98, -0.18]
Ribiero (FM) 2018	1.8	0.06	19	1.75	0.14	19	8.0%	0.45 [-0.19, 1.10]
Total (95% CI)			**649**			**658**	**100.0%**	**0.39 [-0.04, 0.82]**

Heterogeneity: $Tau^2 = 0.49$; $Chi^2 = 118.42$, df = 11 ($P < 0.00001$); $I^2 = 91\%$

Test for overall effect: Z = 1.77 (P = 0.08)

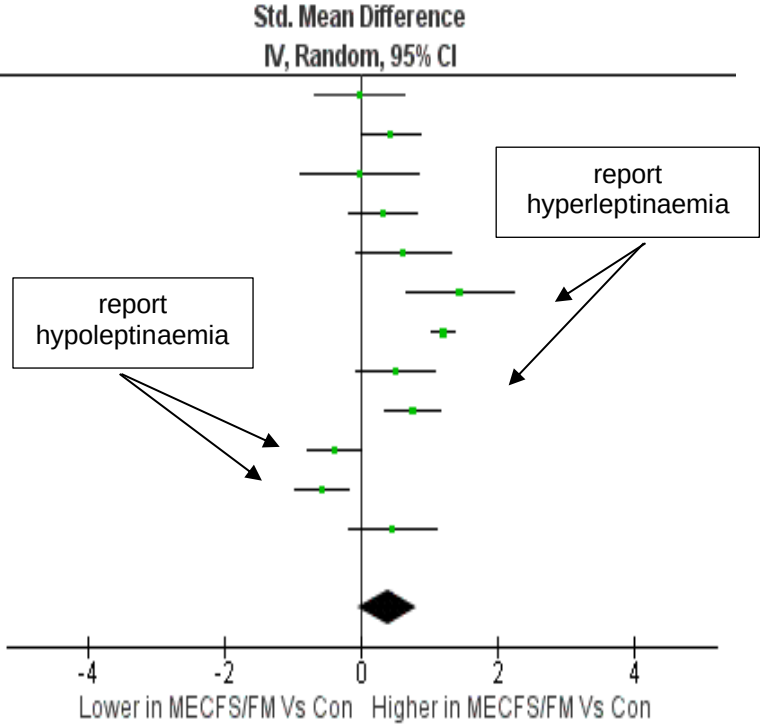


Figure 8 Forest Plot using all 12 Studies Standardised Mean Difference (REVMAN)

Meta-analysis: Forest Plot using Standardised Mean Difference. Heterogeneity $Tau^2 = 0.49$, $Chi^2 = 118.42$, df = 11 ($p<0.00001$); $I^2 = 91\%$.

Test for overall effect: Z=1.77. Overall effect 0.39, 95% CI -0.04, 0.82.

Forest Plot generated in **SUMARI** of all twelve case control studies – **Standardised Mean Difference**

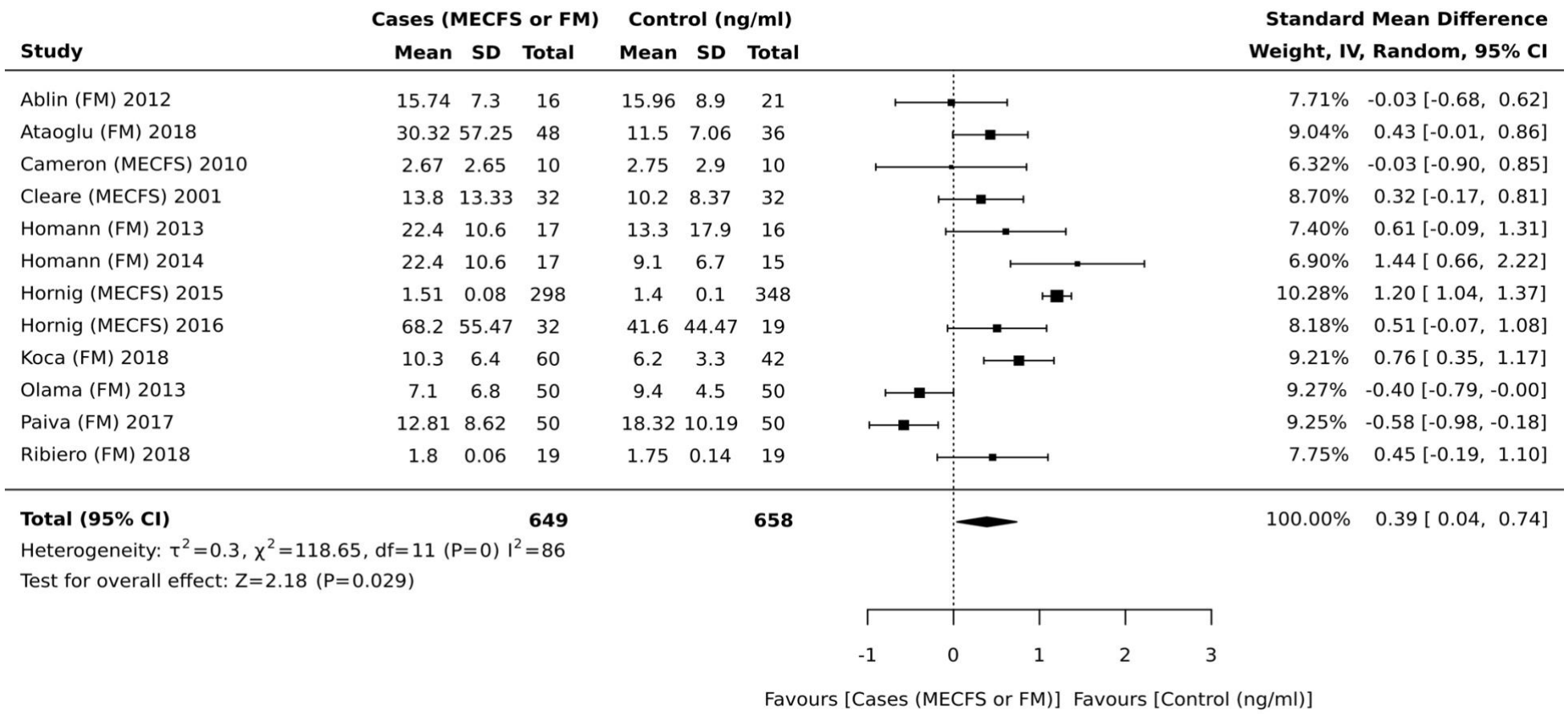


Figure 9 SUMARI Forest plot all 12 Studies Standardised Mean Difference

Forest Plot generated in **SUMARI** of all twelve case control studies – **Mean Difference (natural units)**

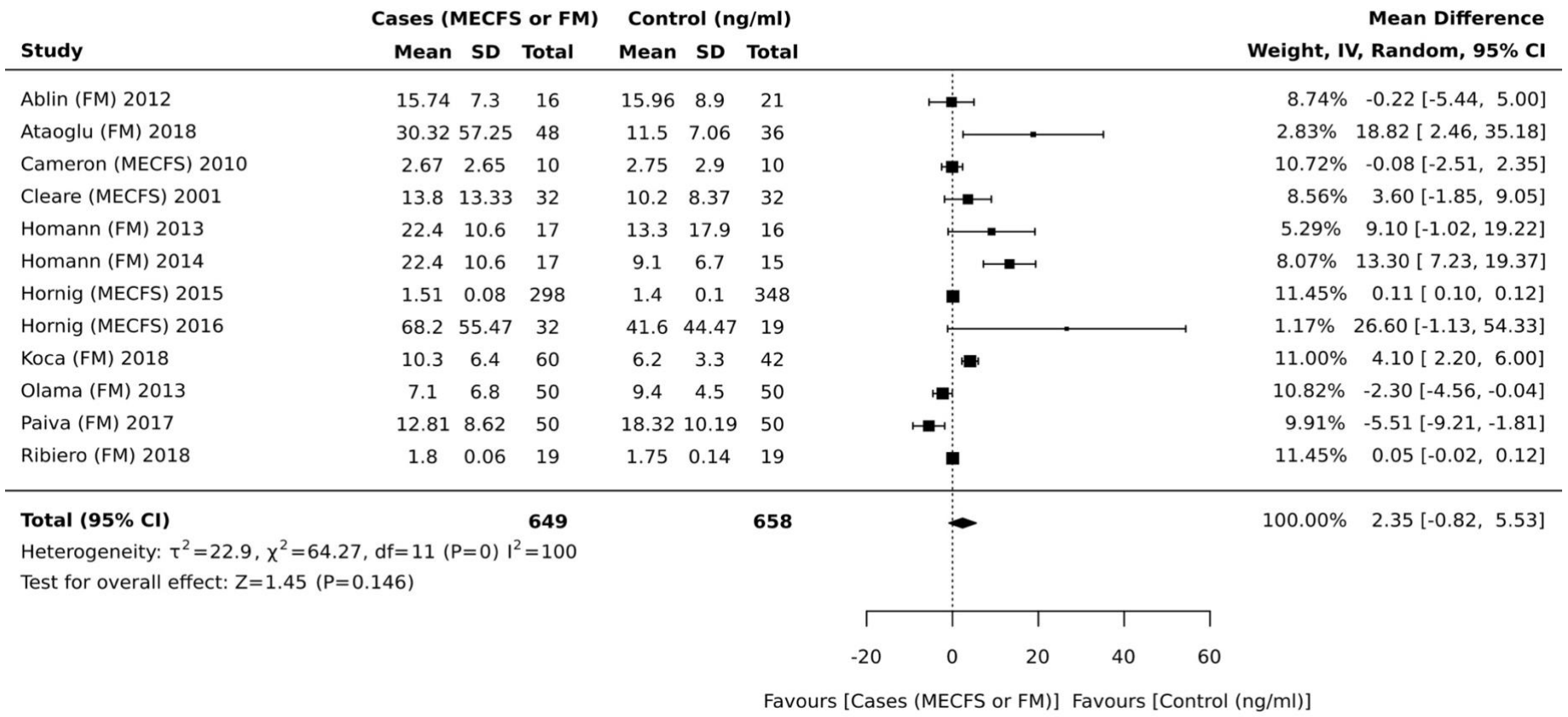


Figure 10 SUMARI Forest plot all 12 Studies Mean Difference

Forest Plot generated in **REVMAN 5** of all twelve case control studies – **Mean Difference (natural units)**

Study or Subgroup	Cases (MECFS or FM) Mean	SD	Total	Control (Healthy) Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ablin (FM) 2012	15.74	7.3	16	15.96	8.9	21	0.2%	-0.22 [-5.44, 5.00]
Ataoglu (FM) 2018	30.32	57.25	48	11.5	7.06	36	0.0%	18.82 [2.46, 35.18]
Cameron (MECFS) 2010	2.67	2.65	10	2.75	2.9	10	1.0%	-0.08 [-2.51, 2.35]
Cleare (MECFS) 2001	13.8	13.33	32	10.2	8.37	32	0.2%	3.60 [-1.85, 9.05]
Homann (FM) 2013	22.4	10.6	17	13.3	17.9	16	0.1%	9.10 [-1.02, 19.22]
Homann (FM) 2014	22.4	10.6	17	9.1	6.7	15	0.2%	13.30 [7.23, 19.37]
Hornig (MECFS) 2015	1.51	0.08	298	1.4	0.1	348	48.3%	0.11 [0.10, 0.12]
Hornig (MECFS) 2016	68.2	55.47	32	41.6	44.47	19	0.0%	26.60 [-1.13, 54.33]
Koca (FM) 2018	10.3	6.4	60	6.2	3.3	42	1.7%	4.10 [2.20, 6.00]
Olama (FM) 2013	7.1	6.8	50	9.4	4.5	50	1.2%	-2.30 [-4.56, -0.04]
Paiva (FM) 2017	12.81	8.62	50	18.32	10.19	50	0.5%	-5.51 [-9.21, -1.81]
Ribiero (FM) 2018	1.8	0.06	19	1.75	0.14	19	46.7%	0.05 [-0.02, 0.12]
Total (95% CI)			**649**			**658**	**100.0%**	**0.13 [-0.12, 0.38]**

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 64.27$, df = 11 ($P < 0.00001$); $I^2 = 83\%$

Test for overall effect: Z = 1.04 (P = 0.30)

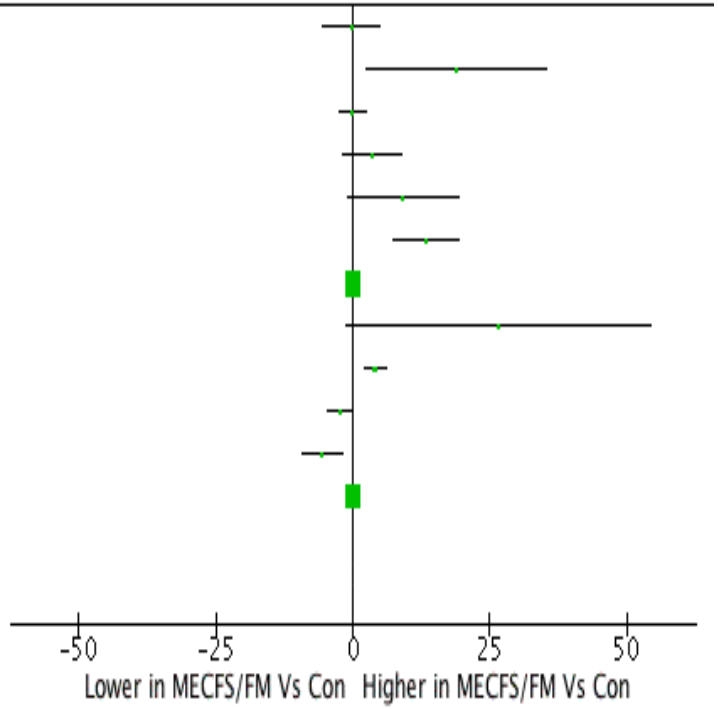


Figure 11 REVMAN Forest plot all 12 Studies Mean Difference

Forest Plot generated in REVMAN 5 - Four **MECFS** studies only – **Standardised Mean Difference**

Study or Subgroup	Cases (MECFS or FM) Mean	SD	Total	Control (Healthy) Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Ablin (FM) 2012	15.74	7.3	16	15.96	8.9	21		Not estimable
Ataoglu (FM) 2018	30.32	57.25	48	11.5	7.06	36		Not estimable
Cameron (MECFS) 2010	2.67	2.65	10	2.75	2.9	10	19.1%	-0.03 [-0.90, 0.85]
Cleare (MECFS) 2001	13.8	13.33	32	10.2	8.37	32	26.0%	0.32 [-0.17, 0.81]
Homann (FM) 2013	22.4	10.6	17	13.3	17.9	16		Not estimable
Homann (FM) 2014	22.4	10.6	17	9.1	6.7	15		Not estimable
Hornig (MECFS) 2015	1.51	0.08	298	1.4	0.1	348	30.4%	1.20 [1.04, 1.37]
Hornig (MECFS) 2016	68.2	55.47	32	41.6	44.47	19	24.5%	0.51 [-0.07, 1.08]
Koca (FM) 2018	10.3	6.4	60	6.2	3.3	42		Not estimable
Olama (FM) 2013	7.1	6.8	50	9.4	4.5	50		Not estimable
Paiva (FM) 2017	12.81	8.62	50	18.32	10.19	50		Not estimable
Ribiero (FM) 2018	1.8	0.06	19	1.75	0.14	19		Not estimable
Total (95% CI)			**372**			**409**	**100.0%**	**0.57 [-0.05, 1.18]**

Heterogeneity: $Tau^2 = 0.32$; $Chi^2 = 20.79$, df = 3 (P = 0.0001); $I^2 = 86\%$

Test for overall effect: Z = 1.81 (P = 0.07)

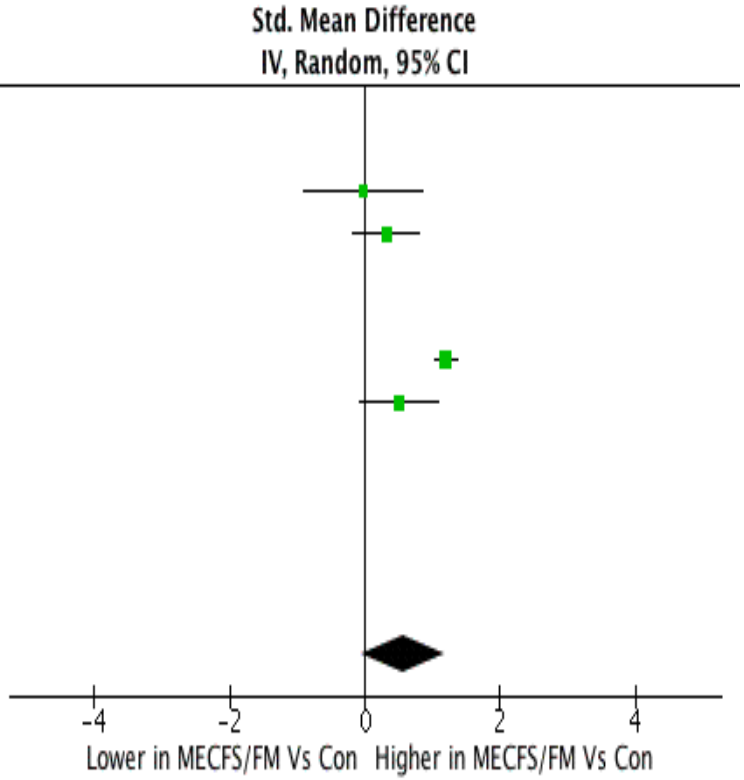


Figure 12 REVMAN Forest plot MECFS Studies only Standardised Mean Difference

Forest Plot generated in REVMAN 5 - Eight **Fibromyalgia** case-control studies – **Standardised Mean Difference**

	Cases (MECFS or FM)			Control (Healthy)				Std. Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
Ablin (FM) 2012	15.74	7.3	16	15.96	8.9	21	11.7%	-0.03 [-0.68, 0.62]
Ataoglu (FM) 2018	30.32	57.25	48	11.5	7.06	36	13.5%	0.43 [-0.01, 0.86]
Cameron (MECFS) 2010	2.67	2.65	10	2.75	2.9	10		Not estimable
Cleare (MECFS) 2001	13.8	13.33	32	10.2	8.37	32		Not estimable
Homann (FM) 2013	22.4	10.6	17	13.3	17.9	16	11.3%	0.61 [-0.09, 1.31]
Homann (FM) 2014	22.4	10.6	17	9.1	6.7	15	10.5%	1.44 [0.65, 2.23]
Hornig (MECFS) 2015	1.51	0.08	298	1.4	0.1	348		Not estimable
Hornig (MECFS) 2016	68.2	55.47	32	41.6	44.47	19		Not estimable
Koca (FM) 2018	10.3	6.4	60	6.2	3.3	42	13.7%	0.76 [0.35, 1.17]
Olama (FM) 2013	7.1	6.8	50	9.4	4.5	50	13.8%	-0.40 [-0.79, 0.00]
Paiva (FM) 2017	12.81	8.62	50	18.32	10.19	50	13.8%	-0.58 [-0.98, -0.18]
Ribiero (FM) 2018	1.8	0.06	19	1.75	0.14	19	11.8%	0.45 [-0.19, 1.10]
Total (95% CI)			**277**			**249**	**100.0%**	**0.30 [-0.15, 0.75]**

Heterogeneity: $Tau^2 = 0.35$; $Chi^2 = 43.20$, df = 7 ($P < 0.00001$); $I^2 = 84\%$

Test for overall effect: Z = 1.29 (P = 0.20)

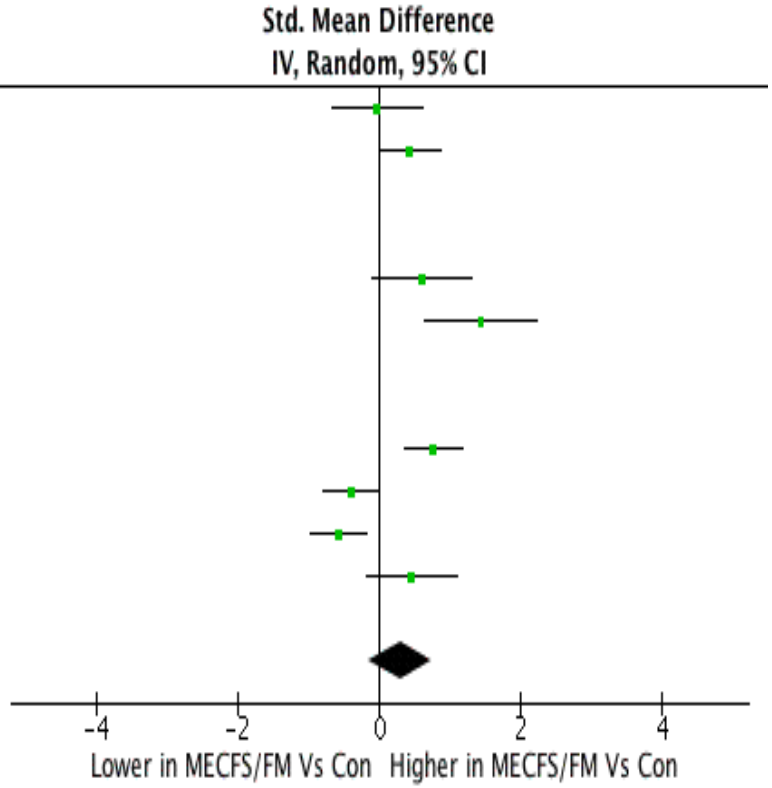


Figure 13 Forest plot REVMAN Fibromyalgia Studies only Standardised Mean Difference

Table 14 Differences in Meta-analysis Type

	Type of Analysis	Studies included	Platform	Difference	Lower CI	Upper CI	Signifi-cance	Heterog-eneity I^2	Z overall effect	Tau^2	Chi^2	df	Cases	Controls	All
1 Fig 8	SMD	All 12	REVMAN	0.39	-0.04	0.82	0.08	91%	1.77	0.49	118.42	11	649	658	1307
2 Fig 9	SMD	All 12	SUMARI	0.39	0.04	0.74	0.029	86%	2.18	0.3	118.65	11	649	658	1307
3 Fig 10	MD	All 12	SUMARI	2.35	-0.82	5.53	0.146	100%	1.45	22.9	64.27	11	649	658	1307
4 Fig 11	MD	All 12	REVMAN	0.13	-0.12	0.38	0.3	83%	1.04	0.03	64.27	11	649	658	1307
5 Fig 12	SMD	MECFS only	REVMAN	0.57	-0.05	1.18	0.07	86%	1.81	0.32	20.79	3	372	409	781
6 Fig 13	SMD	Fibro only	REVMAN	0.3	-0.15	0.75	0.02	84%	1.29	0.35	43.2	7	277	249	526
7 (no figure)	SMD	Without Hornig 2015	REVMAN	0.29	-0.06	-0.06	0.63	78%	1.65	0.25	44.95	10	351	310	661
8 (no figure)	MD	Without Hornig 2015	REVMAN	1.76	-0.5	4.01	0.13	84%	1.53	8.18	61.81	10	351	310	661

Table 14 provides a summary of all forest plot results for quick reference. The first 6 results are provided in the forest plots above (See figure 8-13) and the last two results (table item 7 & 8 – See Table 14) where an additional analysis was completed without the largest study by Hornig et al. 2015 which accounts for almost half of all people in the studies combined, containing 646 participants of the total 1307 participants. The SMD and mean difference (MD) summary results for the remaining 11 studies are provided. Removing this study reduced the pooled difference by just 0.1 (0.39 to 0.29).

3.10 Meta-analysis

The meta-analysis was used to compare baseline leptin results in cases (those studies that described a diagnosis of chronic fatigue and/or fibromyalgia) and controls (healthy participants). It was decided to use a standardised mean difference because the methodology of each study was very different, although they were measuring the same continuous variable circulating leptin in ng/mL. An example of the differences in studies were, the type of circulating fluid used including serum (5 studies), plasma (6 studies) and just one study used CSF (Hornig et al. 2016) (see Table 9 and 16 for more details). This latter study showed that leptin is more concentrated in CSF compared to serum or plasma by approximately 5-fold, which is visually displayed in the histogram (Figure 15 page 102) (Hornig et al. 2016). The other major difference was that this review has combined two syndromes MECFS (4 studies) and fibromyalgia (8 studies); both use similar methodologies and measure the same continuous measure of circulating leptin, but not with enough coherence to use a 'mean difference' or fixed effect.

Of the twelve studies only 5 included male and female participants (Cameron et al. 2010; Cleare, O'Keane & Miell 2001; Hornig et al. 2016; Hornig et al. 2015; Koca et al. 2018) and men were invariably at a lower ratio than women, reflecting the higher frequency of women with MECFS and/or fibromyalgia by gender in the general population. For example, Hornig (2015), the study with the highest population (n=646) in this meta-analysis had a total number of MECFS cases = 298 (female 220) and controls = 348 (females 260). This represented a ratio of around 74% females (n=480) i.e., 3:1. MECFS has a prevalence of around 0.3% in the general population whereas fibromyalgia has a 10 fold higher general population prevalence at around 4% (Natelson 2019).

Prins, van der Meer and Bleijenberg (2006) completed a literature review of the research and also found 75% of participants in their review were female, the mean age was 25-35, and the duration of illness was between 3 to 9 years. There are varying reports on the sex ratio of male to females with fibromyalgia, but a study across 5 different European countries reported a ratio of around 1:3 (Castro-Sanchez et al. 2012). Both illnesses may have a psychobiological basis in the form

of a stress based experience or life event causing neural sensitisation (Prins, van der Meer & Bleijenberg 2006). With reference to diagnostic precision, the heterogenous nature of these syndromes is well reported, and is further highlighted with the results of this meta-analysis.

The forest plots (Figures 8 – 13, page 92) reflect the results of the 12 studies which are also provided in the form of a basic means table shown below (See Table 15). This table provides the raw results for the whole study population 649 cases and 658 controls (total n = 1307) and a simple raw mean difference (without weighting) of cases compared to controls (mean of cases 17.42 ng/mL – mean of controls 11.79 ng/mL) = mean difference of 5.63 ng/mL (raw difference i.e., original units). The total mean of all cases n= 649 cases (17.42 ng/mL) + mean of all controls 658 controls (11.79 ng/mL) = 29.21 ng/mL (cases + controls) and divided by 2 = 14.6 ng/mL (mean of 1307 participants). The overall simple mean is 14.6 ng/mL of circulating leptin. However, because the Hornig et al. (2016) study is using CSF which is 5 times higher, this makes the mean of all 12 studies of 14.6 ng/mL higher than if only blood samples were used. Removing this study from the raw mean provided a mean result of 10.03 ng/mL. This is another example of why the use of SMD was essential.

Table 15 Simple Raw Mean Differences Cases vs Controls (low to high)

	Cases	SD	**Controls**	SD	**Difference**	favours
Hornig MECFS 2015	**1.51**	0.08	**1.4**	0.1	**0.11**	cases
Ribeiro FM 2018	**1.8**	0.06	**1.75**	0.14	**0.05**	cases
Cameron MECFS 2010	**2.67**	2.65	**2.75**	2.9	**-0.08**	controls
Ablin FM 2012	**15.74**	7.3	**15.96**	8.9	**-0.22**	controls
Olama FM 2013	**7.1**	6.8	**9.4**	4.5	**-2.30**	controls
Paiva FM 2017	**12.81**	8.62	**18.32**	10.19	**-5.51**	controls
Koca FM 2018	**10.3**	6.4	**6.2**	3.3	**4.10**	cases
Cleare MECFS 2001	**13.8**	13.33	**10.2**	8.37	**3.60**	cases
Homann FM 2013	**22.4**	10.6	**13.3**	17.9	**9.10**	cases
Homann FM 2014	**22.4**	10.6	**9.1**	6.7	**13.30**	cases
Ataoglu FM 2018	**30.32**	57.25	**11.5**	7.06	**18.82**	cases
Hornig MECFS 2016	**68.2**	55.47	**41.6**	44.47	**26.60**	cases
Mean i.e., Total / 12	**17.42**	14.93	**11.79**	9.54	**5.63**	cases
N (population)	**649**		**658**		**Total**	1307

Difference from the mean in cases (n=649) is (cases 17.42 – sample mean 14.6 = **2.82 ng/mL**) 19.32% difference greater than the total sample mean (n=1307). Difference from mean in controls is (11.79 – 14.93 = -3.14) or 21.51 % lower than the total sample mean. Using the SUMARI meta-analysis calculator, selecting the option of standardised mean difference, including both means and standard deviations provided by each study, this equated to a difference between cases and controls of 0.39 and the 95% confidence intervals are 0.04 to 0.74, of which the lines do not cross the point of significance, so the difference is just significant p=0.029.

The re-expression calculation can be summarised as Standard Deviation (SD) of the control group mean 8.37 ng/mL (represented by the Cleare, O'Keane and Miell (2001) study) multiplied by the Pooled Standardised Mean Difference of 0.39 = 3.26 ng/mL. The re-expressed result in the original units is a close match with the basic raw calculation above i.e., re-expressed result of 3.26 ng/mL higher (0.33 to 6.19) in cases compared to controls. This is very close to the simple calculation (in Table

15) shown earlier demonstrating a difference from the total mean for cases = 2.82 ng/mL, (3.26-2.82 =0.44 ng/mL) suggesting the use of SMD was an effective and representative method for this meta-analysis.

However, when we used REVMAN, this appears to use slightly different weightings in the calculations and the confidence intervals change very slightly to -0.04 to 0.82 resulting in the result now not being significant p=0.08. When the calculations are completed using a mean difference model using **SUMARI** rather than a SMD model, then the difference becomes slightly smaller at 2.35 ng/mL (CI -0.82 to 5.53) p=0.146 but also scored a heterogeneity score of 100% I^2 (See

Figure 14 below).

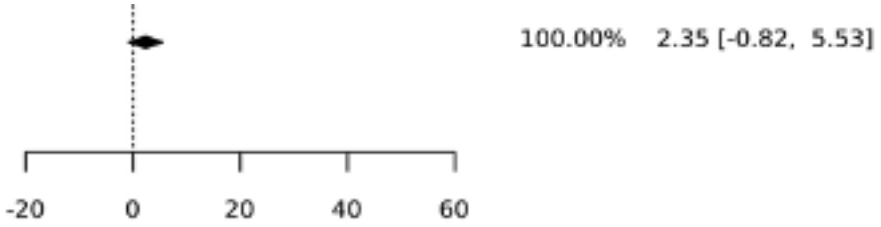


Figure 14 Pooled Difference

All formats favour that leptin is higher in cases than controls in both the Mean Difference (2.35 ng/mL CI -082,5.53) and Standardised Mean Difference (re-expressed 3.26 ng/mL) or even the simple unweighted mean difference as provided in Table 15 (2.82 ng/mL). We can assume the difference to be between 2.35 ng/mL to 3.26 ng/mL with an average of these three results being 2.81 ng/mL. The small difference however of around 2.81 ng/mL could readily be explained by changes in BMI, menstrual phase, or even the changes in leptin across the time of day and therefore this difference is not large enough to be of clinical significance without controlling and accounting for these confounders in all studies (Rafique et al. 2018). So, the results are not convincing and require further research.

Here is a list of 3 different means MD, SMD, and Raw results:

MD result:	2.35 ng/mL
SMD result:	3.26 ng/mL – re-expressed (used Controls)
Raw mean difference:	2.82 ng/mL – (see Table 15)
Average of above:	**2.81ng/mL** – (average of all 3 above)

We can assume the difference falls between 2.35 and 3.26 ng/mL. Cases have a mean leptin at 2.81 ng/mL higher than controls. As more studies are completed in the future, this trend may be clarified.

3.11 Study Design

There were 4 studies completed in Brazil, two in the United States and the remainder from other countries and are displayed in the following table (Table 16). There are only 4 studies that are MECFS related and 8 fibromyalgia related. Most studies had an average of around 109 participants, but some were much lower than the average (the smallest being 20 participants), and the study that stands apart is the Hornig et al. 2015 study with 646 participants (49% of total sample 1307). If we remove the Hornig study the average would be only 60 participants per study.

Every study included females but only 5 studies had male participants, and these were of a much lower proportion of the study population at around 25% males compared to female participants. The 1:3 ratio is representative of the general population prevalence of MECFS as discussed earlier, but most articles had a higher female representation in fibromyalgia studies, but it can be seen that some did not include males at all providing little rationale for this strategy (Natelson 2019). Around half of the studies used plasma (6) and the other half used serum (5), with only one study using CSF. Standard Operating Procedures suggest that plasma

has to be kept on ice as soon as the sample is taken, and it must be centrifuged within approximately half an hour of the sample being obtained (Tuck et al. 2009).

Serum can be left at room temperature as it requires at least 30 min to allow it to clot. The fact that it does not have to be placed on ice or in a freezer immediately makes it easier to manage in a study, but there is evidence that the sooner it is placed on ice following coagulation and stored, the higher the integrity of the sample and the ensuing results (Timms et al. 2007). It was noted that in one study when comparing both serum and CSF, the results for people who had fibromyalgia and were non-smokers had a serum level of 28.8 ng/mL [SD 16.4–48.0] and a CSF level of 165 ng/mL [SD 114–225]: therefore 165 divided by 28.8 = 5.85 times higher in CSF (Bokarewa et al. 2014). The fact that CSF levels of leptin may be over five-fold higher than the serum level of leptin provides another reason for using a standardised mean difference in the analysis because one of the studies only provided the results from circulating CSF (Hornig et al. 2016). Removing this study from the analysis (n=51 of total 1307) made very little difference using the SMD. A table demonstrating study design differences is provided in Table 16.

Table 16 Study design differences of 12 studies

No.	Study Author & Year	Country in which the study conducted	Contains Chronic Fatigue Syndrome	Contains Fibromyalgia	Total number (N)	Cases	Controls	Male in sample	Female in sample	Plasma (medium of sample)	serum	CSF (cerebro-spinal fluid)	Time of day
1	Ablin 2012	Israel	No	Yes	37	16	21	No	Yes	Yes	No	No	Yes
2	Ataoglu 2018	Turkey	No	Yes	84	48	36	No	Yes	No	Yes	No	Yes
3	Cameron 2010	Australia	Yes	No	20	10	10	Yes	Yes	No	Yes	No	No
4	Cleare 2001	UK	Yes	No	64	32	32	Yes	Yes	Yes	No	No	Yes
5	Homann 2013	Brazil	No	Yes	33	17	16	No	Yes	Yes	No	No	Yes
6	Homann 2014	Brazil	No	Yes	32	17	15	No	Yes	Yes	No	No	Yes
7	Hornig 2015	United States	Yes	No	646	298	348	Yes	Yes	Yes	No	No	Yes
8	Hornig 2016	United States	Yes	No	51	32	19	Yes	Yes	No	No	Yes	No
9	Koca 2018	Turkey	No	Yes	102	60	42	Yes	Yes	No	Yes	No	No
10	Olama 2013	Egypt	No	Yes	100	50	50	No	Yes	No	Yes	No	No
11	Paiva 2017	Brazil	No	Yes	100	50	50	No	Yes	No	Yes	No	No
12	Ribeiro 2018	Brazil	No	Yes	38	19	19	No	Yes	Yes	No	No	No
	Totals		4	8	1307	649	658	5	12	6	5	1	6

It helps to provide an initial visual analysis, and when the raw baseline means are plotted in a graph, it shows that 4 studies have negligible difference, and 2 studies favour controls, whereas 6 studies are clearly in favour of cases having a higher level of circulating leptin than controls. The results of the Hornig 2016 study displaying CSF results demonstrate the five-fold increase in leptin levels. This can be seen on the far right of the next graph. See the following page (Figure 15) to explore the visual differences.

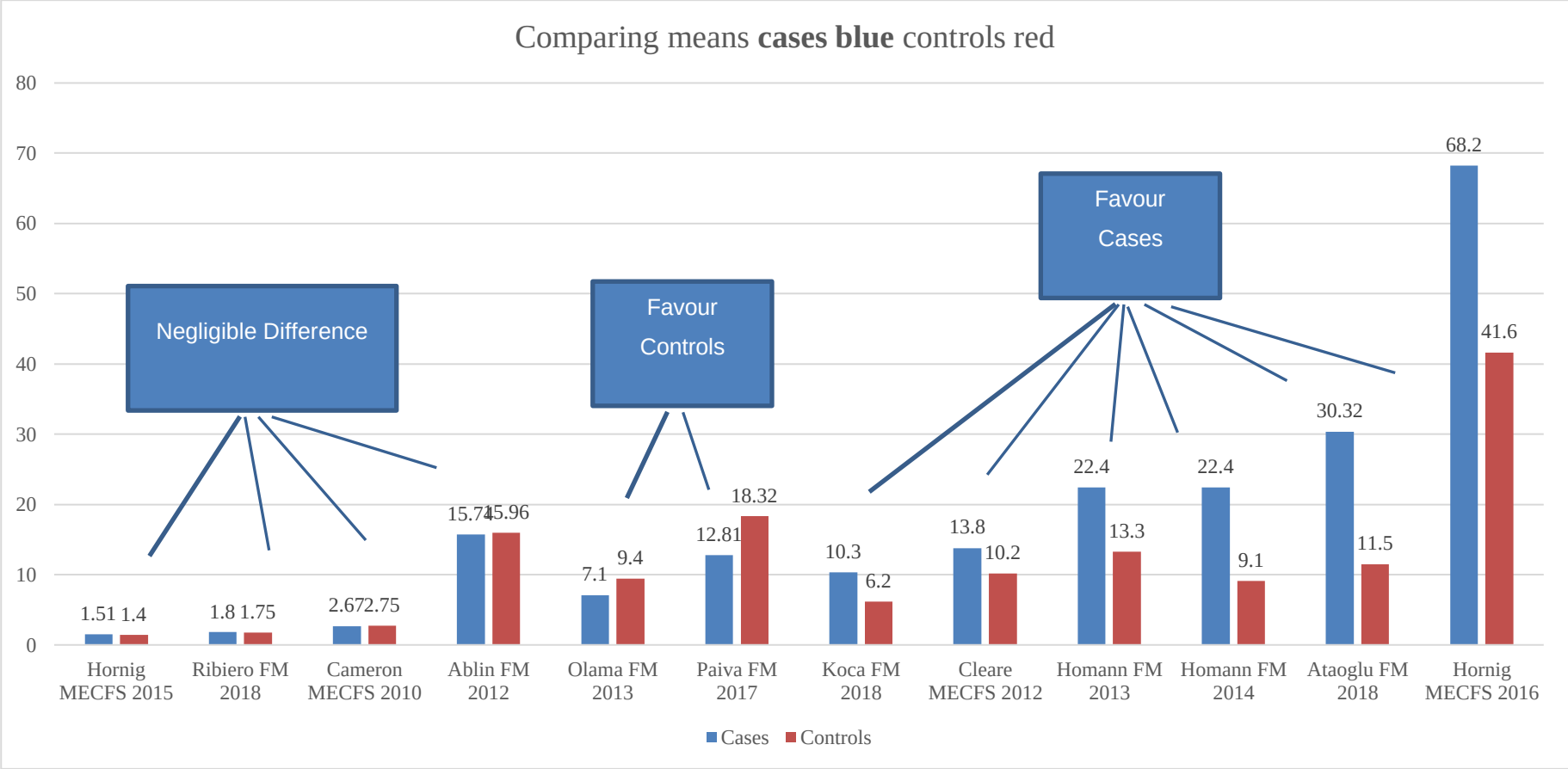


Figure 15 Histogram of Mean Differences of leptin ng/mL (Cases in blue)

Histogram of Mean Differences, no difference (4 studies), controls higher (2), then cases higher (6). NB one CSF study (Hornig 2016)

3.12 Grade and Summary of Findings Table

GRADE is an acronym for Grades of Recommendation, Assessment, Development, and Evaluation. The idea behind GRADE is to provide an assessment of the best available evidence and then to provide a statement of the certainty of that evidence. The principle is usually to review the findings offered in guidelines and to provide a grading of the certainty of the evidence supporting clinicians to assist with decision making (Brozek et al. 2009). A summary of findings table provides a quick reference to assess the overall certainty of the evidence and is now used by professional organisations across the world, as well as high quality journals (See Table 17).

3.13 Assessing Confidence Using Grade

The outcome for the certainty of findings for this systematic review were ‘very low’ (See Table 17, page 112). According to Brozek et al. (2009) p. 672 those studies that are categorised as very low would include:

“Randomised trials with very serious limitations and inconsistent results; Observational studies with serious limitations, unsystematic clinical observations (e.g., case series or case reports).”

The highest quality of research is considered to be pooled findings from randomised controlled trials that have few limitations, are properly blinded and have little imprecision and heterogeneity. Observational studies, such as the ones found in this systematic review, which includes case control studies and cohort studies, would prove difficult to obtain a high certainty rating, before we even start the downgrading process. The inconsistency in the design and methodology resulted in starting at a ‘low level’ but the following issues gradually downgraded the score to ‘very low’ certainty, and any evidence or recommendations from this research would be considered as weak.

There are five dimensions to consider when making an overall certainty rating decision and these include, inconsistency, indirectness, imprecision, risk of bias, and publication bias (Guyatt, G et al. 2013).

A series of publications are available that discusses the issues of quality that may affect certainty, and one of these include issues of imprecision. This can usually be seen in the size of the confidence intervals (Guyatt, GH, Oxman, Kunz, Brozek, et al. 2011). A wide confidence interval suggest the results could fall anywhere within a large range, indicating the real mean result is questionable. In this review the confidence intervals were wide for most of the included studies considering the size of the population investigated (usually <=100 and as small as 20). So, this results in a downgrade of one level to low certainty.

Another issue for consideration is inconsistency which can be seen through the heterogeneity (showing widely differing magnitudes of effect and differences in direction of results), high variation of point estimates and overlapping confidence intervals (Guyatt, GH, Oxman, Kunz, Woodcock, Brozek, Helfand, Alonso-Coello, Glasziou, et al. 2011). A high I^2 represents high heterogeneity which was observed in this systematic review (86% heterogeneity) resulting in a further downgrade of one to very low certainty. When the comparison was completed using just a 'mean difference' the heterogeneity was at the maximum of 100%.

A key issue with comparing the quality of studies in this review was indirectness, which refers to the difficulty in comparison across research studies (Guyatt, GH, Oxman, Kunz, Woodcock, Brozek, Helfand, Alonso-Coello, Falck-Ytter, et al. 2011). The results had to be analysed using a standardised mean difference and random effects model because of apparent differences in methodology, body fluid types used, and the variation in magnitude of results. We cannot downgrade the findings any further, so the GRADE recommendation remains very low for certainty of evidence.

With reference to publication bias, the results included studies with 3 differing outcomes, some indicating low leptin or **hypo**leptinaemia (2), some showing hardly any difference between cases and controls (4), but the majority indicated higher

levels of leptin or **hyper**leptinaemia in cases (6). On balance when the point estimates are published in the form of a funnel plot, you can usually identify patterns of publication bias (Guyatt, GH, Oxman, Montori, et al. 2011). This did not seem to be a problem in this review. See the funnel plot provided in Figure 16 (on page 114), which demonstrates a reasonably symmetrical figure. However, this is limited image as there are only 12 publications to plot.

Lastly, the selected studies for the meta-analysis had some quality issues of concern such as managing confounders consistently, limited details about the method, and protocols, not providing transparent results, and the difficulties in comparing these studies in the natural measurements. A summary of findings table is presented on the following page (See Table 17 below):

Table 17 Summary of Findings Table

Leptin Cases compared to Controls SMD for Myalgic Encephalomyelitis Chronic Fatigue Syndrome and/or Fibromyalgia

Patient or population: [health problem] Chronic Fatigue Syndrome and/or Fibromyalgia vs Controls (Healthy participants)
Setting: Usually a clinic setting or university / community research recruitment
Intervention: Circulating Leptin levels Cases
Comparison: Controls - SMD also re-expressed as original units

Outcomes	**Illustrative comparative risks*** (95% CI) **Leptin in those without ME, CFS or FM**	Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
Leptin Levels ng/mL (circulating leptin levels – plasma / serum or cerebrospinal fluid)	The mean difference in leptin levels in cases was SMD **0.39 (0.04 to 0.74) higher in cases than controls. This is equivalent to a re-expressed result of** 3.26 ng/mL higher (0.33 to 6.19) in cases as compared to controls.*		n=1307 (649 Cases vs 658 Controls) (12 observational studies)	⊕◯◯◯ VERY LOW a,b,c,d	All studies used leptin ng/mL but used different fluids and methods of analysis

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).**CI:** Confidence interval; MD Mean Difference
SMD: Standardised Mean difference. SMD and CI taken from SUMARI analysis.

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations:
a. Downgrade 1 for imprecision Two studies provide evidence of hypoleptinaemia (low leptin), four studies report negligible difference, and 6 studies report hyperleptinaemia (high leptin) in cases.
b. High heterogeneity (I2 = 86%), downgrade 1.
c. Downgraded 1 for risk of bias. Confounders such as BMI and time of day (circadian rhythmicity) are not considered in all studies, and some have adjusted their results whilst others have not. The results vary significantly, indicating the method of laboratory storage, sample handling, assessment and analysis are different between studies.
d. Downgraded 1 level for inconsistency with wide confidence intervals, and outcome results presented in opposite directions.

***Re-expression of SMD to original units**

To translate the Standardised Mean Difference (SMD) (0.39) back to the original measure of ng/mL, it is necessary to take a representative study and take the SD of the control group, which is then multiplied by the SMD. The study Cleare, O'Keane and Miell (2001) as assessed by two reviewers of being of high quality and has a low risk of bias was used. The re-expression calculation can be summarised as Standard Deviation (SD) of the Control group mean 8.37 ng/mL multiplied by the Pooled Standardised Mean Difference of 0.39 = 3.26 ng/mL. Re-expressing SMDs using a familiar instrument in a method recommended by Cochrane is 0.39 x 8.37 ng/mL = 3.26 ng/mL (CI 0.33 ng/mL to 6.19 ng/mL). The CI was calculated using the same formula.

Risk of publication bias

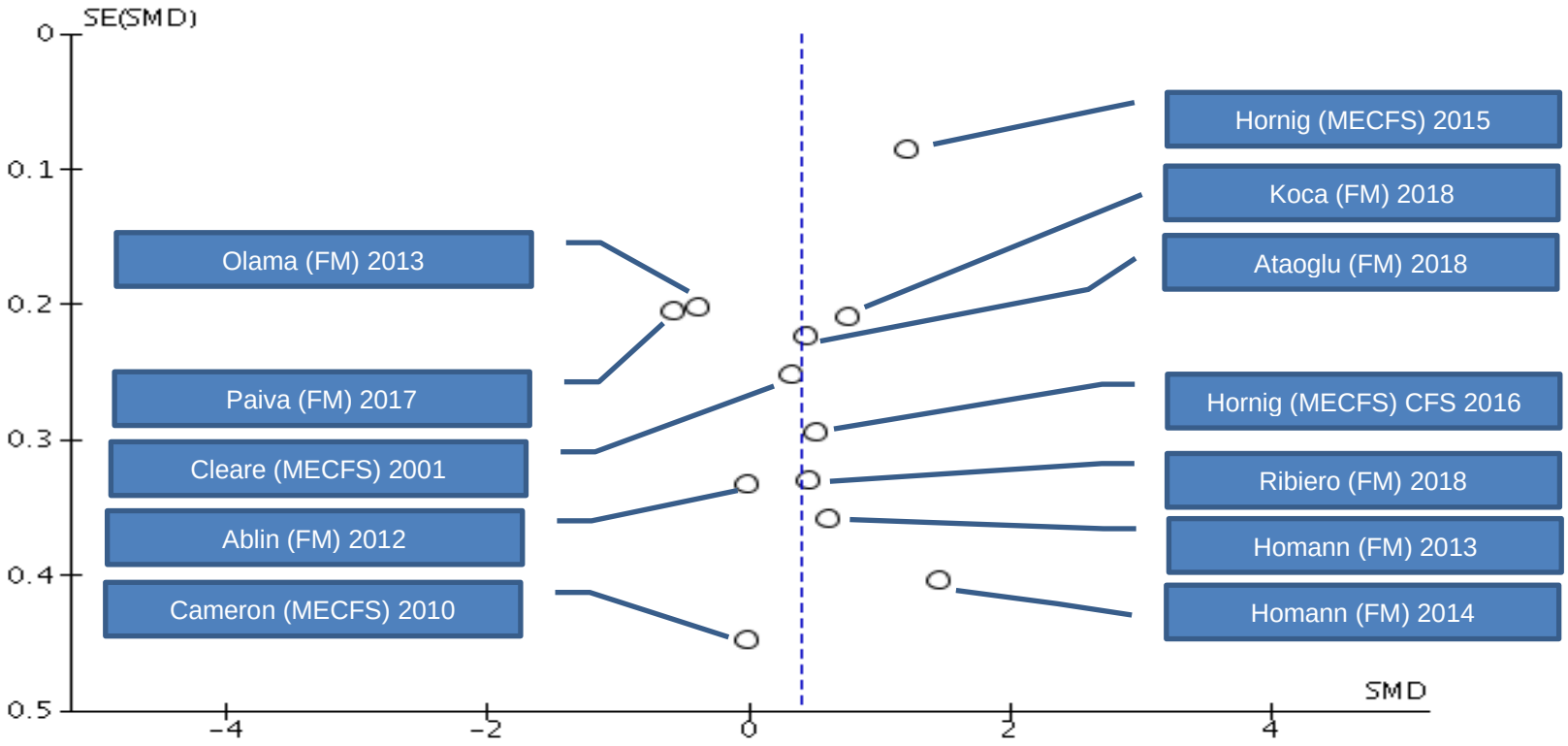


Figure 16 Funnel Plot with Study Details

3.14 Funnel Plot and Publication Bias

The funnel plot above on the previous page indicates that there is a low risk of publication bias. There are the same number of studies on the left of the mean difference, as fall on the right, and an equal number of studies fall in the centre. They are also spread across all quadrants of the funnel plot, which indicates a low risk of publication bias. There are not enough studies to formulate the usual inverted triangle which would normally suggest a low risk of publication bias (Guyatt, GH, Oxman, Montori, et al. 2011).

CHAPTER 4

Cohort Studies

4 Cohort Studies

4.1 Cohort Studies

There were 9 cohort type studies that provided baseline leptin levels, but these were not in the form of a traditional case control study and were not included in the meta-analysis as they had no controls. They do however provide information on baseline leptin, and leptin levels when conditions are changed within a cohort such as when measuring exercise groups across time or when comparing a health condition such as smokers and non-smokers. Table 18 provides a summary of these 9 studies (next page).

A summary of leptin results is provided in the Table 18 below:

Table 18 Summary of Cohort Studies

Study Author & Year	Title	Brief Summary of Article	Baseline Leptin Cases ng/mL	Male	Female	Total N
Bjersing 2013	Exercise and obesity in fibromyalgia (FM): Beneficial roles of IGF-1 and resistin?	The aim was to examine effects of exercise on fatigue, in lean, overweight and obese FM patients -Baseline FIQ fatigue correlated negatively with serum leptin (r = -0.345; P = 0.016). Leptin levels were lowest in lean patients and tended to be higher in overweight patients (P = 0.067) and were highest in obese patients (P <0.001).	Baseline Lean (Median Range) 16ng/mL (10.3 to 25.6 ng/mL); baseline overweight (Median Range) 27.7 ng/mL (19.2 to 45.5 ng/mL); baseline Obese (Median Range); 45.3 (34.5 to 86.3 ng/mL)	No	Yes	48
Bjersing 2017	Benefits of resistance exercise in lean women with FM: Involvement of IGF-1 and leptin	This is a sub-study of a previous registered randomised clinical trial of physical exercise vs relaxation exercise. This published article looked at lean, overweight and obese women with FM and response to exercise. IGF-1 (p= 0.047), IGFBP3 (p= 0.025) and leptin (p= 0.008) were significantly decreased in lean women (n= 18. The clearest clinical response to resistance exercise was found in lean patients with FM.	Median levels reported. All (27.7 ng/mL), lean (21 ng/mL), overweight (39 ng/mL), obese (23 ng/mL).	No	Yes	43

Study Author & Year	Title	Brief Summary of Article	Baseline Leptin Cases ng/mL	Male	Female	Total N
Bokarewa 2013	Smoking is associated with reduced IGF-1 levels and higher pain experience in patients with FM	This is an abstract only and provides Leptin levels for 63 women with FM all aged 52 years. Leptin levels were measured and described in relation to their smoking behaviour of smokers vs non-smokers (those who have given up smoking) = (median: 16.9 vs 34.8 ng/mL, p=0.013). Evaluates the possible relation between pain, smoking and levels of IGF and leptin	Median of 16.9 ng/mL in smokers vs 34.8ng/mL in non-smokers	No	Yes	63
Bokarewa 2014	Smoking is associated with reduced leptin and neuropeptide Y levels and higher pain experience in patients with FM	Present study addressed the effect of cigarette smoking on adipokines and pain parameters, in 62 women with FM pain syndrome with unknown etiology. Current smokers (n= 18) had lower levels of leptin compared to ex-smokers (n= 25). Smoking may be responsible for lower levels of leptin which usually reduces pain levels, but smokers had higher levels of pain as well as lower leptin levels.	Current smokers (n=18) leptin median (IQR) 19.4 ng/mL (11.7-28.7 ng/mL); non-smokers (n=19) leptin median (IQR) 28.8 (16.4-48.0); ex-smokers (n=25) leptin median (IQR) 36.8 (28-55.7 ng/mL)	No	Yes	62

Study Author & Year	Title	Brief Summary of Article	Baseline Leptin Cases ng/mL	Male	Female	Total N
Hornig 2017	Immune network analysis of cerebrospinal fluid (CSF) in myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS) with atypical and classical presentations	Analysed CSF from 32 cases with classical ME/CFS and 27 cases with atypical ME/CFS using a 51-plex cytokine assay. Analysis of CSF in ME/CFS with atypical and classical presentations	Leptin ng/mL atypical CFS duration short <3 yrs. (n=13) 44.07 ng/mL SD 26.65 ng/mL; atypical CFS duration long >=3 yrs. (n=6) 66.35 ng/mL SD 54.02 ng/mL and classical CFS duration short (n=14) 55.44 ng/mL SD 45.25; classical CFS duration long (n=18) 78.13 ng/mL SD 61.80 NB: remember these are CSF samples and are therefore 5 times higher than that of plasma.	Yes	Yes	59
Katz 2017	Leptin, a hypothalamic signalling hormone, is elevated in FM patients	Leptin was found to be higher in FM when compared with rheumatoid arthritis patients.	Leptin levels varied between 1.7 and 70.7 ng/mL (mean 28.0 ng/mL)	N/A	N/A	33

Study Author & Year	Title	Brief Summary of Article	Baseline Leptin Cases ng/mL	Male	Female	Total N
Kurajoh 2016	Plasma leptin concentration is associated with fatigue severity in patients with cardiovascular risk factors- HSCAA study	This cross-sectional study included 347 patients with 1 or more cardiovascular risk factors, who participated in the Hyogo Sleep Cardio-Autonomic Atherosclerosis (HSCAA) Study. Subjects who underwent a complete fatigue scale examination and plasma leptin measurement were included	2.32 ng/mL ± 0.75 ng/mL moderately fatigued	Yes	Yes	347
Quismorio 2014	Elevated serum leptin concentrations in a subset of FM patients with high inflammatory markers	**Abstract Only**: The purpose of the study is to measure biomarkers using the Vectra-DA in FM. In a subset of FM patients with elevated sedimentation rate (ESR) and / or C-reactive protein. The elevated levels of leptin in this **subset of FM pts**, independent of BMI, suggest that factors other than obesity may account for this elevation	45% of subjects had leptin concentrations exceeding the range reported in rheumatoid arthritis (1-45 ng/mL). The mean leptin in FM was 42.3 ng/mL (range 30-81 ng/mL).	6%	94%	33

Study Author & Year	Title	Brief Summary of Article	Baseline Leptin Cases ng/mL	Male	Female	Total N
Younger 2016	Association of Leptin with Body Pain in Women	There are two studies, both cohort studies. Study 1 analyses the daily leptin levels of women with **FM (n=3)** over 25 continuous days, like the Stringer study of 2013. Study 2 is a retrospective study (n=5676) of generally health postmenopausal women, comparing a single blood draw with symptoms of self-reported body pain. Body weight for the three participants was as follows: 60.2, 82.1, and 101.2 kg.	0.7 ng/mL (range 0.2,-2.0),10.2 ng/mL (range 5.8,-17.1), and 20.5 ng/mL (range 11.21-38.7 ng/mL)	No	Yes	3

4.2 Overview of Cohort Studies and Significance of Results

The nine studies are difficult to compare as they have very different study designs and a few of the studies are by the same authors e.g., Bokarewa and Bjersing produced two papers each. These nine studies mostly reported results for fibromyalgia, and two studies did not fit under either syndrome description but were thought to be important studies by both full text reviewers (Kurajoh et al. 2016; Younger et al. 2016). These were included because they provide good benchmark information about the relationship of leptin, pain, and fatigue. The results to each study or pairs of studies are presented in separate tables which summarise the changes in leptin during conditional changes during each research project. These research studies usually have small groups of participants and have been completed over many weeks, or even months. The Younger et al. (2016) study had just 3 participants in the first part of the study, then it divided the article to include a second study which contained an unrelated 5676 participants. This is just one example of how divergent the cohort studies can be from the case-control studies presented earlier. We will now discuss each cohort study in more detail.

4.3 Bjersing et al. (2013 and 2017)

In the first of nine research studies are the two by Jan Bjersing et al. (2013) using resistance exercise in a cohort of 48 patients with fibromyalgia over 30 weeks. There were three different BMI ranges of lean (n=9), overweight (n=26), and obese women (n=13). We are provided with a median and range of leptin levels at baseline, 15 weeks and finally at 30 weeks for women who all had fibromyalgia. There are no control subjects. The results are divided into subgroups of weight range (lean, overweight and obese). The participants were all exposed to one of two types of exercise, randomised across the cohort and these exercises were a moderate-to-high-intensity Nordic walking (NW) program or a supervised low-

intensity walking (LIW) program. The overall results were that leptin significantly negatively correlated with fatigue (r = -0.345; P = 0.016) .

In the Bjersing 2013 study: “Exercise and obesity in fibromyalgia: Beneficial roles of IGF-1 and resistin?”, leptin levels are provided as pg/mL in the tables which have now been converted to ng/mL (See Table 19) for comparison in this review which is calculated by dividing the picogram result by 1000, providing a result in ng/mL. The changes at 15 weeks and 30 weeks are provided; note that this is the change Δ from baseline and not the actual level of leptin. There were no significant changes in leptin, but a reduction was seen by week 30 in lean patients only. It is noteworthy that only 9 of the 13 obese participants were measured at 30 weeks. The large range in the changes in all categories, lean, overweight and obese are so great that these results do not provide confidence. The range of the changes cited compared to the median changes at 15 weeks are so great that it does not allow the researcher to make any clear prediction or to discern any value from these results, for example the range at baseline in the obese cohort of 13 participants was 34.46 to 86.31ng/mL. Therefore, a mean change in the obese group at 15 weeks of between -5.53 to 9.05 ng/mL suggests either a reduction or an increase in leptin. Intuitively, you would assume leptin to lower following 15 weeks of exercise training, as this would coincide with a potential weight loss. The figures presented do not provide clarity about the effectiveness of these interventions in their ability to influence the levels of circulating leptin. See Table 19 on the following page for more details:

Table 19 Serum levels of leptin Bjersing et al. (2013)

Timeline	Lean ng/mL (n=9) Median	Range ng/mL	Overweight (n=26) Median ng/mL	Range ng/mL	Obese (n=13) Median ng/mL	Range ng/mL
Baseline	16.01	10.25 to 25.63	27.66	19.22 to 45.50	45.30	34.46 to 86.31
Changes at Δ 15 weeks	2.14	-4.12 to 5.40	1.42	-6.06 to 6.86	5.27	-5.53 to 9.05
changes at Δ 30 weeks	-4.46	-10.47 to 2.25	1.76	-9.91 to 9.78	1.45	-3.06 to 8.13

Four years later Bjersing et al. (2017) published a similar study: 'Benefits of resistance exercise in lean women with fibromyalgia: Involvement of IGF-1 and leptin' including 43 women with fibromyalgia again using three subgroups of BMI ranges of lean (n=18), overweight (n=17), and obese women (n=8). They had to complete an exercise regimen twice per week for 15 weeks and were measured at baseline and at 15 weeks and the changes in leptin levels can be seen in the table below (Table 20). These results again provided little confidence as the change in lean participants is not very different to the changes in obese participants and have a similar upper and lower interquartile range. Whilst there is no change in overweight participants, it could be argued that this merely shows the fluctuations of leptin across time in 3 groups of people may be high. Whether the exercise routine has made any changes in these levels would be hard to determine without a control cohort who did not do the exercises. In table 2 on page 5 of the article some of the numbers use a comma instead of a period, placing doubt in the interpretation of these results. However, the authors state that leptin was significantly decreased over the course of the 15 weeks in lean women with fibromyalgia ($p = 0.008$), which coincided with a reduction in pain and fatigue. It was not significant in overweight or obese women with fibromyalgia. See Table 20 on the following page:

Table 20 Serum levels of leptin Bjersing et al. (2017)

Time	Lean (n=18) Median/IQR ng/mL	Post Test (IQR) ng/mL	Over-weight (n=17) Median/IQR ng/mL	Post Test (IQR) ng/mL	Obese (n=8) Median /IQR ng/mL	Post Test (IQR) ng/mL
Baseline	**21(86)**		**39(60)**		**23(106)**	
15 wks Δ	**-15.9**	**-23.6 to -0.1**	**0**	**-3 to 19.6**	**-12**	**-23.1 to -2.3**

4.4 Bokarewa et al. (2012 and 2014)

The next two studies were by Bokarewa et al. (2012 and 2014). The first paper (2012): 'Smoking is associated with reduced IGF-1 levels and higher pain experience in patients with fibromyalgia' is only an abstract and appears to be a summary of the following paper published in 2014 as the numbers of participants, division of smokers and non-smokers are the same i.e., 45 non-smokers and 18 current smokers and cites that smokers had lower levels of leptin than non-smokers (ng/mL, median: 16.9 vs 34.8, p=0.013). The abstract provides only limited information about the cohort, so we relied on the full paper for more detailed results and we did not include either studies in the meta-analysis. Why smokers would have lower levels of leptin is unclear, but the difference cited is quite significant, almost halving the amount of leptin compared to those that have never smoked.

The second Bokarewa paper (2014) appears to be a more expansive report on the study provided than that offered in the abstract: 'Smoking is associated with reduced leptin and neuropeptide Y levels and higher pain experience in patients with fibromyalgia' is the full paper on the study and provides much more detail. This study provides results for serum leptin as well as CSF leptin results for the same group. The relationship with serum leptin and CSF leptin suggests that it is more than five-fold higher in CSF compared to serum.

The research includes 62 women with fibromyalgia and reports that current smokers (n=18) have lower levels of leptin compared to those that have never smoked (25). It is noteworthy that the CSF levels provided are for just half of the participants (n=32 of the total 62). The leptin results of smokers, non-smokers and ex-smokers are provided in Table 21 below. The conclusion of the paper is that lower leptin normally has an inverse relationship with neuropeptide Y i.e., the lower the leptin the higher the neuropeptide Y and the lower the pain. However, it was noted that smoking does not reduce the experience of pain and suggested that it may cause a change of the relationship between leptin, neuropeptide Y and pain.

Table 21 Serum & CSF Leptin in Smokers Non-Smokers and Ex-Smokers

Sample Type	Current Smokers (n=18), (CSF n=8) Mean leptin ng/mL	IQ Range (upper and lower IQR) ng/mL	Non-Smokers (n=19), (CSF n=11) Mean leptin ng/mL	IQ Range (upper and lower IQR) ng/mL	Ex-Smokers (n=25), (CSF n=12) Mean leptin ng/mL	IQ Range (upper and lower IQR) ng/mL
Serum	19.4ng/mL	11.7–28.7	28.8	16.4–48.0	36.8	28.0–55.7
CSF	104ng/mL	91–151	165	114–225	178	104–244

4.5 Hornig et al. (2017)

The next cohort study is a paper by Hornig (2017) which compares classical MECFS and atypical MECFS; 'Immune network analysis of CSF in myalgic encephalomyelitis/chronic fatigue syndrome with atypical and classical presentations.' This study included 32 classical cases of MECFS which were previously used in an earlier study (Hornig et al. 2016), and 27 atypical cases which included a more unusual onset, having higher cognitive dysfunction as well as other neurological issues. The atypical group met the full criterion for MECFS (either the Center for Disease Control's Fukuda definition or the 2003 Canadian Consensus Criterion) but also went on to develop other disorders and symptoms like having

seizures or autoinflammatory disorders (Hornig et al. 2017). The two groups were further divided using a 3-year anniversary to determine short-term cases (⩽3 years) or long-term cases (>3 years). Leptin was not found to be significantly different when compared to all other groups with the lowest correlation comparison being atypical short vs classical long (p=0.143). The leptin levels are provided in ng/mL and have been assessed using CSF, which we have previously advised is likely to be five-fold higher than circulating blood levels. Although the results appear to be expressed in ng/mL, the tables provided in the paper have no labels to signify whether these results are ng/mL or pg/mL but comparing this to the previous study mentioned earlier it is highly likely to be expressed in ng/mL. This is a good example of poor labelling which was found in many papers selected for the full text phase which state results without their type of measurement, particularly as they offer the results of other cytokines which are clearly measured in pg/mL. This leaves the reader to assume the type of measurement from the text and method, which is also opaque. See Table 22.

Table 22 CSF leptin typical and atypical cases (assumed ng/mL)

Atypical short duration (N = 13) Mean SD	Atypical long duration (N = 6) Mean SD	Classical short duration (N = 14) Mean SD	Classical long duration (N = 18) Mean SD
44.07 (26.65)	66.35 (54.02)	55.44 (45.25)	78.13 (61.80)

Assumed to be ng/mL but not specified in article.

4.6 Katz (2017)

The study by Katz (2017), 'Leptin, a Hypothalamic Signalling Hormone, Is Elevated in Fibromyalgia Patients' provides the results in an abstract only, with very limited details and was published as part of a poster session. It compared 27 fibromyalgia participants to 6 people with rheumatoid arthritis. Stating that leptin levels in the fibromyalgia participants varied between 1.7 and 70.7 ng/mL (with a mean of 28.0 ng/mL) compared to 0.6 to 33.0 ng/mL in those diagnosed with rheumatoid arthritis. It goes on to state that the leptin levels to BMI ratio for those with fibromyalgia was

1.0 and the ratio for those with rheumatoid arthritis was 0.5. They use these results to suggest that leptin is elevated in those diagnosed with fibromyalgia, but also show that those with rheumatoid arthritis are less obese. See Table 23.

Table 23 Leptin MECFS vs Rheumatoid Arthritis ng/mL

Katz 2017	FM (27)	mean	RA (6)	mean
Leptin levels range ng/mL	1.7-70.7	28	0.6-33.0	10.1
BMI / Leptin Ratio	1.0		0.5	

4.7 Kurajoh et al. (2016)

The next paper does not discus MECFS nor fibromyalgia but does discuss leptin and fatigue. It was agreed by the full text reviewers (MM&ML) to include this study as it provides information about types of fatigue and their relationship with circulating leptin. Kurajoh et al. (2016) in their paper 'Plasma leptin concentration is associated with fatigue severity in patients with cardiovascular risk factors – HSCAA study' which examined the plasma leptin levels of 347 participants with cardiovascular risk. They found that leptin was significantly, positively correlated with a fatigue score. Leptin was higher in the group with moderate fatigue (n=53: 2.33 SD 0.75 ng/mL) than the 'normally-fatigued' group (n=295: 1.85 SD 1.02 ng/mL) $p<0.001$. In reality, this is a tiny difference that could be accounted for by many factors such as sampling error, such as the time when samples were taken, BMI, or even the methodological quality at the laboratory level. The leptin levels provided are quite low across both groups i.e., less than 2.5 ng/mL, which is dramatically lower in comparison to the other cohort studies cited earlier. The paper also provides two models which produce odds ratios (OR) for higher leptin and the risk of those moderately fatigued. In one statistical model they combine the leptin concentration age, male sex, BMI, presence of diabetes, hypertension, dyslipidaemia, past cardiovascular events, current smoking and other covariates such as alcohol to determine an odds ratio of 1.904 (CI 1.130-3.211). Model 2 used leptin levels and alcohol use to calculate an

OR 1.818 (CI 1.069-3.094), then model 3 leptin and an apnoea hypopnea index (AHI) which gave an OR of 1.943 (CI 1.148-3.290). Another five models provided further results in the form of odds ratios – see original article for more details.

Table 24 Leptin in moderate & normally fatigued groups

Kurajoh 2016	Moderately Fatigued n=53	SD	Normally Fatigued n=295	SD
Leptin levels ng/mL	2.33 ng/mL	0.75	1.85 ng/mL	1.02
BMI	25.5	5.5	24.3	4.6

4.8 Quismorio et al. (2014)

The next article is an abstract by Anne Quismorio et al. (2014) and her team, 'Elevated Serum Leptin Concentrations in a Subset of Fibromyalgia Patients with High Inflammatory Markers' in the Journal of Arthritis and Rheumatology which contained thousands of abstracts from the American College of Rheumatology annual meeting held in November 2014. The study reviewed biomarkers of participants with fibromyalgia using a commercially available test kit known as 'Vectra DA' which provides an overall score of 1-100 from the analysis of 12 analytes, and is normally used to assess rheumatoid arthritis (Segurado & Sasso 2014). The test also provides leptin levels which according to the authors was significantly higher when compared to patients with rheumatoid arthritis (1-45 ng/mL). The result for this cohort who have fibromyalgia showed that 45% of the participants had leptin levels exceeding 45 ng/mL with a mean of 42.3 ng/mL (range 30–81 ng/mL). This is an example of how circulating leptin levels can be presented as wildly different from the previous study by Kurajoh et al. (2016) which cites levels of less than 2.5 ng/mL for both cohorts, making it extremely difficult to compare between studies. As this is an abstract only the minimum details of this study are provided which included that participants were of a mean age of 43.5 and 94% were female. As part of the development of this thesis, the author of this review exchanged email correspondence with one of the American team Dr Sam Metyas,

who advised that the Vectra DA is a common test used by rheumatology clinics in America. They were unable to provide the raw results of leptin.

4.9 Younger et al. (2016)

The last cohort study by a team from a group of American universities including Stanford University led by Jarred Younger (2016), 'Association of Leptin with Body Pain in Women' which examined a cohort of just 3 patients, taking blood every day for 25 days, whilst asking the participants to complete a visual analogue scale of pain levels experienced during the daytime on a score of 0 to 100. This was completed using an android phone each evening prior to bed.

The researchers hypothesised that leptin is positively correlated to pain even after controlling for BMI as a confounder. This paper is unusual as it has two research studies in one article, the second examining the results of samples from a biobank donated by 5676 post-menopausal women (aged 50-79) who provided a serum sample. These samples included any patients who had yielded a leptin result and had completed a questionnaire for self-reported pain. For the first study, mean leptin results are provided with BMI and were analysed using a mixed linear model (not described in detail). The authors reported that higher leptin levels were significantly associated with daily pain reports ($F(1, 63) = 12.804$, $p = 0.0007$). Leptin levels were higher and were associated with greater self-reported pain levels. It seems odd that in the first study they provide the weight in kg of each participant instead of their BMI. Yet one of their hypotheses is that BMI (not weight) could be a confounder. They explain how the timing of the sample was controlled, but it remains a little vague in the details. The authors also provided information regarding the needle type and location of the blood draw, but they don't state whether the samples were taken after a period of fasting blood, which is of greater importance. The article provides graphed correlations for pain and leptin levels which are highly correlated at $r=0.77$ ($p=0.0001$) for two of the participants, and $r=0.5$ ($p=0.18$) for the third participant.

The results in the table below (Table 25) are similar to the leptin levels provided in other studies for these weight ranges; all participants are within a range of 0.7 to 38.7 ng/mL. Overall, this paper is well balanced and describes some but not all details of the protocol and method of analysis. It provides the full leptin results as well as the correlations, so it is refreshing to see both are reported and sets a good standard for future research.

Table 25 Study part 1 mean leptin and weight

Participant	participant 1	range	participant 2	range	participant 3	range
Serum leptin ng/mL	**0.7**	**0.2-2.0**	**10.21**	**5.8-17.1**	**20.5**	**11.21-38.7**
Weight kg	**60.2**		**82.1**		**101.2**	

In the second study of the same paper, it appears that both serum and plasma samples were used as it reports collecting samples using various methods of tubes; EDTA (plasma tubes) and citric acid (serum tubes), so the results may be of a combination of either fluid type. It is clearer in study 2 that BMI has been assessed as a confounder and this is described in the statistical analysis and provided in the table. The following table provides the pain level associated with leptin levels, but also the BMI range within each level. It is evident from this table that as BMI measures increase, so does the mean leptin level. The higher the pain level, the more people fall into the combined 'overweight and obese range' (i.e., percentage BMI in the bottom two rows), and these graduate upwards at approximately 10% as it moves up one pain level. See Table 26 on the following page:

Table 26 Study part 2 Pain level vs leptin & BMI

Pain Level	None	Very Mild	Mild	Moderate	Severe
N	1344	2120	1216	1012	49
Leptin ng/mL	**13.8**	**15.3**	**18.3**	**20.3**	**23**
IQR	**16.7**	**18.4**	**22.9**	**23.3**	**29.6**
BMI kg/m2	25.28	26	27.29	27.94	28.92
SD	5.71	6.39	7.36	7.73	10.27

Normal BMI %	46.8	40.8	31.8	27.4	20.7
Overweight %	33.6	34.9	36.2	33.3	36.8
Obese %	17.9	23.1	30.7	38.5	41.4

BMI, body mass index; IQR, interquartile range; SD Standard Deviation

It becomes self-evident that these 9 cohort studies are very different, and the authors approach the issue of circulating leptin and MECFS and/or fibromyalgia from very different perspectives, making it difficult to compare results between studies. The way samples are collected, the study design and the outcome measures use different timescales and interventions. For example, the first two studies by Bjersing are about exercise, fibromyalgia and the experiences of fatigue. The following two studies by Bokarewa are about smokers, fibromyalgia and pain. The remaining studies have other differences which make them unique, but this only allows for a tabular and narrative summary of these studies.

4.10 Critical Appraisal Checklist for Cohort Studies

A critical appraisal checklist was adapted from the JBI Manual of Evidence Synthesis chapter 7: Systematic Reviews of etiology and risk, appendix 7.1 ‘Critical appraisal checklist for cohort studies (Moola, S et al. 2019). The checklist helped to identify low quality studies, and these can be seen with lower scores in Table 25 below, and are mainly assigned to the three papers that were written in the form of abstracts e.g., (Bokarewa et al. 2013; Katz 2017; Quismorio et al. 2014). The abstract from Katz received a score of zero because it essentially just states the results in the abstract without any method or information about recruitment and related factors. The abstracts were kept in the review due to the limited number of studies which included leptin levels in MECFS and/or fibromyalgia. Only one of the nine cohort studies was related to MECFS (Hornig et al. 2017) with the remainders focusing on fibromyalgia.

Further details and information about each study can be found in the previous section, but the critical appraisal checklist provides a quick overview of what areas were covered by each study and those that were not covered. Only one study received an upper score of 8 out of a possible 10 (As one question was not applicable to all studies, as there are 11 questions in the original JBI cohort critical appraisal checklist). The highest scoring paper was by Younger et al. (2016), and the score reflects that it had explained the methods clearly and provided most of the information covered by the questions in the critical appraisal questionnaire. It did not receive a positive response for using similar cohorts because it presented two different studies in the one paper with extremely different sample populations e.g., the first study has 3 participants, and the second cohort had a sample population of 5676. The paper did not try to compare the cohorts but used the two studies to explore body pain and its relationship to circulating leptin in women.

The low scores across the 9 cohort studies reflected the difficulties in trying to compare results between papers, which can be seen from the results tables from each paper displayed in the previous section. The following table are the results of the critical appraisal checklist in full (See Table 27 on next page):

Table 27 Critical appraisal checklist for cohort studies

Study Author & Year (AB = Abstract)	QA1 Cohort Study 1: Were the two groups similar and recruited from the same population?	QA2: Exposures measured similarly to assign people to both exposed and unexposed groups?	QA3: Was the exposure measured in a valid and reliable way?	QA4: Were confounding factors identified?	QA5: Were strategies to deal with confounding factors stated?	QA6: Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	QA7: Were the outcomes measured in a valid and reliable way?	QA8: Was the follow up time reported and sufficient to be long enough for outcomes to occur?	QA9: Was follow-up complete, and if not, were the reasons to loss to follow-up described and explored?	QA10: Were strategies to address incomplete follow-up utilized?	QA11: Was appropriate statistical analysis used?	Total Y
Bjersing 2012	No	N/A	Yes	No	No	N/A	Yes	Yes	Unclear	No	Yes	4
Bjersing 2017	Yes	Yes	Yes	Yes	Unclear	N/A	Yes	Yes	Yes	N/A	Yes	6
Bokarewa 2013 (Ab)	Yes	Yes	Unclear	Yes	No	N/A	Unclear	Unclear	Unclear	N/A	Unclear	3
Bokarewa 2014	Yes	Yes	Yes	No	No	N/A	Yes	N/A	N/A	N/A	Yes	5
Hornig 2017	Yes	Unclear	Unclear	No	No	N/A	Yes	N/A	Yes	N/A	Yes	4
Katz 2017 (Ab)	Unclear	Unclear	Unclear	Unclear	Unclear	No	Unclear	N/A	N/A	N/A	Unclear	0
Kurajoh 2016	Yes	Yes	Unclear	Yes	Yes	N/A	Unclear	N/A	N/A	N/A	Yes	5
Younger 2016	No	No	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	8
Quismorio 2014 (Ab)	No	N/A	Yes	Yes	Yes	Unclear	Unclear	N/A	N/A	N/A	Unclear	4

4.11 Outcomes of Cohort Studies

Several interesting results have been presented in the cohort studies, with each paper offering unique perspectives. Bjersing et al. (2017) provide evidence that regular exercise can influence leptin levels over time and reduce pain. Bokarewa et al. (2014) indicate that smoking interferes with the leptin / neuropeptide Y relationship causing a reduced leptin level in smokers. Where a fall in leptin would normally be expected to reduce the experience of pain, this was found not to be the case with smokers because of the changes in neuropeptide Y which it is thought to have countered this response. Hornig et al. (2017) compared classical MECFS with atypical MECFS and then subdivided these into short-term (less than or equal to 3 years and long-term greater than 3 years), resulting in four categories. No other papers have divided these syndromes in this way, making the results difficult to compare for the purpose of systematic review. Leptin levels were not found to be significantly different across the four groups.

Katz (2017) in an abstract provides the mean difference for participants with fibromyalgia and those with rheumatoid arthritis, indicating that leptin is higher in the cohort with fibromyalgia. Few details are provided about the method and how the results were calculated. Kurajoh et al. (2016) make a comparison between those who experience a normal level of fatigue and those who experience a moderate level of fatigue in a group with heart risk factors. The moderately fatigued group had significantly higher levels of leptin, however the overall mean leptin level for the total population was less than 2 ng/mL with an SD of 0.99. Other studies present leptin levels for participants that are much higher, like a mean of 35 ng/mL, so when comparing these results to this study from a macro multi-study perspective it is difficult to understand how they get such contrastingly low measures of circulating leptin. For comparison, the mean of all cases and controls (n=1307) in ng/mL ± SD combined was 14.61 ± 12.24 (17.42 ± 14.93 cases and 11.79 ± 9.54 controls).

The Quismorio et al. (2014) study is another abstract with limited information and offers similar results to the Katz study discussed earlier, comparing patients with fibromyalgia and those with rheumatoid arthritis. They do provide a little more information about the cohort and the tests involved, whilst citing leptin levels from a

commercially made analysis kit VECTRA DA. These kits allow for the test to be completed at the point of care, but the high levels of leptin presented (30–81 ng/mL) are not seen in most of the case control studies reviewed earlier in this thesis.

Finally, the study by Younger et al. (2016) presents leptin levels in 3 women with fibromyalgia across 25 days, and the higher leptin levels show a high correlation with pain. It should be noted however, that this cohort is small, at only 3 participants. In contrast to their second cohort – referred to as study 2, which is the largest of any cohort or group in all the studies examined in this review with 5676 participants. The team provide a range of pain levels, BMI, and levels of leptin which range from 13.8 ng/mL (mean) in those with no pain and up to 23 ng/mL for those with severe pain. A clear incremental increase of results from those with mild pain to those with severe pain are provided. These differences relate to repeated themes raised throughout the cohort and case control studies including, BMI, symptom severity, pain, fatigue and other symptoms like difficulty with sleep or levels of exercise. The 24 studies selected in this systematic review all offer some insight into the role of leptin, however, many of them use such different methodologies that it makes them difficult to compare. If this area of research is to progress, then the science in the area needs to be more coherent in diagnostic criteria, methodology, process, and outcomes. This would reduce the high level of heterogeneity and allow for effective comparison across cohort and case control studies.

CHAPTER 5

Discussion and Conclusion

5 Discussion and Conclusion

5.1 General Discussion

Heterogeneity in the research population of MECFS and/or fibromyalgia is partly caused by the fact that they are both considered syndromes. A syndrome is a collection of symptoms which are common to a clinical presentation. These diagnoses rely on clinicians to have knowledge and experience of the condition, but because these symptoms are not easily objectively measured, it creates uncertainty in the diagnosis. Another factor that doesn't help the confusion around diagnosis is the changing terminology and social attitudes about the actual existence of these syndromes (reviewed in depth in Chapter 1, section 1.15).

There is a lack of consistency and methodological process in studies which make it difficult to compare results. In an ideal world, future research studies that are investigating MECFS or fibromyalgia will use methodology that can be reproduced by others. It is critical to report the results and that they are presented in full and in a transparent way to enable a simple comparison across research projects. It was challenging in this systematic review to make such comparisons. Not only were the methods and protocols different, but it was extremely hard to find similarities between studies, particularly those of a cohort design (See Chapter 4).

There are several highly cited papers which have been conducted at a research laboratory at Stanford University such as Stringer et al. (2013), Montoya et al. (2017) and Younger et al. (2016) that provide their results using statistical correlations, yet they don't provide the raw circulating leptin levels to allow consumers or other researchers to scrutinise their results. It is not possible to work out the raw leptin levels from the data that is provided, so it is left to the reader to have faith in the authors' output and to assume that these calculations and methods are accurate and precise. It would be helpful for the authors of future studies to publish their results in a similar format which includes the original units of measurement that enable other researchers to perform a meta-analysis. Another difficulty was finding

a comparative level of leptin in humans as this is also inconsistent in the literature. We reported a well-rounded exemplar study that provided a comparative circulating level of leptin alongside other confounding factors (See Table 3, Chapter 1). The example range in this study across gender and BMI range was between 2.2 ± SD 0.03 ng/mL and 23 ± SD 4.0 ng/mL serum leptin (Al-Sultan & Al-Elq 2006). A wide range!

The original a priori question was "**Is there a difference in circulating leptin levels in MECFS and/or fibromyalgia that can be used as an outcome measure in cases and controls?**"

In short, the meta-analysis showed a small difference with cases having higher leptin levels (hyperleptinaemia) than controls, in fact 3.26 ng/mL higher (CI 0.33 ng/mL to 6.19 ng/mL). To put these results in context, those individual research studies that did provide baseline circulating leptin results in their natural units (ng/mL) appear to be using different magnitudes of measurement. These surprisingly large differences reduce the investigators' confidence in the results. For example, one study (n=38) provided a mean result of both cases and controls within a range of between 1.0 ng/mL and 2.0 ng/mL (cases 1.8 ng/mL – controls 1.75 ng/mL) (Ribeiro et al. 2018), whilst another (n=84) reported the results using a range between 11.5 ng/mL to 30.32 ng/mL (Ataoglu et al. 2018). That's a difference of between 10 and 28 ng/mL or a magnitude of 10! Other studies, described earlier, have reported leptin levels as high as 70 ng/mL or even higher.

All case control studies included in this review are measuring human circulating levels of leptin in the blood (11 of 12 studies) but there are great disparities and making sense of these results is difficult from a scientific perspective. It adds doubt to their credulity and usefulness for translation. Not all of these studies can be representative of the average circulating leptin, because they are so different. Each study included in the meta-analysis were using healthy controls, but we must question how the author's measurements of the mean leptin can be so different. These differences in leptin levels point to potential but puzzling discrepancies in methodology, laboratory protocols and analysis as discussed in earlier chapters. When reviewing these published articles, however, these methodological

differences cannot be readily discerned from the material presented. Few details are provided about recruitment, sampling techniques, and laboratory methods.

Imprecision was evident when comparing results. The mean level of circulating leptin of controls across all studies should in theory be around the same level, however, as you can see from the forest plots in chapter 3 (Figure 8 - 13), they are very different. When we compare this to other blood-based analytes such as the level of glucose, insulin or cholesterol then we have a reliable reference level or range to rely on. The average human will be expected to fall within a known range, and the scientific knowledge about these common biomarkers is based around these 'normal' ranges.

These known reference ranges enable a doctor or consumer to make evidenced based decisions about interventions, or about changes in stages of a disease. It is an ideal to strive for in MECFS and/or fibromyalgia research. If you go to your general practitioner for example and have a test for diabetes, this can be achieved through a simple blood test to check your HbA1c (glycated haemoglobin test). When your fasting blood sugar is less than 100 mg/dL (5.6 mmol/L), then we know you are unlikely to have diabetes based on these results. These types of biomarkers are simple and objective, allowing clinicians to benchmark against whole populations with confidence about their clinical implications. In current practice using leptin as a biomarker is far from this ideal due to the inconsistent reports of baseline circulating leptin levels. As an investigator who is researching leptin and trying to advance the understanding of results across studies, it was not easy to work out the justification for the differences reported. We can only assume that investigators are using very different analytical techniques and methodologies. Having inconsistent results which report both hypoleptinaemia and hyperleptinaemia for the same syndrome is unhelpful in advancing this area of research.

To make sense of these outcomes and to make a valid comparison it was necessary to use a standardised mean difference, but this makes it difficult to be certain of the changes of raw circulating leptin levels from the pooled result because the pooled effect has to be re-translated from the SMD outcome back to the original measurement units in ng/mL using one of the controls groups means. This method

is explained in more detail in Chapter 3. Before we can justify further research in MECFS and/or fibromyalgia and leptin, we need to find a way of developing a baseline range which uses the same methodology so that when research is compared between subjects, we have a reference range that we can rely upon similar to the standards of HBa1c test. Once we have a reliable reference range, we can then start to interpret observed effects in relation to the introduction of other independent factors like smoking or exercise, but until we have these benchmarks then the benefit of diverse cohort research of this nature will be limited.

As previously stated, the results of the meta-analysis of the twelve combined case control studies indicated that leptin is slightly higher in cases compared to controls by 3.26 ng/mL (See Table 17, Chapter 3, page 112). This was calculated in SUMARI with an overall pooled difference of 0.39, with a 95% Confidence Interval 0.04 to 074, which was significant (p=0.029) (Tau^2 = 0.3, Chi^2 = 118.65, df = 11 (p<0.00001); with a very high level of heterogeneity at I^2= 86%, Z=2.18. The re-expressed result in the natural units 3.26 ng/mL indicates leptin is higher in cases (CI 0.33 ng/mL to 6.19 ng/mL) compared to controls reflecting the standardised mean difference reference 0.39 (CI 0.04 to 074). Many combinations of subgroup meta-analysis were completed to establish new information.

When comparing results however, we have to consider the precision of these leptin levels because as we have discussed they can change throughout the day, as there are intraday and inter-day differences that can also be vastly influenced by many confounders (See Figure 2 Chapter 1) (Al Maskari & Alnaqdy 2006; Licinio et al. 1997). The pooled difference (3.26 ng/mL) is only a starting point to begin new investigation. If we use the raw mean leptin level for all participants (1307) which was 14.61 ng/mL with an SD (12.24 ng/mL), it provides a benchmark for currently available studies. The SD (12.24) however is high, meaning that an increase of 3.26 ng/mL from the mean level would likely lose any clinical relevance. It would not necessarily support a diagnosis that the person with a higher reading will have MECFS or fibromyalgia, as it may be due to an increased level of BMI or be influenced by the peak phase of the person's menstrual cycle (the luteal phase) (See

Chapter 1, Figure 1). Alternatively, it may be due to their, sex, sleep quality, activity level, or simply unexplained individual difference.

Future research in this area, through good design, should consider all these confounding factors when making comparisons across populations, and only then can we begin to make inferences about the biological effects about the levels of circulating leptin. Any published research needs to be transparent and to provide all the results where practicable, not just correlations and complex statistical extrapolations. The clinical science and scientific method rely on the basic premise of identifying observable changes and effects, but we cannot observe effects if we don't have the data. We need to see the results.

A good example of study design were those studies that had taken multiple samples from the same individual over 24 hours (Licinio et al. 1997; Licinio, Negrao & Wong 2014). Two other studies that were examined at the full text stage of this review, but then excluded due to them not including raw leptin results, demonstrated that there is a correlation with leptin and symptom severity, particularly pain and fatigue. The authors showed that these symptoms correlated to transient levels of leptin across 25 days, as described by Stringer et al. (2013) which is an MECFS study (n=10 MECFS & 10 healthy controls) and Younger et al (Younger et al. 2016) which examined body pain in women (n=3 cases but no controls). There is also evidence to suggest that higher amounts of leptin (hyperleptinaemia) influence other pro-inflammatory cytokines, promoting their production and dispersion, as well as influencing many other immunomodulatory mechanisms (Montoya et al. 2017; Procaccini, Jirillo & Matarese 2012).

So how did the meta-analysis results compare to the separate results of individual studies? Visual inspection of the forest plots (Figures 8 – 9), highlight imprecise, variable results that are inconsistent and have wide confidence intervals with overlapping boundaries. There are few logical patterns other than to state that on average there are more studies showing higher levels of leptin in cases (6 studies), than those showing no difference (4 studies) or an opposing difference (2 studies). Several previous systematic reviews on blood based analytes in MECFS have simply stated whether cytokines are higher or lower in cases or controls (Blundell et

al. 2015). This provides limited information for clinical practice, hence the reason for this current systematic review being completed to find some numerical baseline reference range for circulating leptin in cases of MECFS and/or fibromyalgia and controls. However, as indicated by the systematic review by Blundell et al. (2015), the search for other novel biomarkers is suffering from similar difficulties in establishing a 'normal' or population-based reference range. Whilst science is making rapid advances, the interaction of human cytokines and adipokines is not yet fully understood.

Another key issue that decreases confidence in the results is the very high degree of heterogeneity in the pooled results (I^2=86%). The pooled outcome was 0.39 SMD, however there are wide confidence intervals, and the fact that two studies report the opposite to most of the other studies sows seeds of doubt. The second factor is the inconsistency of research design in each study, leading to the lack of precision with diagnostic categorisation. This may be due in part to the ongoing controversial nature of both syndromes, a lack of funding for this area of clinical practice, or that practitioners question their belief in the existence of these syndromes, creating diagnostic confusion (Natelson 2019).

Thirdly, there is a potential risk of bias caused by confounders not being systematically included and accounted for in most of the full text studies including BMI, age, sex, menstruation, diet, activity and ethnicity. Whilst many authors included BMI statistics, not all did, and all confounding factors have not been systematically considered.

In summary, leptin is potentially higher in cases of MECFS and/or fibromyalgia but we cannot be confident or certain of these results. The clinical value of this small but significant difference is limited. Further research is warranted under more stringent conditions that take account of confounding factors, and further attempt to reduce heterogeneity. A single sample at one time point is likely to be insufficient to account for a person's baseline result, in which to compare those that are symptomatic and those that are well or to show changes in groups. Instead, it is preferred that multiple samples are taken from the same individual within a 24-h period, or even across a series of days. Once consistency in results is achieved,

we can then work toward the behavioural challenges targeted in some of the cohort research such as mild exercise or smoking. BMI differences have been shown to have the biggest impact on circulating leptin, there are already some good examples in the literature of this being completed in healthy cohorts as described earlier (Al Maskari & Alnaqdy 2006; Al-Sultan & Al-Elq 2006). These exemplary studies are few and this area warrants further investigation supporting a systematic review process.

The individual differences in leptin levels may be best limited to 'intra-person' style studies similar to the one described by Stringer et al. (2013). They used the method of including a small sample size but taking several bloods tests across multiple days. 'Intra-person' is a suggested method whereby an individual provides many samples across a set period of time, and the ensuing analysis is done by associating that person's symptoms with their own fluctuating levels of leptin. The two association studies described earlier have only limited participants (as few as 3), but the design is more reliable as the investigators describe the correlated changes in daily leptin levels as opposed to a mean average change for a group (Stringer et al. 2013; Younger et al. 2016). A criticism is that the studies did not provide raw results, but instead used graphical representations pairing leptin levels and standardised correlation figures for each person. There were no leptin levels provided in supplementals either.

Some authors may not have published their raw data because of the inherent variation of leptin between individuals, which can be problematic, as there is no clear understanding for these differences other than key factors like BMI and genetic variation, which are yet to be fully established (Erez et al. 2011). A healthy person may naturally have high leptin levels which can be caused by leptin resistance (Myers et al. 2012), whilst another person who is unwell may have a low leptin level. We need to research the nature of individual and group variability, so we can better understand the pathological disease process or at a minimum develop a useful diagnostic marker. A consistent finding is that the higher the adiposity, or BMI, the higher the leptin level (Izquierdo et al. 2019). Higher leptin levels have been linked to multiple symptoms like neuropathic sensitivity to pain and in the role of fatigue but

further research is required to explain these links (Kurajoh et al. 2016; Maeda et al. 2009).

Was it appropriate to combine both MECFS and fibromyalgia in this systematic review? In several studies the research across both areas examined similar syndrome factors such as pain and fatigue, but not consistently enough to provide a subgroup analysis. It was useful to combine results to be able to report an overall comparison, as well as providing the ability to see the differences between the two types of syndromes. When the results were separated into each syndrome for this subgroups analysis, there was only a small change in the overall outcome, and this was supportive of combining these investigations. One author has even suggested that MECFS and fibromyalgia may be the same phenomenon (Abbi & Natelson 2013). As discussed earlier, authors have previously combined these disorders to compare symptoms in other areas.

5.2 Impact of any limitations and operational definitions

Whilst it seemed like a straightforward idea to do a meta-analysis to compare leptin levels across various research studies, it created many complexities. The lack of certainty in outcomes plagues the research of MECFS and/or fibromyalgia, which is not assisted by the controversies associated with the syndromic names discussed earlier. Organisations like the National Health and Medical Research Institute (NHMRC) and the American College of Rheumatology are working to consolidate the research approach providing an advisory document entitled 'Myalgic Encephalomyelitis / Chronic Fatigue Syndrome Advisory Committee' in 2019 (NHMRC 2019; Wolfe, F et al. 2010). Similarly, the NIH recognised the need to progress cohesive research in this area and formed a collaboration known as the 'Trans-NIH Myalgic Encephalomyelitis / Chronic Fatigue Syndrome Working Group' examining shared interest across agencies and in 2017 announced the 'Centre for myalgic encephalomyelitis/chronic fatigue syndrome research' (NIH 2017).

In 2007 the National Institute of Clinical Excellence produced guidelines in the UK to support treatment approaches and research 'Chronic fatigue syndrome / myalgic encephalomyelitis (or encephalopathy): diagnosis and management' (Hairon & Nice 2007). These guidelines were recently updated in 2020 with a contrasting position to their original recommendations, stating that there is a 'lack of evidence' to support cognitive supportive and graded exercise therapies (Turner-Stokes & Wade 2020). This shows the changing landscape and the controversy that continue to surround these syndromes. The International Consensus Criterion which was developed by an international collaboration of clinicians and researchers identifies the preferred term for Chronic Fatigue Syndrome as Myalgic Encephalomyelitis and the International Consensus Criterion are available from the NHMRC website in the form of a primer document (Carruthers et al. 2011).

Even with issues surrounding the naming consensus the research continues to publish with the term 'Chronic Fatigue Syndrome', so the contemporary literature uses both terms together in the title of publications, which contain either an oblique separating the two terms, or the conditions are merged together in the form of "MECFS", as in the format chosen in the title of this thesis.

This is not necessarily the right format but the preferred term to encompass the literature and it is used for convenience rather than correctness. There are at least a dozen recent articles using the term "Chronic Fatigue Syndrome" and can be found in PubMed as recent as February 2021. Many authors have commented on the labels used in Chronic Fatigue Syndrome but the term CFS has been used since at least 1988 (Twisk 2018). Other issues about the descriptors have been discussed in detail earlier in this thesis.

All studies were included in the meta-analysis regardless of sample size with a range n=20 to 646, with a mean number of participants n=108 across the twelve studies. Including studies with n<=50 participants, which equated to half of all studies.

The consistency of publishing the measurement outcome was problematic. Leptin is an adipokine that can be measured in serum, plasma or CSF but in some papers, it is reported in pg/mL, and in others ng/mL. Where pg was used then a simple calculation to convert the results was used i.e., dividing the result in picograms by

1000 to provide ng. It appeared that some graphs and tables are mislabelled, and one study was excluded because of this obvious error (Covelli et al. 2005).

An issue of concern is that leptin has a pulsatile circadian rhythm, and leptin levels change across the day and therefore the levels reported in many of the articles may not be an accurate representation of an individual's mean circulating leptin. The baseline can also be very different across the population and is dependent on multiple known factors such as BMI, ethnicity, gender, and others (Challet 2015; Licinio et al. 1997). Not all studies that were selected for the full text phase provided enough detail required for analysis, particularly potential factors that may confound the outcome. Leptin affects many different systems in the body but having the ability to cross the blood brain barrier it is known to have an influencing effect on one of our brain's master coordinators, the hypothalamus. This in turn affects the hypothalamic-pituitary-adrenal axis through circulating hormones and our nerve pathways (Challet 2015). Making assumptions about leptin mean values are precarious in that people have different baselines (Zhou & Chen 2018).

5.3 Implications for practice

The inspiration behind this thesis was the need to find a 'normal' level of leptin to compare with MECFS and/or fibromyalgia patients to see if there was an obvious difference, with the hope it would be a potential biomarker for research. The implications for practice are that the current evidence on leptin in MECFS and/or fibromyalgia is weak. To support advances in treatment it would be helpful to know how leptin behaves and how it contributes to the disease process. If we were able to modify the frequency that we measure leptin across populations then we would have a better measure of how and why changes might be occurring.

Considering its pleiotropic, hormonal, immunological and neurological influences, we need to know how this adipokine is affecting the 'normal' human body before we can make assumptions about its effects within the disease process. As leptin was only discovered in 1994, when it was declared to be a major factor in obesity and

weight changes, we have come a long way in realising the importance of this adipokine and its pleiotropic effects on our system. A basic search for the word leptin in pub med reveals 38,490 hits, indicating that a lot of new research is underway. Much of the work on leptin has been done in animal research, which is where the animal and human homologue of the original *Ob* gene (*Lep* gene) was discovered (Zhang et al. 1994). A way forward for clinical practice may be to include leptin levels in the standard blood test used during an essential annual health check. For example, just as cholesterol, iron and glucose levels are tested, leptin levels could also be tested. This would establish a population benchmark to work from. In the meantime, mass data obtained from international biobanks to generate some baseline comparators could another approach. To this end, the author has contacted the UK Biobank to register a research request.

5.4 Implications for research

Large cohort and case control studies should be performed to discover the normal range of leptin in healthy humans before we can investigate changes in a syndrome such as MECFS and/or fibromyalgia. Many current studies rarely amount to more than 100 participants. With large biobanks being developed in different countries, this population could be accessed to obtain some comparative data for leptin. It is necessary to be able to distinguish and predict changes between the effects of confounders such as BMI, age, sex and adiposity before we can start to investigate outside these parameters. Evidence is growing that links hyperleptinemia with the sensitisation of pain and the outcome of symptoms like fatigue, but the research is often completed in diseased cohorts such as arthritis patients, cardiovascular disease, or inflammatory diseases like hepatitis, diabetes and cancer. Sifting out which symptoms that might be caused by leptin is lost in the noise of these comorbid diseases. Indeed, in this systematic review we came across interesting and novel research into exercise, smoking, and obesity which exhibited effects on baseline leptin between cohorts across time, but the results are of limited for translation into clinical practice. However, the research completed does provides some guidance

to future experiments, providing preliminary data and timelines for similar sized cohorts.

5.5 Recommendations for future research

Following an exploration of the literature for this systematic review the author has created some practice protocol recommendations for future publications and research. These 10 recommendations consider some of the issues and challenges experienced during this review:

1. Confounders are identified such as BMI, adiposity, age, ethnicity, sex, lifestyle (e.g., level of fitness and activity, comorbid issues, and historical factors such as previous major illness). The phase of the menstrual cycle is rarely mentioned but has been reported to significantly alter leptin levels across time. Leptin levels in men and women should be investigated separately, as there is a threefold difference in women. Use international biobank data such as the UK Biobank to obtain datasets on these factors, e.g., BMI, sex, age, and leptin ranges.
2. Consistent use of circulating fluid type: Many studies have used serum, but a consistency of fluid type would be helpful. When trying to compare serum levels in one study against plasma or CSF in another remains problematic. Serum is more stable, is less time critical and more practical to collect and allows for 30 min at room temperature before the need to aliquot and freeze.
3. Clarify time parameters: because leptin has circadian changes across 24 h, supporting the body's time clock is one of its functions, so it is important to know at what time the person started their fasting and what time the sample was taken. When the sample is taken, it should be within a time window and the same for everybody in the study i.e., between 9-10 am, or at another time which is made very clear in the protocol. For some studies samples have

been taken daily for 25 days. A consistent time should be used and needs to be reported in the results. The method of taking a series of samples across 24 h will provide a more accurate reflection of the individual's leptin levels.

4. Measure activity and diet: these are basic communication pathways that affect hormones within our body, therefore leptin levels need to be linked to these factors. For example, one participant may be active, and another bedridden. These will have major effects on the leptin outcome. Diet is another factor because leptin is a hormone that controls activity such as food consumption in animals and humans. Some people are believed to have leptin resistance – not dissimilar to the process of insulin resistance. It has an interplay with other hormones such as ghrelin, insulin and circadian linked controlling clock mechanisms.

5. Specify frequency of sampling: If samples are going to be taken across time, then evidence has been provided that the pulsatile nature of leptin means the levels change approximately every 32 min but has been taken as frequently as every 7 min in some studies for a 24-h period. The highest level of leptin occurs between 10 pm and 4 am in the morning, yet samples are taken at the known trough which is around 8 am until noon. This would be the least representative time to take a leptin sample, yet most studies have taken the levels at this time. It would be more representative to take a sample in each of the time zones, but this is impractical and difficult to do if the person is assessed after fasting. We know that fasting has little effect on leptin levels, so further investigation is required to see if it needs to be part of the research design. To obtain a representative serum level it is recommended to take a minimum group of samples across the four quadrants in the 24-h period.

6. Method of intra-person or inter-person measures: leptin is different between individuals and the pattern in humans is inconsistent. Therefore, comparing measures across a group of people may not be the best approach, but instead comparing relative changes as part of one individual's leptin profile may be a way of disambiguating the results. This has been done in several studies where they took results once per day for just a few individuals. An

exemplar is the study by Licinio et al. (1997) which measured individuals across 24 h and provided the leptin level changes every 7 min (but not in MECFS or fibromyalgia). They also demonstrated it in another study with a group of healthy controls collecting 2070 samples from 10 healthy participants over 24 h (approx. 207 each) (Licinio, Negrao & Wong 2014). Whilst this may appear excessive, from a research perspective this method may be more productive than taking just one sample per day. A compromise would be an increase in frequency of measures within a day e.g., a minimum of 4 to 12 timepoints across 24 h. Some studies have taken one sample at 15 weeks and 30 weeks, but as leptin fluctuates from day to day, this may be a fruitless method, and an intraday sampling at these timepoints needs to be considered. It is important to include the measures of other biological and activity changes which are confounding factors such as weight, diet and activity which may have all changed across time e.g., in someone dieting.

7. Accurate sampling, sample handling, storage and analysis: Every study has slight differences in how the sample is taken, how long it takes from sampling to centrifugation, what speed and length of time centrifugation occurs (including simple factors like G-Force), and how quickly the serum, plasma or CSF is frozen or unfrozen. How long samples are stored and how many times they have been defrosted is relevant to the level of analytes measured. There are also different types of analysis such as radioimmunoassay as well as enzyme linked immunosorbent assay. There are different commercial kits, providing potentially different results for the same group of samples. It is recommended that wherever possible similar methods are used across studies.

8. Regulate the use of questionnaires used to assess levels of symptoms and comorbid issues: these need to be the same across these types of syndromes, but also so that symptoms can be compared across syndromes and other diseases. An example of a consistent measure is the Fibromyalgia Impact Questionnaire (Revised) or using a visual analogue scale of pain on

a scale of 1-100. Whatever measures are selected they need to be consistent across studies to enable systematic symptom data investigation.

9. Publishing results with transparency: Some research papers have not made their raw data available, that is the levels of leptin and other analytes, in their natural form of measurement such as nanograms or picograms per mL. Whilst associations are useful to link severity within a study, unless the raw results are provided it limits the researcher's ability to compare across studies, reduces transparency, increases the risk of bias, and makes the evidence difficult to translate.
10. Increase objective measures: The controversy of diagnosis remains, and a greater reliance on objective evidence rather than self-report measures need to be developed. It's a cyclical argument but we need to used discovery research to identify these biomarkers before we can use them objectively, supported by consistent scientific methods which provide objective measures. The full armoury of current technology needs to be used including CAT scans, regular blood measures, ECG, EEG's, genetic variants and epigenetic mRNA changes (both mitochondrial and cellular), and a greater reliance on objective evidence from wearable devices. Another method is performing post-mortem examinations of organ changes such as the brain, and heart, which can be performed at both the macro and cellular level. This method has been used for other diseases like depression, Parkinsonism and Alzheimer's.

5.6 Conclusion

Based on the combined results of this systematic review, leptin is slightly higher in cases in MECFS and/or fibromyalgia syndrome patients than healthy controls. The degree of confidence in these findings is of very low certainty, and other confounding factors may be an explanation for such difference. Following an initial scoping of the literature, it was hypothesised that cases would indeed be higher than controls,

as this was foreshadowed in several articles on the pro-inflammatory effects of leptin. However, upon completing this review, two studies reported results in the opposite direction, which has left us with a landscape of confusion. That is, two studies reported hypoleptinaemia (lower leptin), in cases compared to healthy controls. The cohort studies were so unique that they offered little use for comparison across this type of study design and were not used in the case control meta-analysis. For example, trying to compare the difference in patients with fibromyalgia who are smokers, and the difference in people who exercise would not make sense. Others compared arthritic patients, patients with depression or multiple sclerosis to fibromyalgia patients, and some of these articles were of low quality in the form of abstracts which only offered limited evidence or design detail.

Much of the literature describes the role of leptin as being part of an auto-immune process, but upon reviewing the evidence presented in this systematic review, it can only be concluded that leptin there is no clear evidence to support this. Therefore, though regularly reported, the relationship to inflammatory processes could not be substantiated by this review. As discussed earlier, the inconsistency of results was disconcerting, as there were two studies that reported lower leptin in cases compared to controls, and four studies that showed no discernible difference, and six studies that indicated significantly higher levels of leptin in cases.

There are too few studies of similar design to draw confidence from the current available evidence. The methodology is very different in each study, causing some difficulties in comparison. A series of benchmarks and protocols need to be developed if exploration of biomarkers in these syndromes is to yield useful results. To provide clarity, clinical investigators need to use similar standard practices and methods to aid comparison of circulating leptin levels. This challenge includes the way results are reported and published, but as a minimum the publication of the baseline leptin levels need to be provided. Additionally, a standard list of confounders including age, sex, ethnicity, BMI, diet, activity level, menstrual cycle phase, and an accurate time of when the sample was taken.

The preferred design would be to provide a minimum number of measures from one individual across a 24-h period to capture the zenith (peak) and nadir (trough) levels of circulating leptin. Further to this, it would be better to examine changes of an individual's fluctuations of leptin in relation to other behavioural parameters or introduced changes than across a group. The mean amplitudes of these individual results still lend themselves to for comparison across similar groups (e.g., lean, overweight and obese).

Our current understanding of circulating leptin differences across subpopulations is insufficient, as an intra-day, intra-person method is the only objective design that makes any scientific sense. If a person's baseline leptin is consistently measured at 8 am, 4 pm, 10 pm and 4 am, then we could add new parameters such as exercise for that individual, once their usual levels are known. Further work about disease longevity, how long the person has suffered from the syndrome, is required to determine if there are differences in short term and long-term cases. Where studies are completed across time, then it is important to note changes in daily activity against these differences. That is, consideration of the environment, life experiences and biological interactions over time. The person environment interaction. Researchers should publish all the details including the cofounders, the raw measurements as well as the effect of the parameter being investigated. The method described earlier in this thesis of taking samples every 7 min may be too frequent as we know that leptin changes only every 32 min with a similar number of pulses across 24 h (Licinio, Negrao & Wong 2014). There are two consistently reported diurnal changes with low leptin in the morning, and high leptin in the middle of the night, but a few additional measures would provide helpful interquartile changes. The ideal frequency to obtain a mean average 24-h leptin would require further investigation, but once per day is clearly not enough.

The controversy surrounding the diagnosis of both MECFS and fibromyalgia is problematic and further investigation and discovery of biological objective measures may help overcome these challenges. Pain and fatigue have been identified to be some of the key symptoms correlated with circulating leptin. A symptom focused

approach could be a way forward. However, severity of symptoms are usually obtained from self-report, and more objective measurements for these symptoms would be helpful. There may be better objective and technological ways to measure symptoms like pain such as changes in EEG, or muscle changes, and for fatigue we could use heart and breathing rate collected by wearable devices to obtain changes in VO2 Max (a measure that indicates the amount of oxygen you can access during exercise) (Keller, Pryor & Giloteaux 2014; Nelson et al. 2019).

Whilst the literature review did not provide strong evidence for leptin being the biomarker of choice for investigating these syndromes, it did highlight the need for objective, empirical scientific evidence and a reduction in heterogeneity for the investigation of MECFS and/or fibromyalgia. This thesis has presented the difficulties that will continue to ensue if consistent protocols for future research and publication are not developed. There needs to be a consensus for the investigation methods in MECFS and/or Fibromyalgia. The sooner we achieve some shared standards then we can form a foundation of evidence for understanding the biological mechanisms in these syndromes.

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7 Appendix

APPENDICES

Table 28 All Database Searches

Embase, PsycINFO, PubMed, Science Direct, and Scopus on 3rd April 2020 (updated on 27/7/20). All databases were searched on the same date.

Search Database	Search Terms - Exact	Comments	Found
Pub Med	((“fatigue syndrome, Chronic"[Mesh] OR “Chronic Fatigue Syndrome” OR Fibromyalgia OR Myalgic Encephalomyelitis OR ME/CFS OR MECFS OR ME-CFS OR Febricula OR “Neurocirculatory Asthenia” OR “Atypical Poliomyelitis” OR Akureyri OR “Royal Free” OR Tapanui OR “Chronic Epstein Barr” OR “Systemic Exertion Intolerance Disease” OR "postural orthostatic tachycardia syndrome" OR POTS) AND (Leptin OR "Leptin"[Mesh]))	Search is exact	52
Embase	('chronic fatigue syndrome'/exp OR 'chronic fatigue syndrome' OR 'fibromyalgia'/exp OR fibromyalgia OR 'myalgic encephalomyelitis' OR 'me cfs' OR mecfs OR ME-CFS OR febricula OR 'neurocirculatory asthenia' OR 'atypical poliomyelitis' OR akureyri OR 'royal free' OR tapanui OR 'epstein barr virus infection'/de OR 'chronic epstein barr' OR 'systemic exertion intolerance disease' OR pots OR 'postural orthostatic tachycardia syndrome'/de OR 'postural orthostatic tachycardia syndrome') AND ('leptin'/exp OR leptin)	Removed ME as this is an abbreviation but can be mistaken for he word me.	133
Psych INFO	((Chronic Fatigue Syndrome or Fibromyalgia or Myalgic Encephalomyelitis or MECFS or ME-CFS or Febricula or Neurocirculatory Asthenia or Atypical Poliomyelitis or Akureyri or Royal Free or Tapanui or Chronic Epstein Barr or Systemic	ME was only searched in title as it is too short and can be confused for the pronoun ‘me’. All other	273

Search Database	Search Terms - Exact	Comments	Found
	Exertion Intolerance or POTS).af. or ME.ti.) and Leptin.af.	words search in all fields.	
Scopus	('chronic fatigue syndrome'/exp OR 'chronic fatigue syndrome' OR 'fibromyalgia'/exp OR fibromyalgia OR 'myalgic encephalomyelitis' OR 'me cfs' OR mecfs OR ME-CFS OR febricula OR 'neurocirculatory asthenia' OR 'atypical poliomyelitis' OR akureyri OR 'royal free' OR tapanui OR 'epstein barr virus infection'/de OR 'chronic epstein barr' OR 'systemic exertion intolerance disease' OR pots OR 'postural orthostatic tachycardia syndrome'/de OR 'postural orthostatic tachycardia syndrome') AND ('leptin'/exp OR leptin) Science Direct had to be searched as per below:		133
Science Direct	"Chronic Fatigue Syndrome" AND Leptin = 81 Fibromyalgia AND Leptin =137 Leptin AND "Myalgic Encephalomyelitis" = 10 Leptin AND ME-CFS = 9 Leptin AND MECFS = 0 Leptin AND Febricula = 0 Leptin AND “Neurocirculatory Asthenia" = 0 Leptin AND “Atypical Poliomyelitis" = 0 Leptin AND Akureyri = 0 Leptin AND "Royal Free" = 57 Leptin AND Tapanui = 0 Leptin AND "Chronic Epstein Barr" = 1	Only selected research articles and review articles and each term was separately matched with leptin. It allowed an oblique to be used so ME/CFS (3) was included as MECFS returned 0.	368

Search Database	Search Terms - Exact	Comments	Found
	Leptin AND "Systemic Exertion Intolerance Disease" = 0 Leptin AND POTS = 123 "postural orthostatic tachycardia syndrome" AND Leptin = 7	Science Direct only accepts 8 boolean operators as a maximum and returned too many results so it has been broken down to smaller searches. Each pair needs to be search separately or you get less rather than more.	
		duplicates	94
subtotal			959(865)

Table 29 All 85 Excluded Studies

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
1	The co-ordinated cytokine/hormone response to acute injury incorporates leptin	Wallace, A. M.; Sattar, N.; McMillan, D. C.	Recent studies have implicated leptin as a 'stress' hormone and highlighted its association with increases in inflammatory cytokines, C-reactive protein and cortisol. In order to investigate the exact temporal leptin response to stress we undertook a detailed longitudinal study of circulating leptin concentrations during the well-defined surgical injury of cholecystectomy. Circulating concentrations of cortisol, free fatty acids, leptin and C-reactive protein were measured at 3, 6, 9, 12, 18, 24, 48 and 72 h from the start of surgery in nine patients. There was a significant correlation between baseline concentrations of leptin and BMI ($r = 0.893$, $P < 0.001$). Over the 72 h from the start of surgery there were significant ($P < 0.05$) increases in the concentrations of all analytes (peak median concentrations); cortisol (6 h), free fatty acids (9 h), leptin (18 h) and C-reactive protein (48 h). Interestingly the timing of the leptin peak at approximately 18 h after an acute inflammatory stimulus is exactly the same as previously reported for interleukin 6.	2000	Cytokine	#284	Exclusion reason: Wrong patient population; Martin Lewis (2020-04-29 14:27:22)(Select): Study 2. Large number of postmenopausal women. Maybe provide a good reference for variability etc. ; Michael Musker (2020-04-22 16:44:45)(Select): Exclude: Refers to plasma levels following acute surgery. Wrong study population. provides leptin levels over 24 hours following surgery and suggests leptin levels mirror the relationship between cortisol and IL6.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
2	Chronic fatigue syndrome and circulating cytokines: A systematic review	Blundell, S.; Ray, K. K.; Buckland, M.; White, P. D.	There has been much interest in the role of the immune system in the pathophysiology of chronic fatigue syndrome (CFS), as CFS may develop following an infection and cytokines are known to induce acute sickness behaviour, with similar symptoms to CFS. Using the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) guidelines, a search was conducted on PubMed, Web of Science, Embase and PsycINFO, for CFS related-terms in combination with cytokine-related terms. Cases had to meet established criteria for CFS and be compared with healthy controls. Papers retrieved were assessed for both inclusionary criteria and quality. 38 papers met the inclusionary criteria. The quality of the studies varied. 77 serum or plasma cytokines were measured without immune stimulation. Cases of CFS had significantly elevated concentrations of transforming growth factor-beta (TGF-Œ≤) in five out of eight (63%) studies. No other cytokines were present in abnormal concentrations in the majority of studies, although insufficient data were available for some cytokines.	2015	Brain, Behavior, and Immunity	#728	Exclusion reason: Systematic Review - exclude; Michael Musker (2020-04-19 16:28:54)(Select): After further discussion, I will exclude this as it is a systematic review - see table 1 (although it only cites other papers as it is a systematic review and then only states that results are higher or lower). See Hornig et al 2015 reference table 1); Martin Lewis (2020-04-29 11:25:35)(Select): I assume other reviews need to be acknowledged;

3	Autoinflammation and Immunomodulation in Inflammatory Fibromyalgia Syndrome-A Review	Metyas, S.; Rezk, T.; Arkfeld, D.; Leptich, T.	Generalized pain with tender points in specific areas accompanied by systemic symptoms such as fatigue and stiffness is characteristic of fibromyalgia (FM) syndrome. The genesis of FM is still being investigated with conflicting data on factors including autonomic dysfunction, neurotransmitters, and hormones often in combination with external stressful events. However, recent research is starting to suggest that there is a previously underappreciated subtype of fibromyalgia called inflammatory Fibromyalgia (iFM). Recent studies have described cytokines, inflammatory markers, sleep disorders, hyperalgesia, cognitive dysfunction, serum leptin levels and other inflammatory indicators as potential markers for iFM. This article will; 1) review the inflammatory markers and abnormal levels of other laboratory indicators that can help to identify the subgroup of patients that fall into the new category of Inflammatory Fibromyalgia [1-5] and 2) review all completed trials that were focused on treating this new category of disease. Through this review it is hoped that and further understanding of the complexity of the etiology of fibromyalgia can be explored.	2017	Curr Rheumatol Rev	#223	Exclusion reason: No leptin results; Michael Musker (2020-10-04 21:48:44)(Select): After reviewing the paper, there are no results. I contacted the author who said they used a multitest assay that only provides a summary result.; Michael Musker (2020-09-16 10:15:53)(Select): I was able to obtain the results from the author who advised that they don't have specific leptin results as part of this study. No leptin results in article so exclude. The paper refers to the Quismorio abstract, but provides no results just says Leptin is higher in cases using this reference (20), and in the diagram refers to reference 21 but references only go up to 20.; Michael Musker

4	Obesity and pain	Mason, Peggy et al.	Today, most Americans are overweight, with a steadily increasing proportion of the population qualifying as obese. Most Americans also experience some form of pain, such as lower back pain or headache, and often the condition is chronic. Thus, it is no surprise that a large number of overweight individuals experience chronic pain. The probability of two very common conditions occurring concurrently is simply the product of their individual probabilities. Clearly, the trick here is to tease apart whether a significant relationship exists between weight and pain and to discern any causal factors involved. It may be, as is often assumed, that extra weight leads to conditions such as lower back pain. It may also be that the pain experience alters neural control of energy balance, resulting in either under- or overweight individuals. Alternatively, there may be a common factor that predisposes individuals to both an abnormal energy balance state, either obesity or an underweight condition, and chronic pain. Finally, there may be different answers for different types of pain and for different energy imbalances. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2012	Pain comorbidities: Understanding and treating the complex patient.	#330	Exclusion reason: No results provided; Michael Musker (2020-09-16 10:14:18)(Select): This text has only verbal reference to the role of leptin but has not results within the text of this chapter. - Exclude; Michael Musker (2020-04-22 22:56:40)(Select): The book is available in Barr Smith, will retrieve book and review chapter;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
5	Leptin, An Adipokine With Central Importance in the Global Obesity Problem	Mechanick, Jeffrey I.; Zhao, Shan; Garvey, W. Timothy	Leptin has central importance in the global obesity and cardiovascular disease problem. Leptin is principally secreted by adipocytes and acts in the hypothalamus to suppress appetite and food intake, increase energy expenditure, and regulate body weight. Based on clinical translation of specific and networked actions, leptin affects the cardiovascular system and may be a marker and driver of cardiometabolic risk factors with interventions that are actionable by cardiologists. Leptin subnetwork analysis demonstrates a statistically significant role for ethnoculturally and socioeconomically appropriate lifestyle intervention in cardiovascular disease. Emergent mechanistic components and potential diagnostic or therapeutic targets include hexokinase 3, urocortins, clusterin, sialic acid-binding immunoglobulin-like lectin 6, C-reactive protein, platelet glycoprotein VI, albumin, pentraxin 3, ghrelin, obestatin prepropeptide, leptin receptor, neuropeptide Y, and corticotropin-releasing factor receptor 1. Emergent associated symptoms include weight change, eating disorders, vascular necrosis, chronic fatigue, and chest pain. Leptin-targeted therapies are reported for lipodystrophy and leptin deficiency, but they are investigational for leptin resistance, obesity, and other chronic diseases.	2018	Global Heart	#228	Exclusion reason: No leptin results; Michael Musker (2020-04-22 00:18:24)(Select): Exclude: No results here. Another extensive discussion on the interplay of leptin and the neuroimmune system - great article: 'Leptin is a pleiotropic stress- an ancient anorexigen, immunomodulator, and growth factor, with signaling pathways and neural substrates conserved over 350 million years, having a key physiologic role during starvation, and now situated as a central player in cardiometabolic networking research;

6	Role of neuroendocrine and neuroimmune mechanisms in chronic inflammatory rheumatic diseases, The 10-year update	Straub, Rainer H.; Bijlsma, Johannes W. J.; Masi, Alfonse; Cutolo, Maurizio	Background Neuroendocrine immunology in musculoskeletal diseases is an emerging scientific field. It deals with the aspects of efferent neuronal and neurohormonal bearing on the peripheral immune and musculoskeletal systems. This review aims to add new information that appeared since 2001. Search strategy in a continuous process, year by year, this search strategy yielded relevant papers that were screened and collected in a database, which build the platform of this review. Results The main findings are the anti-inflammatory role of androgens, the loss of androgens (androgen drain), the bimodal role of estrogens (support B cells and inhibit macrophages and T cells), increased conversion of androgens to estrogens in inflammation (androgen drain), disturbances of the gonadal axis, inadequate amount of HPA axis hormones relative to inflammation (disproportion principle), biologics partly improve neuroendocrine axes, anti-corticotropin-releasing hormone therapies improve inflammation (antalarmin), bimodal role of the sympathetic nervous system (proinflammatory early, anti-inflammatory late, most probably due to catecholamine-producing local cells), anti-inflammatory role of alpha melanocyte-stimulating hormone, vasoactive intestinal peptide, and the Vagus nerve via nicotinergic receptors.	2013	Seminars in Arthritis and Rheumatism	#29	Exclusion reason: No leptin results; Michael Musker (2020-04-22 16:19:15)(Select): Exclude: An explanatory document, with no results provided. It specifically states in the introduction 'We do not touch non-inflammatory fibromyalgia or stress-related aspects of rheumatic diseases because these subjects are demonstrated elsewhere in extensive form';

7	The Relationship of Endocannabinoidome Lipid Mediators with Pain and Psychological Stress in Women with Fibromyalgia: A Case-Control Study	Stensson, Niclas; Ghafouri, Nazdar; Ernberg, Malin; Mannerkorpi, Kaisa; Kosek, Eva; Gerdle, Ghafouri, Bijar	Characterized by chronic widespread pain, generalized hyperalgesia, and psychological stress, fibromyalgia (FM) is difficult to diagnose and lacks effective treatments. Endocannabinoids, -arachidonoylethanolamide (AEA), 2-arachidonoylglycerol (2-AG), and the related oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and stearoylethanolamide (SEA), are endogenous lipid mediators with analgesic and anti-inflammatory characteristics, in company with psychological modulating properties (eg, stress and anxiety), and are included in a new emerging "ome," the endocannabinoidome. . This case-control study compared the concentration differences of AEA, OEA, PEA, SEA, and 2-AG in 104 women with FM and 116 healthy control subjects. All participants rated their pain, anxiety, depression, and current health status. The relationships between the lipid concentrations and the clinical assessments were investigated using powerful multivariate data analysis and traditional bivariate statistics. The concentrations of OEA, PEA, SEA, and 2-AG were significantly higher in women with FM than in healthy control subjects; significance remained for OEA and SEA after controlling for body mass index and age.	2018	The Journal of Pain	#33	Exclusion reason: No leptin results; Michael Musker (2020-04-22 22:15:52)(Select): Exclude: Discusses many lipids except leptin with fibromyalgia ' Endocannabinoids, arachidonoylethanolamide (AEA), 2-arachidonoylglycerol (2-AG), and the related oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and stearoylethanolamide (SEA),are endogenous lipid mediators with analgesic and anti-inflammatory characteristics, in company with psychological modulating properties (eg, stress and anxiety), and are included in a new emerging ,ome,, the endocannabinoidome. No leptin results provided;

8	Vectra DA for the objective measurement of disease activity in patients with rheumatoid arthritis	Segurado, O. G.; Sasso, E. H.	Quantitative and regular assessment of disease activity in rheumatoid arthritis (RA) is required to achieve treatment targets such as remission and to optimise clinical outcomes. To assess inflammation accurately, predict joint damage and monitor treatment response, a measure of disease activity in RA should reflect the pathological processes resulting in irreversible joint damage and functional disability. The Vectra DA blood test is an objective measure of disease activity for patients with RA. Vectra DA provides an accurate, reproducible score on a scale of 1 to 100 based on the concentrations of 12 biomarkers that reflect the pathophysiologic diversity of RA. The analytical validity, clinical validity, and clinical utility of Vectra DA have been evaluated for patients with RA in registries and prospective and retrospective clinical studies. As a biomarker-based instrument for assessing disease activity in RA, the Vectra DA test can help monitor therapeutic response to methotrexate and biologic agents and assess clinically challenging situations, such as when clinical measures are confounded by non-inflammatory pain from fibromyalgia. Vectra DA scores correlate with imaging of joint inflammation and are predictive for radiographic progression, with high Vectra DA scores being associated with more frequent and severe progression and low scores being predictive for non-progression. In summary,	2014	Clinical and Experimental Rheumatology	#75	Exclusion reason: No leptin results; Michael Musker (2020-04-22 15:53:52)(Select): Exclude: only mentions leptin as one of 12 biomarkers that make up the commercial Vectra DA test. No results provided on leptin.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
9	Leptin, adiponectin, and insulin resistance	Sarigianni, M.; Tsapas, A.; Paletas, K.	Leptin has also been shown to induce changes in monocyte properties that are potentially involved in atherogenesis. Furthermore, leptin is also thought to have pleiotropic actions contributing to obesity complications besides body weight regulation. Adiponectin is another hormone produced by adipose tissue and is inversely correlated with body fat mass, insulin resistance, and risk for type 2 diabetes mellitus. In conclusion, some of the effects of leptin and adiponectin on insulin sensitivity could be mediated via changes in NHE-1 activity	2011	Angiology	#92	Exclusion reason: No results provided; Michael Musker (2020-04-22 15:47:23)(Select): Exclude: A brief about a previous study, but contains no results or information on MECFS or Fibromyalgia - 'Leptin has also been shown to induce changes in monocyte properties that are potentially involved in atherogenesis. Furthermore, leptin is also thought to have pleiotropic actions contributing to obesity complications besides body weight regulation.';

10	Involvement of signaling molecules on Na+/H+ exchanger-1 activity in human monocytes	Sarigianni, M.; Tsapas, A.; Mikhailidis, D. P.; Kaloyianni, M.; Koliakos, G.; Paletas, K.	Background: Sodium/hydrogen exchanger-1 (NHE-1) contributes to maintaining intracellular pH (pHi). We assessed the effect of glucose, insulin, leptin and adrenaline on NHE-1 activity in human monocytes in vitro. These cells play a role in atherogenesis and disturbances in the hormones evaluated are associated with obesity and diabetes. Methods and Results: Monocytes were isolated from 16 healthy obese and 10 lean healthy subjects. NHE-1 activity was estimated by measuring pHi with a fluorescent dye. pHi was assessed pre- and post-incubation with glucose, insulin, leptin and adrenaline. Experiments were repeated after adding a NHE-1 inhibitor (cariporide) or an inhibitor of protein kinase C (PKC), nitric oxide synthase (NOS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, phosphoi- nositide 3-kinases (PI3K) or actin polymerization. Within the whole study population, glucose enhanced NHE-1 activity by a processes involving PKC, NOS, PI3K and actin polymerization (p = 0.0006 to 0.01). IInsulin-mediated activation of NHE-1 (p = <0.0001 to 0.02) required the classical isoforms of PKC, NOS, NADPH oxidase and PI3K. Leptin increased NHE-1 activity (p = 0.0004 to 0.04) through the involvement of PKC and actin polymerization.	2010	Open Cardiovascular Medicine Journal	#93	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 15:43:57)(Select): Exclude: no reference to MECFS or other diseases such as fibromyalgia. Article focuses on the intracellular effects of leptin such as intracellular PH and other effects on cells in obese and lean health subjects.;

11	Epidemiological evidence against a role for C-reactive protein causing leptin resistance	Rutter, M. K.; Sattar, N.; Tajar, A.; O'Neill, T. W.; Lee, D. M.; Bartfai, G.; Boonen, S.; Casanueva, F. F.; Finn, J. D.; Forti, G.; Giwercman, A.; Han, T. S.; Huhtaniemi, I. T.; Kula, K.; Lean, M. E. J.; Pendleton, N.; Punab, M.; Silman, A. J.; et al.	Objective: It has been suggested that elevated levels of C-reactive protein (CRP) might interfere with leptin signalling and contribute to leptin resistance. Our aim was to assess whether plasma levels of CRP influence leptin resistance in humans, and our hypothesis was that CRP levels would modify the cross-sectional relationships between leptin and measures of adiposity. Design and methods: We assessed four measures of adiposity: BMI, waist circumference, fat mass and body fat (%) in 2113 British Regional Heart Study (BRHS) men (mean (S.D.) age 69 (5) years), with replication in 760 (age 69 (6) years) European Male Ageing Study (EMAS) subjects. Results: In BRHS subjects, leptin correlated with CRP (Spearman's r=0.22, P<0.0001). Leptin and CRP correlated with all four measures of adiposity (r value range: 0.22-0.57, all P<0.0001). Ageadjusted mean levels for adiposity measures increased in relation to leptin levels, but CRP level did not consistently influence the Œ≤-coefficients of the regression lines in a CRP-stratified analysis. In BRHS subjects, the BMI vs leptin relationship demonstrated a weak statistical interaction with CRP (P=0.04). We observed no similar interaction in EMAS subjects and no significant interactions with other measures of adiposity in BRHS or EMAS cohorts.	2013	European Journal of Endocrinology	#103	Exclusion reason: Another Major Disease; Michael Musker (2020-04-22 15:36:30)(Select): Exclude: provides leptin levels between patients with cardiac issues and diabetics - no mention of mecfs or fibromyalgia;

12	Physical activity protects the human brain against metabolic stress induced by a postprandial and chronic inflammation	Pruimboom, Leo; Raison, Charles L.; Muskiet, Frits A. J.; Abedini, Abizaid Scheurink Kleinridders Kotrschal Ridgel Cuevas Knab Petersen Navarrete Peters Pedersen et al.	In recent years, it has become clear that chronic systemic low-grade inflammation is at the root of many, if not all, typically Western diseases associated with the metabolic syndrome. While much focus has been given to sedentary lifestyle as a cause of chronic inflammation, it is less often appreciated that chronic inflammation may also promote a sedentary lifestyle, which in turn causes chronic inflammation. Given that even minor increases in chronic inflammation reduce brain volume in otherwise healthy individuals, the bidirectional relationship between inflammation and sedentary behaviour may explain why humans have lost brain volume in the last 30,000 years and also intelligence in the last 30 years. We review evidence that lack of physical activity induces chronic low-grade inflammation and, consequently, an energy conflict between the selfish immune system and the selfish brain. Although the notion that increased physical activity would improve health in the modern world is widespread, here we provide a novel perspective on this truism by providing evidence that recovery of normal human behaviour, such as spontaneous physical activity, would calm proinflammatory activity, thereby allocating more energy to the brain and other organs, and by doing so would improve human health. (PsycINFO Database Record (c) 2017 APA, all rights reserved)	2015	Behavioural Neurology	#149	Exclusion reason: No results provided; Michael Musker (2020-04-22 15:00:47)(Select): Exclude: Another explanatory document that has no results but mentions leptin and inflammations: 'The major neuropeptide systems that have been studied relative to spontaneous physical activity include cholecystokinin, corticotropin-releasing hormone, neuromedin U, neuropeptide Y, leptin, agouti-related protein, orexins, and ghrelin. All these systems influence dopaminergic signalling'.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
13	Thyroid and sympathetic influences on plasma leptin in hypothyroidism and hyperthyroidism	Pinkney, J. H.; Goodrick, S. J.; Katz, J. R.; Johnson, A. B.; Lightman, S. L.; Coppack, S. W.; Medbak, S.; Mohamed-Ali, V.	Objectives: To determine the dependence of plasma leptin concentrations upon circulating noradrenaline (NA) and thyroid hormones (TH) in humans. Design: Cross-sectional study in 40 newly diagnosed untreated patients with primary thyroid disease, and 69 lean and obese euthyroid control subjects. Measurements: Plasma leptin, NA, free T3 (fT3) and TSH in the fasting state. Anthropometry and % body fat (electrical bioimpedance). Results: Leptin levels were highest in 37 obese euthyroid and 22 hypothyroid (median [interquartiles] 31.5 [19.0-48.0], 19.2 [11.5-31.5] ng ml-1), and lowest in 32 lean euthyroid and 18 hyperthyroid subjects (6.6 [3.9-14.4], 8.9 [5.5-11.1]; ANOVA, P < 0.0001). Plasma NA was similar in all groups (P = n.s.). In obese controls. TSH correlated with % body fat and leptin (r = 0.67, r = 0.61; P < 0.001). Treatment of hypothyroidism (n = 10) with T4 reduced leptin from 20.8 [11.8-31.6] to 12.9 [4.6-21.2] (P = 0.005) with no change in BMI. Conclusions: Thyroid status modifies leptin secretion independently of adiposity and NA. The data suggest leptin thyroid interactions at hypothalamic and adipocyte level.	2000	International Journal of Obesity	#164	Exclusion reason: Another Major Disease; Michael Musker (2020-04-22 14:55:57)(Select): Exclude: tracks patients treated for hypothyroidism across 6 months: Conclusions: Thyroid status modifies leptin secretion independently of adiposity and NA. The data suggest leptin ¬± thyroid interactions at hypothalamic and adipocyte level. No mention of CFS or fibromyalgia;

14	Leptin and the pituitary-thyroid axis: A comparative study in lean, obese, hypothyroid and hyperthyroid subjects	Pinkney, J. H.; Goodrick, S. J.; Katz, J.; Johnson, A. B.; Lightman, S. L.; Coppack, S. W.; Mohamed-Ali, V.	OBJECTIVE: To study interactions between leptin and the pituitary- thyroid axis, both in euthyroid and dysthyroid states. SUBJECTS AND MEASUREMENTS: We investigated the relationships of plasma leptin to levels of free thyroid hormones and TSH in 18 patients with newly diagnosed hyperthyroidism, 22 with newly diagnosed primary hypothyroidism, and 32 lean (body mass index [BMI] <30) and 37 obese (BMI> 30 kg/m2) euthyroid subjects. Hypothyroid patients were restudied during thyroxine replacement treatment. RESULTS: Median [interquartile range] plasma leptin concentrations were highest in obese euthyroid subjects (31-5 [19.0-48.0] and in untreated hypothyroid patients (19.2 [11.5-31.5]), and lowest levels in untreated hyperthyroid patients (8.9 [5.5-11.1])and lean euthyroid control subjects (6.6 [3.9-14.4]g/l (Kruskall-Wallis one-way analysis of variance; P<0.0001). In euthyroid subjects, plasma leptin levels were higher in obese than in lean subjects (P<0.00001). In obese subjects plasma levels of TSH correlated With percentage body fat (r=0.67; P<0.001) and plasma leptin (r=0.61; P<0.001). In untreated hyperthyroid subjects plasma leptin was unrelated to free T3, and in untreated hypothyroidism plasma leptin was unrelated to either free T3 or TSH concentrations (all P=NS).	1998	Clin Endocrinol (Oxf)	#165	Exclusion reason: Another Major Disease; Michael Musker (2020-04-22 14:46:07)(Select): Exclude: Provides leptin results for euthyroid, hyperthyroidism and hypothyroidism in obese and lean patients. No mention of MECFS or fibromyalgia.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
15	Pathogenetic mechanisms and therapeutic targets of fibromyalgia	Pile, K.	All rheumatologists care for patients with the Ofibromyalgic syndrome O, but the diagnosis of fibromyalgia (FM) remains a controversial. This presentation will review pathogenetic mechanisms as a starting point to discuss actual and conceptual interventions that may improve the current and future quality of life of those presenting. FM is a common chronic pain condition, characterised by widespread pain and decreased pain threshold, with allodynia and hyperalgesia. Additionally disturbed non-restorative sleep patterns, fatigue, mood changes, and irritability of bladder and bowel are commonly described. The absence of peripheral pathology has focussed research on central nervous system pain sensitisation with altered pain modulation at the level of spine and brain. Education, physical, psychological, and pharmacological are utilised in the multi-faceted armamentarium of therapy. Genetically suggested mechanisms will be discussed as will the potential role of cytokines leptin, and brain-derived neurotrophic factor. Therapeutic targets include alpha2-delta ligands, reuptake inhibition of serotonin, noradrenaline, and dopamine. Antagonism of substance- P, and opioid will be reviewed, in additions to medications targeting sleep.	2014	Int J Rheum Dis	#167	Exclusion reason: No leptin results; Michael Musker (2020-04-22 11:46:16)(Select): Exclude: only an abstract with no results and the extent of this is: Genetically suggested mechanisms will be discussed as will the potential role of leptin, and brain-derived neurotrophic factor. Therapeutic targets ligands, reuptake inhibition of serotonin, noradrenaline, and dopamine. Antagonism of substance-P, and opioid will be reviewed, in additions to medications targeting sleep.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covide nce #	Reasons for exclusion following consensus
16	The role of psychobiol ogical and neuroendo crine mechanism s in appetite regulation and obesity	Paspala, I.; Katsiki, N.; Kapoukrani dou, D.; Mikhailidis, D. P.; Tsiligiroglo u-Fachantido u, A.	Obesity is a multifactorial disease. Among its causes are physical inactivity and overeating. In addition, other factors may play an important role in the development of overweight/obesity. For example, certain hormones including leptin, insulin and ghrelin, may influence appetite and consequently body weight. Obesity frequently co-exists with metabolic disorders including dyslipidemia, hypertension and insulin resistance, thus constituting the metabolic syndrome which is characterized by increased cardiovascular risk. Lack of comprehensive knowledge on obesity-related issues makes both prevention and treatment difficult. This review considers the psychobiological and neuroendocrine mechanisms of appetite and food intake. Whether these factors, in terms of obesity prevention and treatment, will prove to be relevant in clinical practice (including reducing the cardiovas-cular risk associated with obesity) remains to be established. ¬© Paspala et al.	2012	Open Cardiovas cular Medicine Journal	#176	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 11:42:42)(Select): Exclude: article focuses on obesity, and discusses the role of leptin, ghrelin and insulin - no cfs or fibromyalgia in article;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
17	Leptin: A promising therapeutic target with pleiotropic action besides body weight regulation	Paraskevas, K. I.; Liapis, C. D.; Mikhailidis, D. P.	Leptin seems to regulate various physiological mechanisms besides body weight. Leptin plays a role in vascular biology and pathology as well as renal function. In addition, leptin has been implicated in the regulation of fertility and reproduction. The effect of pharmaceutical agents on circulating plasma leptin levels has been assessed. Among the drugs investigated are glitazones, statins, fibrates, serotonin reuptake inhibitors and cannabinoid-1 receptor antagonists. Since these agents are used to treat pathological conditions there is a potential role for leptin in these states.	2006	Current Drug Targets	#182	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 22:06:03)(Select): Exclude: only discusses interaction with other drugs and hormones, but no mention of chronic fatigue or fibromyalgia;

18	Glucocorticoid receptor mediated negative feedback in chronic fatigue syndrome using the low dose (0.5 mg) dexamethasone suppression test	Papadopoulos, Andrew; Ebrecht, Marcel; Roberts, Amanda D. L.; Poon, Lucia; Rohleder, Nicolas; Cleare, Anthony J.; Beck, Buysse Candy Chalder Dinan Fukuda Gaab et al.	Background: Chronic fatigue syndrome (CFS) is associated with hypocortisolism, but it is not yet clear the extent to which enhanced negative feedback may underlie this finding. Methods: We undertook a low-dose dexamethasone (0.5 mg) suppression test in 18 CFS patients and 20 matched, healthy controls. We measured salivary cortisol levels at 0800 h, 1200 h, 1600 h and 2000 h before and after the administration of 0.5 mg of dexamethasone. Results: Basal cortisol output was raised in this group of CFS patients compared to controls. Overall, the percentage suppression following dexamethasone administration was no different between CFS (mean +/- sem: 80.4 +/- 4.4%) and controls (76.2 +/- 4.9 %). However, the sub-group of patients with CFS and comorbid depression (n = 9) showed a significant hypersuppression of salivary cortisol in response to dexamethasone (89.0 +/- 1.9%; p < 0.05 v controls). Limitations: The sub-group analysis was on small numbers and should be considered preliminary. Dexamethasone probes only glucocorticoid medicated negative feedback but does not probe mineralocorticoid feedback, the other main physiological feedback mechanism.	2009	Journal of Affective Disorders	#185	Exclusion reason: No leptin results; Michael Musker (2020-04-22 11:04:31)(Select): Exclude: focuses on cortisol in mecfs and controls - no leptin results: Results: Basal cortisol output was raised in this group of CFS patients compared to controls. Overall, the percentage suppression following dexamethasone administration was no different between CFS (mean ¬± sem: 80.4 ¬± 4.4%) and controls (76.2 ¬± 4.9 %). However, the sub-group of patients with CFS and comorbid depression (n= 9) showed a significant hypersuppression of salivary cortisol in response to dexamethasone (89.0 ¬± 1.9%; pb0.05 v controls).;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
19	The association between chronic pain and obesity	Okifuji, A.; Hare, B. D.	Obesity and pain present serious public health concerns in our society. Evidence strongly suggests that comorbid obesity is common in chronic pain conditions, and pain complaints are common in obese individuals. In this paper, we review the association between obesity and pain in the general population as well as chronic pain patients. We also review the relationship between obesity and pain response to noxious stimulation in animals and humans. Based upon the existing research, we present several potential mechanisms that may link the two phenomena, including mechanical/structural factors, chemical mediators, depression, sleep, and lifestyle. We discuss the clinical implications of obesity and pain, focusing on the effect of weight loss, both surgical and noninvasive, on pain. The literature suggests that the two conditions are significant comorbidities, adversely impacting each other. The nature of the relationship however is not likely to be direct, but many interacting factors appear to contribute. Weight loss for obese pain patients appears to be an important aspect of overall pain rehabilitation, although more efforts are needed to determine strategies to maintain long-term benefit.	2015	Journal of Pain Research	#197	Exclusion reason: No leptin results; Michael Musker (2020-04-22 10:48:09)(Select): Exclude: only discusses leptin in the context of join pain, but does not have any results. This article is not about MECFS either. 'Leptin is a hormone that signals energy intake and stores to the brain, and obesity is associated with high leptin levels.83 In end-stage OA, joint pain was significantly associated with synovial leptin level.84 Increased leptin level in OA

20	Stress as a pathophysiological factor in functional somatic syndromes	Nater, Urs M.; Fischer, Susanne; Ehlert, Ulrike; Aaron, Abraham Adeyemi Adler Adler Ahles Alfven Amel Kashipaz Anderberg Bach Barsky Bazzichi Berntson Bigal Biondi Blanchard Bohmelt Boisset-Pioro Borish et al.	Functional somatic syndromes (FSS) are defined by a constellation of symptoms for which after thorough medical examination no structural pathology and no proportional tissue abnormalities can be identified. Pathophysiology of these syndromes has remained elusive and treatment options are limited. Current research efforts acknowledge the importance of stress as a potential risk factor in the manifestation and maintenance of FSS. A substantial body of research has focused on psychological stress factors as well as alterations of the endocrine stress system (the hypothalamic-pituitary-adrenal, HPA axis, in particular), the immune system, and the autonomic nervous system (ANS). Dysregulation of these systems might explain some of the symptoms of FSS. In this review, we describe studies reporting stress-related findings in three of the most prevalent and well-described FSS, i.e. chronic fatigue syndrome (CFS), irritable bowel syndrome (IBS), and fibromyalgia syndrome (FMS). Psychobiological processes which seem to play a role in the translation of stress into functional symptoms and syndromes are discussed. (PsycINFO Database Record (c) 2020 APA, all rights reserved)	2011	Current Psychiatry Reviews	#212	Exclusion reason: No Leptin in abstract; Michael Musker (2020-04-24 09:13:32)(Select): Exclude: this article reviews the psychological impacts of Chronic Fatigue Syndrome, Fibromyalgia and IBS. It does note that these three syndromes have similar symptoms, and it may depend on the first professional seen that determines the diagnosis eg a neurologist CFS, a rheumatologist Fibromyalgia, and a gastroenterologist IBS. Lists of studies of each are provided along with their psychological outcomes.;

21	Leaky brain in neurological and psychiatric disorders: Drivers and consequences	Morris, Gerwyn; Fernandes, Brisa S.; Puri, Basant K.; Walker, Adam J.; Carvalho, Andre F.; Berk, Michael; Abbott, Abbott Acuna Castroviejo Aid Aird Akanuma Al-Sadi Al-Shabrawey Albert Albrecht Aliev Aliev Alluri Alluri Alvi et al.	Background: The blood-brain barrier acts as a highly regulated interface; its dysfunction may exacerbate, and perhaps initiate, neurological and neuropsychiatric disorders. Methods: In this narrative review, focussing on redox, inflammatory and mitochondrial pathways and their effects on the blood-brain barrier, a model is proposed detailing mechanisms which might explain how increases in blood-brain barrier permeability occur and can be maintained with increasing inflammatory and oxidative and nitrosative stress being the initial drivers. Results: Peripheral inflammation, which is causatively implicated in the pathogenesis of major psychiatric disorders, is associated with elevated peripheral pro-inflammatory cytokines, which in turn cause increased blood-brain barrier permeability. Reactive oxygen species, such as superoxide radicals and hydrogen peroxide, and reactive nitrogen species, such as nitric oxide and peroxynitrite, play essential roles in normal brain capillary endothelial cell functioning; however, chronically elevated oxidative and nitrosative stress can lead to mitochondrial dysfunction and damage to the blood-brain barrier. Activated microglia, redox control of which is mediated by nitric oxide synthases and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, secrete neurotoxic molecules such as reactive	2018	Australian and New Zealand Journal of Psychiatry	#539	Exclusion reason: No leptin results; Michael Musker (2020-04-22 00:36:05)(Select): Exclude: An exploratory discussion on the role of the immune system and the blood brain barrier and neurotoxic effects of chemokines: ' Activated microglia secrete a range of neurotoxic molecules such as ROS, NO, PGE, cyclooxygenase (COX)-2, quinolinic acid, several chemokines such as monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 1 (CXCL-1) and macrophage inflammatory protein 1Œ± (MIP-1Œ±), and the PICs IL-6, TNF-Œ± and IL-1Œ≤, which all exert a detrimental effect on the integrity and function of the BBB';

22	The emerging role of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs)	Morris, G. Berk, M. Galecki, P. Maes, M.	The World Health Organization classifies myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs) as a nervous system disease. Together with other diseases under the G93 heading, ME/cfs shares a triad of abnormalities involving elevated oxidative and nitrosative stress (O&NS), activation of immuno-inflammatory pathways, and mitochondrial dysfunctions with depleted levels of adenosine triphosphate (ATP) synthesis. There is also abundant evidence that many patients with ME/cfs (up to around 60 %) may suffer from autoimmune responses. Awide range of reported abnormalities in ME/cfs are highly pertinent to the generation of autoimmunity. Here we review the potential sources of autoimmunity which are observed in people with ME/cfs. The increased levels of pro-inflammatory cytokines, e.g., interleukin-1 and tumor necrosis factor-alpha, and increased levels of nuclear factor-kappaB predispose to an autoimmune environment. Many cytokine abnormalities conspire to produce a predominance of effector B cells and autoreactive T cells. The common observation of reduced natural killer cell function in ME/cfs is a source of disrupted homeostasis and prolonged effector T cell survival. B cells may be pathogenic by playing a role in autoimmunity independent of their ability to produce antibodies.	2014	Molecular Neurobiology	#540	Exclusion reason: No leptin results; Michael Musker (2020-04-22 00:29:00)(Select): Exclude: A discussion document on Signs and drivers of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome. A detailed discussion and explanatory document but no results provided.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
23	A narrative review on the similarities and dissimilarities between myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and sickness behavior	Morris, G.; Anderson, G.; Galecki, P.; Berk, M.; Maes, M.	It is of importance whether myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a variant of sickness behavior. The latter is induced by acute infections/injury being principally mediated through proinflammatory cytokines. Sickness is a beneficial behavioral response that serves to enhance recovery, conserves energy and plays a role in the resolution of inflammation. There are behavioral/symptomatic similarities (for example, fatigue, malaise, hyperalgesia) and dissimilarities (gastrointestinal symptoms, anorexia and weight loss) between sickness and ME/CFS. While sickness is an adaptive response induced by proinflammatory cytokines, ME/CFS is a chronic, disabling disorder, where the pathophysiology is related to activation of immunoinflammatory and oxidative pathways and autoimmune responses. While sickness behavior is a state of energy conservation, which plays a role in combating pathogens, ME/CFS is a chronic disease underpinned by a state of energy depletion. While sickness is an acute response to infection/injury, the trigger factors in ME/CFS are less well defined	2013	BMC Medicine	#541	Exclusion reason: No leptin results; Michael Musker (2020-04-22 10:00:59)(Select): Exclude: This article discusses the role of sickness behaviour and neuroimmune responses. There is no data provided. It reviews theoretical links to sickness behaviour and the role of cytokines.;

24	Brain-immune interactions and disease susceptibility	be, Adcock Adcock Aksentijevich Altshuler Amsterdam Andonopoulos Arato Axelson Bahls Banki Banki Barnes Bartalena Bauer Baumgartner Bianchi Bisbocci Blalock Bologa Bonavera Boswell B et al.	Many studies have established the routes by which the immune and central nervous (CNS) systems communicate. This network of connections permits the CNS to regulate the immune system through both neuroendocrine and neuronal pathways. In turn, the immune system signals the CNS through neuronal and humoral routes, via immune mediators and cytokines. This regulatory system between the immune system and CNS plays an important role in susceptibility and resistance to autoimmune, inflammatory, infectious and allergic diseases. This review focuses on the regulation of the immune system via the neuroendocrine system, and underlines the link between neuroendocrine dysregulation and development of major depressive disorders, autoimmune diseases and osteoporosis.	2005	Molecular Psychiatry	#336	Exclusion reason: No leptin results; Michael Musker (2020-04-22 00:07:57)(Select): Exclude: This is another discussion paper on the interplay between the HPA axis and the immune system: 'Alterations in endocrine and immunological system, such as hypercortisolism, increased levels of cytokines, particularly interleukin-6 (IL-6), and increased levels of leptin, are possible mechanisms of bone loss in patients with depression. Cytokines including IL-6, IL-1, TNFa and leptin are important local factors that regulate the bone metabolism. IL-6 is implicated in bone turnover and stimulates differentiation and proliferation of osteoclasts. IL-1 and TNF

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
25	The effect of body mass index on pain levels of patients with fibromyalgia	Torres-Gutiérrez, C. J. Merriweather, E. Rakel, B. Dailey, D. Muenters, L. A. Abdelhamid, R. Darghosian, L. Vance, C. Crofford, L. Sluka, K.	Fibromyalgia (FM) is a chronic pain syndrome characterized by widespread pain, depression, and mood and sleep disturbances. There is currently no consensus on the pathogenesis of FM. Overweight women are 60-70% more likely to develop fibromyalgia and experience more severe pain symptoms than normal women. Adipose cells release pro-inflammatory cytokines (IL-6, TNF-Œ±, Leptin) that are linked to pain pathogenesis. This study aims to determine the relationship between weight or body mass index (BMI) and pain in fibromyalgia. A sample of 93 women was recruited for participation in a set of tests which classify the pain felt by the participants. Pearson Product Moment Correlation (r) analyses were done between BMI and the pain levels. One-way analysis of variance (ANOVA) was used to determine group differences in pain measures. For a sub-analysis consisting of 27 patients, blood samples were acquired from the participants. The Peripheral Blood Mononuclear Cell layer (PBMC) was isolated. Monocyte phenotype was determined using fluorescence-activated cell sorting (FACS) and a Pearson Product Moment Correlation (r) analysis was done. We found that there is no relationship between BMI, Pain levels and Monocyte phenotype.	2016	FASEB Journal	#311	Exclusion reason: No leptin results; Michael Musker (2020-04-21 16:24:11)(Select): Exclude: This is only an abstract and does not contain Leptin results. You can access the abstract here: https://www.fasebj.org/doi/abs/10.1096/fasebj.30.1_supplement.1179.10;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
26	Serum ghrelin levels but not GH, IGF-1 and IGFBP-3 levels are altered in patients with fibromyalgia syndrome	Tander, Berna; Atmaca, Aysegul; Aliyazicioglu, Yuksel; Canturk, Ferhan	Introduction Both hypothalamo-pituitary-insulin-like growth factor-1 (IGF-1) axis and ghrelin levels may be altered in fibromyalgia syndrome (FMS) due to increased somatostatin tone. The aim of this study is to compare hypothalamo-pituitary-IGF-1 axis, ghrelin concentrations and their relations in premenopausal women with FMS and premenopausal healthy controls. Methods Seventy-five women (47 FMS and 28 healthy women) were enrolled in the study. Fasting plasma glucose, serum growth hormone (GH), insulin, C-peptide, IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3) and ghrelin levels were measured. Depressive symptoms were assessed using beck depression inventory. Pain intensity and sleep disturbance were recorded on a visual analog scale. The activity of daily living was assessed by fibromyalgia impact questionnaire. Results There were no significant differences in GH, IGF-1, IGFBP-3, glucose, insulin, and C-peptide levels between patients and controls ($p>0.05$), whereas ghrelin levels were significantly lower in patients than controls ($p<0.05$). Ghrelin levels were not correlated with GH, IGF-1, IGFBP-3, glucose, insulin,	2007	Joint Bone Spine	#14	Exclusion reason: No leptin results; Michael Musker (2020-04-22 16:41:25)(Select): Exclude: No leptin results provided, but it does provide results for the other items in the title. ghrelin, GH, IGF-1 and IGFBP-3 levels;

27	Neuropeptides in Hypothalamic Neuronal Disorders	Swaab, Dick F.	A few examples of hypothalamic, peptidergic disorders leading to clinical signs and symptoms are presented in this review. Increased activity of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) and decreased activity of the vasopressin neurons in the biological clock and of the thyroxine-releasing hormone (TRH) neurons in the PVN contribute to the signs and symptoms of depression. In men, the central nucleus of the bed nucleus of the stria terminalis (BSTc) is about twice as large and contains twice as many somatostatin neurons as in women. In transsexuals this sex difference is reversed, pointing to a role of this structure in gender. Luteinizing hormone-releasing hormone (LHRH) neurons are formed in the fetal olfactory placade and migrate along the terminal nerve fibers into the hypothalamus. In Kallmann's syndrome the migration process of the LHRH (gonadotropin-releasing hormone) neurons is aborted, which explains the joint occurrence of hypogonadotropic hypogonadism and anosmia in this syndrome. In postmenopausal women, the neurons of the infundibular nucleus hypertrophy and become hyperactive because of the disappearance of the estrogen feedback and contain hyperactive peptidergic neurons. Climacteric flushes may be caused by hyperactivity of the neurokinin-B or LHRH neurons in this nucleus.	2004	International Review of Cytology	#18	Exclusion reason: No results provided; Michael Musker (2020-04-22 16:39:02)(Select): Exclude: An explanatory document examining the role of leptin and relationship with the hypothalamus and other brain regions.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
28	Crosstalk between the immune, endocrine, and nervous systems in immunotoxicology	Manley, Kevin; Han, Weiguo; Zelin, Grant; Lawrence, David A.	The interconnected nature of the endocrine, immune and nervous systems has been well established. The central nervous system innervates the primary and secondary immune organs and the endocrine glands through direct axonal contact. Hormonal signals from the endocrine system influence both the nervous and immune systems with differential effects based on sex and environmental exposures. Immune cells can communicate by either cytokine or neurotransmitter release to signal the endocrine, gastrointestinal, and nervous systems. This review focuses on the effects of environmental toxicants on the inter-play between these systems with inclusion of effects from the microbiome and exposome. Endocrine disrupting chemicals and xenoestrogenic compounds affect the hypothalamic-pituitary-adrenal or gonadal axis, which can lead to hormonal alterations of communication between the nervous and immune systems. Immune responses within the nervous system and the delicate balance between appropriate response to infection/disease and detrimental effects through inappropriate response are discussed. Environmental chemicals, maternal immune	2018	Current Opinion in Toxicology	#340	Exclusion reason: No results provided; Michael Musker (2020-04-21 23:46:18)(Select): Exclude: This article provides a detailed description of the interplay of immune neuroendocrine network (INEN) but has no leptin results - 'Macrophages and mast cells in white adipose fat can enhance inflammation leading to release of the appetite-regulating hormone leptin that signals the hypothalamus; leptin also enhances some autoimmune diseases and promotes inflammation leading to cardiovascular diseases';

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
29	Fatigue in Other Medical Disorders	Majid, Hashir; Shabbir-Moosajee, Munira; Nadeem, Sarah	KEY POINTS Fatigue associated with medical disorders can have a significant impact on functional status, quality of life, and clinical outcomes. A variety of medical diseases can be associated with fatigue, including renal, hematological, and endocrine pathologies. The pathophysiology of fatigue and sleepiness in the setting of some common medical problems is discussed in this review. Treatment focuses on correction of the underlying medical disorder.	2013	Sleep Medicine Clinics	#348	Exclusion reason: Another Major Disease; Michael Musker (2020-04-22 21:49:36)(Select): Exclude: no leptin results included and this article focuses on major disease like kidney disease and cancer;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
30	Oxidative stress in exercise training: the involvement of inflammation and peripheral signals	Magherini, F.; Fiaschi, T.; Marzocchini, R.; Mannelli, M.; Gamberi, T.; Modesti, P. A.; Modesti, A.	The evidence about the health benefits of regular physical activity is well established. Exercise intensity is a significant variable and structured high-intensity interval training (HIIT) has been demonstrated to improve both whole-body and skeletal muscle metabolic health in different populations. Conversely, fatigue accumulation, if not resolved, leads to overwork, chronic fatigue syndrome (CFS), overtraining syndrome up to alterations of endocrine function, immune, systemic inflammation, and organic diseases with health threat. In response to temporary increases in stress during training, some athletes are unable to maintain sufficient caloric intake, thus suffering a negative energy balance that causes further stress. The regulation of the energy balance is controlled by the central nervous system through an elaborate interaction of the signalling that involves different tissues such as leptin, adiponectin and ghrelin whose provide important feedback to the hypothalamus to regulate the energy balance.	2019	Free Radic Res	#351	Exclusion reason: No leptin results; Michael Musker (2020-04-21 23:33:03)(Select): Exclude: This article discusses the role of leptin, adiponectin and ghrelin's role during ;

31	Associations between the gammainterferon gene (IFNG3), asthenia, and obesity	MacMurray, J.; Wu, S.; Muhleman, D.; Gade-Andavolu, R.; Blake, H.; Peters, W.; Johnson, J. P.; Saucier, G.; Comings, D. E.	Objectives: Asthenia, or heightened fatiguability, is typically characterized by a positive energy balance, often disposes to weight gain, and is comorbid with depression. Conversely, cachexia is characterized by increased energy expenditure despite falling caloric intake, resulting in loss of adipose tissue and muscle mass. The cytokine gammainterferon (IFNG) has been implicated both in chronic fatigue syndrome and cachexia, suggesting that it may exert modulatory influence over satiety and/or energy homeostatic mechanisms such as leptin or neuropeptide Y. In the present study, we sought to determine the possible role of the biallelic IFNG genetic polymorphism (IFNG3) in asthenia and obesity. Methods: We examined the IFNG3 marker in 163 non-Hispanic Caucasian subjects (college students) evaluated for asthenia, and an additional 62 middle-aged and older non-Hispanic Caucasian females recruited as part of an obesity study. Results: In the college student sample, the IFNG3 marker was found to be associated with asthenia (mean asthenia scores/sample: 11 = 55.6(34], 12 = 50.8(79], 22 = 48.0[50];P< .0025). In the obesity sample, a similar correspondence was found between IFNG3 and body-mass index (11 = 35.6[12], 12 = 30.0[29], 22 = 26.7[21]; P < .005).	1998	American Journal of Medical Genetics - Neuropsychiatric Genetics	#352	Exclusion reason: No leptin results; Michael Musker (2020-04-22 23:05:52)(Select): Exclude: This is an abstract that only reports on gammainterferon IFNG but makes references to leptin but provides no results on leptin in this study.;

32	Perceived fatigue and energy are independent unipolar states: Supporting evidence	Loy, Bryan D.; Cameron, Michelle H.; O'Connor, Patrick J.	Persistent fatigue is a common problem (,à¤20,Äì45% of U.S. population), with higher prevalence and severity in people with medical conditions such as cancer, depression, fibromyalgia, heart failure, sleep apnea and multiple sclerosis. There are few FDA-approved treatments for fatigue and great disagreement on how to measure fatigue, with over 250 instruments used in research. Many instruments define fatigue as ,Äúa lack of energy,Äù, thus viewing energy and fatigue states as opposites on a single bipolar continuum. In this paper, we hypothesize that energy and fatigue are distinct perceptual states, should be measured using separate unipolar scales, have different mechanisms, and deficits should be treated using tailored therapies. Energy and fatigue independence has been found in both exploratory and confirmatory factor analysis studies. Experiments in various fields, including behavioral pharmacology and exercise science, often find changes in energy and not fatigue, or vice versa. If the hypothesis that energy and fatigue are independent is correct, there are likely different mechanisms that drive energy and fatigue changes. Energy could be increased by elevated dopamine and norepinephrine transmission and binding. Fatigue could be increased by elevated brain serotonin and inflammatory cytokines and reduced histamine binding.	2018	Med Hypotheses	#360	Exclusion reason: No results provided; Michael Musker (2020-04-21 08:43:09)(Select): Exclude: this article discusses the use of energy based questions in fatigue questionnaires and only mentions leptin on page 7 referring to the Stringer 2013 study.;

33	Application of a multi-biomarker disease activity (vectra,Ñ¢ ta) score for assessing rheumatoid arthritis patients with low CRP or fibromyalgia	Lee, Y. C.; Haney, D.; Alexander, C.; Frits, M. L.; Iannaccone, C. K.; Shadick, N. A.; Segurado, O. G.; Weinblatt, M. E.; Sasso, E. H.	Background Clinical assessment of rheumatoid arthritis (RA) patients may be challenging when objective measures, such as C-reactive protein (CRP), do not show elevated disease activity or when patients have concomitant, non-inflammatory pain, as occurs with fibromyalgia (FM). A multi-biomarker disease activity (MBDA) blood test has been developed to assess RA disease activity with a score, ranging from 1-100, that is calculated using a validated algorithm with 12 serum protein biomarkers (VCAM-1, EGF, VEGF-A, IL-6, TNF-RI, YKL-40, MMP-1, MMP-3, leptin, resistin, SAA, CRP). Objectives To evaluate the role of the MBDA score in the assessment of RA disease activity in a cohort of established RA patients, including patients with low CRP levels and those with and without concomitant FM. Methods 208 RA patients from BRASS, a large prospective observational cohort, were randomly selected for a substudy of pain in RA. For the present cross-sectional study, DAS28-CRP components, the Widespread Pain Index (to diagnose FM by a modified version of the 2010 ACR Diagnostic Criteria for FM), and the MBDA blood test were obtained at the initial substudy visit. 198 of 208 patients had complete data and were included in this analysis.	2013	Annals of the Rheumatic Diseases	#370	Exclusion reason: No results provided; Michael Musker (2020-04-21 00:42:08)(Select): Exclude: This study focuses on rheumatoid arthritis patients. Its is only an abstract and provides no data on leptin.;

34	Application of a multi-biomarker disease activity (vectra¬Æ DA) score for assessing rheumatoid arthritis patients with fibromyalgia or low C-reactive protein	Lee, Y. C.; Hackett, J.; Alexander, C.; Frits, M. A.; Iannaccone, C. K.; Shadick, N. A.; Weinblatt, M. E.; Segurado, O.; Sasso, E. H.	Background/Purpose: Clinical assessment of rheumatoid arthritis (RA) may be challenging if patients have fibromyalgia (FM) or if C-reactive protein (CRP) is low (≤ 1 mg/dL). A multi-biomarker disease activity (MBDA) blood test has been developed to assess RA disease activity with a score (range: 1-100) that is calculated using a validated algorithm for 12 serum protein biomarkers (VCAM-1, EGF, VEGF-A, IL-6, TNF-RI, MMP-1, MMP-3, YKL-40, leptin, resistin, SAA, CRP). The present study evaluated the role of the MBDA score for assessing disease activity in a cohort of established RA patients, including patients with concomitant FM or low CRP. Methods: 208 RA patients from a prospective observational cohort were randomly selected for a substudy of pain in RA. For the present cross-sectional study, DAS28-CRP components, the Widespread Pain Index (to diagnose FM by a modified version of the 2010 ACR Diagnostic Criteria for FM), and the MBDA (Vectra‰ DA) score were evaluated for the initial substudy visit. 198 patients with non-missing baseline MBDA score and DAS28-CRP components were included. Measures of disease activity were compared between patients with RA+FM vs. RA without FM using: t-test or Wilcoxon rank sum tests; multivariate adjustment for age, sex, race, BMI,	2013	Arthritis and Rheumatism	#371	Exclusion reason: No results provided; Michael Musker (2020-04-21 00:33:16)(Select): Exclude: Hard to find in abstracts so need to use page reference 1145 to find. This study is only an abstract and are all patients suffering from rheumatoid arthritis. It mentions leptin is part of a 12 item test, but only provides results for crp. Background/Purpose: Clinical assessment of rheumatoid arthritis (RA) may be challenging if patients have fibromyalgia (FM) or if C-reactive protein (CRP) is low (1 mg/dL). A multi-biomarker disease activity (MBDA) blood test has been developed to assess RA disease activity with a score (range: 1,to 100) that is calculated using a validated algorithm for 12 serum protein biomarkers

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
35	Commensal flora and the regulation of inflammatory and autoimmune responses	Kranich, Jan; Maslowski, Kendle M.; Mackay, Charles R.	The gut microbiota has recently been recognized for its role in immune regulation, and changes in gut microbiota may be the basis for an increased incidence of autoimmune diseases and asthma in developed countries. Beneficial microbes produce factors that are distributed systemically, and therefore can influence peripheral inflammatory responses. Such symbiosis factors are important for the control and resolution of inflammation and autoimmune diseases. Here we discuss immune regulation by recently identified symbiosis factors and how certain environmental factors favor their production and influence the composition of the gut microflora.	2011	Seminars in Immunology	#388	Exclusion reason: No results provided; Michael Musker (2020-04-21 00:08:13)(Select): Exclude: only mentions leptin in relation to gut microbiota of OB/OB mice and provides info on the different microflora.;

36	Patients with Fibromyalgia and Chronic Fatigue Syndrome show increased hsCRP compared to healthy controls	Groven, Nina; Fors, Egil A.; Reitan, Solveig Klaebo; Ablin, Ataoglu Balbaloglu Carruthers Chalder Clauw Cleeland Fedewa Feinberg Fors Fukuda Giloteaux Groeger Groven Hornig Kent Klepstad et al.	Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM) are both chronic disorders that have a devastating effect on the lives of the affected patients and their families. Both conditions have overlapping clinical features that partly resemble those of inflammatory disorders. The etiology is still not understood, and it is suggested that the immune system might be a contributing factor. So far, the results are inconclusive. The purpose of this study was to compare the two conditions and investigate the level of the inflammatory marker high-sensitivity CRP (hsCRP) in CFS and FM patients compared to healthy controls. Female participants aged 18-60 years were enrolled in this study. The group consisted of 49 CFS patients, 57 FM patients, and 54 healthy controls. hsCRP levels were significantly higher for both the CFS and the FM groups compared to healthy controls when adjusting for age, smoking, and BMI ($p < .001$). There was no difference between the two patient groups. The level of hsCRP was affected by BMI but not by age and smoking. Patients with CFS and FM have higher concentrations of hsCRP compared to healthy controls. This remains significant even after adjusting for BMI. CFS and FM cannot be distinguished from each other on the basis of hsCRP in our study.	2019	Brain, Behavior, and Immunity	#510	Exclusion reason: Wrong outcomes; Michael Musker (2020-04-20 12:44:01)(Select): Exclude: only mentions leptin to refer to the atogalu study but no results on leptin in this article: 'The purpose of this study was to compare the two conditions and investigate the level of the inflammatory marker high-sensitivity CRP (hsCRP) in CFS and FM patients compared to healthy controls.';

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
37	Stimulation of interleukin-6 release by interleukin-1Œ≤ from isolated human adipocytes	Flower, Louise; Gray, Rosaire; Pinkney, Jonathan; Mohamed-Ali, Vidya	The secretion of interleukin-6 (IL-6) is modulated by immune, hormonal and metabolic stimuli in a cell-specific manner. We investigated the effect of cytokines, TNFŒ± and IL-1Œ≤, and insulin on IL-6 release from human adipocytes and peripheral blood cells (PBC). Adipocytes released IL-6 constitutively (after 5h: 5.64 [1.61,Äì15.30]pgml,àí1, after 10h: 15.95 [2.34,Äì45.59]pgml,àí1, p=0.007), while PBC secretion did not change significantly over this period. LPS stimulated IL-6 secretion in PBC after 5h but was without effect on adipocytes. TNFŒ± and insulin induced IL-6 production from PBC, but had no effect on adipocytes. IL-1Œ≤, however, induced a substantial increase in IL-6 release in adipocytes and PBC (all p<0.05). Adipose tissue production of IL-1Œ≤ was assessed in vivo by measuring arterio-venous differences across the subcutaneous abdominal adipose bed. Net release of IL-1Œ≤ was not observed, suggesting that under basal conditions there is no detectable release of this cytokine into the circulation from this depot. In conclusion (1) PBC demonstrate regulated IL-6 release, while the adipocyte release has a large constitutive component;	2003	Cytokine	#564	Exclusion reason: Wrong comparator; Michael Musker (2020-04-20 12:20:05)(Select): Exclude: this is an important article on IL1Beta and IL6 but only makes reference to Leptin, no results provided. Nor does it have any references to Chronic Fatigue or Fibromyalgia;

38	Plasma neuropeptide Y: A biomarker for symptom severity in chronic fatigue syndrome	Fletcher, M. A. Rosenthal, M. Antoni, M. Ironson, G. Zeng, X. R. Barnes, Z. Harvey, J. M. Hurwitz, B. Levis, S. Broderick, G. Klimas, N. G.	Background: Chronic fatigue syndrome (CFS) is a complex, multi-symptom illness with a multisystem pathogenesis involving alterations in the nervous, endocrine and immune systems. Abnormalities in stress responses have been identified as potential triggers or mediators of CFS symptoms. This study focused on the stress mediator neuropeptide Y (NPY). We hypothesized that NPY would be a useful biomarker for CFS. Methods: The CFS patients (n = 93) were from the Chronic Fatigue and Related Disorders Clinic at the University of Miami and met the 1994 case definition of Fukuda and colleagues. Healthy sedentary controls (n = 100)) were from NIH or VA funded studies. Another fatiguing, multi-symptom illness, Gulf War Illness (GWI), was also compared to CFS. We measured NPY in plasma using a radioimmunoassay (RIA). Psychometric measures, available for a subset of CFS patients included: Perceived Stress Scale, Profile of Mood States, ATQ Positive & Negative Self-Talk Scores, the COPE, the Beck Depression Inventory, Fatigue Symptom Inventory, Cognitive Capacity Screening Examination, Medical Outcomes Survey Short Form-36, and the Quality of Life Scale. Results: Plasma NPY was elevated in CFS subjects, compared to controls (p = .000) and to GWI cases (p = .000). Receiver operating characteristics (ROC) curve analyses indicated that the predictive ability of plasma	2010	Behavioral and Brain Functions	#566	Exclusion reason: No leptin in article; Michael Musker (2020-04-20 12:15:10)(Select): Exclude: there is no discussion of Leptin in this article.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
39	Counterbalance between leptin and cortisol may be associated with fibromyalgia [2]	Fietta, P.; Fietta, P.	We read with interest the article by Liao et al., suggesting that the counterbalance between leptin and cortisol may be associated with comorbid depression and anxiety. Leptin, the product of the obese gene mainly integrating metabolic, secreted by adipocytes, controls energy balance and exerts pleiotropic actions, immune, neuroendocrine and behavioral responses.2 Leptin may provide negative feedback inhibition to the hypothalamus–pituitary–adrenal (HPA) axis, which is crucial for adapting to chronic stress, and psychopathology would be the result if such a mechanism of counterbalance was impaired. Liao et al. proposed leptin as valid neuroendocrinologic marker for the hypervigilant state. Fibromyalgia (FM) is a common clinical condition defined as persistent, widespread musculoskeletal pain, in the presence of tender points at specific anatomical sites. Fibromyalgia is more than just a pain syndrome; it includes a protean series of disturbances, mainly involving autonomic, neuroendocrine and neuropsychic systems.	2006	Psychiatry Clin Neurosci	#571	Exclusion reason: No results provided; Michael Musker (2020-04-20 12:12:16)(Select): Exclude: This is a letter to the editor about another article by Liao et al but this letter contains no leptin results but does refer to a study on FM patients and leptin stating that leptin levels were higher in participants with Fibromyalgia - but the referencing is unclear possibly Fietta P. Focus on leptin, a pleiotropic hormone. Minerva Med. 2005; 96: 65,to75.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
40	Comment on "Diurnal excretion of urinary cortisol, cortisone, and cortisol metabolites in chronic fatigue syndrome."	Fenske, Martin; Bailey, Beisel Fenske Jerjes Klerman Kobberling Lewicka Linkowski Lloyd Purnell	Comments on an article by Walid K Jerjes et al (see record 2006-01484-009). The authors stated that the aim of their study was to obtain comprehensive information on basal hypothalamic-pituitary-adrenal (HPA) axis activity in chronic fatigue syndrome (CFS) patients. In my opinion, however, some shortcomings make the interpretation of their results difficult. Firstly, data on urinary free cortisol (UFC) excretion are at variance with early findings, which reported increased UFC excretion in the early morning and a low excretion rate of UFC at later daytime. Interpretation of urinary free cortison (UFCn) data is further complicated by the fact that UFC and UFCn excretion changes with daytime. Thus, it may be expected from the aforementioned findings that urinary free cortisone (UFCn) values in their study were quite variable and that they did not give information as to whether chronic fatigue syndrome may affect the renal metabolism of cortisone. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2006	Journal of Psychosomatic Research	#577	Exclusion reason: No leptin in article; Michael Musker (2020-04-20 12:01:39)(Select): Exclude: this article is only a comment letter to the editor and neither this article or the original article make reference to Leptin. It focuses only on urinary free cortisone UFC.;

41	Sleep disturbances of adult women suffering from fibromyalgia: A systematic review of observational studies	Diaz-Piedra, Carolina; Di Stasi, Leandro L.; Baldwin, Carol M.; Buela-Casal, Gualberto; Catena, Andres; Abad, Affleck Akdogan Akkaya Bagge Bennett Carpenter et al.	Although sleep complaints are often reported in patients with fibromyalgia syndrome (FMS), there is no conclusive evidence that these complaints represent symptomatic disorders of sleep physiology. Thus, the question of the role of sleep disturbances as an etiological or maintenance factor in FMS remains open. This study identifies the subjective and objective characteristics of sleep disturbances in adult women diagnosed with FMS. We carried out a systematic review of publications since 1990, the publication year of the American College of Rheumatology criteria of FMS. We selected empirical studies comparing sleep characteristics of adult women with FMS and healthy women or women with rheumatic diseases. We identified 42 articles. Patients with FMS were more likely to exhibit sleep complaints and also a less efficient, lighter and fragmented sleep. The evidence of a FMS signature on objective measures of sleep is inconsistent, however, as the majority of studies lacks statistical power. Current evidence cannot confirm the role played by sleep physiology in the pathogenesis or maintenance of FMS symptoms; nonetheless, it is clear that sleep disturbances are present in this syndrome. (PsycINFO Database Record (c) 2019 APA, all rights reserved)	2015	Sleep Medicine Reviews	#604	Exclusion reason: No Leptin in abstract; Michael Musker (2020-04-20 11:38:36)(Select): Exclude: a systematic review of sleep and fibromyalgia listing studies in relation to sleep ictal patterns. No mention of Leptin throughout article. Except for 1 Olama reference.;

42	A new model for chronic diseases	Diani, S. D. Sara	Chronic diseases are defined diseases whose symptoms last for at least six months and tend to worsen over time. In Europe, they cause at least 86% of deaths. In this speculative unifying model I set a new hypothesis for the etiology of the majority of chronic diseases. The main aim is to put order and observe our organism in a systemic way, connecting pathologies we now see as disconnected phenomena, with the conceptual frameworks of complex systems and network medicine. Chronic diseases could be caused by a first unsolved acute infection. In case the pathogen cannot be completely eliminated, it becomes a persistent infectious. After the acute episode, some mild symptoms will occur and probably disappear; the chronic disease will remain latent over time. It will manifest even after years or decades, in the presence of another acute infection, a particular stress, trauma, or another event. The presence of the persistent infectious elicits changes in the immune and systemic regulation, and these processes degenerate over time. They will assume their rules and patterns, being independent from the initial stimulus. The key to understand the dynamics and individuality of chronic diseases is the immune system and its networks. The immune mechanisms that can lead to the persistent response are mainly the switch from the Th1 to the Th2 immunity and the molecular mimicry.	2018	Med Hypotheses	#605	Exclusion reason: No results provided; Michael Musker (2020-04-20 11:33:36)(Select): Exclude: an explanatory document but no leptin results 'The leptin crosses the BBB and regulates appetite and energy balance. In addition, leptin produced by fat cells and lymphocytes fits into the Th1 pro-inflammatory network, and may contribute to inflammation of the CNS';

43	Leptin hormone and other biochemical influences on systemic inflammation	DeLany, Judith	Summary Over the past 30 years, a sharp rise in the prevalence of overweight and obesity has been noted in both children and adults. Health consequences include biomechanical, biochemical and psychosocial factors, with broad implications toward central adiposity and a number of conditions with which it relates. Substantial new information has surfaced within the last decade that alters previous concepts regarding the role of adipose tissue in health and in disease. This literature review explores the role that white adipose tissue (WAT) plays within a cascade of endocrine interfaces that have significant health consequences. WAT is now known to be an active participant in regulating physiological and pathological processes, including immunity and inflammation and to play a primary role in the development of a triad of hormonal imbalance (leptin resistance, adrenaline resistance, insulin resistance). Particular focus is placed on leptin hormone and its potential influences on inflammation and a host of other metabolic disturbances.	2008	Journal of Bodywork and Movement Therapies	#610	Exclusion reason: No results provided; Michael Musker (2020-04-19 18:54:29)(Select): Exclude: good graphics and explanation of the role of leptin: 'The many faces of leptin hormone First discovered in 1994, leptin hormone may verywell be the most important hormone studied todate. Though first thought to signal satiety (hungersatisfaction), peripheral actions of leptin are nowknown to interface in insulin biosynthesis and, withleptin receptors present on the pancreas, inpancreatic secretion (Fehmann et al., 1997). Inreturn, insulin stimulates leptin secretion fromadipose tissue (Havel, 2002; Trayhurn et al.,1999),

44	Influence of a periodized circuit training protocol on intermuscular adipose tissue of patients with knee osteoarthritis: protocol for a randomized controlled trial	de Almeida, A. C.; Pedroso, M. G.; Aily, J. B.; Goncalves, G. H.; Pastre, C. M.; Mattiello, S. M.	BACKGROUND: The objective of this study is to analyze the influence of a 14-week periodized circuit training protocol on patients with knee osteoarthritis (OA), in randomized intervention groups, on thigh intermuscular adipose tissue (interMAT), body composition, systemic inflammation, cartilage degradation, and its repercussion on pain, functional performance and quality of life. METHODS: This study presents a protocol for a randomized controlled trial. Sixty selected participants diagnosed with knee OA grades II and III, 40-65 years old and BMI < 30 kg/m(2,) will be randomly divided into three groups:periodized circuit training, strength training, and educational protocol. The circuit training and strength training protocols consist of 14-week training protocols conducted 3 times a week. The circuit training group will perform selected exercises previously stratified as light, moderate, and intense, arranged progressively in a circuit model, the strength group will perform regular strength exercises, and the educational protocol group will participate in a 14-week protocol with lectures twice a month about healthy lifestyles. Baseline and follow-up evaluations will be conducted for thigh interMAT (computed tomography), body composition (DXA), inflammation (IL-1beta, IL-6, IL-10, TNF-alpha, leptin, and adiponectin),	2018	BMC Musculoskelet Disord	#615	Exclusion reason: Another Major Disease; Michael Musker (2020-04-19 18:49:05)(Select): Exclude: no leptin results provided;

45	Neuroendocrine dysfunction		Notes that over the past decade, numerous studies have attempted to link chronic fatigue syndrome (CFS) or similar states to neuroendocrine dysfunction. This chapter provides an overview of the accumulated evidence. It includes not only studies looking directly at endocrine function, but also those using endocrine tests to assess other etiologic factors, such as monoamine dysfunction. (PsycINFO Database Record (c) 2019 APA, all rights reserved)	2003	Handbook of chronic fatigue syndrome.	#652	Exclusion reason: No Leptin in abstract; Michael Musker (2020-04-19 18:40:21)(Select): Exclude: unable to locate this article, but it likely again to refer back to 2001 study. It is a book chapter, but unable to locate book chapter even on google and all search platforms.;

46	Levels of DHEA and DHEAS and responses to CRH stimulation and hydrocortisone treatment in chronic fatigue syndrome	Cleare, A. J.; O'Keane, V.; Miell, J. P.; Arlt, Barrett-Connor Barrett-Connor Chalder Becker Demitrack Feldman Fukuda Gaab Goldberg Goodyer Hamilos Himmel Holsboer Hunt et al.	Investigated basal levels of dehydroepiandrostenedione (DHEA) and its sulphate (DHEA-S), the cortisol/DHEA molar ratio and the responsiveness of DHEA to stimulation by corticotrophin-releasing hormone (CRH). We also assessed these parameters prior to and following treatment with low dose oral hydrocortisone. Basal levels of serum DHEA, DHEAS and cortisol were measured in 16 patients with CFS without depression and in 16 controls matched for age, gender, weight, body mass index and menstrual history. Basal levels of DHEA were higher in the patient, compared to the control, group, while levels of DHEAS in patients were not different from controls. Higher DHEA levels were correlated with higher disability scores. Levels of DHEA and DHEAS were lower in patients following treatment with hydrocortisone. There was a rise in DHEA responsiveness to CRH in the patients after treatment but this did not attain significance. DHEA levels are raised in CFS and correlate with the degree of self-reported disability. Hydrocortisone therapy leads to a reduction in these levels towards normal, and an increased DHEA response to CRH, most marked in those who show a clinical response to this therapy. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2004	Psychoneuroendocrinology	#649	Exclusion reason: No results provided; Michael Musker (2020-04-19 18:31:00)(Select): Exclude: only refers to previous 2001 study: 'Thus, the larger increases in leptin after hydrocortisone in treatment responders, as seen in this previous study, may mirror the larger alterations in DHEA levels and responses to challenge seen in the present study.'

No.	Title	Authors	Abstract	Publi Year	Journal	Covide nce #	Reasons for exclusion following consensus
47	The HPA axis and the genesis of chronic fatigue syndrome	Cleare, A. J.	Many studies of patients with long-standing chronic fatigue syndrome (CFS) have found alterations to the hypothalamo-pituitary-adrenal (HPA) axis, including mild hypocortisolism, heightened negative feedback and blunted responses to challenge. However, recent prospective studies of high-risk cohorts suggest that there are no HPA axis changes present during the early stages of the genesis of fatiguing illnesses. Moreover, HPA axis changes can be reversed by modifying behavioural features of the illness, such as inactivity, deconditioning and sleep disturbance. Nevertheless, raising levels of cortisol pharmacologically can temporarily alleviate symptoms of fatigue. This article presents the case that there is no specific change to the HPA axis in CFS and that the observed changes are of multifactorial aetiology, with some factors occurring as a consequence of the illness. Nevertheless, the HPA axis might play a role in exacerbating or perpetuating symptoms late on in the course of the illness.	2004	Trends in Endocrinology and Metabolism	#651	Exclusion reason: No results provided; Michael Musker (2020-04-19 18:25:08)(Select): Exclude: only makes reference to their 2001 study but provides no new results 'Plasma leptin in chronic fatigue syndrome, and a placebo-controlled study of the effects of low-dose hydrocortisone on leptin secretion.';

48	Is leptin a marker for arthritis in hypothyroidism?	Ciurtin, C.; Dabu, M.; Dabu, B.; Stoica, V.	Background. Previous literature data have pointed out the potential role of leptin in the modulation of inflammatory processes associated with arthritis. It is considered that leptin is implicated in bone remodelling, has protective qualities in the complex pathogenesis of septic arthritis, could be considered as an additional marker of inflammation in rheumatoid arthritis and also an independent regulator of the TSH levels. Objectives. Our study was focused on the investigation of the leptin potential role in differentiating between the patterns of arthritis associated with hypothyroidism. Methods. We have investigated 19 patients with joint effusions and clinical signs of hypothyroidism in comparison with 21 patients with clinical hypothyroidism and absence of arthritic signs. All the patients were evaluated before the initiation of hormone replacement therapy. The two groups were matched for sex (11.1 % vs. 13.8% males), age (54.45¬±7.8 vs. 56,79¬±11.6 years old) and BMI (28.67¬± 2.45 vs. 29.7¬±3.52kg/m2). The leptin serum levels were quantified using a human leptin EIA kit. Results. The free T4 levels for the two groups were 0.25¬±0.18 vs. 0.29¬±O. 13 ng/dl and the TSH levels were 11.7¬±2.26vs. 12.9¬±3.75 mU/1,	2009	Clinical and Experimental Rheumatology	#654	Exclusion reason: Wrong patient population; Michael Musker (2020-04-19 18:14:06)(Select): Exclude: unable to locate article and on further consideration, this relates to hypothyroidism;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
49	Western Diet and the Immune System: An Inflammatory Connection	Christ, Anette; Lauterbach, Mario; Latz, Eicke	The consumption of Western-type calorically rich diets combined with chronic overnutrition and a sedentary lifestyle in Western societies evokes a state of chronic metabolic inflammation, termed metaflammation. Metaflammation contributes to the development of many prevalent non-communicable diseases (NCDs), and these lifestyle-associated pathologies represent a rising public health problem with global epidemic dimensions. A better understanding of how modern lifestyle and Western diet (WD) activate immune cells is essential for the development of efficient preventive and therapeutic strategies for common NCDs. Here, we review the current mechanistic understanding of how the Western lifestyle can induce metaflammation, and we discuss how this knowledge can be translated to protect the public from the health burden associated with their selected lifestyle.	2019	Immunity	#661	Exclusion reason: No results provided; Michael Musker (2020-04-19 17:58:10)(Select): Exclude: this is only a discussion on inflammatory pathways in chronic metabolic conditions. No mention of CFS and only mentions Leptin sensitivity.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covide nce #	Reasons for exclusion following consensus
50	Fatigue in neurologica l disorders	Chaudhuri, Abhijit; Behan, Peter O.	Summary Chronic fatigue is a typical symptom of neurological diseases, and is most disabling in multiple sclerosis, postpoliomyelitis, poststroke, and in chronic fatigue syndrome. Disorders of neuromuscular junction transmission and metabolic diseases cause muscle fatigability, which is characterised by failure to sustain the force of muscle contraction (peripheral fatigue). Fatigue is also seen in diseases that affect the central, peripheral, and autonomic nervous systems (central fatigue). Enhanced perception of effort and limited endurance of sustained physical and mental activities are the main characteristics of central fatigue. Metabolic and structural lesions that disrupt the usual process of activation in pathways interconnecting the basal ganglia, thalamus, limbic system, and higher cortical centre are implicated in the pathophysiological process of central fatigue. A state of pre-existing relative hypocortisolaemia might sensitise the hypothalamic-pituitary-adrenal axis to development of persistent central fatigue after stress. The contributions of physiological, cognitive, and affective changes underlying fatigue are variable, and treatment is largely symptomatic and rehabilitative.	2004	The Lancet	#671	Exclusion reason: No leptin in article; Michael Musker (2020-04-19 17:41:22)(Select): Exclude: only an explanatory document no leptin results provided;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
51	Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition	Calder, Philip C.; Bosco, Nabil; Bourdet-Sicard, RaphaV´lle; Capuron, Lucile; Delzenne, Nathalie; DorV©, Joel; Franceschi, Claudio; Lehtinen, Markus J.; Recker, Tobias; Salvioli, Stefano; Visioli, Francesco	Ageing of the global population has become a public health concern with an important socio-economic dimension. Ageing is characterized by an increase in the concentration of inflammatory markers in the bloodstream, a phenomenon that has been termed ,inflammageing, The inflammatory response is beneficial as an acute, transient reaction to harmful conditions, facilitating the defense, repair, turnover and adaptation of many tissues. However, chronic and low grade inflammation is likely to be detrimental for many tissues and for normal functions. We provide an overview of low grade inflammation (LGI) and determine the potential drivers and the effects of the ,'inflamed,' phenotype observed in the elderly. We discuss the role of gut microbiota and immune system crosstalk and the gut-brain axis. Then, we focus on major health complications associated with LGI in the elderly, including mental health and wellbeing, metabolic abnormalities and infections. Finally, we discuss the possibility of manipulating LGI in the elderly by nutritional interventions. We provide an overview of the evidence that exists in the elderly for omega-3 fatty acid, probiotic, prebiotic, antioxidant and polyphenol interventions as a means to influence LGI.	2017	Ageing Research Reviews	#691	Exclusion reason: No Leptin in abstract; Michael Musker (2020-04-19 17:10:41)(Select): Exclude: It discusses the roles of other cytokines but no mention of leptin throughout article.;

52	Leptin and psychiatric disorders	Balci Sengul, Ceyhan; Sengul, Cem; Okay, Tuncer; Dilbaz, Nesrin; Allison, Antonijevic Asakawa At maca Atmaca Baptista Baratta Bromel Caro Cleare De Vos Deuschle Eder Friedman Fulton ying et al.	For a long time biologic markers have been searched and alterations of these markers with treatment were studied. Zang discovered Leptin in 1994, since then many studies were performed in general medicine and psychiatry. Leptin draw attention because of its central and peripheric effects. Leptin is synthesized in adipose tissue as a 167-aminoacide polypeptide and then transform in to a 146-aminoacide polypeptide in circulatory system It is also found at hypothalamus and amygdala in brain. In recent researches, some evidences were determined that leptin is not only involved in food intake and energy consumption but also involved in sleep regulation sexual behavior, and impulsivity. These evidences gave rise to a lot of studies, which had been performed to designate the role of leptin in psychiatric disorders and conditions like depression, bipolar disorder, suicidality and eating disorder. In this article researches of leptin in psychiatry literature were reviewed.	2004	Leptin ve psikiyatrik bozuklukl ar.	#755	Exclusion reason: Foreign Language; Martin Lewis (2020-04-29 10:58:04)(Select): English version not good. Seems off topic; Michael Musker (2020-04-19 16:47:02)(Select): Exclude as this is in turkish and going from the English abstract this is about psychiatric disorders.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
53	Key developments in endocrinology	Rogers, D.; Vanderpump, M.	Endocrine problems are common and varied, and often require multidisciplinary management. In our unit, for example, we have a specialist clinic for patients with thyroid and parathyroid disorders, and patients are managed jointly by an endocrinologist and otolaryngologist. This combination of specialists means that management decisions are based on consensus opinions covering all aspects of the endocrine disease. We have therefore written the following article covering new developments in both the medical and surgical aspects of endocrine disease. * Primary hyperparathyroidism: when to operate? An increased awareness of hypercalcaemia has developed since the introduction of the serum autoanalyser in the 1970s. This, along with the wide availability of parathyroid hormone (PTH) assays, has led to more frequent and earlier diagnosis of primary hyperparathyroidism (PHPT). Also, while in the past most patients diagnosed with PHPT were symptomatic, nowadays most are asymptomatic. Symptomatic patients may have renal stones, bone pain, pathologic fractures, proximal muscle weakness, depression, lethargy and vague aches and pains. In patients with `asymptomatic' PHPT, the future risk of	2000	Practitioner	#115	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 15:31:47)(Select): Exclude: no mention of leptin, CFS or fibromyalgia in this article;

No.	Title	Authors	Abstract	Publi Year	Journal	Covide nce #	Reasons for exclusion following consensus
			developing complications of PHPT ranges from 23 to 62 per cent at ten years.1 The most likely risks are progressive, silent bone loss and nephrolithiasis or nephrocalcinosis with renal dysfunction.				

54	Relationship between fibromyalgia and obesity in pain, function, mood, and sleep	Okifuji, Akiko; Donaldson, Gary W.; Barck, Lynn; Fine, Perry G.; Arendt-Nielsen, Baumstark Bennett Bernstein Bigal Bjorntorp Bjorvatn Bluher Branco et al.	Fibromyalgia syndrome (FMS) is a prevalent and disabling chronic pain disorder. Past research suggests that obesity is a common comorbidity and may be related to the severity of FMS. The main objective of the present study was to evaluate the relationships between FMS and obesity in the multiple FMS-related domains: hyperalgesia, symptoms, physical abilities, and sleep. A total of 215 FMS patients completed a set of self-report inventories to assess FMS-related symptoms and underwent the tender point (TP) examination, physical performance testing, and 7-day home sleep assessment. Forty-seven percent of our sample was obese and an additional 30% was overweight. Obesity was related significantly to greater pain sensitivity to TP palpation particularly in the lower body areas, reduced physical strength and lower-body flexibility, shorter sleep duration, and greater restlessness during sleep. The results confirmed that obesity is a prevalent comorbidity of FMS that may contribute to the severity of the problem. Potential mechanisms underlying the relationship are discussed. Perspective: This report presents how obesity may be interrelated to fibromyalgia pain, disability, and sleep. We found that obesity is common in FMS.	2010	The Journal of Pain	#198	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 10:51:48)(Select): Exclude: leptin is not discussed in article nor are any biological results. Focuses on symptom questionnaires of pain, mood and sleep in obese and overweight people with fibromyalgia.;

55	Chronic fatigue syndrome	Nater, Urs M.; Heim, Christine M.; Raison, Charles; Aaron, Abbot Afari Altemus Amold Antoni Appel Ax Barsky Bates Bierl Blakely Borish Bou-Holaigah Bounous Briggs Buchwald et al.	Chronic fatigue syndrome (CFS) is a complex illness defined by unexplained disabling fatigue as its core feature and a combination of other accompanying symptoms, such as diffuse pain, subjective cognitive impairment, and sleep problems. Medical conditions that may explain the prolonged fatigue as well as a number of psychiatric diagnoses exclude a patient from the diagnosis of CFS (Reeves et al., 2003). Recently, efforts have been made to assess case-defining symptoms of CFS objectively. Persons are classified as having CFS if they meet the following three empirically derived criteria as assessed by psychometrically evaluated questionnaires (Reeves et al., 2005): (1) severe fatigue; (2) substantial functional impairment; and (3) presence of substantial accompanying symptoms. Because CFS is a diagnosis of exclusion, a thorough medical history and assessment are required before the diagnosis can be formally established. This chapter discusses the prevalence, pathophysiology, risk factors, comorbidity, and treatment of CFS. (PsycINFO Database Record (c) 2019 APA, all rights reserved)	2012	Neurobiology of psychiatric disorders.	#211	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 10:42:47)(Select): Exclude: An excellent summary and list of references of what CFS is. No leptin discussed in chapter. No results.;

56	Leveraging Prior Knowledge of Endocrine Immune Regulation in the Therapeutically Relevant Phenotyping of Women With Chronic Fatigue Syndrome	Morris, Matthew C.; Cooney, Katherine E.; Sedghamiz, Hooman; Abreu, Maria; Collado, Fanny; Balbin, Elizabeth G.; Craddock, Travis J. A.; Klimas, Nancy G.; Broderick, Gordon; Fletcher, Mary Ann	Purpose The complex and varied presentation of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) has made it difficult to diagnose, study, and treat. Its symptoms and likely etiology involve multiple components of endocrine and immune regulation, including the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-gonadal axis, and their interactive oversight of immune function. We propose that the persistence of ME/CFS may involve changes in the regulatory interactions across these physiological axes. We also propose that the robustness of this new pathogenic equilibrium may at least in part explain the limited success of conventional single-target therapies. Methods A comprehensive model was constructed of female endocrine,Äìimmune signaling consisting of 28 markers linked by 214 documented regulatory interactions. This detailed model was then constrained to adhere to experimental measurements in a subset of 17 candidate immune markers measured in peripheral blood of patients with ME/CFS and healthy control subjects before, during, and after a maximal exercise challenge. A set of 26 competing numerical models satisfied these data to within 5% error.	2019	Clinical Therapeutics	#536	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 00:51:18)(Select): Exclude: No leptin in article but useful for other cytokine comparison Plasma concentrations of interferon-g (IFN-g), IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-10, IL-13, IL-15, IL-17, IL-23, and TNF-a were measured by using a Q-Plex multiplex ELISA (Quansys Biosciences, Logan, Utah). Although any raw results are not provided and results are presented graphically and difficult to interpret.;

57	The deleterious effects of oxidative and nitrosative stress on palmitoylation, membrane lipid rafts and lipid-based cellular signalling: New drug targets in neuroimmune disorders	Morris, Gerwyn; Walder, Ken; Puri, Basant K.; Berk, Michael; Maes, Michael; Aggarwal, Aicart-Ramos Ajami Akhtar Alfano Allen Allen Allende Amminger Anderson Axelsen Babina Baker Balagopalan Ballek Barberger-Gateau et al.	Oxidative and nitrosative stress (O&NS) is causatively implicated in the pathogenesis of Alzheimer's and Parkinson's disease, multiple sclerosis, chronic fatigue syndrome, schizophrenia and depression. Many of the consequences stemming from O&NS, including damage to proteins, lipids and DNA, are well known, whereas the effects of O&NS on lipoprotein-based cellular signalling involving palmitoylation and plasma membrane lipid rafts are less well documented. The aim of this narrative review is to discuss the mechanisms involved in lipid-based signalling, including palmitoylation, membrane/lipid raft (MLR) and n-3 polyunsaturated fatty acid (PUFA) functions, the effects of O&NS processes on these processes and their role in the abovementioned diseases. S-palmitoylation is a post-translational modification, which regulates protein trafficking and association with the plasma membrane, protein subcellular location and functions. Palmitoylation and MRLs play a key role in neuronal functions, including glutamatergic neurotransmission, and immune-inflammatory responses. Palmitoylation, MLRs and n-3 PUFAs are vulnerable to the corruptive effects of O&NS.	2016	Molecular Neurobiology	#537	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 00:37:40)(Select): Exclude: no mention of leptin in this article and no results of any kind.;

58	Impact of sleep debt on physiological rhythms	Spiegel, K.; Leproult, R.; van Cauter, E.; Achermann Ahima Allan Bliwise Bonnet Borbely Brabant Broman Buysse Chin-Chance Czeisler Dallman Desir Dinges Franceschini Friedman et al.	We measured sleep and 24-hour hormonal profiles in 11 healthy young males after 6 days of sleep restriction and after 6 days of sleep recovery. At the end of sleep restriction, we observed reduced amounts of slow wave sleep (SWS) and rapid eye movement (REM) sleep and an alteration in the temporal distribution of these sleep stages, i.e. an increased pressure for REM sleep at the beginning of the sleep period and a decrease in the amount of slow wave activity (SWA) during the first sleep cycle. These later abnormalities are usually observed in depression. In addition, numerous alterations in the 24-hour hormonal profiles were observed in the state of sleep debt. The amount of melatonin secreted was reduced because of a delay in the onset of the nocturnal secretion and a reduction in the value of the acrophase. The 24-hour mean TSH levels were reduced and the nocturnal TSH elevation was markedly dampened, most likely as a result of elevated levels of thyroid hormones. Since these alterations are qualitatively and quantitatively similar to those observed during aging and sometimes during depression, a state of sleep debt is likely to increase the severity of depression and widespread age-related chronic conditions such as obesity, diabetes and hypertension. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2003	Impact d'une dette de sommeil sur les rythmes physiologiques.	#40	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 16:15:03)(Select): Exclude: On closer inspection of the abstract this article focuses healthy young males and different sleep patterns.;

59	Structural brain abnormalities in patients with inflammatory illness acquired following exposure to water-damaged buildings: A volumetric MRI study using NeuroQuant¬Æ	Shoemaker, Ritchie C.; House, Dennis; Ryan, James C.	Executive cognitive and neurologic abnormalities are commonly seen in patients with a chronic inflammatory response syndrome (CIRS) acquired following exposure to the interior environment of water-damaged buildings (WDB), but a clear delineation of the physiologic or structural basis for these abnormalities has not been defined. Symptoms of affected patients routinely include headache, difficulty with recent memory, concentration, word finding, numbness, tingling, metallic taste and vertigo. Additionally, persistent proteomic abnormalities in inflammatory parameters that can alter permeability of the blood,Äìbrain barrier, such as C4a, TGFB1, MMP9 and VEGF, are notably present in cases of CIRS-WDB compared to controls, suggesting a consequent inflammatory injury to the central nervous system. Findings of gliotic areas in MRI scans in over 45% of CIRS-WDB cases compared to 5% of controls, as well as elevated lactate and depressed ratios of glutamate to glutamine, are regularly seen in MR spectroscopy of cases. This study used the volumetric software program NeuroQuant¬Æ (NQ) to determine specific brain structure volumes in consecutive patients (N=17) seen in a medical clinic specializing in inflammatory illness. Each of these patients presented for evaluation of an illness thought to be associated with exposure to WDB, and received an MRI that was evaluated by NQ.	2014	Neurotoxicol Teratol	#58	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 16:09:41)(Select): Exclude: Again focuses on patients exposed to water-damaged buildings and in fact distinguishes this group who may be wrongly diagnosed with fibromyalgia.;

60	A time-series study of sick building syndrome: chronic, biotoxin-associated illness from exposure to water-damaged buildings	Shoemaker, R. C.; House, D. E.	The human health risk for chronic illnesses involving multiple body systems following inhalation exposure to the indoor environments of water-damaged buildings (WDBs) has remained poorly characterized and the subject of intense controversy. The current study assessed the hypothesis that exposure to the indoor environments of WDBs with visible microbial colonization was associated with illness. The study used a cross-sectional design with assessments at five time points, and the interventions of cholestyramine (CSM) therapy, exposure avoidance following therapy, and reexposure to the buildings after illness resolution. The methodological approach included oral administration of questionnaires, medical examinations, laboratory analyses, pulmonary function testing, and measurements of visual function. Of the 21 study volunteers, 19 completed assessment at each of the five time points. Data at Time Point 1 indicated multiple symptoms involving at least four organ systems in all study participants, a restrictive respiratory condition in four participants, and abnormally low visual contrast sensitivity (VCS) in 18 participants. Serum leptin levels were abnormally high and alpha melanocyte stimulating hormone (MSH) levels were abnormally low.	2005	Neurotoxicol Teratol	#57	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 16:07:17)(Select): Exclude: This article has no clear cohort and it is difficult to attribute to a group of fibromyalgia patients.;

61	Sick building syndrome (SBS) and exposure to water-damaged buildings: time series study, clinical trial and mechanisms	Shoemaker, R. C.; House, D. E.	Occupants of water-damaged buildings (WDBs) with evidence of microbial amplification often describe a syndrome involving multiple organ systems, commonly referred to as "sick building syndrome" (SBS), following chronic exposure to the indoor air. Studies have demonstrated that the indoor air of WDBs often contains a complex mixture of fungi, mycotoxins, bacteria, endotoxins, antigens, lipopolysaccharides, and biologically produced volatile compounds. A case-series study with medical assessments at five time points was conducted to characterize the syndrome after a double-blinded, placebo-controlled clinical trial conducted among a group of study participants investigated the efficacy of cholestyramine (CSM) therapy. The general hypothesis of the time series study was that chronic exposure to the indoor air of WDBs is associated with SBS. Consecutive clinical patients were screened for diagnosis of SBS using criteria of exposure potential, symptoms involving at least five organ systems, and the absence of confounding factors. Twenty-eight cases signed voluntary consent forms for participation in the time-series study and provided samples of microbial contaminants from water-damaged areas in the buildings they occupied. Twenty-six participants with a group-mean duration of illness of 11 months	2006	Neurotoxicol Teratol	#56	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 16:52:10)(Select): Exclude: provide leptin results across 4 timepoints but the cohort relates back to a mixture of participants that all have Sick building syndrome.;

62	The increase of alpha-melanocyte-stimulating hormone in the plasma of chronic fatigue syndrome patients	Shishioh-Ikejima, Nobue; Ogawa, Tokiko; Yamaguti, Kouzi; Watanabe, Yasuyoshi; Kuratsune, Hirohiko; Kiyama, Hiroshi; Airaghi, Catania Cleare Donahoo Erlwein Fletcher Fukuda Hoggard Jason et al.	Background: Despite extensive research, no reliable biological marker for chronic fatigue syndrome (CFS) has yet been identified. However, hyperactivation of melanotrophs in the pituitary gland and increased levels of plasma alpha-melanocyte-stimulating hormone (alpha-MSH) have recently been detected in an animal model of chronic stress. Because CFS is considered to be caused partly by chronic stress events, increased alpha-MSH plasma levels may also occur in CFS patients. We therefore examined alpha-MSH levels in CFS patients. Methods: Fifty-five CFS patients, who were previously diagnosed within 10 years of with the disease, were enrolled in this study. Thirty healthy volunteers were studied as controls. Fasting bloods samples were collected in the morning and evaluated for their plasma levels of alpha-MSH, adrenocorticotropic hormone (ACTH), serum cortisol and dehydroepiandrosterone sulfate (DHEA-S). Mean levels of alpha-MSH were compared between the CFS and control groups using Welch's t test. Results: The mean plasma alpha-MSH concentration in the CFS group (17.9 +/- 1.0 pg/mL) was significantly higher than that in healthy controls (14.5 +/- 1.0 pg/mL, p = 0.02). However, there was a wide range of values in the CFS group. The factors correlated with the plasma alpha-	2010	BMC Neurology	#60	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 15:58:46)(Select): Exclude: no mention of leptin in article. Focuses only on alpha-melanocyte-stimulating hormone results and DHEA;

63	Daily fluctuations of progesterone and testosterone are associated with fibromyalgia pain severity	Schertzinger, Meredith; Wesson-Sides, Kate; Parkitny, Luke; Younger, Jarred; Akkus, Albert Aloisi Bartley Bartley Bramwell Cairns Coronel Coulombe de Kruijf de Nicola Deng et al.	The purpose of this longitudinal blood sampling study was to examine relationships between sex hormones and fibromyalgia pain. Eight women meeting case definition criteria for fibromyalgia provided venous blood samples and reported their fibromyalgia pain severity over 25 consecutive days. All women exhibited normal menstrual cycles and were not taking oral contraceptives. Cortisol, and the sex hormones estradiol, progesterone, and testosterone, were assayed from serum. A linear mixed model was used to determine if fluctuations of sex hormones were associated with changes in pain severity. In the entire sample, day to day changes in progesterone (P = .002) as well as testosterone (P = .015) were significantly and inversely correlated with pain severity. There was no relationship between estradiol and pain (P = .551) or cortisol and pain (P = .633). These results suggest that progesterone and testosterone play a protective role in fibromyalgia pain severity. Sex and other hormones may serve to increase as well as decrease fibromyalgia pain severity. Perspective: Sex hormones fluctuate normally in women with fibromyalgia, but may still contribute to pain severity. (PsycINFO Database Record (c) 2018 APA, all rights reserved)	2018	The Journal of Pain	#85	Exclusion reason: No leptin in article; Michael Musker (2020-04-22 15:49:07)(Select): Exclude: Leptin not mentioned in article. Links Progesterone and Testosterone to pain outcomes.;

64	Improved fibrinolytic activity during exercise may be an effect of the adipocyte-derived hormones leptin and adiponectin	Eriksson, M.; Johnson, O.; Boman, K.; Hallmans, G.; Hellsten, G.; Nilsson, T. K.; Soderberg, S.	INTRODUCTION: Physical activity is associated with improved fibrinolytic activity and reduced risk for cardiovascular disease. High levels of leptin and low levels of adiponectin, both adipocyte-derived hormones, or adipokines, are related to dysfibrinolysis and risk for cardiovascular disease. In this study, we explored if improved fibrinolytic activity during exercise could be linked to changes in leptin and adiponectin levels. MATERIALS AND METHODS: Twenty healthy men (mean age 36 years) participated in a 14-day long skiing expedition in the Swedish mountains. They were randomly assigned to either a 40% or a 30% fat-based diet. Anthropometry, lipids, fibrinolytic activity (PAI-1 activity, tPA activity and mass) and adipokines (leptin and adiponectin) were measured before, during and six weeks after the expedition. RESULTS: PAI-1 activity and circulating levels of leptin decreased whereas levels of adiponectin increased during exercise. The fall in PAI-1 activity showed a strong linear association with changes in leptin and adiponectin levels ($p = 0.001$ and $p < 0.001$, respectively). Changes in leptin and adiponectin levels were independent of decreasing waist circumference. However, the association between anthropometric measures and adipokines changed considerably during the expedition.	2008	Thromb Res	#585	Exclusion reason: Wrong patient population; Michael Musker (2020-04-20 11:44:40)(Select): Exclude: this focuses fibrinolytic activity which is about blood clotting time and extreme exercise. Leptin results but not relevant cohort.;

65	Differences in circulating concentrations of total, free and bound leptin relate to gender and body composition in adult humans	McConway, M. G.; Johnson, D.; Kelly, A.; Griffin, D.; Smith, J.; Wallace, A. M.	We describe a radioimmunoassay (RIA) for total leptin and a gel filtration procedure for the separation of free and bound leptin in human serum. The RIA, based on a locally prepared antibody, has a minimum detection limit of 0.9 ng/mL, a working range (CV < 10%) of 2.5-50 ng/mL, inter-assay precision of 10.2, 7.2 and 8.9%CV at 7.9, 15.4 and 30.0 ng/mL, respectively, 94% recovery of exogenous leptin (range 81.1-120.6%), exhibited parallelism and demonstrated no significant cross-reactivity or interferences. A difference plot of results from this method and those from a commercially available kit (Linco Research) demonstrated satisfactory agreement up to concentrations of 50 ng/mL total leptin, with no significant bias. A gender-dependent correlation was obtained between body mass index (BMI) and total leptin (r = 0.91, P <0.001, n = 75 for men; r = 0.79, P < 0.001, n = 72 for women), with women having higher leptin concentrations than men for any given BMI. Gel filtration studies (inter-assay precision: 4.7%CV, n=18) demonstrated that a variable fraction (between 10% and 40%) of total leptin in serum was bound with high affinity (K(eq)= 1.0-1.45 <x> 109 L/mol) to a non-albumin, non-lipid macromolecule. Binding affinities were found to be similar irrespective of gender or fat mass.	2000	Annals of Clinical Biochemistry	#319	Exclusion reason: Wrong patient population; Michael Musker (2020-04-22 00:11:33)(Select): Exclude: Excellent reference of leptin levels in men and women but is not related to MECFS or fibromyalgia in any way;

66	Biomarkers for chronic fatigue	Klimas, Nancy G.; Broderick, Gordon; Fletcher, Mary Ann; Al-shair, Albright Allin Baraniuk Basu Ben-Zvi Boncler Bou-Holaigah Bower Brenu Broderick Byrnes Carruthers Crouse et al.	Fatigue that persists for 6 months or more is termed chronic fatigue. Chronic fatigue (CF) in combination with a minimum of 4 of 8 symptoms and the absence of diseases that could explain these symptoms, constitute the case definition for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Inflammation, immune system activation, autonomic dysfunction, impaired functioning in the hypothalamic-pituitary-adrenal axis, and neuroendocrine dysregulation have all been suggested as root causes of fatigue. The identification of objective markers consistently associated with CFS/ME is an important goal in relation to diagnosis and treatment, as the current case definitions are based entirely on physical signs and symptoms. This review is focused on the recent literature related to biomarkers for fatigue associated with CFS/ME and, for comparison, those associated with other diseases. These markers are distributed across several of the body's core regulatory systems. A complex construct of symptoms emerges from alterations and/or dysfunctions in the nervous, endocrine and immune systems. We propose that new insight will depend on our ability to develop and deploy an integrative profiling of CFS/ME pathogenesis at the molecular level. Until such a molecular signature is obtained efforts to develop effective treatments will continue to be severely limited.	2012	Brain, Behavior, and Immunity	#395	Exclusion reason: No leptin in article; Michael Musker (2020-04-21 00:01:21)(Select): Exclude: no mention of leptin in article, only a reference to an article 'Behavioral recovery from acute hypoxia is reliant on leptin' in references;

67	Cytokines and pathological sleep	Kapsimalis, Fotis; Basta, Maria; Varouchakis, George; Gourgoulianis, Konstantinos; Vgontzas, Alexandros; Kryger, Meir; Alberti, Andreozzi Anisman Arter Bauer Benca Bixler Blalock Brower Chen et al.	Cytokines are proteins produced by leukocytes and other cells that function as intercellular mediators acting on several target tissues, resulting in multiple biologic actions. Over the last decade, medical research has explored the interaction between cytokines and sleep disorders. The aim of this review is to illustrate recent advances in knowledge about the relationship between cytokines and disorders of excessive sleepiness. Cytokines may have an important role in mediating excessive daytime sleepiness in sleep loss or insomnia. Alterations of the immune system have also been associated with narcolepsy. The relationship between cytokines and hormonal regulatory mechanisms may explain symptoms like fatigue and sleepiness in chronic inflammatory diseases. Cytokines may play an important role in the pathogenesis of obstructive sleep apnea and cardiovascular consequences of this condition. New biologic treatments targeting cytokines have been investigated in conditions characterized by sleep disturbance.	2008	Sleep Medicine	#420	Exclusion reason: No results provided; Michael Musker (2020-04-20 23:52:23)(Select): Exclude: This article makes numerous references to leptin and sleep but no results are provided. Provides lots of references on leptin and sleep, interesting links to IL1beta, IL6 and Leptin. Possible links then with MECFS - sleep and adipokines.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covide nce #	Reasons for exclusion following consensus
68	Allostatic load biomarkers of chronic stress and impact on health and cognition	Juster, Robert-Paul; McEwen, Bruce S.; Lupien, Sonia J.	The allostatic load model expands the stress-disease literature by proposing a temporal cascade of multi-systemic physiological dysregulations that contribute to disease trajectories. By incorporating an allostatic load index representing neuroendocrine, immune, metabolic, and cardiovascular system functioning, numerous studies have demonstrated greater prediction of morbidity and mortality over and beyond traditional detection methods employed in biomedical practice. This article reviews theoretical and empirical work using the allostatic load model vis-√†-vis the effects of chronic stress on physical and mental health. Specific risk and protective factors associated with increased allostatic load are elucidated and policies for promoting successful aging are proposed.	2010	Neuroscie nce & Biobehavi oral Reviews	#428	Exclusion reason: No results provided; Michael Musker (2020-04-20 23:47:01)(Select): Exclude: this article is about Allostatic load(AL) represents the ‚Äòwear and tear‚Äô the bodyexperiences when repeated allostatic responses are activatedduring stressful situations - only one reference to leptin suggesting that lipids should be included in future analyses.;

69	Chronic fatigue: An evolutionary concept analysis	Jorgensen, Roberta; Aaronson, Asbring Barnett Bianchi Cella Chwastiak Clarke Evans Evans Falk Flechtner Glacken Hagerty Hart Hart Hewlett Hilsabeck Hjermstad Johnson Jones Jorgensen Kato Kim Kralik Lee et al.	Aim: This paper is a report of a concept analysis of chronic fatigue. Background: Fatigue is a prevalent symptom encompassing both acute and chronic manifestations. It is chronic fatigue that is most problematic because of its duration and impact on life quality. The rise in prevalence of chronic conditions will result in a need to address coexistent symptoms, clarification of which is needed. Chronic fatigue is one of the most common symptoms in chronic illness. Clarification of the concept and an understanding of its use by discipline are needed. Data sources: The evolutionary method of concept analysis was used to ascertain the attributes, antecedents, consequences and surrogate terms for chronic fatigue. A review of the literature published between 1966 and 2007 was carried out to determine the contextual use of the concept of chronic fatigue among disciplines. Sources used for this analysis included CINAHL, Medline, PsychINFO and Social Work Abstracts and the search yielded 66 papers. Results: The chronic fatigue experience is associated with a multitude of physical, psychological and social factors. The defining attributes of chronic fatigue are constancy, abnormality, whole-body experience, inexplicability and disabling. The antecedents of chronic fatigue are physical disease, psychopathology, female gender and a history of abuse.	2008	Journal of Advanced Nursing	#432	Exclusion reason: No leptin in article; Michael Musker (2020-04-20 23:42:24)(Select): Exclude: leptin is not mentioned in the article only in a reference, which refers to 'Fatigue in irritable bowel syndrome: characterization and putative role of leptin. ';

70	Enhanced feedback sensitivity to prednisolone in chronic fatigue syndrome	Jerjes, Walid K.; Taylor, Norman F.; Wood, Peter J.; Cleare, Anthony J.; Altemus, Bourgeois Buysse Candy Cassidy Cevik Chalder Clauw Cleare Cordon-Cardo DeKloet et al.	Objective: Enhancement of negative feedback control of the HPA axis in patients with chronic fatigue syndrome (CFS) has been reported using the low dose dexamethasone suppression test. We have developed the use of prednisolone (5 mg) as a more physiologically appropriate alternative to dexamethasone in the investigation of mild degrees of glucocorticoid resistance or supersensitivity. The objective of the study was to use this test to look for alterations in negative feedback control of the HPA axis in CFS patients. Methods: Fifteen patients with CFS were recruited after fulfilling strict criteria including the absence of comorbid psychiatric diagnosis. They collected urine between 0900 and 1800 h and saliva at 0900 h pre-prednisolone. At midnight, they took prednisolone (5 mg) orally and then collected urine and saliva at the same intervals the following day. Results: Salivary cortisol was lower in CFS subjects pre-prednisolone than controls. Urinary cortisol metabolites were lower in CFS subjects pre-prednisolone, but did not reach significance. Both measures were significantly lower in CFS subjects post-dose. Mean percentage suppression of both salivary cortisol and urinary cortisol metabolites was significantly higher in CFS compared to controls.	2007	Psychoneuroendocrinology	#440	Exclusion reason: No leptin in article; Michael Musker (2020-04-20 23:37:15)(Select): Exclude: only has a reference to Cleare paper - no other mention of leptin in abstract or article.;

71	Myalgic encephalomyelitis: Symptoms and biomarkers	Jason, Leonard A.; Zinn, Marcie L.; Zinn, Mark A.; Aalbers, Agliari Ali Anastasi Armitage Arnett Arnold Arnsten Bachstetter Badawy Badham Bakheit Baram et al.	Myalgic Encephalomyelitis (ME) continues to cause significant morbidity worldwide with an estimated one million cases in the United States. Hurdles to establishing consensus to achieve accurate evaluation of patients with ME continue, fueled by poor agreement about case definitions, slow progress in development of standardized diagnostic approaches, and issues surrounding research priorities. Because there are other medical problems, such as early MS and Parkinson's Disease, which have some similar clinical presentations, it is critical to accurately diagnose ME to make a differential diagnosis. In this article, we explore and summarize advances in the physiological and neurological approaches to understanding, diagnosing, and treating ME. We identify key areas and approaches to elucidate the core and secondary symptom clusters in ME so as to provide some practical suggestions in evaluation of ME for clinicians and researchers. This review, therefore, represents a synthesis of key discussions in the literature, and has important implications for a better understanding of ME, its biological markers, and diagnostic criteria. There is a clear need for more longitudinal studies in this area with larger data sets, which correct for multiple testing. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2015	Current Neuropharmacology	#442	Exclusion reason: No results provided; Michael Musker (2020-04-20 23:33:51)(Select): Exclusion: only 1 reference to leptin when referring to Stringer et al study. No data provided.;

72	Defining essential features of myalgic encephalo myelitis and chronic fatigue syndrome	Jason, Leonard A.; Sunnquist, Madison; Brown, Abigail; Reed, Jordan; Arroll, Brenu Broderick Brown Brurberg Cairns Carruthers David Dowsett Euba Friedberg Fukuda et al.	Considerable debate surrounds the search for the defining features of patients with myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS). Current case definitions were created through clinical consensus. Failure to operationalize these case definitions has led to considerable variability in the identification of patients. In addition, some case definitions do not require cardinal symptoms of this illness, whereas other case definitions do require core symptoms of this illness, and these latter case criteria appear to identify a more impaired group of patients. Criterion variance is most likely to occur when operationally explicit criteria do not exist for diagnostic categories, or when there are varying criteria for contrasting case definitions, which is an impediment to the research in this field. To deal with this problem, it is possible to differentiate those that meet more loosely defined criteria from those that are more narrowly and defined, thus differentiating CFS from ME. In order to progress the search for biological markers and effective treatments, essential features need to be operationalized and broadly used to increase the probability that individuals included in samples have the same underlying illness. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2015	Journal of Human Behavior in the Social Environment	#443	Exclusion reason: No results provided; Michael Musker (2020-04-20 23:28:59)(Select): Exclude: this only mentions the stringer study and references it. No other mentions of leptin.;

73	Chronic fatigue immune dysfunction syndrome: Neuroendocrine alterations	Izgi, Hasan Basri; SofuogIu, Seher; Asdemir, Akif; Allain, Altemus Antoni Arlt Avgerinos Bakheit Barret-Connor Baschetti Baulieu Bou-Holaigah Brooks Brosnan Chaudhuri Checkley Cleare p et al.	Chronic fatigue immune dysfunction syndrome (CFIDS) is a heterogenous problem with an ambiguous origin and characterized by a severe disabling physical and mental fatigue that is exacerbating by minor strain. There has been a great deal of interest in neuroendocrinology on this challenging syndrome and neuroendocrinologic data obtained so far will be reviewed in this paper. Many studies had been performed to investigate the function of the hypothalamic-pituitary-adrenal (HPA) axis in CFIDS but the results are quite conflicting. Overall evidence of those neuroendocrinologic studies and hormonal treatment will be discussed in this article. Many of the HPA axis studies indicate a reduced cortisol output and symptom production are correlated in at least some CFIDS patients. There is some evidence for heightened negative feedback and changes in glucocorticoid receptor function for impaired ACTH and cortisol responses. Furthermore, a mutation in the gene which controls the production of corticosteroid-binding globulin (CBG) which is associated with complete loss of function of CBG was identified recently in CFIDS. However, there is no consensus on a specific dysfunction of HPA axis in CFIDS.	2006	Kronik Yorgunluk Immun Disfonksiyon Sendromu: Noroendokrin Degisiklikler.	#452	Exclusion reason: Foreign Language; Michael Musker (2020-04-20 23:25:19)(Select): Exclude: Title and abstract does not contain Leptin and article is not available through all sources. It is a turkish journal so is not in English.;

74	Leptin and mood disorders	Di Lorenzo, D.; Barbato, G.; Conte, F.; Costanzo, A.; Della Monica, C.; Serio, M.; Ficca, G.; Almanza-Perez, Antonijevic Atmaca Balthasar Bjorbaek Brunetti Campfield Chehab et al.	Objectives: Leptin is an important factor involved in the regulation of feeding behavior, and its discovery has opened new perspectives in the study of obesity. Given that patients with a diagnosis of affective disorders frequently show appetite loss, weight decrease and dysfunctions of the hypothalamic-pituitary-adrenal axis, and that leptin seems to induce anorexia by interacting with specific neuroendocrine and neurotransmission systems involved in mood regulation, a possible relationship between leptin serum levels and mood disorders can be hypothesized. The aim of this article is to provide the reader with a comprehensive review addressing this issue. Methods: A systematic review of the literature on the relationships between leptin, ghrelin and mood disorders has been carried by searching PubMed for all studies on this topic in the period 1995-2010. Results: Interestingly, it was found that still very few studies have directly investigated leptin plasma levels in affective disorders. Significant reduction of leptin plasma levels in depressed patients has been occasionally reported, however other studies have not confirmed this finding (Table I). Conclusions:	2011	Leptina e disturbi dell'umore.	#606	Exclusion reason: Foreign Language; Michael Musker (2020-04-19 18:59:23)(Select): Exclude: only the abstract is available in English and it focuses on affective disorders. However the references listed in the table may be worth looking up.;

75	A Systematic Review of Drug Therapies for Chronic Fatigue Syndrome/ Myalgic Encephalo myelitis	Collatz, Ansel; Johnston, Samantha C.; Staines, Donald R.; Marshall-Gradisnik, Sonya M.	Purpose The pathogenesis of chronic fatigue syndrome or myalgic encephalomyelitis (CFS/ME) is complex and remains poorly understood. Evidence regarding the use of drug therapies in CFS/ME is currently limited and conflicting. The aim of this systematic review was to examine the existing evidence on the efficacy of drug therapies and determine whether any can be recommended for patients with CFS/ME. Methods MEDLINE, EMBASE, and PubMed databases were searched from the start of their records to March 2016 to identify relevant studies. Randomized controlled trials focusing solely on drug therapy to alleviate and/or eliminate chronic fatigue symptoms were included in the review. Any trials that considered graded exercise therapy, cognitive behavior therapy, adaptive pacing, or any other nonpharmaceutical treatment plans were excluded. The inclusion criteria were examined to ensure that study participants met specific CFS/ME diagnostic criteria. Study size, intervention, and end point outcome domains were summarized. Findings A total of 1039 studies were identified with the search terms; 26 studies met all the criteria and were considered suitable for review. Three different diagnostic criteria were identified: the Holmes criteria, International Consensus Criteria, and the Fukuda criteria.	2016	Clinical Therapeu tics	#643	Exclusion reason: No results provided; Michael Musker (2020-04-19 18:45:16)(Select): exclude: no results provided for Leptin;

76	Physical or mental fatigue and immunode pression	Castell, Linda M.; Acworth, Bailey Bardwell Blomstrand Brown Budgett Calders Cannon Carmichael Castell Gleeson et al.	Fatigue is an integral component of illness and sickness behavior. This chapter reviews possible links between fatigue, mood states, immunodepression, and the incidence of illness, particularly upper respiratory tract infections. In terms of fatigue, our work has focused mainly on the biochemical markers (tryptophan, branched chain amino acids) involved in central fatigue in endurance athletes (with/without unexplained underperformance syndrome) in chronic fatigue syndrome, and in postoperative fatigue. We have also looked at tryptophan from a different perspective by undertaking a functional magnetic resonance imaging (fMRI) study on the brain in healthy humans, with/without tryptophan supplementation, undertaking a cognition function task. The availability of tryptophan has consequences for immune function. In prolonged exercise, mucosal protection may be impaired as a consequence of oronasal breathing. Individuals involved in our studies have often had a high incidence of minor illnesses, particularly upper respiratory tract infections (URTI), after prolonged, exhaustive exercise such as a marathon race, or intensive training.	2008	Fatigue science for human health.	#680	Exclusion reason: No results provided; Michael Musker (2020-04-19 17:31:23)(Select): Exclude this is a book chapter that discusses the concepts, looking at the preview of the chapter available here, no leptin results provided: https://link.springer.com/chapter/10.1007/978-4-431-73464-2_13 ;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
77	Evaluation of serum leptin, basal cortisol, basal dehydroepiandrosterone sulfate levels in primary fibromyalgia and their association with psychological status and quality of life	Çaliş, M. Tufan, Ö Akgül, Ö Bahadir, N. Çaliş, H. T.	Objective: The aim of this study is to investigate leptin, basal cortisol, basal dehydroepiandrosterone sulfate (DHEAS) levels in female patients with fibromyalgia and their relationship with psychological status and quality of life. Materials-Methods: The study was performed in 49 female patients and 35 healthy controls aged between 20-55 years. Serum leptin, basal cortisol and basal DHEAS levels were compared between patient and control group. The patients and the controls were assessed using Hamilton Anxiety Rating Scale, Hamilton Depression Scale, SCL90-R (Symptom Check List), Fibromyalgia Impact Questionnaire, Visual Analog Scale (VAS), Modified Fatigue Impact Scale, Nottingham Health Profile. The relationship of these scales with leptin, cortisol and DHEAS levels were investigated Results: There were no significant differences in the mean levels of basal cortisol and DHEAS between the fibromyalgia and control groups ($p>0.05$), but leptin levels were significantly higher in patients with primary fibromyalgia ($p<0.05$). HAM-A scores were positively correlated with the leptin levels in fibromyalgia group ($p<0.05$).	2013	Turkiye Fiziksel Tip ve Rehabilitasyon Dergisi	#690	Exclusion reason: No results provided; Michael Musker (2020-04-19 17:22:20)(Select): Exclude: This is only and abstract and states that leptin levels were significantly higher in patients with primary fibromyalgia ($p<0.05$) but provides no results.;

78	Guidelines for biomarkers in autoimmune rheumatic diseases - evidence based analysis	Giacomelli, Roberto; Afeltra, Antonella; Alunno, Alessia; Bartoloni-Bocci, Elena; Berardicurti, Onorina; Bombardieri, Michele; Bortoluzzi, Alessandra; Caporali, Roberto; Caso, Francesco; Cervera, Ricard; Chimenti, Maria Sole; et al.	Autoimmune rheumatic diseases are characterised by an abnormal immune system response, complement activation, cytokines dysregulation and inflammation. In last years, despite many progresses in managing these patients, it has been shown that clinical remission is reached in less than 50% of patients and a personalised and tailored therapeutic approach is still lacking resulting in a significant gap between guidelines and real-world practice. In this context, the need for biomarkers facilitating early diagnosis and profiling those individuals at the highest risk for a poor outcome has become of crucial interest. A biomarker generally refers to a measured characteristic which may be used as an indicator of some biological state or condition. Three different types of medical biomarkers has been suggested: i. mechanistic markers; ii. clinical disease markers; iii. therapeutic markers. A combination of biomarkers from these different groups could be used for an ideal more accurate diagnosis and treatment. However, although a growing body of evidence is focused on improving biomarkers, a significant amount of this information is not integrated on standard clinical care. The overarching aim of this work was to clarify the meaning of specific biomarkers during autoimmune diseases; their possible role in confirming diagnosis, predicting outcome and suggesting specific treatments.	2019	Autoimmun Rev	#526	Exclusion reason: Wrong patient population; Michael Musker (2020-04-20 12:39:23)(Select): Exclude: only a brief mention of Leptin in 4.2 of article, but not relevant.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
79	Some Unresolved Issues for the Study of Fatigue: The Way Forward	Gerber, Lynn H.	This section of the supplement “Fatigue: A New Frontier” will discuss 3 issues that have significant import for elucidating mechanisms by which fatigue occurs and its evaluation and treatment. These issues are clearly not the only ones that are important for future research or clinical practice, but they are the most prevalent issues raised in the articles of this supplement. They present significant challenges in both research and clinical arenas and offer substantial research opportunity. There exists a body of published data that leave a number of unresolved key issues. These include: 1) What are our best metrics for measuring fatigue? 2) Do the constructs of peripheral and central fatigue advance our understanding of fatigue syndromes or create arbitrary limitations? 3) Will a better understanding of the interactions among immunoregulation and metabolic control of energy production and utilization (and performance and perception) help us derive a comprehensive approach to the management of fatigue?	2010	PM&R	#529	Exclusion reason: No results provided; Michael Musker (2020-04-20 12:34:33)(Select): Exclude: This article makes only 1 reference to Leptin but provides no results 'Further, linkages between the pro-inflammatory cytokines and prostaglandins, substance P, and leptins suggest significant communication between the central nervous system and regulation of inflammation' - more of an opinion piece.;

80	Organization and integration of the endocrine system: The arousal and sleep perspective	Chrousos, George P.; Abou-Samra, Aghajanian Aguilera Akira Albanese Albelson Andreis Antoni Bardeleben Behan Beitins Bellinger Benarroch Benker Bernadini Bernton Besedovsky Gool et al.	This article focuses on the neuroendocrine infrastructure of the adaptive response to stress and on its effects on the major endocrine axes in the body. Also discussed is the altered regulation or dysregulation of the adaptive response in various physiologic and pathophysiologic states, which may influence the growth and development of an individual and define the vulnerability of this individual to endocrine, psychiatric, or immunologic disease. (PsycINFO Database Record (c) 2017 APA, all rights reserved)	2007	Sleep Medicine Clinics	#660	Exclusion reason: Wrong patient population; Michael Musker (2020-04-19 18:01:36)(Select): Exclude: only mentions the role of leptin in anorexia, but no results provided.;

81	Ictal adipokines are associated with pain severity and treatment response in episodic migraine	Chai, Nu Cindy; Gelaye, Bizu; Tietjen, Gretchen E.; Dash, Paul D.; Gower, Barbara A.; White, Linda W.; Ward, Thomas N.; Scher, Ann I.; Peterlin, B. Lee; Avraham, Banks Bedaiwy Berilgen Bernecker Brown Chai Considine Doherty Duarte Elias et al.	Objective: To evaluate ictal adipokine levels in episodic migraineurs and their association with pain severity and treatment response. Methods: This was a double-blind, placebo-controlled trial evaluating peripheral blood specimens from episodic migraineurs at acute pain onset and 30 to 120 minutes after treatment with sumatriptan/naproxen sodium vs placebo. Total adiponectin (T-ADP), ADP multimers (high molecular weight [HMW], middle molecular weight, and low molecular weight [LMW]), leptin, and resistin levels were evaluated by immunoassays. Results: Thirty-four participants (17 responders, 17 nonresponders) were included. In all participants, pretreatment pain severity increased with every quartile increase in both the HMW:T-ADP ratio (coefficient of variation [CV] 0.51; 95%confidence interval [CI]: 0.08, 0.93; p = 0.019) and resistin levels (CV 0.58; 95% CI: 0.21, 0.96; p = 0.002), but was not associated with quartile changes in leptin levels. In responders, T-ADP (CV 20.98; 95% CI: 21.88, 20.08; p = 0.031) and resistin (CV 20.95; 95%CI: 21.83, 20.07; p = 0.034) levels decreased 120 minutes after treatment as compared with pretreatment. In addition, in responders, the HMW:T-ADP r	2015	Neurology	#676	Exclusion reason: Wrong patient population; Michael Musker (2020-04-19 17:37:26)(Select): Exclude: this focuses on migrain treatment responses and is the wrong patient group.;

82	Leptin regulates dopamine responses to sustained stress in humans	Burghardt, Paul R.; Love, Tiffany M.; Stohler, Christian S.; Hodgkinson, Colin; Shen, Pei-Hong; Enoch, Mary-Anne; Goldman, David; Zubieta, Jon-Kar; Abercrombie, Amato Appelhans Arvaniti Baliki Berridge Born et al.	Neural systems that identify and respond to salient stimuli are critical for survival in a complex and changing environment. In addition, interindividual differences, including genetic variation and hormonal and metabolic status likely influence the behavioral strategies and neuronal responses to environmental challenges. Here, we examined the relationship between leptin allelic variation and plasma leptin levels with DAD2/3R availability in vivo as measured with [11C]raclopride PET at baseline and during a standardized pain stress challenge. Allelic variation in the leptin gene was associated with varying levels of dopamine release in response to the pain stressor, but not with baseline D2/3 receptor availability. Circulating leptin was also positively associated with stress-induced dopamine release. These results show that leptin serves as a regulator of neuronal function in humans and provides an etiological mechanism for differences in dopamine neurotransmission in response to salient stimuli as related to metabolic function. The capacity for leptin to influence stress-induced dopaminergic function is of importance for pathological states where dopamine is thought to play an integral role, such as mood, substance-use disorders, eating disorders, and obesity. (PsycINFO Database Record (c) 2016 APA, all rights reserved)	2012	The Journal of Neuroscience	#695	Exclusion reason: Wrong patient population; Michael Musker (2020-04-19 17:06:55)(Select): Exclude: this article compares leptin results with dopamine in pain release, but does not compare between a cohort (only males and females). It has details of Lep gene and variants.;

83	Altered immune pathway activity under exercise challenge in Gulf War Illness: An exploratory analysis	Broderick, Gordon; Ben-Hamo, Rotem; Vashishtha, Saurabh; Efroni, Sol; Nathanson, Lubov; Barnes, Zachary; Fletcher, Mary Ann; Klimas, Nancy	Though potentially linked to the basic physiology of stress response we still have no clear understanding of Gulf War Illness (GWI), a debilitating illness presenting with a complex constellation of immune, endocrine and neurological symptoms. Here we compared male GWI (n=20) with healthy veterans (n=22) and subjects with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) (n=7). Blood was drawn during a Graded eXercise Test (GXT) prior to exercise, at peak effort (VO2 max) and 4-h post exercise. Affymetrix HG U133 plus 2.0 microarray gene expression profiling in peripheral blood mononuclear cells (PBMCs) was used to estimate activation of over 500 documented pathways. This was cast against ELISA-based measurement of 16 cytokines in plasma and flow cytometric assessment of lymphocyte populations and cytotoxicity. A 2-way ANOVA corrected for multiple comparisons (q statistic <0.05) indicated significant increases in neuroendocrine-immune signaling and inflammatory activity in GWI, with decreased apoptotic signaling. Conversely, cell cycle progression and immune signaling were broadly subdued in CFS. Partial correlation networks linking pathways with symptom severity via changes in immune cell abundance, function and signaling were constructed.	2013	Brain, Behavior, and Immunity	#703	Exclusion reason: No results provided; Martin Lewis (2020-04-29 14:31:51)(Select): No leptin data; Michael Musker (2020-04-19 17:00:02)(Select): Exclude this article does contain CFS participants but does not analyse Leptin, it does however state that the immune response from CFS participants is subdued.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
84	Integrative model of chronically activated immune-hormonal pathways important in the generation of fibromyalgia	Breeding, P. C.; Russell, N. C.; Nicolson, G. L.	Clinicians are often challenged by patients presenting with a syndrome of chronic and diffuse full body pain with long standing fatigue and a cluster of related symptoms. Fibromyalgia has become the commonly accepted term for this syndrome. Diagnosis is established through recognized subjective symptoms, such as tender points and other indicators of chronic full body pain and fatigue. Suspected triggers have included bacterial and viral infections, toxins, allergens, and emotional and physical trauma. Unknown causes limit the prescription of effective treatments; however, neuropathic pain and fatigue have been identified as key components so dual reuptake inhibitors and anti-convulsants have shown some effectiveness for some patients. Based upon laboratory and clinical studies of the last decade, this article proposes a model for a subset of fibromyalgia patients who have prolonged immune activation with related oxidative and nitrogenous stress leading to multiple hormonal repression, disrupted collagen physiology, neuropathic pain and fatigue. This integrative model of fibromyalgia is based on chronic up-regulation of the immune system with subsequent hormonal, connective tissue and nervous system implications.	2012	British Journal of Medical Practitioners	#706	Exclusion reason: No results provided; Martin Lewis (2020-04-29 14:30:02)(Select): No data; Michael Musker (2020-04-19 16:53:02)(Select): This discusses Leptin in the context of metabolic syndrome and it's relationship with IL6 on page 2 but no results are provided.;

No.	Title	Authors	Abstract	Publi Year	Journal	Covidence #	Reasons for exclusion following consensus
85	The neuroendocrinology of chronic fatigue syndrome	Cleare, A. J.	Chronic fatigue syndrome (CFS) is a common and disabling problem; although most likely of biopsychosocial origin, the nature of the pathophysiological components remains unclear. There has been a wealth of interest in the endocrinology of this condition, which will be reviewed in this article. Most studied has been the hypothalamic-pituitary-adrenal (HPA) axis; although the quality of many studies is poor, the overall balance of evidence points to reduced cortisol output in at least some patients, with some evidence that this is linked to symptom production or persistence. There is evidence for heightened negative feedback and glucocorticoid receptor function and for impaired ACTH and cortisol responses to a variety of challenges. However, there is no evidence for a specific or uniform dysfunction of the HPA axis. Given the many factors that may impinge on the HPA axis in CFS, such as inactivity, sleep disturbance, psychiatric comorbidity, medication, and ongoing stress, it seems likely that HPA axis disturbance is heterogeneous and of multifactorial etiology in CFS. Studies assessing GH, dehydroepiandrostenedione and its sulfate, melatonin, leptin, and neuroendocrine-monoamine interactions are also reviewed.	2003	Endocr Rev	#653	Exclusion reason: No results provided; Michael Musker (2020-04-19 18:18:38)(Select): Exclude: it mentions another study (ref83) but has no leptin results. Article lists other results such as cortisol comparisons;